www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article
DOI: 10.46309/biodicon.2026.1719691
19/1 (2026) 46-54

Nano-enabled agriculture: Effects of graphene on the development of Bread Wheat (*Triticum aestivum* L.) genotypes

Murat OLGUN [1], Suat PAT [2], Murat ARDIÇ [3], Okan SEZER [*3]
ORCID: 0000-0001-6981-4545**;** 0000-0001-9301-8880**;** 0000-0001-8734-3038**;** 0000-0001-7304-1346

[1] Eskişehir Osmangazi University, Agricultural Faculty, Field Crop Department, 26040 Eskişehir, Türkiye
[2] Eskişehir Osmangazi University, Faculty of Science, Department of Physics, 26040 Eskişehir, Türkiye
[3] Eskişehir Osmangazi University, Faculty of Science, Department of Biology, 26040 Eskişehir, Türkiye

Abstract

Purpose: This study aimed to investigate the effects of graphene oxide (GO) seed coating on the morphological, physiological, and spectral characteristics of bread wheat (*Triticum aestivum* L.) genotypes. The objective was to assess whether GO applications could enhance early-stage plant growth, photosynthetic efficiency, and overall plant health, and to determine genotype-specific responses to this nanotechnological intervention.
Method: The experiment was conducted using six bread wheat genotypes (Nacibey, Rumeli, KateA, Ekiz, Esperia, Ahmetağa, and Sönmez) under controlled laboratory conditions at Eskişehir Osmangazi University. A completely randomized design with three replications was used. Seeds were surface-sterilized and coated with powdered graphene oxide, while control groups received no coating. Various morphological (root, stem, leaf height and biomass), physiological (SPAD values), and spectral (NDVI, SR, OSAVI, PRI, SIPI) parameters were measured. Statistical analyses were performed using JMP 5.0 software and LSD tests.
Findings: Graphene oxide seed coating significantly enhanced several physiological and spectral parameters, including SPAD, NDVI, SR, OSAVI, PRI, and SIPI, indicating improved chlorophyll content, photosynthetic capacity, and stress resilience. Among the genotypes, Ekiz showed the highest photosynthetic efficiency, while Sönmez exhibited notable improvements in root and leaf biomass. The Rumeli genotype demonstrated superior vegetative growth. However, the seed coating had limited effects on certain morphological traits such as root height and stem biomass, which appeared to be more genotype-dependent. Significant genotype × coating interactions were observed in several parameters, highlighting the importance of genetic background in response to treatment.
Conclusion: Graphene oxide seed coating emerges as a promising nanotechnological tool to enhance early growth and photosynthetic traits in bread wheat. Its effectiveness, however, varies with genotype, underlining the need for genotype-specific application strategies. While GO improved plant vitality and photosynthetic efficiency, especially in genotypes like Ekiz and Sönmez, optimal dosages and further research under field conditions are necessary to ensure safe and sustainable use in agriculture.

Keywords: graphene oxide, seed coating, wheat genotypes, spectral indices

---------- * ----------

Nano-teknoloji destekli tarım: Grafenin ekmeklik buğday (*Triticum aestivum* L.) genotiplerinin gelişimi üzerine etkileri

Özet

Amaç: Bu çalışmanın amacı, grafen oksit (GO) ile yapılan tohum kaplamasının ekmeklik buğday (*Triticum aestivum* L.) genotiplerinin morfolojik, fizyolojik ve spektral özellikleri üzerindeki etkilerini araştırmaktır. Çalışma ile GO

---

[*] *Corresponding author*: Tel.: +902222393750/2840; Fax.: +902222393578; E-mail: oksezer@ogu.edu.tr
Received: 14.06.2025; Published: 15.03.2026 BioDiCon. 1204-140625

uygulamalarının erken dönem bitki gelişimini, fotosentetik verimliliği ve genel bitki sağlığını artırıp artırmadığı ve bu uygulamaya genotiplerin verdikleri yanıtların belirlenmesi hedeflenmiştir.
Metod: Deneme, Eskişehir Osmangazi Üniversitesi'nde kontrollü laboratuvar koşullarında altı ekmeklik buğday genotipi (Nacibey, Rumeli, KateA, Ekiz, Esperia, Ahmetağa ve Sönmez) kullanılarak yürütülmüştür. Araştırma, üç tekerrürlü tesadüf parselleri deneme desenine göre planlanmıştır. Tohumlar yüzey sterilizasyonunun ardından grafen oksit tozu ile kaplanmış, kontrol grubundaki tohumlara ise kaplama uygulanmamıştır. Kök, gövde ve yaprak uzunluğu ve biyokütle gibi morfolojik özellikler; SPAD değerleri gibi fizyolojik ve NDVI, SR, OSAVI, PRI, SIPI gibi spektral parametreler ölçülmüştür. Veriler JMP 5.0 yazılımı ile analiz edilmiş ve LSD testi kullanılmıştır.
Bulgular: Grafen oksit tohum kaplaması, SPAD, NDVI, SR, OSAVI, PRI ve SIPI gibi fizyolojik ve spektral parametrelerde anlamlı artış sağlamıştır. Bu artışlar, klorofil içeriği, fotosentetik kapasite ve stres dayanıklılığının arttığını göstermektedir. Genotipler arasında, Ekiz genotipi en yüksek fotosentetik verimliliği sergilerken, Sönmez genotipi kök ve yaprak biyokütlesinde önemli gelişmeler göstermiştir. Rumeli genotipi ise özellikle vejetatif büyüme (yaprak ve gövde uzunluğu) açısından öne çıkmıştır. Öte yandan, kök uzunluğu ve gövde biyokütlesi gibi bazı morfolojik özellikler üzerinde kaplamanın sınırlı etkisi olmuş; bu özelliklerin daha çok genotipe bağlı olduğu görülmüştür. Bazı özelliklerde tohum kaplaması ile genotip arasındaki etkileşim anlamlı çıkmıştır; bu durum, genetik yapının tedaviye verilen yanıtta belirleyici olduğunu ortaya koymaktadır.
Sonuç: Grafen oksit tohum kaplaması, ekmeklik buğdayda erken büyüme ve fotosentetik özellikleri iyileştiren umut verici bir nanoteknolojik uygulama olarak öne çıkmaktadır. Ancak uygulamanın etkinliği genotipe bağlı olarak değiştiğinden, genotip özelinde stratejiler geliştirilmelidir. Ekiz ve Sönmez genotiplerinde olumlu etkiler görülmekle birlikte, grafen oksitin tarımda güvenli ve sürdürülebilir şekilde kullanılabilmesi için uygun dozların belirlenmesi ve farklı çevre koşullarında yapılacak ilave çalışmalara ihtiyaç vardır.

