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Research Article

Prediction of Ear Weight, Kernel Weight and Viability in Maize Using Image Analysis

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Abstract

In maize breeding studies, it is becoming common to determine the ear and kernel characteristics by image analysis. While current methods focus on measurements that can be obtained directly by image analysis, it has not been adequately addressed whether different parameters such as weight and viability can be estimated using these measurements. This study aimed to determine whether it is possible to estimate the ear weight (g), kernel weight (g), single kernel weight (g) and viability (1/0) status of maize with the help of features (area, perimeter, width, length) extracted from images of the ear and kernel samples. In this study, 233 ear and 1242 grain samples belonging to 13 maize genotypes were used as material. Digital images of the ear samples were taken with a 5 MP camera and from the kernel samples with a desktop scanner. The ear weight reference data (DV1) and the kernel weight reference data (DV2) were obtained by weighing each sample on a precision balance. Single kernel reference data (DV3) was obtained with the measurements of single kernel weights. Kernel samples underwent paper germination test and reference data (DV4) related to viability was created. Regression models were developed by using the features obtained from image analysis (area, perimeter, width, height) for each reference data set as the predictor variable. As a result of the study, it was seen that the ear weight and kernel weight can be estimated with the help of the parameters extracted from the image analysis. While moderate success was achieved in the determination of single seed weight, it was difficult to determine the viability status based on the morphometric measurements of a single kernel in maize.
Keywords: *Zea mays*, Regression, Morphometric measurements

Görüntü Analizi Kullanılarak Mısırda Koçan Ağırlığı, Tane Ağırlığı ve Canlılığın Tahmini

Öz

Mısır ıslah çalışmalarında, görüntü analizleri ile koçan ve tane özelliklerinin belirlenmesi yaygınlaşmaktadır. Mevcut yöntemler, doğrudan görüntü analizi ile elde edilebilecek ölçümlere odaklanırken, ağırlık ve canlılık gibi farklı parametrelerin bu ölçümler kullanılarak tahmin edilip edilemeyeceği yeterince ele alınmamıştır. Bu çalışmanın amacı,görüntü analizlerinden çıkarılan özellikler (alan, çevre, çevre, genişlik, uzunluk) kullanılarak mısırda koçan ağırlığı (g), tane ağırlığı (g), tek tane ağırlığı (g) ve canlılık (1/0) durumunun tahminlenip tahminlenemeyeceğinin belirlenmesidir. Çalışmada 13 mısır genotipine ait 233 koçan ve 1242 tane örneği materyal olarak kullanılmıştır. Dijital görüntüler koçan örneklerinden 5 MP kamera ile, tane örneklerinden ise masaüstü tarayıcı ile alınmıştır. Koçan ağırlığı referans verileri (DV1) ve tane ağırlığı referans verileri (DV2), her numunenin hassas terazide tartılmasıyla elde edilmiştir. Tek tane ağırlıklarının ölçümleri ile tek tohum referans verileri (DV3) elde edilmiştir. Tane örnekleri kağıt çimlenme testine tabi tutulmuş ve canlılığa ilişkin referans veriler (DV4) oluşturulmuştur. Her bir referans veri seti için görüntü analizinden elde edilen özellikler (alan, çevre, genişlik, yükseklik) tahminleyici değişken olarak kullanılarak regresyon modelleri geliştirilmiştir. Çalışma sonucunda, görüntü analizinden çıkarılan parametreler yardımıyla koçan ağırlığı ve tane ağırlığı tahmin edilebildiği görülmüştür. Tek tane ağırlığının belirlenmesinde orta düzeyde başarı elde edilirken, mısırdatek taneden alınan morfometrik ölçümlere dayalı canlılık durumunun belirlenmesinin mümkün olmadığı tespit edilmiştir.
Anahtar Kelimeler:*Zea mays*, Regresyon, morfometrik ölçümler

