

Biological Diversity and Conservation

ISSN 1308-5301 Print

Research article/Araştırma makalesi DOI: 10.46309/biodicon.. 2025.1656264 18/3 (2025) 349-359

Identification of wheat seeds from bran layer using optical microscopy and deep learning

Yildiray ANAGUN *1, Sahin ISIK1, Murat OLGUN2, Okan SEZER3

ORCID: 0000-0002-7743-0709; 0000-0003-1768-7104; 0000-0001-6981-4545; 0000-0001-7304-1346

¹Department of Computer Engineering, Eskisehir Osmangazi University, 26040, Eskisehir, Turkey ²Department of Field Crops, Eskisehir Osmangazi University, 26040, Eskisehir, Turkey ³Department of Botanic, Eskisehir Osmangazi University, 26040, Eskisehir, Turkey

Abstract

Purpose: This study aims to automate the identification of grain varieties and select the most suitable wheat genotypes for specific ecological conditions using Artificial Intelligence (AI)-based systems. The goal is to facilitate high-yield and high-quality production through pre-sowing analysis.

Method: Seeds from nine wheat genotypes with different qualities were used, and cross-sections of the wheat genotypes were photographed under a light microscope to create a specialized dataset. A Convolutional Neural Network (CNN)-based automated wheat identification framework was then proposed, utilizing both shallow and deep architectures.

Findings: The experiments confirm that CNN-based methods are highly effective in extracting distinctive features from wheat bran and accurately identifying wheat seed varieties.

Conclusion: The research successfully distinguished nine varieties and found that a simpler model (ResNet18) outperformed deeper networks, offering a practical solution for agricultural verification.

Keywords: deep learning; optical microscopy; seed analysis; wheat classification

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

Optik mikroskopi ve derin öğrenme kullanılarak kepek katmanından buğday tohumlarının tanımlanması

Özet

Amaç: Bu çalışma, Yapay Zeka (YZ) sistemleri kullanarak tahıl ürünlerini otomatik olarak tanımayı ve belirli bir ekim alanına en uygun buğday genotiplerini seçmeyi amaçlamaktadır. Bu sayede, yüksek verimli ve kaliteli üretim hedeflenmektedir.

Metod: Farklı kalitelere sahip dokuz buğday genotipinden alınan tohumlar kullanılmış ve özel bir veri seti oluşturmak için buğday genotiplerinin enine kesitleri bir ışık mikroskobu altında fotoğraflanmıştır. Daha sonra, hem sığ hem de derin mimarilerden yararlanan, Evrişimli Sinir Ağı (CNN) tabanlı otomatik bir buğday tanımlama çerçevesi önerilmiştir.

Bulgular: Yapılan deneyler, CNN tabanlı yöntemlerin buğday kepeğinden ayırt edici özellikler çıkarmada ve buğday tohumu çeşitlerini doğru bir şekilde tanımlamada oldukça etkili olduğunu doğrulamaktadır.

Sonuç: Araştırma, dokuz çeşidi başarıyla ayırt etmiş ve daha basit bir modelin (ResNet18) daha derin ağlardan daha iyi performans göstererek tarımsal doğrulama için pratik bir çözüm sunduğunu ortaya koymuştur.

Anahtar kelimeler: derin öğrenme; optik mikroskopi; tohum analizi; buğday;sınıflandırma

-

1. Introduction

Automatic grain sorting systems can reduce labor and administrative costs while minimizing human error and improving efficiency. In plant taxonomy, macro- and micro-morphological characteristics are often used alongside molecular techniques for diagnosis. However, identifying a taxon based on these traits requires extensive expertise and can only be performed by specialists. Another challenge is that plant identification typically requires the entire plant or at least specific parts with distinguishable characteristics. For wheat genotypes, seed identification is particularly difficult, since producers often supply seeds directly for planting. Accurate, cost-effective, and non-specialized identification of a specific genotype requires an in-depth botanical and seed analysis. For this purpose, several studies have investigated various wheat seed characteristics [1-4]. However, there remains a significant research gap in terms of both (1) determining which characteristics of wheat genotypes yield more distinct or precise results and (2) identifying which classification technique is most effective for these distinctive features [5; 6]. Therefore, the identification of wheat seeds is a critical factor in determining their chemical and physical properties during grain processing [7]. Additionally, categorizing wheat grain genotypes at the lowest possible cost directly impacts their sales price in the food industry.

The outer layer of the wheat grain, known as the bran, is composed of three main anatomical structures: the pericarp, testa, and aleurone layers, all of which are rich in structural polysaccharides (cellulose and hemicellulose) and phenolic compounds. The thickness of these layers, cell wall architecture, phenolic composition, and degree of lignification exhibit substantial variation depending on the wheat genotype [8]. In particular, the cellular arrangement, thickness, and phenolic profile of the aleurone layer emerge as key parameters with strong potential for genotype discrimination [9]. Moreover, significant differences in antioxidant capacity and phenolic content of the bran layer have been observed among different wheat genotypes, highlighting their potential for biochemical genotype differentiation [10]. These anatomical and chemical differences can be characterized through microscopic analyses, spectroscopic techniques, and profiling of bioactive compounds, and may serve as biomarkers for genotype identification. In conclusion, the morphological and chemical structural features of the bran layer hold considerable potential for the classification of wheat genotypes and for applications in quality assessment studies.