Anahtar kelimeler: grafen oksit, tohum kaplama, buğday genotipleri, spektral indeksler

1. Introduction

Bread wheat (*Triticum aestivum* L.) is one of the cornerstone crops for global food security, serving as a fundamental source of carbohydrates and proteins in human nutrition [1]. According to Shewry and Hey (2015), wheat provides approximately 20% of the protein intake in the human diet and accounts for more than 50% of daily caloric intake, particularly in developing countries [2]. Based on FAO (2023), global wheat production reached 781 million tons in 2023 season, representing 29% of total global cereal production [1]. The phenological development of wheat is closely linked to the concept of thermal time (degree-days) [3].

Modern wheat breeding programs focus on developing varieties that are adaptable to climate change [4]. Reynolds et al. (2012) documented that varieties with high water-use efficiency can achieve 15–20% higher yields under drought conditions [5]. Among molecular breeding techniques, genomic selection plays a critical role in accelerating wheat improvement [4]. Local varieties developed under rainfed conditions possess a high capacity for adaptation to climate variability; however, molecular breeding techniques are necessary to improve yield stability. Agronomic practices in wheat cultivation such as proper soil preparation, sowing time, fertilization, and irrigation strategies directly affect both yield and quality. In recent years, there has been a growing interest in the use of nanomaterials, such as graphene oxide, to enhance wheat productivity. Ren et al. (2020) demonstrated that graphene oxide (GO) applications at appropriate dosages can improve seed germination rates by 15–30%. Graphene has shown positive effects on root development, enhancing the plant's ability to uptake water and nutrients [6]. Its protective effect under stress conditions has also been investigated. Zhao et al. (2022) found that graphene oxide applications increased plant resilience under salinity and drought stress. Studies on yield enhancement have shown promising results [7]. Chen et al. (2018) demonstrated that graphene oxide can enhance wheat seedling development at low doses, but may lead to phytotoxic effects at higher concentrations [8]. Li et al. (2018) found that GO and its derivatives reduce the contents of protein, starch, and minerals key components determining wheat grain quality while increasing the level of soluble sugars. These effects are particularly evident at high concentrations [9]. Moreover, further research is needed to assess the environmental impact and cost-effectiveness of graphene oxide use.

In conclusion, to increase yield and quality in bread wheat cultivation, it is essential to develop climate-resilient varieties, promote precision agriculture technologies, adopt soil health-preserving practices, implement integrated nutrient management strategies, and optimize the use of nanotechnological approaches such as graphene. Multidisciplinary research in this field is of great importance for the future of sustainable wheat production. This study investigates the effects of graphene oxide (GO) seed coating on the morphological, physiological, and spectral characteristics of bread wheat (*Triticum aestivum* L.) genotypes.

2. Materials and methods

This study was conducted in the laboratories of the Faculty of Science, Department of Biology, at Eskişehir Osmangazi University (ESOGÜ). Six bread wheat (*Triticum aestivum* L.) genotypes (Nacibey, Rumeli, KateA, Ekiz, Esperia, Ahmetağa and Sönmez) were used in the experiment. Room temperature was maintained between 24-26°C throughout the study. The experiment was arranged in a completely randomized design with three replications. Pots measuring 30 × 30 cm were filled with a soil mixture composed of 50% peat and 50% soil. Ten wheat seeds were sown in each pot, and after germination, seedlings were thinned to five plants per pot. Along with sowing, 1.5 g of pure P_2O_5 and 1.5 g of pure N were applied to each pot. Plants were irrigated every five days to maintain vitality. The pots were placed into growth chambers providing a light intensity of 300-500 µmol m^{-2} s^{-1} (light/dark-16/8 hours) [10], and the entire study was carried out within these chambers. Plants were harvested 40 days after sowing. The growth chamber was divided into two sections: one for seed coating and one as a control. The ion generator was placed inside the treatment chamber at a distance of 50-60 cm from the plants. In the experiment, wheat seeds (*Triticum aestivum* L.) were first subjected to surface sterilization by immersion in 70% ethanol for 1 minute, followed by treatment with a 2% sodium hypochlorite solution for 10 minutes. The seeds were then thoroughly rinsed with sterile distilled water. After sterilization, GO solution of 400 mg L^{-1} made by dissolving GO powder in ultrapure distilled water using ultrasonication at 20 kHz for 4 hours. The grains were soaked for 24 hours before planting in pots [11]. The control group seeds were only exposed to distilled water under the same conditions. Following the treatment, both the control group and the graphene oxide coated seeds were sown into pots containing soil. All pots were maintained under identical environmental conditions for further observation and evaluation of plant growth. Measurements were taken for root biomass [12], stem biomass [12], leaf biomass [12], NDVI (Normalized Difference Vegetation Index) [13, 14], SR (Simple Ratio Index) [14], OSAVI (Optimized Soil-Adjusted Vegetation Index) [14], PRI (Photochemical Reflectance Index) [15], SIPI (Structure Insensitive Pigment Index) [16], and SPAD (Soil Plant Analysis Development) [17]. Data obtained from the experiment were subjected to statistical analysis using the JMP 5.0 software package and compared using the LSD (Least Significant Difference) test.