Introduction

Maize (*Zea mays*) is a herbaceous and highly cross-pollinated plant from the Poaceae family. The homeland of the maize plant is South America. This crop is an important grain that is used as a raw material for industry, as well as for human and animal nutrition (Prasanna et al., 2012). As a result, maize breeding studies are becoming widespread in our country. In maize breeding studies, kernel and ear characteristics are important phenotypic traits for maize breeding (Hallauer et al., 1988). The measurement of these traits requires time and labor. With developing technology, studies were carried out about the use of different techniques in order to shorten the duration of these measurements. Image analysis is considered to be a modern and alternative method among these techniques. Traditional image analysis methods are more suitable than other methods because they are cheap and practical (Wu et al., 2018). Image analysis has many uses in agriculture (Doğan et al., 2018). The main areas for use of image analysis in kernel measurements are; characterization and identification, classification and grading, physiological tests, detection of mechanical or disease damage, determination of color and morphological features (Kiratiratanapruk and Sinthupinyo, 2011; Kapadia et al., 2017; Yafie et al., 2020; Beyaz and Gerdan, 2021). Among these purposes, the use of image analysis in morphometric measurements has become quite widespread.

When morphometric measurements are made by humans, they require high labor and time. In addition, the human error rate in these measurements is quite high. In order to eliminate these negative methods, new approaches have been developed. Among these approaches, analysis based on image processing is used in many different fields today. These techniques are also actively used in seed analysis. Tanabata et al. (2012) developed a software called SmartGrain for image analysis of seeds of different plant species. With this software, measurements such as length, width and depth of the seed can be made in a much shorter time and with less labor compared to manual measurements, but this software cannot extract seed color information (Tanabata et al., 2012). With the plug-in called SeedAnalyser developed on the ImageJ platform, a tool was developed that can group seeds by image classification by extracting morphological, structural and color features from seed images (Loddo et al., 2022). With this tool, seeds with different characteristics can be separated with high success by using image analysis and machine learning technique. In the MATLAB environment, Zhu et al. (2021), extracted seed area, perimeter, width, length, circularity and central point as well as color characteristics in a short time.

This software was designed to perform measurements for direct ear or grain morphometry. Different studies were conducted to compare the reference results for morphometric measurements with the results obtained from image analysis (Gierz et al. 2021; Cirit et al., 2022). Again, a remarkable number of studies have been conducted about the comparison of morphometry results obtained from different image processing software (Makanza et al. 2018; Loddo et al., 2022). There are limited studies about whether morphometric measurements can be made using simple parameters (area, perimeter, width, height) obtained by image analysis.

The objective of this study was to investigate the possibility of determining ear weight, total kernel weight per ear, single kernel weight, and kernel viability through the utilization of some morphometric measurements obtained from image analysis.

Material and Methods

In the study, ear (n=231) and grain (n=1223) samples of 10 different local corn samples and 3 standard genotypes found in the Department of Field Crops, Faculty of Agriculture, Çanakkale Onsekiz Mart University were used. These materials were sampled from breeding trials conducted in the Field Crops Department in 2021.

Data were collected related to dependent variables in the research of ear weight, kernel weight, single kernel weight and viability. Three different image analyses were carried out in the study. In the first stage, the images of cob samples for each genotype were taken with the EceGöz imaging system at 5 MP resolution. Results for reference measurements were obtained from digital images with the ImageJ program (Abràmoff et al., 2004).

Figure 1. Digital and processed images of ear samples using ImageJ software

The materials used in the research were scanned at 300 dpi resolution, with 50 to 100 seeds from each population using a desktop scanner. Population sequence and seed numbers in these images were recorded on Excel files to be used in germination tests and image processing analysis. The seed morphology of the populations was examined with SmartGrain (Tanabata et al., 2012) software.