In this study, a Convolutional Neural Network (CNN)-based framework is developed to automatically classify nine different wheat grain genotypes (Yubileynaya, Bayraktar-2000, Esperia, Konya-18, Krasunia, Maden-2, Misiia, Nacibey, and Syrenia) using microscopic bran images. These genotypes are widely used for commercial purposes in Turkey's food industry. To capture the distinguishing characteristics of each wheat genotype, a custom dataset was created by imaging seed cross-sections from the selected varieties under a light microscope (LM) at 400X magnification. Precise determination of the bran layer's variation ranges for each genotype is essential for differentiation based on bran features. After preparing the dataset to represent these variation ranges, a denoising filter was applied during preprocessing. To enhance the discriminative features of the microscopic bran images in the CNN, the dataset was augmented using various image processing techniques, including flipping, horizontal/vertical shifts, rotation, and others. Key contributions of this study:

- 1 Presenting a cost-effective and practical CNN-based framework for wheat genotype identification using microscopic bran images.
- 2 Establishing a foundation for selecting high-quality genotypes based on bran characteristics for future research.
- 3 Evaluating the performance of different CNN architectures and recommending the most reliable one for wheat genotype classification.

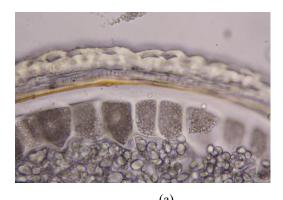
Image classification is one of the most widely used automatic identification and labeling techniques in computer vision and is now successfully applied in both industrial production processes and agriculture. It is also commonly used in automated grain classification, seed testing, and certification processes. Machine learning-supported image classification techniques can generally be divided into two categories: traditional methods and Deep Learning (DL)-based methods. While discriminative features are manually determined in traditional methods, DL-based methods automatically extract image features through convolutional layers, max-pooling, and fully connected layers. With the increasing prevalence of agricultural modernization in recent years, DL-based approaches have found widespread application in agricultural cultivation, including species classification, plant disease detection [11], and crop yield estimation [12].

CNN-based methods have achieved significant progress in end-to-end artificial intelligence for wheat genotype categorization [13; 14]. These methods can efficiently handle large amounts of microstructural image data by leveraging powerful computational capabilities. [15] adopted a different approach using CNN technology to identify nine wheat genotypes from grain surface images captured via Scanning Electron Microscopy (SEM). In SEM, spatial resolution depends on both the size of the electron beam and the volume of the sample interacting with it. However, SEM imaging is costly and lacks portability. The CNN methodology requires careful hyperparameter tuning, which involves training multiple models with different configurations and selecting the optimal one. During training, it is essential to ensure that the model converges while avoiding overfitting or underfitting. Some recent state-of-the-art CNN architectures include Very Deep Convolutional Networks (VGGNet) [16], Dense Convolutional Networks (DenseNet) [17], Residual Neural Networks (ResNet) [18], MobileNet [19], and EfficientNet [20].

The remainder of the paper is structured as follows: Section 2 provides a detailed description of the proposed framework, including preprocessing steps, experiments conducted using both perceptual and classical quality criteria, and the methodology's effectiveness. Section 3 presents a comparative analysis of the results against existing wheat classification methods. Finally, Section 4 summarizes the key contributions and suggests potential directions for future research.

2. Materials and methods

Inspired by previous studies, we propose a CNN-based identification method that offers a simple and low-cost technique for wheat identification using microscopic wheat bran images. A new microscopic wheat seed dataset was created using wheat bran samples, comprising nine wheat genotypes and a total of 1368 images. Figure 1 shows representative microscopic wheat bran samples. The selected wheat cultivars cover a wide range of bran types and grain size distributions. Within the scope of this study, the Nikon 80i light microscope, which supports various imaging modes such as bright field, dark field, phase contrast, and polarized light, was utilized for the visualization of the bran layer. This advanced optical system is widely preferred for the examination, imaging, and quantitative analysis of structural features in cross-sections derived from wheat grains as well as other biological specimens prepared through diverse histological techniques. The system offers a significant advantage in enabling clear morphological differentiation of histological layers within the bran, such as the pericarp, testa, and aleurone, particularly in transverse sections of wheat, owing to its highcontrast imaging capability. Through the integration of a digital camera and analysis software, high-resolution images of the samples can be acquired, allowing objective measurements and archiving of visual data. However, one of the primary limitations encountered during measurement is the presence of artifacts originating from sample preparation. In particular, uneven section thickness, improper placement on slides, or tissue deformation may compromise the clarity of layer boundaries, thereby reducing measurement accuracy. For this reason, special attention was given during sectioning to ensure uniform thickness and homogeneity of the tissue samples in alignment with the objectives of the study. Crosssections of wheat grains were manually prepared from the examined cultivars using a scalpel. All sections were then photographed with a Nikon 80i light microscope. To account for structural variability in the wheat bran, 25-50 microphotographs were taken from each genotype.