3. Results

The use of graphene oxide and its derivatives in agriculture has attracted significant attention in recent years. In particular, graphene oxide (GO) and reduced graphene oxide (rGO) are being tested on various plant species due to their potential to either promote or inhibit plant growth. Graphene oxide applications represent an innovative approach to enhancing yield and stress tolerance in wheat cultivation. When applied at controlled doses, GO accelerates seed germination, supports root development, and promotes overall plant growth. Derivatives such as graphene oxide facilitate water and nutrient uptake, thereby increasing resistance to drought and salt stress. Moreover, GO improves the soil's water retention capacity and provides protection against plant diseases through its antimicrobial properties.

However, excessive doses may cause toxic effects, highlighting the need to determine optimal and safe application rates. While further research is needed to support the widespread adoption of this technology in agriculture, it is clear that graphene oxide holds great promise for wheat production. The effects of graphene oxide application on the morphological, physiological, and spectral characteristics of bread wheat genotypes are presented in Table 1.

Table 1. Effects of graphene oxide application on selected characteristics of bread wheat (*Triticum aestivum* L.) genotypes

	D.F.	**Stem Height**		**Root Height**		**Leaf Height**		**Root Weight**	
		MS	**F_{Value}**	**MS**	**F_{Value}**	**MS**	**F_{Value}**	**MS**	**F_{Value}**
Seed Coating	**1**	15,363	23,197**	0,412	0,055ns	28,749	2,134ns	0,004	3,571ns
Error$_1$	**28**	0,662		7,447		13,471		0,001	
Genotypes	**6**	6,840	6,763**	74,191	14,211**	81,814	4,965**	0,022	19,527**
Seed Co. x Gen	**6**	1,924	1,902ns	44,127	8,452**	92,467	5,612**	0,006	5,423**
Error$_2$	**168**	1,011		5,221		16,477		0,001	
Total	**209**	1,227		8,593		20,190		0,002	
C.V. (%)	**209**		**19,719**		**31,164**		**23,106**		**82,044**

	D.F.	**Stem Weight**		**Leaf Weight**		**SPAD**		**NDVI**	
		MS	**F_{Value}**	**MS**	**F_{Value}**	**MS**	**F_{Value}**	**MS**	**F_{Value}**
Seed Coating	**1**	0,003	0,523ns	0,001	0,014ns	110,110	8,167**	0,017	16,094**
Error$_1$	**28**	0,005		0,001		13,483		0,001	
Genotypes	**6**	0,009	1,545ns	0,010	5,998**	54,432	4,615**	0,014	5,967**
Seed Co. x Gen	**6**	0,005	0,846ns	0,003	1,887ns	80,857	6,856**	0,007	3,057**
Error$_2$	**168**	0,006		0,002		11,794		0,002	
Total	**209**	0,929		0,002		15,697		0,003	
C.V. (%)			**130,135**		**36,001**		**12,096**		**10,697**

	D.F.	**SR**		**OSAVI**		**PRI**		**SIPI**	
		MS	**F_{Value}**	**MS**	**F_{Value}**	**MS**	**F_{Value}**	**MS**	**F_{Value}**
Seed Coating	**1**	0,832	16,314**	0,016	13,020**	0,001	11,334**	0,006	6,933*
Error$_1$	**28**	0,051		0,001		0,000		0,001	
Genotypes	**6**	0,711	6,208**	0,018	6,913**	0,001	2,595*	0,013	6,618**
SeCo x Gen	**6**	0,376	3,283**	0,009	3,410**	0,001	1,657ns	0,006	3,184**
Error$_2$	**168**	0,115		0,003		0,001		0,002	
Total	**209**	0,134		0,003		0,001		0,002	
C.V. (%)			**12,684**		**11,565**		**40,227**		**9,031**

The seed coating treatment showed significant effects only on certain traits, including stem height, SPAD value (reflecting chlorophyll content), NDVI (indicative of plant vigor), and some spectral indices (SR, OSAVI, PRI, and

SIPI). These findings suggest that seed coating is particularly effective in influencing early-stage plant growth and physiological development. The application significantly increased stem height (F = 23.197**), which indicates that the coating may facilitate water uptake by the seed, thus accelerating early post-germination growth. Halmer (2008) noted that seed coatings can enhance water retention capacity, thereby supporting germination and seedling development [18]. Both SPAD and NDVI values were significantly affected by the seed coating application (F = 8.167** and F = 16.094**, respectively). This implies a positive influence of coating on photosynthetic capacity and an improvement in chlorophyll synthesis and general plant health. Taylor et al. (1998) reported that micronutrient-enriched seed coatings support early chlorophyll production and plant vigor [19]. Among the spectral indices, SR (Simple Ratio), OSAVI (Optimized Soil Adjusted Vegetation Index), PRI (Photochemical Reflectance Index), and SIPI (Structure Insensitive Pigment Index) were also significantly influenced by seed coating. These indices are associated with plant water status, photosynthetic activity, and pigment content. The coatings appear to enhance plant resilience to environmental stress, which is reflected in the spectral responses. Beeri & Peled (2006) emphasized that spectral indices respond sensitively to agricultural practices [20].

In contrast, seed coating had no statistically significant effect on morphological traits such as root length, leaf length, root weight, stem and leaf biomass. This suggests that the treatment mainly supports early shoot growth (e.g., stem height), but does not independently influence more complex developmental processes such as root growth or biomass accumulation. These processes are known to be more affected by genotype-environment interactions. The genotype factor, however, showed statistically significant effects on nearly all measured traits. Significant differences were observed between genotypes in morphological traits such as root height, leaf height, root and leaf weight, as well as in physiological traits like SPAD and NDVI, and all spectral indices. These findings highlight the central role of genetic variation in plant physiology and demonstrate that different genotypes respond differently to environmental or treatment-based variables. Chaves et al. (2009) emphasized that genotypic differences play a key role in determining plant yield and physiological responses under drought and other environmental stress conditions [21].