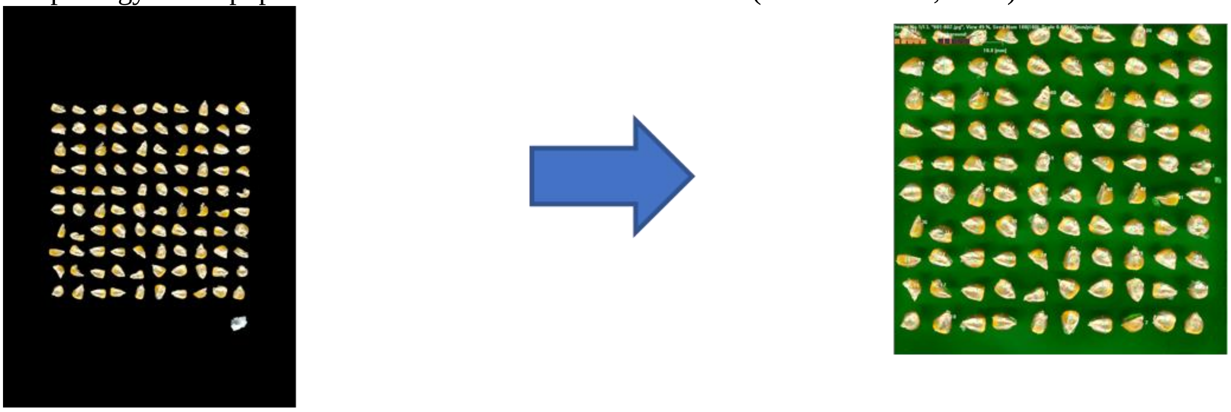

Figure 2. Digital and processed images of kernel samples SmartGrain software

Samples from which seed images were taken underwent a germination test. In the germination test, the seeds were arranged on germination papers as they were imaged. Seeds lined up on germination papers were wetted with distilled water. For germination, the seeds were kept at 24 degrees for 7 days. At the end of this period, germinated and non-germinated seeds were counted, and viable seeds were determined and recorded without mixing their order. In the study, data about ear weight (DV1), kernel weight per ear (DV2), single kernel weight (DV3) and kernel viability (DV4) were collected as dependent variables. Nine samples of kernel data were discarded because of they had extreme values of color spaces.

The statistical analyses in the study were performed using the R statistical package program (R Core Team, 2019). The differences in the genotypes used as material in terms of the examined characteristics were analyzed with the analysis of variance technique. As a result of variance analysis, the differences between genotypes were compared with the t test. The relationships between the image processing analyses for seed characteristics were measured as a reference analysis and the results obtained were examined with regression analyses.

Results and Discussion

The results of the regression analysis for estimation of ear weight using the image analysis features are presented in Table 1. All of the different models created based on area, perimeter, length and diameter data were found to be statistically significant. These models had success rate of over 80% and the ear weight can be determined by using the image analysis outputs (Table 1). However, when only the ear area data was used as an estimator, an R^2 value of 82.9% was calculated, while it was determined that the addition of other features to the model did not have a significant effect on increasing prediction success.

Additionally, predicting ear weight can be useful for breeders and farmers who are interested in selecting plants with higher yields, as it allows them to identify individual ears with high weights and use them for further breeding or production. Predicting ear weight using image analysis is a more straightforward process, as it only requires measuring the weight of the entire ear. Ear weight is less likely to be influenced by variations in kernel size or number and is a more direct indicator of the yield of the crop. Previous studies generated some mathematical models that use several ear features, such as length, width, and shape parameters, to predict ear weight with high accuracy. In our study the best results were obtained for ear weight prediction using image analysis. However, it is noteworthy that the results obtained in our study have partly lower success than other studies. The main reason for this could be attributed to several issues. Firstly, morphometric features alone obtained from image analysis may not be effective for predicting ear weight in maize because ear weight is a complex trait influenced by a variety of factors. While morphometric features such as ear length, diameter, and shape may be correlated with ear weight, they may not be the only or even the most important factors influencing ear weight. In our study we predict ear weight by only morphometric features, and we do not take into account the number of kernels on the ear. Also, we cannot consider the weight of cobs to predict ear weight. These two factors have significant effect on the changes the ear weight in maize. Drienovsky et al (2019) noticed that total weight of ear highly correlated with the total weight of kernel in maize. We can not capture the number of kernel or features of cob using image analysis in our study. Additionally, image analysis may not insufficient to determine the moisture content of ear samples. Undoubtedly, these issues caused the relatively low estimation success in our study when comparing the results of previous studies.

Table 1. Results of regression models for ear weight (DV1) estimation using the morphometric features of ear samples extracted from image analysis.

Features/Stat.	**Model 1**	**Model 2**	**Model 3**	**Model 4**
Area	3.020***	4.735***	4.738***	4.781***
Perimeter		-5.058***	-5.367***	-5.362***
Length			0.626	0.466
Width				-0.660
Constant	-69.269***	26.316**	27.500**	30.401
N	233	233	233	233
R^2	0.829	0.888	0.888	0.888
Adjusted R^2	0.829	0.887	0.886	0.886
Std. Err.	29.191	23.725	23.772	23.824
	(df = 231)	(df = 230)	(df = 229)	(df = 228)
F Statistic	1.121.822***	908.933***	603.588***	450.724***
	(df = 1; 231)	(df = 2; 230)	(df = 3; 229)	(df = 4; 228)

* p<0,05, ** p<0,01, *** p<0,001

The results of the regression analysis for estimation of the grain weight using the image analysis parameters are presented in Table 2. All of the different models created based on area, perimeter, length and diameter data were statistically significant. It was observed that these models had success rate of over 70% and the ear weight can be determined by using the image analysis outputs (Table 2). However, an R^2 value of 75.2% was calculated when only the cob area data was used as an estimator, while this value reached 83.7% when the perimeter variable was included. It was determined that the addition of other variables to the model did not have a significant effect on the estimation success (Table 2).

