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NON-DESTRUCTIVELY DETERMINING BLUEBERRY (*Vaccinium corymbosum* L.) LEAF AREA USING DPI-BASED SOFTWARE

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Abstract: Blueberries (*Vaccinium* spp.) are a popular crop all throughout the world. Blueberry (*Vaccinium corymbosum* L.) leaves were randomly selected from the experimental area of Ondokuz Mayis University, Faculty of Agriculture as a research material. Blueberries are high in polyphenolic chemicals, particularly anthocyanins, which are antioxidants and anti-inflammatory. A total of 1500 leaves were collected, with 100 for each cultivar, to represent the variety of sizes found on 15 plants of each cultivar. Manual leaf area measurement was made with a digital planimeter. The area was measured using a 300dpi resolution image read from the relevant file in such a way that every one of them is defined by 8bits in RGB color space. A weighted sum of the RGB components of the image is used to convert RGB values to grayscale values. This three-dimensional gray image generates a binary image. The Otsu method was used to determine the threshold value required to minimize the in-class variation of the threshold black and white pixels. Simulink was used for easy use of the end-user with the developed software. This software can be used for the area measurement of all plant leaves.

Keywords: Blueberry, Leaf area, Software, *Vaccinium corymbosum* L.

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1. Introduction

Leaves are one of the fundamental physiological organs of a plant which photosynthesis and transpiration take place and they are the most important organs that plants have. They contribute to the absorption of nutrients and water from the soil through the roots and allow the translocation of photosynthetic products from the source to the sink organs. Moreover, within a life cycle of a plant, leaves pass from a juvenile heterotrophic state to a mature autotrophic condition determining source-to-sink transitions (Turgeon, 1989; Shakya and Lal, 2018). Since leaf size is an adaptation and response to the environment, it can be considered an indicator of the conditions plants grow in: climate, topography, soils, etc. These relationships are interesting for geneticists, ecologists, and agronomists. Agricultural practices aim to improve growing conditions for plants. Leaf area is one of the aspects measured by agricultural scientists, especially agronomists, to establish the best agricultural practices. Similarly, farmers can check if they are creating an ideal environment for their annual and perennial crops (Uzun and Çelik, 1999; Çelik and Odabas, 2011; Trimble, 2019).

Leaf area measurement is a reliable parameter in studying the impact of the environment on plants in the disciplines of ecology, genetics, and crop management. Eco-physiologists, geneticists, botanists, ecologists, environmental scientists, and agronomists are some of the occupations that use leaf area measurements. Leaf area measurement is extremely useful to scientists and growers. Therefore, an understanding of leaf area and the different methods to measure it is important (NeSmith, 1991; Montero et al., 2000). There are destructive and non-destructive methods of measuring leaf area. Some of the common methods are direct measurements, millimeter graph paper method, planimeter, image processing and digital scanners. In the past decade, scientist developed several equations for estimating the actual leaf area nondestructively by several linear measurements (Uzun and Çelik, 1999; Pandey and Singh, 2011).

A non-destructive leaf area calculation method is essential as the blueberry leaves cannot be used for scientific studies. Blueberry leaves are also important both for berry enlargement and ripening as well as their chemical properties. According to the Retamales and Hancock (2018), the whole-canopy leaf area affects fruit quality. They also revealed that the rate of leaf area development of blueberry bushes is greater than in annual crops. And it is clear that maintaining a balance between vegetative and reproductive growth is needed to optimize blueberry yield and quality. Carbohydrates are produced, even after harvesting to color change and leaf fall (Goncalves et al., 2002).

According to leaf size and shape, direct leaf area determination in the field is generally unrealistic because it needs sophisticated leaf area meter devices (Uzun and Çelik, 1999; Odabas et al., 2009). The non-destructive leaf area prediction via simple equations is an inexpensive and rapid tool, and can provide many advantages to researchers for horticultural experiments. Non-destructive methods, which do not require the leaves to be detached, are useful because they allow measurements to be repeated during the plant's growth period, and reduce the variability associated with destructive sampling procedures (Çelik and Uzun, 2002). Moreover, the simple mathematical models enable us to measure leaf area on the same plants during growth periods and may reduce variability in experiments.

Development of a mathematical model to predict leaf area with the dimensions of leaf length, width and petiole length, or some combinations of these variables, which usually have high accuracy, has been a common approach (Kersteins and Hawes, 1994; Montero et al, 2000). Various mathematical models for indirect estimation of leaf area can be revealed separately for cultivars, species and genotypes, or a model can be applied for several cultivars and different species (Potdar, 1991; Öztürk et al., 2019). It is obvious that without proper experimental and statistical validation, models which attempt to predict leaf area of the plants must be viewed with caution (Rao et al., 1978).