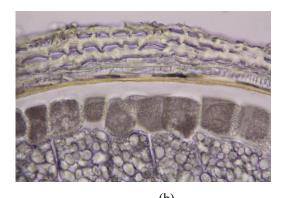


Figure 1. Wheat bran samples analyzed in this study (a) Yubileynaya and (b) Esperia samples

Input images are fed into shallow models (EfficientNetB0 and ResNet18) and deeper models (EfficientNetB4, EfficientNetV2S, Inception-v4, and EfficientNetV2M) to train a large number of filters of increasing depth within the given CNN architectures. The performance of the CNN models was enhanced through data augmentation and image processing techniques. Additionally, objective evaluation metrics (accuracy, sensitivity (recall), specificity, precision, and F1-score) were calculated to ensure a fair assessment.

CNNs represent the most advanced structure of ANNs (Artificial Neural Networks), inspired by the human brain, and have been widely adopted in signal and image processing applications. This article presents a CNN-based recognition method capable of extracting discriminative features for wheat genotype identification from microscopic bran images. Figure 2 illustrates the methodological workflow of the automated wheat seed prediction system based on bran characteristics. The subsections cover the following:

- 1 A custom dataset of microscopic wheat bran images was generated, demonstrating its potential as a benchmark for DL-based applications in breeding research.
- The proposed wheat genotype categorization approach was outlined, with feature extraction performed using residual networks, CNN groups, and inception networks. Data augmentation techniques were applied before feeding the input images into the models.

3

2.1 Data Acquisition

Data was collected at the Transitional Zone Agricultural Research Institute of the Republic of Turkey's Ministry of Agriculture and Forestry. The study included nine different bread wheat genotypes: Yubileynaya, Bayraktar-2000, Esperia, Konya-18, Krasunia, Maden-2, Misiia, Nacibey, and Syrenia. Among these, four genotypes (Bayraktar-2000, Konya-18, Maden-2, and Nacibey) are of Turkish origin, while the other five (Yubileynaya, Esperia, Krasunia, Misiia, and Syrenia) are of foreign origin. Six of the genotypes (Yubileynaya, Bayraktar-2000, Krasunia, Maden-2, Misiia, and Nacibey) are suitable for dryland agriculture, whereas the remaining three (Esperia, Konya-18, and Syrenia) are suitable for irrigated agriculture.

2.2 Data pre-processing

The microscopic raw images have a resolution of 4272 × 2848 pixels, a density of 300 DPI, and are in JPEG format. Instead of extracting square patches, we extracted non-overlapping patches of size 356 × 890 × 3 from each image to measure wheat bran thickness. In deep learning, splitting the dataset into training, testing, and validation subsets is crucial for evaluating model performance and generalization ability. Due to the limited dataset size, a smaller portion was allocated for validation and testing, allowing more data to be used for training. The dataset consisted of 1368 bran images, divided into training, testing, and validation sets in an 8:1:1 ratio specifically, 1094 patches for training, and 136 patches each for testing and validation. Before training, image pixels were normalized to the [0, 1] range to ensure numerical stability. The initial stage of deep learning typically involves input preprocessing. To enhance classification stability and i.e., to extract meaningful features, we applied image processing as cropping and denoising before feeding the data into the CNN model. Unnecessary regions were removed via cropping, and a bilateral filter was used for noise reduction while preserving image details.

2.3 Data Augmentation

Data augmentation is another strategy to reduce the risk of overfitting and underfitting while learning invariant features that improve model performance and robustness. Therefore, we thoroughly evaluated the data augmentation process before training. To stabilize the training process, we applied four distinct data augmentation techniques: rotation, normalization, and horizontal and vertical flipping. By implementing these four augmentation methods (detailed in Table 1), we expanded the original dataset to 4704 brain images to achieve the desired accuracy. The second and third columns of the table specify the function names and their corresponding invariant parameters, respectively.

Table 1 Data	augmentation	techniques	with thei	r narameters
Table 1. Data	augmentation	teeminques	WITH THE	i parameters

Technique	Augmentation	Parameters			
Number	technique				
1	Rotation	-30° to 30°			
2	Horizontal Flip	0.5			
3	Vertical Flip	0.5			
4	Normalization	mean	:	0.485, 0.456, 0.406	
		std	:	0.229, 0.224, 0.225	

In deep learning, data augmentation is a highly effective technique for improving a model's generalization ability and preventing overfitting. However, the optimal augmentation rate depends on the dataset, model complexity, and the nature of the problem. Due to the small size of the dataset used in this study and the tendency of complex models like Inception-v4 and EfficientNetV2M to overfit, we adopted an aggressive data augmentation strategy, expanding the dataset by 300%. However, excessive augmentation can hinder learning and lead to underfitting.