The previous studies showed that total kernel weight could be estimated by different morphometric features obtained with image analysis. Sandhya et al. (2021) developed a model to predict total kernel weight using image analysis outputs. They used kernel number per ear and kernel length as predictor variables in their prediction model. They found high similarity (R^2> 0.9815) between estimated kernel weight and measured values. In this study, the aim was to predict total kernel weight using ear features. Although this method seems acceptable, the regression coefficient for the model was lower than the value in the study by Sandhya et al (2021). In our study, one limitation of using morphometric features for predicting total kernel weight is that these features do not take into account the number of kernels on the ear. Two ears with the same average kernel size and shape may have different weights if one has more kernels than the other. Total kernel weight can also be affected by some other factors such as moisture content of ear, which may not be fully captured with morphometric features obtained from image analysis. Therefore, these factors could cause biased calculation of total kernel weight using image analysis in our study.

Table 2. Results of regression models for total kernel weight (DV2) using the morphometric features of ear samples extracted from image analysis.

Features/Stats.	Model 1	Model 2	Model 3	Model 4
Area	2.583***	4.440***	4.454***	4.206***
Perimeter		-5.478***	-6.863***	-6.891***
Length			2.807	3.735
Width				3.831
Constant	-63.314***	40.212***	45.519***	28.691
N	233	233	233	233
R^2	0.752	0.837	0.838	0.838
Adjusted R^2	0.751	0.835	0.836	0.835
Std. Err.	31.6 (df = 231)	25.7 (df = 230)	25.6 (df = 229)	25.7 (df = 228)
F Statistic	698.9*** (df = 1; 231)	588.7*** (df = 2; 230)	393.9*** (df = 3; 229)	294.3*** (df = 4; 228)

* p<0,05, ** p<0,01, *** p<0,001

Results of the regression analysis related to the estimation of single kernel weight using image analysis parameters are presented in Table 3. All of the different models created based on area, perimeter, length and diameter data were found to be statistically significant. In these models, single seed weight could be determined with over 60% success by using all the variables in the image analysis outputs (Table 3). When kernel area data is used, the R^2 value was 46.7%, adding the area variable did not significantly increase the R^2 value, and adding the kernel width increased the R^2 value to over 60% (Table 3).

Table 3. Results of regression models single kernel weight (DV3) using the morphometric features of ear samples extracted from image analysis.

Features/Stats.	Model 1	Model 2	Model 3	Model 4
Area	0.004***	0.005***	0.004***	0.002***
Perimeter		-0.006***	0.015***	0.012***
Length			-0.045***	-0.030***
Width				0.017***
Constant	0.055***	0.146***	0.078***	-0.033
N	1,223	1,223	1,223	1,223
R^2	0.467	0.477	0.625	0.631
Adjusted R^2	0.467	0.476	0.624	0.629
Std. Err.	0.059 (df = 1221)	0.059 (df = 1220)	0.050 (df = 1219)	0.049 (df = 1218)
F Statistic	1,070.205*** (df = 1; 1221)	556.318*** (df = 2; 1220)	677.789*** (df = 3; 1219)	520.008*** (df = 4; 1218)