The interaction between seed coating and genotype (Seed Coating × Genotype) was statistically significant for certain parameters, including root height, leaf height, root weight, SPAD, NDVI, SR, OSAVI, and SIPI. This indicates that the response to seed coating is genotype-specific, and some genotypes may benefit more than others from the treatment. Such interactions reveal that genetic background is a determining factor in the success of agricultural practices. Different genotypes exhibit variability in both morphological and physiological responses to seed coating applications. Moreover, genetic diversity significantly influences the physiological defense mechanisms plants develop against abiotic stress. In summary, this analysis demonstrates that seed coating is particularly effective on physiological and spectral parameters, but its effectiveness is shaped by genotypic differences. Therefore, developing genotype-specific strategies in agricultural applications is important for increasing the effectiveness of coating technologies. While genotype effects dominate most morphological traits, physiological and spectral responses are shaped by both seed coating and genotype, and in some cases, synergistic effects arise from the interaction of these two factors. The mean values of the traits examined in bread wheat genotypes exposed to graphene oxide treatment are presented in Table 2.

In this study, the effects of seed coating application and different genotypes on various morphological, physiological, and spectral traits of wheat plants were investigated. Additionally, the interaction between these two factors and its impact on the variation of these traits was analyzed. The results indicated that seed coating application, particularly, led to significant improvements in physiological and spectral parameters, there were meaningful differences between genotypes, and genotype × application interaction was significant for certain traits. Regarding morphological traits, the seed coating application did not have a significant effect on stem height, with no meaningful difference observed between coated and uncoated (control) groups. The average stem height was found to be 5.888 cm for coated seeds and 5.347 cm for the control group. Similarly, the effect of seed coating on root height was limited, with averages being very close to each other (9.450 cm for coated, 9.362 cm for control). However, when evaluated by genotype, the Rumeli genotype showed a significantly higher root height (13.813 cm) compared to others. These findings suggest that root and stem morphology are largely determined by genetic factors, and seed coating has a limited effect in these areas. As noted by Mo et al. (2020), morphological traits are shaped by the plant's genetic structure and show less response to environmental interventions [22]. A positive effect of seed coating application was observed for leaf height. With the coating, the average leaf height increased to 19.816 cm, while it remained at 19.076 cm in the control group. Among the genotypes, Esperia and Ahmetağa exhibited the highest values at 22.247 cm and 20,907 cm, respectively. Moreover, the genotype × seed coating interaction was significant for this trait. Seed coating has had negative effects on some genotypes. For example, the Sönmez genotype showed a significant decrease in leaf heigth, from 22.587 cm to 13.680 cm. This suggests that seed coating exerts differential effects on vegetative growth in different genotypes. Seed coating was also effective in biomass indicators such as root weight and leaf weight. In the coated group, root weight averaged 0.057 g, while in the control group, it was 0.049 g. The highest root weight was observed in the Rumeli genotype (0.120 g in control), followed by Sönmez (0.090 g in coated). Leaf weight was also higher in coated seeds (0.159 g for Ahmetağa) compared to the control (0.142 g for Rumeli). In particular, in the Esperia genotype, leaf weight increased from 0.085 g to 0.139 g with the coating. Halmer (2008) stated that seed coating regulates the relationship between seeds and the microbial environment, facilitating faster nutrient uptake and supporting biomass increase during early developmental stages [18]. Stem weight measurements indicate that the coating treatment caused a decrease in weight in four genotypes. The largest decrease occurred in KateA, from 0.082 g to 0.050 g. However, the coating treatment caused an increase in weight in two genotypes. A significant increase, particularly in the Esperia genotype, from 0.031 g to 0.093 g, is noticeable.

Table 2. Mean values of the examined traits in bread wheat genotypes exposed to graphene oxide treatment