2.4 Experiments

Optimizing CNN models is crucial for managing hardware resources and energy requirements. Therefore, considering the parameter sizes of the models, the input images were resized to 300×300 pixels for ResNet18, EfficientNetB0, EfficientNetV2S, and Inception-v4, and 150×150 pixels for the larger models, EfficientNetB4 and EfficientNetV2M, to ensure compatibility with each network. All networks were pretrained using 14 million annotated images from the noisy student database [21]. To construct the optimal model, a 5-fold Cross-Validation (CV) technique was employed. In traditional training processes for DL-based models, the most common methods to improve performance include increasing the width, depth, and resolution of the inputs, as well as selecting the most appropriate hyperparameters, such as batch size, epoch count, and learning rate. All experiments were conducted in Python v3.7 on a platform equipped with a 2.0 GHz Intel Xeon CPU and an NVIDIA Tesla T4 GPU (13 GB). The training process used

the following hyperparameters: 40 epochs, a batch size of 16, and an initial learning rate of 1e-4. The Adam optimizer was selected to achieve the best results from the network.

An activation function determines the output value of a neuron based on its input and introduces nonlinearity into ANNs. For example, a rectified linear unit (ReLU) serves as the default activation function in ResNet18 and Inception-v4, whereas EfficientNetB0/EfficientNetB4 and EfficientNetV2S/EfficientNetV2M use a sigmoid linear unit (SiLU). However, ReLU has some weaknesses, including a non-zero mean, the vanishing gradient problem, and unbounded output. These issues are less significant in shallow networks with only a few layers. However, in deeper neural networks, they can lead to trivial gradients and reduced generalization ability.

3. Results

Upon reviewing the experiments in Section 2, we conducted extensive quantitative performance evaluations of bran characterization using microscopic imaging for wheat genotype identification. For this purpose, based on CNN algorithms, we not only performed feature recognition and evaluation but also analyzed the results using objective performance criteria.

3.1 Performance analyses and verification

Since wheat genotypes are very similar, CNNs often require large amounts of annotated image data to accurately learn features and reliably distinguish between classes due to the complexity of the problem. We conducted extensive experiments using six different state-of-the-art, pre-trained deep CNN architectures with Noisy Student weights on the same dataset. Notably, we used identical train/test sets across all networks in each analysis, along with a 5-fold cross-validation method (averaged over five iterations) to determine the optimal model.

We obtained the classification performance from 5 replicate tests with accuracy, sensitivity (recall), specificity, precision and f1-score and presented them as a combination of mean(μ)±std. Mathematical expressions of the statistical metrics are given followings:

$$Accuracy = (TP + TN) / (TP + TN + FP + FN)$$
(1)

Sensitivity(Recall) =
$$TP/(TP+FN)$$
 (2)

$$Specifity = TN / (TN + FP)$$
(3)

$$Precision = TP / (TP + FP)$$
(4)

$$F1 - Score = 2 \times (Precision \times Re\ call) / (Precision + Re\ call)$$
(5)

True positives (TP) and True Negatives (TN) represent correctly identified positive and negative samples, while False Positives (FP) and False Negatives (FN) correspond to incorrectly identified negative and positive samples, respectively. The accuracy of the independent test data was calculated using Equation (1). Sensitivity measures how well a CNN model detects positive instances, whereas specificity refers to the proportion of actual negatives predicted as negative (true negatives). Sensitivity (recall) and specificity were calculated using Equations (2) and (3), respectively. The F1 score is given in Equations (5); it is a statistical metric that evaluates model accuracy and is defined as the harmonic mean of precision (Eq. 4) and recall (Eq. 2). Table 2 presents the parameter sizes, training times, and statistical metrics for each CNN model, along with a comparison of results based on activation functions.

Table 2. Identification performance of the CNN models

Model	Parameter Size	Training Time (min)	Accuracy	Sensitivity	Specificity	Precision	F1-score
EfficientNetB0	4.0M	28.92	97.16±0.43	87.44±2.03	98.38±0.25	88.24±1.83	87.51±1.91
ResNet18	11.18M	16.96	97.67±0.15	90.03±0.67	98.79±0.08	90.50±0.83	90.06±0.70
EfficientNetB4	17.56M	23.12	95.81±0.42	81.47±2.06	97.63±0.23	81.74±2.13	81.29±2.07
EfficientNetV2S	20.19M	49.50	97.56±0.25	89.33±1.32	98.61±0.14	90.10±1.31	89.45±1.33
Inception-v4	41.15M	64.67	96.83±0.37	86.01±1.48	98.20±0.20	86.72±2.43	85.97±1.76
EfficientNetV2M	52.87M	31.82	96.59±0.17	85.01±0.98	98.06±0.10	85.78±0.61	85.04±0.72

As the table shows, deeper models resulted in longer training times, with the Inception-v4 model taking 64.63 minutes. In contrast, ResNet18 with the default ReLU activation function achieved better performance scores than other CNN models while having the shortest training time (16.93 minutes). Interestingly, the ResNet18 model demonstrates

better convergence despite having a parameter size close to that of EfficientNetB4. The test accuracies of ResNet18 and EfficientNetB4 are 97.67 ± 0.15 and 95.81 ± 0.42 , respectively. It should be noted that all test images are independent of the training sets. Moreover, EfficientNetB4 has the lowest sensitivity score (81.47 ± 2.06) and F1-score (81.29 ± 2.07) . On the other hand, deeper models like EfficientNetV2M and Inception-v4 exhibit worse precision (85.78 ± 0.61) and 86.72 ± 2.43 , respectively) compared to shallower models such as ResNet18 (90.50 ± 0.83) and EfficientNetB0 (88.24 ± 1.83) .