* p<0,05, ** p<0,01, *** p<0,001

In maize, single kernel weight has an important effect on plant development. Revilla et al (2011) stated that parents with heavier kernels had better early vigor and earlier flowering dates. Therefore, it is desirable to increase single kernel weight in maize. Previous studies showed that this trait could be detected by image analysis in maize (Makanza et al., 2018). Moderate success was achieved for this feature in our study. Morphometric features obtained from image analysis may not be effective for predicting single kernel weight in maize because weight is a complex trait that is influenced by a variety of factors, including genetic, environmental, and developmental factors. While morphometric features such as kernel size and shape may be correlated with weight, they may not be the only or even the most important factors influencing weight. One limitation of using morphometric features for predicting weight is that these features do not take into account the internal density or composition of the kernel, which can vary depending on factors such as water content, starch content, and protein content. Two kernels with the same size and shape may have different weights if one has higher density due to differences in composition. Furthermore, kernel weight can be influenced by the position of the kernel on the ear, as well as by interactions between kernels on the same ear. Kernels near the base of the ear, for example, may be larger and heavier than kernels near the tip of the ear. Similarly, kernels on the same ear may compete for resources, which can affect their growth and weight. Kernel weight can also be affected by post-harvest factors such as moisture content, storage conditions, and processing methods, which may not be fully captured by morphometric features obtained from image analysis. Therefore, our model has moderate accuracy for prediction of single kernel weight using morphometric features extracted image analysis.

The results of the regression models created for the determination of kernel viability using image analysis parameters in the study are presented in Table 4. According to these results, kernel viability could not be determined by morphological measurements based on image analysis. As a matter of fact, R^2 values for all models were found to be very low. There are several studies showing that kernel viability can be detected based on image analysis. In these studies, several kernel features, such as color, size, and shape parameters, were extracted from images of individual kernels and these features were used as inputs for machine learning algorithms. Yaman and Kahriman (2022) achieved high success (accuracy=0.91) from a machine learning model in which spectral data and morphological features were used together in their studies on the determination of kernel viability in maize with morphological features, image data and spectral data. In our study, it was understood that kernel viability could not be determined using morphometric features alone. Image analysis features may not be effective for predicting viability in maize for a few reasons. Firstly, image analysis typically captures morphological features of the kernel such as size, shape, and color. While these features may be correlated with viability, they do not provide direct information about the physiological state of the kernel. Other factors such as seed vigor, genetic factors, and environmental conditions can also influence kernel viability, which may not be fully captured by image analysis features. Kernel viability may also depend on internal factors such as the integrity of the cell membrane, which is not visible from the outside of the kernel. Therefore, image analysis may not be able to capture all the necessary information for predicting viability. Image analysis features may not be effective for predicting viability in maize because they may not provide direct information about the physiological state of the kernel, may vary depending on the stage of kernel development, and may not capture all the necessary information for predicting viability. On the other hand, the modeling technique used for viability detection also affects the results of the study. The majority of successful studies used deep learning or machine learning methods combined with machine vision. The linear regression modeling technique used in our study may not have been insufficient in this regard. In addition, the success of the models created in this study may be low, since the predictive variables consisted only of morphological features.

Table 4. Results of regression models for kernel viability (DV4) using the morphometric features of ear samples extracted from image analysis

Features/Stats.	Model 1	Model 2	Model 3	Model 4
Area	0.001**	0.004**	0.004***	0.002
Perimeter		-0.009	-0.015**	-0.019**
Length			0.013	0.034
Width				0.023
Constant	0.829***	0.978***	0.997***	0.848***
N	1,223	1,223	1,223	1,223
R^2	0.005	0.007	0.009	0.009
Adjusted R^2	0.004	0.006	0.006	0.006
Std. Err.	0.280	0.279	0.279	0.279
	(df = 1221)	(df = 1220)	(df = 1219)	(df = 1218)
F Statistic	6.498**	4.603**	3.501**	2.880**
	(df = 1; 1221)	(df = 2; 1220)	(df = 3; 1219)	(df = 4; 1218)

* p<0,05, ** p<0,01, *** p<0,001

Conclusion

In this study, the results are given for prediction of ear weight, grain weight per ear, single kernel weight and kernel viability, which cannot be obtained by direct image analysis, using image analysis parameters related to morphometric features. It is generally more accurate to predict ear weight than total kernel weight in maize using image analysis because ear weight is a more direct measure. For the models created to determine the single ear weight through image analysis results, it was observed that 82.9% accuracy could be achieved when only the ear area was used, while the regression coefficient increased to 88.8% when the ear perimeter variable was also included. Total kernel weight is calculated by summing the weight of all the kernels on an ear, while ear weight is the weight of the entire ear, including the ear and husks. Single kernel weight could be predicted with kernel morphometric features in our study. It was understood that the kernel viability cannot be predicted by using the linear regression method only on kernel morphology.

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Authors' Contributions

The authors declare that they have contributed equally to the article.

Conflicts of Interest Statement

The authors declare that they have no conflict of interest.

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