The importance of rapid and accurate measurements of leaf area in agronomic and physiological studies is well known, but literature revealed little information available for highbush blueberries. A leaf area estimation model on rabbiteye blueberry was reported only by Nesmith (1991). Northern highbush blueberries have been getting the most popular small fruits in the northern part of Anatolia and several physiological and quantitative studies are going to be adjusted on it. Therefore, the objective of this study was to develop regression models that would accurately predict northern highbush blueberry cultivars leaf area using physically determinable points of measurement. Area measurements of leaves are generally used with devices that take up a large area or portable (measuring according to the sensitivity of the user) devices. However, the costs of these devices are high.

In the present study, a software has been developed to measure the leaf width, height and area of the plant with high accuracy using a database of blueberry leaves. Image processing and artificial neural networks are used in this software. This software can be used not only for blueberry leaves, but also for calculating leaf width, height and area of other plants.

2. Material and Methods

2.1. Plant Material

Pot grown 6 years old blueberry plants were randomly selected from the experimental area of the University of Ondokuz Mayis, Faculty of Agriculture. Twelve northern highbush (*Vaccinium corymbosum* L.) (Bluecrop, Denise Blue, Bluegold, Patriot, Nui, Brigitta, Hortblue Poppins, Goldtraube, Aurora, Liberty, Northland and Reka) and 3 southern highbush (*Vaccinium corymbosum* x *Vaccinium darrowii* hybrids) Misty, O'Neil and Jubilee were used to develop leaf area prediction models. Leaves on the first flushes were considered, and a total of 1500 leaves, 100 for each cultivar, were collected representing the range of sizes present on 15 plants of each cultivar. The leaves were processed in the following manner in order to develop the best fitting model for predicting the highbush blueberry leaf area individually and overall. After collecting the leaves, they were placed in sealed plastic bags, stored at 15 °C, moved to laboratory conditions, finally placed on the photocopier desktop by holding them flat and secure, and scanned with 300 dpi.

In agricultural trials, leaf area estimate algorithms that try to forecast leaf area non-destructively can bring various advantages to researchers. Furthermore, these models allow researchers to measure leaf area on the same plants over the duration of a study, resulting in lower experimental variability. Expensive tools and/or predictive methods can be used to determine leaf area (Figure 1).

Figure 1. Digital planimeter.

2.2. Image Processing

The 300dpi resolution image required to calculate the area was read from the relevant file in a way that each of them is represented by 8bits in RGB color space. RGB values are converted to grayscale values using a weighted sum of the RGB components of the image (0.2989 x R + 0.5870 x G + 0.1140 x B). A binary image is obtained from this 3-dimensional gray image. Here, the threshold value required to minimize the in-class variance of the threshold black and white pixels was determined by the Otsu method (Otsu, 1979).

It is aimed to separate the compound leaves from each other by using the morphological image processing method on this binary image. For this purpose, erosion and then dilation is applied to the image with a disk-shaped structural element. Any areas with small pixels from the image are extracted and the gaps (white areas) are filled. Thus, an image is obtained in which the leaf areas are white and the other areas are black. The number of leaves is determined by labeling the connected components from the pixels of the image. Also, the actual number of pixels in this linked white region is calculated as a scalar. Since 1 Inch=2.54 cm, the area for a DPI_X=DPI_Y=300 image is calculated with the equation 1.

$$LA=PN/[(DPI\_X/2.54)x(DPI\_Y/2.54)] \quad (1)$$

where; LA is leaf area, PN is pixel number, X is leaf width and Y is leaf length. The Otsu method is used for automatic image thresholding in computer vision and image processing. The method returns a single intensity threshold that divides pixels into two classes: foreground and background, in its most basic form. The density within-class variance is minimized, or the variance between classes is maximized, to calculate this threshold.

2.3. Graphical User Interface (GUI)

Graphical User Interface (GUI) designs are created to assist electronic devices in using icons, icons, and other visual graphics. On older computers and electronic devices with GUI, pre-command-based operating systems, the command line was utilized to accomplish any task. Users used the keyboard and commands to carry out all computer operations. Within the computer screen, the GUI consists of windows, icons, and control windows. Users can use computers without having to input even a single command line in this manner.

The computer can be handled with a mouse and operations may be completed rapidly using shortcuts and keys thanks to the GUI. Unlike command-based systems like IOS, Unix, or MS-DOS, the GUI is significantly easier to learn and use. To accomplish actions in command-based systems, users must both write and memorize codes. In GUI operating systems, however, the computer can be utilized without knowing a single line of command code. Another benefit that graphical interfaces provide to consumers is that they do not require knowledge of any programming languages. Because all systems with a graphical user interface are now created with the needs of the end-users in mind (Anonymous, 2022).