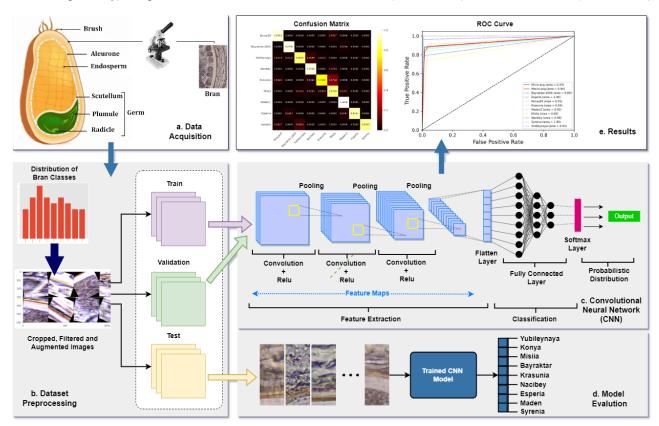


Figure 2. Overview of the CNN-based wheat genotype prediction system (a) Collection of wheat bran images from nine wheat genotypes. (b) Data augmentation as a pre-processing step. (c) The architecture/structure of the CNN network. (d) Evaluation of the model on test images. (e) Results

3.2 Statistical Comparison

A Receiver Operating Characteristic (ROC) curve is a graphical illustration used to evaluate the discriminative power of a test and identify a suitable threshold value. It is plotted by connecting the points corresponding to the true positive rate and false positive rate at each cutoff point. When the curve approaches the upper left corner, the diagnostic test maintains a higher true positive rate, indicating well-established specificity and sensitivity. Additionally, the Area Under the Curve (AUC) provides a numerical representation of the overall effectiveness of the diagnostic method. In Figure 3, the AUC values were calculated for each class to evaluate the performance of the models and were plotted along with the ROC curve. Based on the ROC curves, the EfficientNetV2S model achieved the highest macro-average AUC of 0.99. The AUC values for the Bayraktar and Nacibey cultivars were 1.00 across all models. Although the Inception-v4 model yielded the lowest AUC value (0.96) for the Yubileynaya genotype, the EfficientNetV2S and ResNet18 models achieved the highest value (0.98).

3.3 Comparison with state-of-the-art

This section provides a brief discussion of common wheat seed classification approaches documented in the literature, as summarized in Table 3. [14] developed a CNN-based wheat classification method using 15-class RGB images. While they achieved a test accuracy of 97.33%, they noted that convolutional layers are slow and computationally expensive. As a result, they recommended using a smaller batch size to speed up the training process. Among the most common CNN architectures for wheat seed recognition are DenseNet201, InceptionV3, and MobileNet. [13] demonstrated the feasibility of identifying wheat varieties based on the dorsoventral view of the grain, regardless of origin, achieving test accuracies of 95.68%, 95.62%, and 95.49%, respectively. While DenseNet201 and InceptionV3 showed better compatibility with the input data, MobileNet exhibited overfitting after 50 epochs.

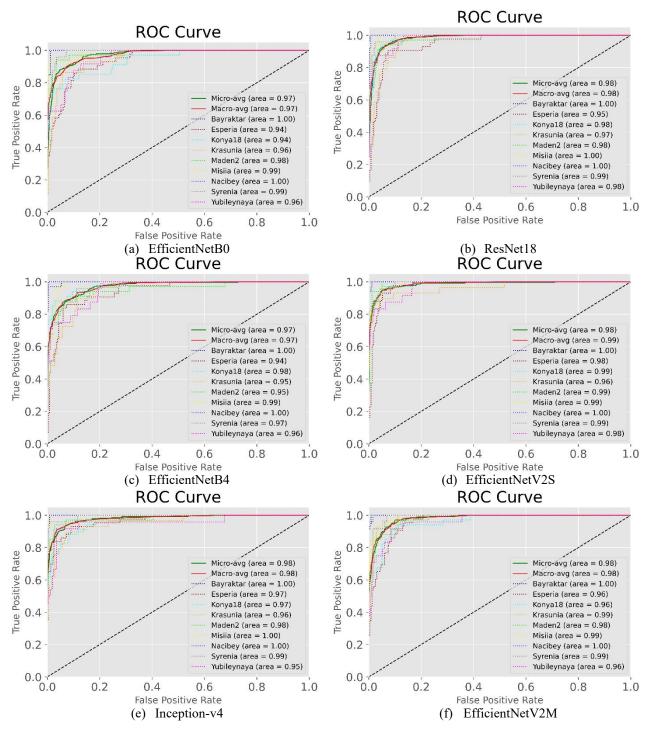


Figure 3. The ROC curves of the CNN models on the test dataset: (a) EfficientNetB0, (b) ResNet18, (c) EfficientNetB4, (d) EfficientNetV2S, (e) Inception-v4, and (f) EfficientNetV2M