		Stem Height			**Root Height**			**Leaf Height**	
	Seed Coat.	**Control**	**Mean**	**Seed Coat.**	**Control**	**Mean**	**Seed Coat.**	**Control**	**Mean**
Nacibey	5,340	5,720	4,843 **C**	10,333	7,920	9,717 **AB**	19,033	20,287	17,450 **BC**
Rumeli	6,107	6,173	5,857 **A**	13,813	9,347	8,947 **B**	20,533	19,607	21,267 **A**
KateA	5,267	6,200	6,010 **A**	8,807	8,087	11,920 **A**	19,947	20,267	19,287 **A-C**
Ekiz	6,407	4,347	5,770 **AB**	7,847	9,100	10,623 **AB**	19,040	15,867	20,257 **A**
Esperia	5,993	5,913	5,103 **BC**	9,973	10,027	8,930 **B**	22,247	18,040	20,077 **AB**
Ahmetağa	5,367	4,940	5,600 **AB**	11,900	9,053	6,937 **C**	20,907	20,207	16,973 **C**
Sönmez	5,000	5,867	6,137 **A**	5,787	9,693	8,770 **B**	13,680	22,587	20,813 **A**
Mean	5,888 **A**	5,347 **B**	5,617	9,450	9,362	9,406	19,816	19,076	19,446
L.S.D.(%)	**SeedCo.:**0,310;**Gen:**0,682			**Gen:**1,549;**SeedCo.x Gen:**0,310			**Gen:**2,752;**SeedCo.x Gen:**3,892		
		Root Weight			**Stem Weight**			**Leaf Weight**	
	Seed Coat.	**Control**	**Mean**	**Seed Coat.**	**Control**	**Mean**	**Seed Coat.**	**Control**	**Mean**
Nacibey	0,037	0,044	0,031 **C**	0,047	0,048	0,040	0,140	0,128	0,122 **B**
Rumeli	0,036	0,120	0,052 **BC**	0,045	0,056	0,070	0,097	0,142	0,133 **AB**
KateA	0,075	0,056	0,033 **C**	0,050	0,082	0,038	0,119	0,122	0,091 **C**
Ekiz	0,032	0,025	0,104 **A**	0,046	0,034	0,056	0,103	0,104	0,151 **A**
Esperia	0,059	0,029	0,049 **C**	0,093	0,031	0,045	0,139	0,085	0,121 **B**
Ahmetağa	0,087	0,023	0,073 **B**	0,057	0,039	0,083	0,159	0,123	0,116 **BC**
Sönmez	0,090	0,027	0,030 **C**	0,083	0,087	0,066	0,110	0,126	0,114 **BC**
Mean	0,057	0,049	0,053	0,053	0,061	0,057	0,121	0,120	0,121
L.S.D.(%)	**Gen:**0,023;**SeedCo.x Gen:**0,032						**Gen:**0,028		
		SPAD			**NDVI**			**SR**	
	Seed Coat.	**Control**	**Mean**	**Seed Coat.**	**Control**	**Mean**	**Seed Coat.**	**Control**	**Mean**
Nacibey	30,751	32,438	31,729 **BC**	0,489	0,481	0,463 **C**	2,958	2,872	2,771 **C**
Rumeli	29,567	36,055	31,854 **BC**	0,469	0,503	0,480 **BC**	2,791	3,032	2,865 **BC**
KateA	35,698	33,605	31,234 **C**	0,483	0,479	0,479 **BC**	2,897	2,860	2,854 **C**
Ekiz	36,238	32,707	34,995 **A**	0,525	0,437	0,513 **A**	3,242	2,584	3,117 **A**
Esperia	31,271	32,902	32,678 **A-C**	0,478	0,488	0,458 **C**	2,858	2,917	2,732 **C**
Ahmetağa	33,934	29,658	32,738 **A-C**	0,523	0,434	0,467 **C**	3,201	2,567	2,785 **C**
Sönmez	31,871	31,871	34,054 **AB**	0,454	0,488	0,507 **AB**	2,711	2,932	3,087 **AB**
Mean	33,479 A	32,031 B	32,755	0,490 **A**	0,472 **B**	0,481	2,950 **A**	2,824 **B**	2,887
L.S.D.(%)	**Seed Co**:1,400, **Gen:**2,329; **SeedCo.x Gen:**3,293			**Seed** Co:0,013, **Gen:**0,032; **SeedCo.x Gen:**0,046			**Seed Co:**0,086, **Gen:**0,230; **SeedCo.x Gen**:0,325		
	OSAVI			**PRI**			**SIPI**		
	Seed Coat.	**Control**	**Mean**	**Seed Coat.**	**Control**	**Mean**	**Seed Coat.**	**Control**	**Mean**
Nacibey	0,499	0,474	0,467 **C**	0,012	0,011	0,010 **a-c**	0,543	0,522	0,517 **B**
Rumeli	0,465	0,504	0,472 **C**	0,011	0,011	0,010 **ab**	0,519	0,549	0,525 **B**
KateA	0,472	0,482	0,475 **BC**	0,010	0,009	0,011 **ab**	0,526	0,531	0,528 **B**
Ekiz	0,522	0,435	0,518 **A**	0,012	0,007	0,012 **a**	0,573	0,491	0,564 **A**
Esperia	0,469	0,485	0,446 **C**	0,010	0,011	0,008 **c**	0,527	0,537	0,511 **B**
Ahmetağa	0,532	0,421	0,474 **BC**	0,012	0,007	0,010 **bc**	0,579	0,497	0,521 **B**
Sönmez	0,465	0,490	0,506 **AB**	0,010	0,010	0,011 **ab**	0,511	0,547	0,560 **A**
Mean	0,489 **A**	0,471 **B**	0,480	0,011 **B**	1,153 **A**	0,010	0,538 **a**	0,527 **b**	0,532
L.S.D.(%)	**Seed Co:**0,014, **Gen:**0,034; **SeedCo.x Gen:**0,049			**Seed Co:**0,001, **Gen:**0,002;			**Seed Co:**0,008, **Gen:**0,030; **SeedCo.x Gen:**0,043		

In terms of physiological traits, the SPAD value (leaf chlorophyll content) was positively influenced by seed coating. The average SPAD value in the coated seeds was 33.479, while in the control group, it was 32.031. Among the genotypes, the highest SPAD values were observed in Ekiz (34.995) and Sönmez (34.054). The genotype × application interaction was also significant for this parameter, with the SPAD value in the Ekiz genotype rising from 32.707 to 36.238 with seed coating. The increase in chlorophyll content indicates enhanced photosynthetic capacity and overall plant health [21]. Significant positive results were obtained for spectral indices such as NDVI, SR, OSAVI, PRI, and SIPI with the seed coating application. The NDVI (Normalized Difference Vegetation Index) value was 0.490 in the coated group and 0.472 in the control group. This increase suggests that the seed coating positively affected the green biomass and overall health of the plants. Among the genotypes, Ekiz (0.513) and Sönmez (0.499) exhibited the highest NDVI values. In the Ekiz genotype, seed coating increased the NDVI value from 0.437 to 0.525, showing a strong interaction. The Ekiz genotype led with a value of 2.584, and this value increased to 3.242 with coating. SR is an indicator of plant health based on the ratio of infrared to red light reflected by the leaves; higher values indicate greater photosynthetic activity [16]. The Ahmetağa genotype performed best in terms of OSAVI (0.532 in coated). A similar increase was observed for PRI (Photochemical Reflectance Index) and SIPI (Structure-Insensitive Pigment Index). PRI was 0.011 in the coated group and 1.153 in the control group, while SIPI was 0.538 in the coated group and 0.527 in the control group. PRI values increased with coating in all genotypes except one. The highest increase, with an increase of 0.005, was determined in Ekiz and Ahmetağa genotypes. Particularly in SIPI, the effect of coating increased the value in Ekiz and Ahmetağa genotypes. These indices reflect the balance of carotenoid and chlorophyll content in plants and light use efficiency.