For leaf and fruit measurements, new equipment, tools, and machines, such as hand scanners and laser optic apparatuses, have recently been developed. For both basic and simple studies, these are quite expensive and sophisticated apparatus. Furthermore, when compared to geometric measurements, non-destructive calculation of leaf area saves time (Odabas et al., 2009).

Artificial neural networks, image processing, and GUI were implemented using the program MATLAB software (Matlab ® R2013a). Whether the developed software measures correctly or not has been verified with a square with a certain width and length (Figure 2).

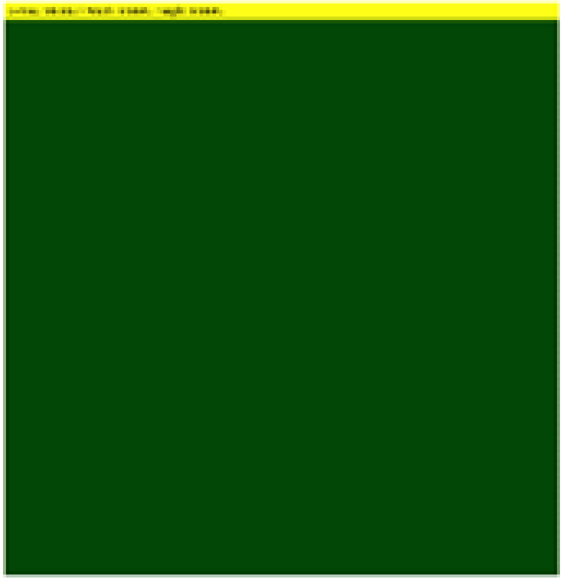

Figure 2. Square used for software area measurement verification (10cm × 10cm).

With the help of the developed interface, the areas of all leaf types can be easily measured. For the system, first of all, the leaves must be scanned at 300dpi resolution. The scanned image should be saved as a jpeg or tiff file. Then the program processes the raw image first. Image processing application is used by the software for this process. (Figure 3).

3. Results and Discussion

The leaf area is a measurement of plant health and potential crop yields that is directly tied to time-dependent crop growth (Baar et al., 2022). Leaf width, leaf length, and leaf areas are automatically calculated for each leaf, with precise borders determined by the software (Yin et al., 2022). The photosynthetic rate, dry matter buildup, and crop development are all influenced by the leaf. One of the criteria used to evaluate plant vegetative growth is leaf area. Leaf area index is used in crop modeling, as well as crop model calibration and validation (Shabani et al., 2017).

Leaf width and leaf length were used as input parameters for the ANN that calculated leaf area, while leaf area was used as an output parameter. A comparison of the results obtained with the software with the planimeter measurements is shown in Table 1. The software developed in terms of measurement values gives higher accuracy and faster results. Measuring with a planimeter is both time-consuming and makes a significant difference in precision. There is no uniformity between measurements. This causes errors in the leaf area calculation.

The planimeter measurements shown in Table 1 took approximately 20 minutes. The same process was carried out in less than one second with the help of software. This is another proof that the developed software is quite effective.