Wheat quality varies depending on the genotypes used for cultivation. [22] developed SVM and ANN-based methods to classify seven Iranian wheat genotypes using texture features extracted via Local Binary Pattern (LBP), Gray-Level Co-Occurrence Matrix (GLCM), and Gray-Level Run-Length Matrix (GLRM) algorithms. Their experimental results showed test accuracies of 90.33% for SVM and 96.19% for ANN. While ANN suffers from limitations such as longer training times, convergence challenges, and fixed-size parametric architectures, SVM's classification performance is constrained by its reliance on a single feature vector. Consequently, SVM underperforms compared to ANN in this context. [23] used a CNN and SVM to extract deep features and evaluated the performance of different CNN architectures for wheat classification. However, in most cases, data collection poses challenges due to factors such as time constraints, storage limitations, or data-related issues. In their study, they achieved the highest accuracy rate of 98.1% using DenseNet201. [24] employed Inception-V3, MobileNet-V2, and ResNet18 to classify five different bread wheat genotypes, attaining the best performance (97.67%) with ResNet18. They applied a segmentation process, which

introduces additional computational costs, before feeding the wheat RGB images into the CNN model. [25] compared four traditional machine learning methods (SVM, kNN, MLP, and naïve Bayes) and obtained the highest accuracy (93.0%) with SVM. Since these methods rely on feature selection, challenges such as data rate and diversity key characteristics of big data arise during the process. These studies demonstrate high accuracy in identifying wheat genotypes from images containing multiple seeds of a single genotype. However, in commercial settings, seeds from different genotypes may be mixed and sold together. In such cases, single-seed images must be used for genotype identification to determine the varieties and their proportions in the mixture. When using single-seed images, the reference data is limited to features derived from the seed's external appearance, restricting data diversity. Therefore, for genotype determination from a single seed or a small set of seed images, there is a need to identify new, reliable, and highly discriminative features to enrich the dataset.

Table 3. A detailed comparison of wheat seed classification methods

Authors	Method	Dataset/Classes	Data Type	Performance (Accuracy)
[13]	- DenseNet201 - Inceptionv3 - MobileNet	4-classes and a total of 31.606 samples	RGB	- 95.68% - 95.62% - 95.49%
[14]	CNN	15-classes	RGB	- 97.53% acc.
[22]	SVM, ANN	7-classes	RGB	SVM: 90.33% ANN: 96.19%
[23]	CNN+SVM (AlexNet, ResNet18, ResNet50, ResNet101, Inceptionv3, DenseNet201, Inceptionresnetv2)	4-classes	RGB	DenseNet201 98.1% max acc.
[24]	- Inception-V3 - Mobilenet-V2 Resnet18	5-classes and a total of 8354 samples.	RGB	- 97.37% - 97.07% - 97.67%
[25]	SVM, kNN, MLP, Naïve Bayes (NB)	2-classes (fresh and rotten)	RGB	SVM: 93.0% NB: 65.0%
Proposed Framework	EfficientNetB0, ResNet18 EfficientNetB4, EfficientNetv2s, Inception-v4, EfficientNetv2m	9-classes of microscopic wheat bran images	RGB	ResNet18 - 97.67% max acc.

In our study, we evaluated the usability of bran layer images obtained via light microscopy from seed cross-sections for genotype identification. The bran layer was chosen as a reference because it can be easily photographed under light microscopy without special preparation. Additionally, images taken from different sections of a single seed provide richer data per grain. In contrast, macro photographs and stereomicroscope images offer limited data due to lower magnification. SEM is impractical due to its high cost and specialized preparation requirements. Thus, bran layer imaging emerges as a crucial reference for future wheat genotype identification, combining practicality with high data richness.

4. Conclusions and discussion

Today, different wheat genotypes are registered and sold commercially with breeding studies conducted by public and private institutions. Unfortunately, the supply of seeds of wheat genotypes that are not suitable for the ecological conditions of the region to be planted causes serious economic losses to the farmers. Therefore, a feasible computer-aided method is required to avoid economic damage and accurately distinguish the supplied genotype. We introduced an end-to-end CNN-based approach for wheat genotype identification, utilizing microscopic wheat bran images that capture the organism's characteristic features, and uncovered the following key findings:

- Nine wheat varieties with different qualities such as milling extraction, dough balance, baking performance, color and texture were automatically identified from the bran layer.
- By conducting a series of experiments on microscopic bran images, we examined several advanced CNN models, which had not been studied in earlier research.
- We suggested convenient choices for identification in agriculture and pointed that the ResNet18 shallow model performance is better than the deeper CNN models.

Although the proposed framework can be implemented cheaper and less costly manner with fewer samples, it also has some drawbacks. One of the major drawbacks is the difficulty of sectioning a wheat seed in the laboratory. If a CNN consists of multiple layers, the training process can take a particularly long time due to operations such as max

pooling. The second drawback is the unguaranteed convergence of neural networks, which can also underfitting or overfitting. Despite these disadvantages, CNNs have great performance while classifying images that are very similar to the dataset. In conclusion, this study revealed that CNN algorithms are feasible in detecting wheat varieties from microscopic bran images. We will refine the recognition outputs by considering a lightweight CNN architecture. We also plan to use the data obtained from microscopic wheat bran images to distinguish wheat diseases in our future studies.