In overall, the seed coating application was found to have significant effects on plant development, particularly on morphological traits such as leaf height, root and leaf weight, as well as physiological and spectral parameters like SPAD, NDVI, SR, OSAVI, PRI, and SIPI. While no significant difference was observed in traits like stem height and root height, seed coating particularly supported parameters that enhance the plant's photosynthetic capacity, pigment structure, and overall vegetative development. Several studies in the literature support these findings. For instance, Taylor et al. (1998) reported that seed coatings have positive effects on chlorophyll content and leaf development [23]. Similarly, Halmer (2008) emphasized that coating materials can increase water and nutrient uptake, thereby enhancing root and leaf biomass [18]. The increases in spectral indices such as NDVI and PRI align with the plant health evaluation criteria proposed by Sims and Gamon (2002) [16]. At the genotype level, the Ekiz genotype showed the highest values for physiological and spectral traits such as SPAD, NDVI, SR, PRI, and SIPI, making it the most superior genotype overall. Ekiz's particularly high performance in terms of photosynthetic efficiency and chlorophyll-carotenoid balance indicates that this variety responded well to the seed coating application. The Rumeli genotype stood out, especially in terms of leaf and stem height, gaining an advantage in vegetative growth. This highlights that genotypes can exhibit various strengths for different traits and can respond differently to environmental applications. Chaves et al. (2009) also noted that genetic diversity plays a determining role in plant responses to environmental stress factors [21]. In conclusion, seed coating application provides positive effects on many morphological and physiological parameters that support plant development. These effects vary depending on genotype, with some genotypes responding more effectively to this application. The Ekiz and Sönmez genotypes benefited the most from the seed coating, while the Rumeli genotype excelled in vegetative growth. Considering the potential of seed coating to enhance plant health and yield, it should be regarded as a significant agricultural practice.

3.1. PCA (Principal Component Analysis)

The PCA analysis and Path analysis, which explain the performance of the elements examined in bread wheat genotypes exposed to graphene oxide application, are provided in Table 3 and Figure 1. In this study, the PCA results show that the first two components together explain 65.8% of the total variance. This ratio indicates that the data can be represented in a two-dimensional plane without significant loss. The first principal component, PC_1, explains 50.1% of the total variance. Variables, showig a high positive correlation with PC_1, include spectral plant indices such as NDVI (0.402), SR (0.399), OSAVI (0.395), SIPI (0.393), PRI (0.362), and SPAD (0.243). These indices provide information about the plant's photosynthetic activity, green tissue density, stress level, and overall health. For example, NDVI (Normalized Difference Vegetation Index) is commonly used to assess the density and development of vegetation, while OSAVI (Optimized Soil Adjusted Vegetation Index) serves a similar purpose by minimizing soil effects. SR (Simple Ratio) and SIPI (Structure Insensitive Pigment Index) are related to chlorophyll and carotenoid pigment content. PRI (Photochemical Reflectance Index) is associated with light-use efficiency and is used to assess the plant's stress level. The SPAD value reflects the measurement of chlorophyll content and provides information about the plant's nitrogen status. The high loading values of these indices on the PC_1 component in PCA suggest that PC_1 is an axis directly related to the physiological condition of the plants, especially photosynthetic activity and chlorophyll density. OSAVI makes plant indices more sensitive by reducing the effect of soil background. Tucker (1979) demonstrated that NDVI is a reliable indicator for assessing plant health [13]. Also PRI is directly related to photosynthetic efficiency.

Table 3. PCA analysis explaining the performance of the elements examined in bread wheat genotypes exposed to graphene oxide application

	PC_1	**PC_2**	**Eigenanalysis of the Correlation Matrix**						
Eigenvalue	6,015	1,886	**Variables**	**PC_1**	**PC_2**	**Variables**	**PC_1**	**PC_2**
Proportion	0,501	0,157	**LeafHe**	0,172	0,498	NDVI	0,402	0,004
Cumulative	0,501	0,658	**RootWe**	0,171	-0,429	SR	0,399	-0,01
Variables	**PC_1**	**PC_2**	**StemWe**	0,075	-0,126	OSAVI	0,395	-0,051
StemHe	0,277	0,192	**LeafWe**	0,176	-0,015	PRI	0,362	0,035
RoorHe	0,079	0,583	**SPAD**	0,243	-0,412	SIPI	0,393	0,026

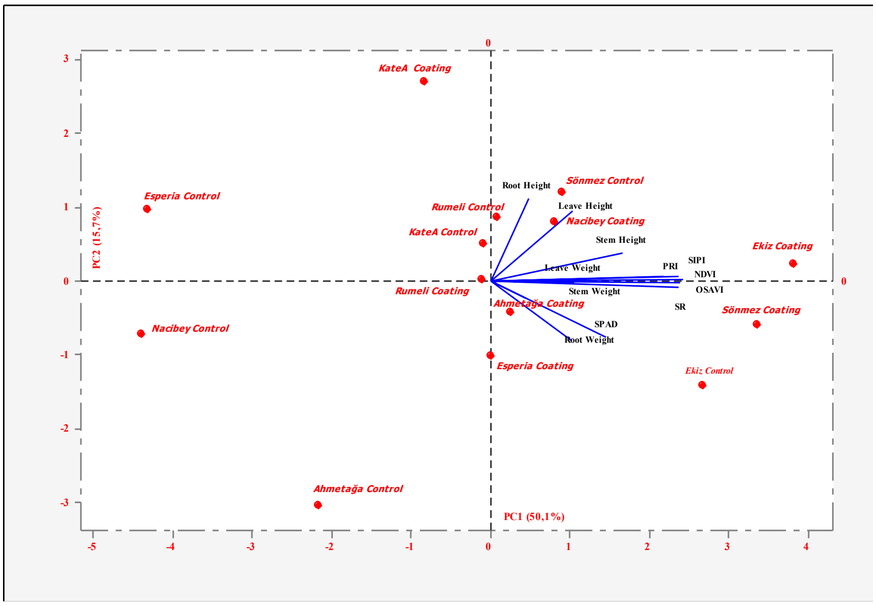

Figure 1. BiPlot graph in path analysis explaining the performance of the elements examined in bread wheat genotypes exposed to graphene oxide application