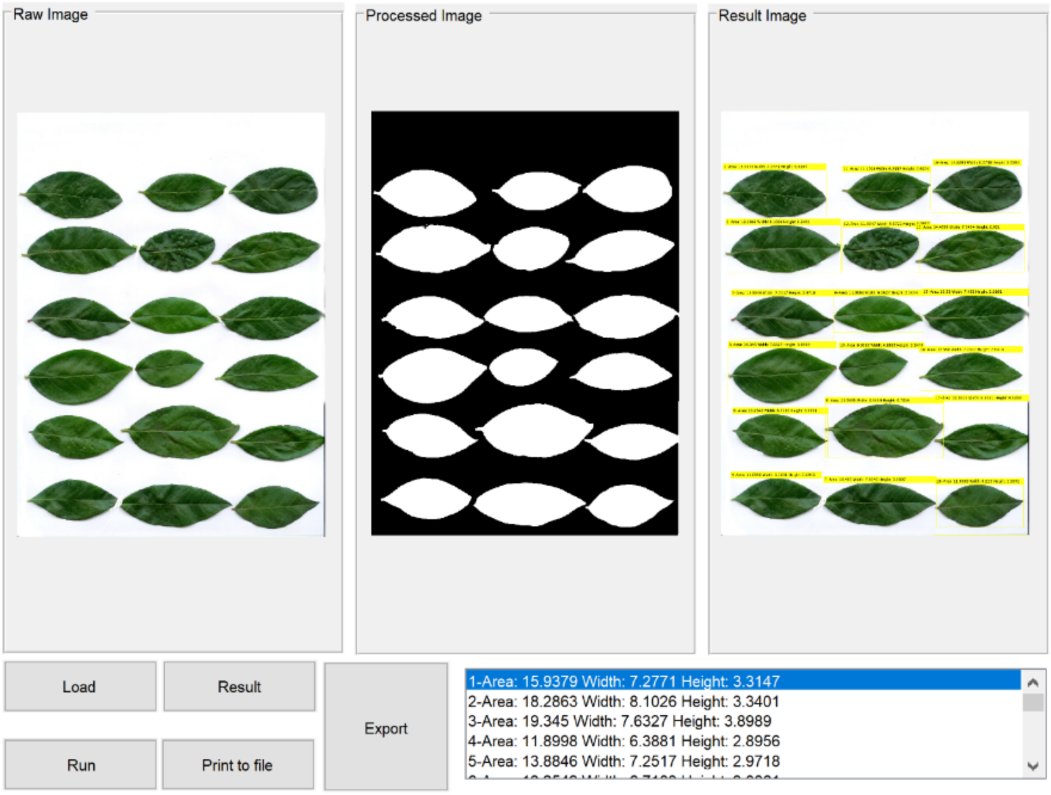

Figure 3. The developed GUI was used to calculate the leaf width, leaf length and leaf area of the samples.

Table1. Comparison of software results and digital planimeter measurement

Software			Digital planimeter		
Area (cm^2)	Width (cm)	Height (cm)	Area (cm^2)	Width (cm)	Height (cm)
15.8	7.2	3.3	14.0	6.3	3.0
18.2	8.1	3.3	16.4	7.2	3.1
19.3	7.6	3.9	16.1	6.8	3.5
13.7	7.2	3.0	11.8	6.5	2.7
11.6	6.3	2.9	9.7	5.7	2.6
13.1	6.7	3.1	11.1	6.0	2.7
16.1	7.9	3.1	14.4	7.3	2.7
20.8	8.3	3.8	18.3	7.2	3.5
10.9	6.3	2.6	9.5	5.7	2.3
8.9	4.8	2.7	8.0	4.5	2.4
11.1	6.2	2.7	10.0	5.6	2.5
11.7	5.4	3.0	10.4	5.0	2.7
14.1	7.6	2.9	12.4	6.7	2.5
12.3	7.2	2.6	10.7	6.4	2.4
15.3	7.3	3.1	13.0	6.9	2.7
14.6	6.2	3.3	12.1	5.6	3.0
11.6	6.7	9.3	9.7	5.8	2.3
11.5	6.1	2.9	10.3	5.0	2.7

One of the similar research, the proposed enhancement was to automate the image segmentation process using a two-level thresholding stage, removing the need to manually enter setup values. The findings imply that this technology is cost-effective and simple to execute in field situations, paving the way for future deployment on portable and traditional devices such as smartphones and tablets (Mora et al., 2016).

4. Conclusion

A plant's leaf area is a key factor of its growth. The material produced by the plant in an interval of time is dependent on the size of the leaves assimilating system. Particularly in the early stages of growth, it is comparatively easy to measure leaf area directly allows for the use of a small number of plants. In the later stages, however, when the leaves become numerous and large, measuring the leaf area of every leaf may become extremely laborious. This difficulty is amplified when working with field crops. Because of the crop's high variability, all observations must be done on a large number of random samples, each including numerous plants, in order to correctly quantify growth changes and the degree of experimental errors. Directly measuring the leaf area of such large plant samples would be impractical due to the time and effort required to measure hundreds, if not thousands, of leaves at each sampling interval. In this study, it is aimed to calculate the leaf area, which is important in plant physiology studies. In the calculation of the leaf area, the width and length of the leaf are considered as the basic criteria. Area calculation was carried out by using artificial neural networks in the leaf database created by image processing. The results obtained have created an interface that end users can use more easily via the GUI. Computer-assisted prediction is possible when image processing and a neural network technique are coupled. As a result, the image processing and neural network technique is more reliable, faster, and allows for high-precision prediction.

Author Contributions

M.S.O. (25%), G.K. (25%), H.Ç. (25%) and R.O. (25%) design of study. M.S.O. (25%), G.K. (25%), H.Ç. (25%) and R.O. (25%) data acquisition and analysis. M.S.O. (25%), G.K. (25%), H.Ç. (25%) and R.O. (25%) writing up. M.S.O. (25%), G.K. (25%), H.Ç. (25%) and R.O. (25%) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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