Today, various wheat genotypes are registered and sold commercially through breeding studies conducted by public and private institutions. Unfortunately, supplying seeds of wheat genotypes unsuitable for the ecological conditions of the target region leads to significant economic losses for farmers. Therefore, a practical, computer-aided method is needed to prevent economic damage and accurately identify the supplied genotype. We propose an end-to-end CNN-based approach for wheat genotype identification using microscopic wheat bran images, which capture the organism's distinctive features. Our study yielded the following key findings:

- Nine wheat genotypes with differing qualities such as milling extraction, dough balance, baking performance, color, and texture were automatically identified from the bran layer.
- Through a series of experiments on microscopic bran images, we evaluated several advanced CNN models that had not been explored in prior research.
- We identified practical solutions for agricultural identification and demonstrated that the ResNet18 shallow model outperforms deeper CNN architectures.

Although the proposed framework can be implemented cheaper and less costly manner with fewer samples, it also has some challenges. One of the major challenges encountered during the selection of wheat genotypes for agricultural activities is the lack of a simple, rapid, low-cost, and non-specialist method for verifying the authenticity of the varieties provided by suppliers. As a result, farmers often rely solely on the information given by the supplier when purchasing seeds. If a supplier provides a genotype that is not suitable for the cultivation environment under the name of a suitable one (e.g., supplying a genotype adapted to irrigated conditions for cultivation in a drought-prone area), significant yield losses may occur. This study aims to address this gap by developing a simple, cost-effective, and userfriendly diagnostic system for wheat genotype identification. It represents a pioneering effort in the development of such systems. Therefore, the study does not include any data on the suitability of the selected genotypes for specific cultivation environments, as this lies outside the scope of its primary objective. In short, the purpose of the study is to verify whether the seeds provided by the supplier indeed correspond to the high-yielding genotypes suitable for the target cultivation environment. In the manuscript, references to environmental suitability are made in the context of potential yield losses that may result from genotype misidentification. The second one is the unguaranteed convergence of neural networks, which can also underfitting or overfitting. Despite these disadvantages, CNNs have great performance while classifying images that are very similar to the dataset. In conclusion, this study revealed that CNN algorithms are feasible in detecting wheat genotypes from microscopic bran images. We will refine the recognition outputs by considering a lightweight CNN architecture. We also plan to use the data obtained from microscopic wheat bran images to distinguish wheat diseases in our future studies.

Conflicts of interest: The authors declare that they have no competing interests.

Funding: No financial benefit or support was received from any institution or person for this research.

Ethical statement: This study does not require ethical approval.

Author contributions: Author contributions to the study were as follows: Yildiray ANAGUN: Conceptualization, study design, and organization. Sahin ISIK: Data analysis and processing, including writing subprograms and utilizing AI tools. Murat OLGUN: Formal analysis, supervision, and investigation. Okan SEZER: Data preparation, writing, review & editing. All authors contributed to the final revision and approval of the manuscript.

References

- [1] Arslan, A., Aygun, Y. Z., Turkmen, M., Celiktas, N., & Mert, M. (2025). Combining non-destructive devices and multivariate analysis as a tool to quantify the fatty acid profiles of linseed genotypes. *Talanta*, 281, 126798. doi:https://doi.org/10.1016/j.talanta.2024.126798
- [2] Bao, Y., Mi, C., Wu, N., Liu, F., & He, Y. (2019). Rapid Classification of Wheat Grain Varieties Using Hyperspectral Imaging and Chemometrics. *Applied Sciences*, 9(19), 4119. doi:https://doi.org/10.3390/app9194119