The second principal component, PC_2, explains 15.7% of the total variance. Variables that show a strong positive correlation with PC_2 including morphological characteristics such as LeafHe (0.498) and RootHe (0.583), while variables such as RootWe (-0.429) and SPAD (-0.412) have negative loadings. This indicates that PC_2 is particularly related to the plant's physical and structural development. Variables such as root and leaf height are morphological measures that directly reflect the plant's growth potential. However, the negative loading of SPAD on this component suggests that there may be an inverse relationship between morphological structure and chlorophyll content. Ali et al. (2007) noted that characteristics like plant height and leaf height interact with genetic variation and environmental factors and emphasized that these morphological measures are important variables in yield analysis [24]. Uddling et al. (2007) showed that measurements taken with the SPAD device provide reliable information about the chlorophyll content in plants and that this information is related to factors such as nitrogen status and stress [25]. Therefore, PC_2 can be considered a secondary significant component that represents the differentiation between the plant's structural size and physiological contents.

In the PCA analysis, the obtained PC_1 and PC_2 components together present a meaningful structure through plant indices and morphological measurements. Certain distinctions, found in the plant development, is highly valuable in agricultural studies for conducting holistic evaluations of plant development, stress analysis, and yield prediction. The use of PCA in this manner is widely recommended in the literature and provides significant results, especially in studies that combine spectral data with morphological measurements [23]. Graphene oxide seed coating is a promising nanotechnological approach to increase yield and quality in wheat farming. The positive effects observed, particularly in the Ekiz, Sönmez, and Rumeli genotypes, suggest that with targeted use, this technology could make significant contributions to the future of agricultural production. The biplot graph visualizes the positions of genotypes and measured traits according to two main components (PC_1 and PC_2), showing both the variation between genotypes and which variables contribute to this variation. This analysis is important for genetic selection, variety development, and identifying suitable genotypes. The biplot graph is a multivariate statistical analysis method that positions genotypes and measured traits according to two main components (PC_1 and PC_2). This graph is frequently used to evaluate the performance of genetic material, identify superior genotypes, and guide selection decisions. According to the biplot analysis, genotypes in each region show varying performances in specific traits. The evaluation of these genotypes is summarized below:

Genotype	**Superior Traits**	**Region**
Sönmez Coating, Ekiz Coating	Stem height, plant height, fresh weight, etc.	1st
Ekiz Control	Root weight, structural resistance	4th
KeteA Coating	Root height	2nd
Nacibey Control, Ahmetağa Control	Low performance	3rd

Results explain that, the genotypes Sönmez Coating, Nacibey Coating, and Ekiz Coating are taken into consideration as high-yielding and environmentally adaptive genotypes. These genotypes can be considered as primary materials in future breeding programs. Besides, low-performing genotypes can either be eliminated or be used breefing programmes under different environmental conditions to be evaluated.

4. Conclusions and discussion

This study thoroughly examined the effects of graphene oxide (GO) seed coating on the morphological, physiological, and spectral characteristics of bread wheat (*Triticum aestivum* L.) genotypes. The findings reveal that graphene oxide application notably improves parameters related to plant health and photosynthetic efficiency. Seed coating led to significant increases in morphological characteristics such as leaf height, root and leaf weight, as well as spectral indices like SPAD value (chlorophyll content), NDVI (plant vitality), SR, OSAVI, PRI, and SIPI. These results indicate that graphene oxide enhances boosts photosynthetic activity, and improves stress tolerance, enhancing water and nutrient uptake during the early development stage.

Among the genotypes, Ekiz, Nacibey, and Sönmez showed the higher performance by graphene oxide application. The Ekiz genotype demonstrated superior growth in photosynthetic efficiency with high SPAD, NDVI, and spectral index values, while the Sönmez genotype showed significant improvements in morphological features such as root and leaf weight. The Rumeli genotype excelled in vegetative growth parameters (leaf and stem height). These findings emphasize that genotypes respond positively and differently to graphene oxide application. One of the most striking aspects of this study is the strong effect of graphene oxide coating on spectral indices. The increase in NDVI and OSAVI suggests improvements in plant health, while changes in PRI and SIPI indicate enhanced photosynthetic efficiency and stress resistance. These results indicate that graphene oxide could be integrated with remote sensing technologies in agriculture to monitor plant health. It should be taken into consideration that over dosages of optimal level, suggested, may cause toxic effects, and cause detrimental effect on plant growth and health. Therefore, future studies investigating the effects of graphene oxide under different environmental conditions and across various genotypes are essential for advancing sustainable agricultural practices.

Conflicts of interest: No Conflict of Interest.

Funding: No Funding Received.

Ethical statement: This study does not require ethical approval.

Author contributions: Conceptualization, design, supervision, literature review, and critical revision: all authors; methodology, data collection, materials, and resources: M.O.; analysis, interpretation, and writing-original draft: M.O. and O.S. All authors read and approved the final manuscript.

References

[1] Food and Agriculture Organization (FAO). (2023). World food and agriculture: Statistical yearbook 2023. FAO. https://doi.org/10.4060/cc8166en

[2] Shewry, P.R. & Hey, S.J. (2015). The contribution of wheat to human diet and health. *Food and Energy Security*, 4(3), 178–202. https://doi.org/10.1002/fes3.64

[3] Porter, J.R. & Gawith, M. (1999). Temperatures and the growth and development of wheat: A review. *European Journal of Agronomy*, 10(1), 23–36. https://doi.org/10.1016/S1161-0301(98)00047-1

[4] Crossa, J., Pérez-Rodríguez, P., Cuevas, J., Montesinos-López, O., Jarquín, D., de los Campos, G. & Gianola, D. (2017). Genomic selection in plant breeding: Methods, models, and perspectives. *Trends in Plant Science*, 22(11), 961–975. https://doi.org/10.1016/j.tplants.2017.08.011

[5] Reynolds, M.P., Pask, A.J.D. & Mullan, D.M. (2012). *Physiological breeding I: Interdisciplinary approaches to improve crop adaptation*. CIMMYT.