- [3] Charytanowicz, M., Kulczycki, P., Kowalski, P. A., Łukasik, S., & Czabak-Garbacz, R. (2018). An evaluation of utilizing geometric features for wheat grain classification using X-ray images. *Computers and Electronics in Agriculture*, 144, 260-268. doi:https://doi.org/10.1016/j.compag.2017.12.004
- [4] Diederichsen, A., & Raney, J. P. (2006). Seed colour, seed weight and seed oil content in Linum usitatissimum accessions held by Plant Gene Resources of Canada. *Plant Breeding*, 125(4), 372-377. doi:https://doi.org/10.1111/j.1439-0523.2006.01231.x
- [5] Rahman, A., & Cho, B.-K. (2016). Assessment of seed quality using non-destructive measurement techniques: a review. *Seed Science Research*, 26(4), 285-305. doi:https://10.1017/S0960258516000234
- [6] Wang, Q. G., Zhu, Q. B., Qin, J., & Huang, G. (2015). Review of seed quality and safety tests using optical sensing technologies. *Seed Science and Technology*, 43, 1-30. doi:https://doi.org/10.15258/sst.2015.43.3.16
- [7] Olgun, M., Köse, A., Belen, S., Karaduman, Y., Budak Başçiftçi, Z., Ayter Arpacıoğlu, N. G., & Turan, M. (2024). Characterization of whole seeds lipids, extracted lipids composition in bread wheat (T.aestivum L.) genotypes grown in Eskisehir province in Türkiye. *Biological Diversity and Conservation*, 17(2), 175-189. doi:https://doi.org/10.46309/biodicon.2024.1394551
- [8] Moss, R. (1973). Conditioning studies on Australian wheat. II. Morphology of wheat and its relationship to conditioning. *Journal of the Science of Food and Agriculture*, 24(9), 1067-1076. doi:https://doi.org/10.1002/jsfa.2740240908
- [9] Chen, Z., Mense, A. L., Brewer, L. R., & Shi, Y. C. (2024). Wheat bran layers: composition, structure, fractionation, and potential uses in foods. *Crit Rev Food Sci Nutr*, 64(19), 6636-6659. doi:https://doi.org/10.1080/10408398.2023.2171962
- [10] Saidani, S., Zairi, M., Meziani, S., Labga, L., Tiboura, G., Menadi, N., & Demmouche, A. (2022). Bioactive Compounds and Antioxidant Activity of Peripheral Layers of Soft Wheat Grown in Algeria During Seed Filling. *Food and Environment Safety Journal*, 21. doi:https://doi.org/10.4316/fens.2022.009
- [11] Deniz, E., & Serttaş, S. (2023). Disease detection in bean leaves using deep learning. *Communications Faculty of Sciences University of Ankara Series A2-A3 Physical Sciences and Engineering*, 65(2), 115-129. doi:https://doi.org/10.33769/aupse.1247233
- [12] Özkan, K., Seke, E., & Işık, Ş. (2021). Wheat kernels classification using visible-near infrared camera based on deep learning. *Pamukkale University Journal of Engineering Sciences*, 27(5), 618-626. doi:https://dx.doi.org/10.5505/pajes.2020.80774
- [13] Laabassi, K., Belarbi, M. A., Mahmoudi, S., Mahmoudi, S. A., & Ferhat, K. (2021). Wheat varieties identification based on a deep learning approach. *Journal of the Saudi Society of Agricultural Sciences*, 20(5), 281-289. doi:https://doi.org/10.1016/j.jssas.2021.02.008
- [14] Lingwal, S., Bhatia, K., & Tomer, M. (2021). Image-based wheat grain classification using convolutional neural network. *Multimedia Tools and Applications*, 80, 1-25. doi:https://doi.org/10.1007/s11042-020-10174-3
- [15] Anagun, Y., Isik, S., Olgun, M., Sezer, O., Basciftci, Z. B., & Arpacioglu, N. G. A. (2023). The classification of wheat species based on deep convolutional neural networks using scanning electron microscope (SEM) imaging. *European Food Research and Technology*, 249(4), 1023-1034. doi:https://doi.org/10.1007/s00217-022-04192-8
- [16] Simonyan, K., & Zisserman, A. (2015). *Very Deep Convolutional Networks for Large-Scale Image Recognition*. Paper presented at the 3rd International Conference on Learning Representations (ICLR 2015).
- [17] Huang, G., Liu, Z., Maaten, L. V. D., & Weinberger, K. Q. (2017, 21-26 July 2017). *Densely Connected Convolutional Networks*. Paper presented at the 2017 IEEE Conference on Computer Vision and Pattern Recognition (CVPR).
- [18] He, K., Zhang, X., Ren, S., & Sun, J. (2016, 27-30 June 2016). *Deep Residual Learning for Image Recognition*. Paper presented at the 2016 IEEE Conference on Computer Vision and Pattern Recognition (CVPR).
- [19] Sandler, M., Howard, A., Zhu, M., Zhmoginov, A., & Chen, L. C. (2018, 18-23 June 2018). *MobileNetV2: Inverted Residuals and Linear Bottlenecks*. Paper presented at the 2018 IEEE/CVF Conference on Computer Vision and Pattern Recognition.
- [20] Tan, M., & Le, Q. V. (2019, 9-15 June 2019). *EfficientNet: Rethinking Model Scaling for Convolutional Neural Networks*. Paper presented at the 36th International Conference on Machine Learning (ICML 2019), Long Beach.
- [21] Russakovsky, O., Deng, J., Su, H., Krause, J., Satheesh, S., Ma, S., . . . Fei-Fei, L. (2015). ImageNet Large Scale Visual Recognition Challenge. *International Journal of Computer Vision*, 115(3), 211-252. doi:https://doi.org/10.1007/s11263-015-0816-y
- [22] Khojastehnazhand, M., & Roostaei, M. (2022). Classification of seven Iranian wheat varieties using texture features. *Expert Systems with Applications*, 199, 117014. doi:https://doi.org/10.1016/j.eswa.2022.117014
- Unlersen, M., Sönmez, M., Aslan, M., Demir, B., Aydin, N., Sabanci, K., & Ropelewska, E. (2022). CNN-SVM hybrid model for varietal classification of wheat based on bulk samples. *European Food Research and Technology*, 248. doi:https://doi.org/10.1007/s00217-022-04029-4
- [24] Yasar, A. (2022). Benchmarking analysis of CNN models for bread wheat varieties. *European Food Research and Technology*, 249. doi:https://doi.org/10.1007/s00217-022-04172-y

[25] Agarwal, D., Sweta, & Bachan, P. (2023). Machine learning approach for the classification of wheat grains. *Smart Agricultural Technology*, *3*, 100136. doi:https://doi.org/10.1016/j.atech.2022.100136