[6] Ren, W., Chang, H., Li, L. & Teng, Y. (2020). Effect of graphene oxide on growth of wheat seedlings: Insights from oxidative stress and physiological flux. *Bulletin of Environmental Contamination and Toxicology*, 105, 139–145. https://doi.org/10.1007/s00128-020-02888-9

[7] Zhao, L., Wang, W., Fu, X., Liu, A., Cao, J. & Liu, J. (2022). Graphene oxide, a novel nanomaterial as soil water retention agent, dramatically enhances drought stress tolerance in soybean plants. *Frontiers in plant science*, 13, 810905. https://doi.org/10.3389/fpls.2022.810905

[8] Chen, J., Yang, L., Li, S. & Ding, W. (2018). Various physiological response to graphene oxide and amine-functionalized graphene oxide in wheat (*Triticum aestivum*). *Molecules*, 23(5), 1104. https://doi.org/10.3390/molecules23051104

[9] Li, X., Mu, L., Li, D., Ouyang, S., He, C. & Hu, X. (2018). Effects of the size and oxidation of graphene oxide on crop quality and specific molecular pathways. *Carbon*, 140, 352–361. https://doi.org/10.1016/j.carbon.2018.08.063

[10] Monostori, I., Heilmann, M., Kocsy, G., Rakszegi, M., Ahres, M., Altenbach, S.B., ... & Darko, É. (2018). LED lighting–modification of growth, metabolism, yield and flour composition in wheat by spectral quality and intensity. *Frontiers in Plant Science*, 9, 605. https://doi.org/10.3389/fpls.2018.00605

[11] Ibrahim, M. I. M., Awad, E. S. A. M., Dahdouh, S. M. M., & El-Etr, W. M. T. (2025). Graphene Oxide Effect on the Wheat Plant (Triticum aestivum) under Different Application and Concentration Techniques. Asian Soil Research Journal, 9(4), 38–54.

[12] Poorter, H., Niklas, K.J., Reich, P.B., Oleksyn, J., Poot, P. & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New phytologist*, 193(1), 30–50. https://doi.org/10.1111/j.1469-8137.2011.03952.x

[13] Tucker, C.J. (1979). Red and photographic infrared linear combinations for monitoring vegetation. *Remote Sensing of Environment*, 8(2), 127–150. https://doi.org/10.1016/0034-4257(79)90013-0

[14] Mulla, D.J. (2013). Twenty-five years of remote sensing in precision agriculture: Key advances and remaining knowledge gaps. *Biosystems Engineering*, 114(4), 358–371. https://doi.org/10.1016/j.biosystemseng.2012.08.009

[15] Gamon, J., Serrano, L. & Surfus, J S. (1997). The photochemical reflectance index: an optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient levels. *Oecologia*, 112, 492–501. https://doi.org/10.1007/s004420050337

[16] Sims, D.A. & Gamon, J.A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote sensing of environment*, 81(2-3), 337–354. https://doi.org/10.1016/S0034-4257(02)00010-X

[17] Maiti, D. & Das, D.K. (2006). Management of nitrogen through the use of Leaf Colour Chart (LCC) and Soil Plant Analysis Development (SPAD) in wheat under irrigated ecosystem: (Stickstoffbemessung mittels Blattfärbungstabelle (LCC) und analyse der Boden-Pflanze Entwicklung (SPAD) in Bewässerungs-Weizen Ökosystemen). *Archives of Agronomy and Soil Science*, 52(1), 105–112. https://doi.org/10.1080/03650340500460875

[18] Halmer, P. (2008). Seed technology and seed enhancement. In B. A. Whitaker & R. A. C. Jones (Eds.), *Encyclopedia of applied plant sciences* (pp. 132–142). Elsevier.

[19] Taylor, A.G., Allen, P.S., Bennett, M.A., Bradford, K.J., Burris, J.S. & Misra, M.K. (1998). Seed enhancements. *Seed Science Research*, 8(2), 245–256. https://doi.org/10.1017/S0960258500004141

[20] Beeri, O. & Peled, A. (2006). Spectral indices for precise agriculture monitoring. *International Journal of Remote Sensing*, 27(10), 2039-2047. https://doi.org/10.1080/01431160612331392950

[21] Chaves, M.M., Flexas, J, & Pinheiro, C. (2009). Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Annals of Botany*, 103(4), 551–560. https://doi.org/10.1093/aob/mcn125

[22] Mo, Q., Wang, W., Chen, Y., Peng, Z. & Zhou, Q. (2020). Response of foliar functional traits to experimental N and P addition among overstory and understory species in a tropical secondary forest. *Global Ecology and Conservation*, 23, e01109. https://doi.org/10.1016/j.gecco.2020.e01109

[23] Jolliffe, I. T. & Cadima, J. (2016). Principal component analysis: A review and recent developments. *Philosophical Transactions of the Royal Society A*, 374(2065), 20150202. https://doi.org/10.1098/rsta.2015.0202

[24] Ali, S., Shah, S.A., Hassnain, A., Shah, Z. & Munir, I. (2007). Genotypic variation for yield and morphological traits in wheat. *Sarhad Journal of Agriculture*, 23(4), 943.

[25] Uddling, J., Gelang-Alfredsson, J., Piikki, K. & Pleijel, H. (2007). Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosynthesis Research*, 91(1), 37–46. https://doi.org/10.1007/s11120-006-9077-5