biology and life sciences forum

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Proceeding Paper

Image Based Phenotyping of Shell Thickness Revealed Strong Association with Kernel Recovery in Macadamia †

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† Presented at the 2nd International Electronic Conference on Plant Sciences—10th Anniversary of Journal Plants, 1–15 December 2021; Available online: https://iecps2021.sciforum.net/.

Abstract: Phenotyping in macadamia breeding programs is laborious, time-consuming, and costly. Developing rapid and cost-effective phenotyping technologies can reduce costs and increase breeding efficiency. This project assessed the possibility of using nutshell thickness to predict kernel recovery (KR) by applying a rapid and cost-effective method. Pictures of samples were captured with a digital camera and processed with Image J. The outcomes indicated that shell thicknesses can be used as a predictor for KR and image-based measurements offered higher prediction accuracy of KR than manual measurements.

Keywords: rapid phenotyping; macadamia breeding; image processing

Citation: Nguyen, T.; Bruce, T.; Alam, M. Image Based Phenotyping of Shell Thickness Revealed Strong Association with Kernel recovery in Macadamia. *Biol. Life Sci. Forum* **2021**, *1*, x. https://doi.org/10.3390/xxxxx

Academic Editor: Iker Aranjuelo

Published: 2 December 2021

1. Introduction

Four species of the genus Macadamia belonging to the *Proteaceae* family were found in the east coast of Australia. The species are *Macadamia. integrifolia*, *M.tetraphylla*, *M. ternifolia* and *M. jansenii* [1]. Only two macadamia species that produce edible kernels are *M. integrifolia* and *M. tetraphylla*. These two species and their hybrids are cultivated around the world with almost half of production located in Australia and South Africa [2]. Kernels can be used as snack food or in bakery goods and their oil has high commercial values for various purposes such as oil cooking and cosmetics [1]. Significant efforts and investments are being made into breeding programs of macadamia to improve profitability [1]. However, phenotyping activities of macadamia remains costly and laborious due to limitations of conventional phenotyping technology. In recent time, rapid phenotyping technologies have emerged as an effective approach to reduce the cost of breeding and improve breeding efficiency. Particularly, image-based rapid phenotyping techniques have been developed in other plants to characterise growth traits such as plant height, diameter at breast height, leaf areas and nut traits such as nut length and width. This report will explore the possibility of applying image-based rapid phenotyping techniques on nut traits of macadamia.

Macadamia nuts contains protective hard nutshell surroundings kernel. The thickness of shell wall ranged from 1mm to 4mm depending on the locations of measurements [3]. Kernel performance is commonly measured by the amount of total kernel in a nut in shell (NIS), also called kernel recovery (KR). Kernel recovery is one of the key indicators of farm profitability. There was a general consensus, without scientific records, that a thicker nutshell results in a smaller kernel, thus lower kernel recovery. This study assessed the relationships between nutshell thickness and nut yield indicators including kernel recovery. The cost of accurate measurement of KR across a large number of nuts is costly. Therefore, another focus of this study is to assess the kernel recovery using a rapid and cost-effective method.

Various rapid phenotyping techniques were developed to characterise plant traits. Two-dimensional image-based methodologies were applied to soybean and cereal seeds [4–6]. Image J software was employed extract morphological information from pictures of soybean seeds taken by a digital camera and recorded a high correlation coefficient (R = 0.94) between image-based measurements and manual measurements [6]. A software called Grain Scan was utilised to analyse pictures of wheat seed scanned by a desktop scanner and recorded a high Pearson correlation between image-based measurements and actual measurements (p = 0.981–0.996) [5]. Three-dimensional image processing techniques were also developed. 3D point clouds were constructed from Structure from Motion algorithms and were applied to extract growth traits such as tree stem circumferences and bole volume [7]; number of plant leaves [8]; root volume [9]; steam diameter at breast height [10]. However, due to the low resolutions of 3D point clouds, methods to extract micro-dissections in nuts was not established [11]. 3D image techniques with Computed Tomography scanner were developed for nut phenotyping due to their high resolutions [11], although the costs of purchase and maintenance were extremely high. For these reasons, 2-D image processing techniques are still commonly applied due to their low cost and high level of accuracy.

The aims of this study are to (i) identify the relationships of shell traits with kernel recovery, (ii) develop a 2D-image based rapid phenotyping techniques to characterise macadamia nut traits and (iii) explore the efficiency of image-based phenotyping of nutshell to use as a predictor of kernel recovery.

2. Materials and Methods

2.1. Plant Materials

Nut samples were collected from 50 second generation macadamia breeding progeny grown in Bundaberg station of Department of Agriculture and Fisheries. These progeny trees were planted from 2013 to 2016.

2.2. Sample Preparation for Nut Characterisation

Twenty nuts from each tree were collected, de-husked and dried for measurements. The drying task took six days including two days at 35 °C, two days at 45 °C and two days at 55 °C in order to achieve 1.5% moisture in content. Each nut was cracked into two halves by nut crackers in order to measure nut and kernel characteristics.

2.3. Manual Measurements

A ToolPRO digital slide calliper was used to measure nut length (hilum to micropyle), nut width (equator), nutshell thickness of each half of an individual nut at three positions, including hilum, micropyle and equators. A scale was used to measure the weight of whole nut, nutshell and kernel. Data of measurements were recorded in Excel spreadsheet. Data Analysis function was applied on data of each trait to generate the descriptive statistical information including minimum, maximum, mean and standard deviation.

2.4. Image-Based Measurements

For image-based analysis, a sub-sample consisting of ten progenies was measured. Samples were categorised into different groups based on the measurements of shell thickness at the equator, including less than 2 mm, 2–3 mm, 3–4 mm, more than 4 mm. At least two progenies were selected from each group. Pictures of nut samples from each tree were captured with a digital camera (Canon 7D mark II, lens 18-135mm f/3.5–5.6 IS) held by a tripod and processed with ImageJ. A ruler was positioned in the pictures as a reference for measurement. Polygon selections function in ImageJ was applied to create a polygon around outer-bound and inner-bound of nutshell. The software extracted several information from the polygons including outershell's area (OA), perimeter (OP), length (OL), width (OW), circularity (OC), roundness (OR) and innershell's area (IA), perimeter (IP), length (IL), width (IW), circularity (IC), roundness (IR). Nut section area (NSA) is calculated from the difference between outer-shell area and inner-shell area. Average shell thickness (AST) is calculated in accordance with this formula:

$$AST = \frac{NSA}{0.5 \times (Outershell\ perimeter + Innershell\ perimeter)} \quad (1)$$

2.5. Trait Correlations

The relationships among nut traits were examined by calculating correlation efficient in Data Analysis on Excel. When the coefficient approached close to 1 or -1, this indicated a strong positive or negative correlation between two variables. Shell thickness at different positions, nut length and nut width were measured against whole nut weight, shell weight, kernel weight and kernel recovery.

3. Results and Discussion

3.1. Extent of Variation across Genotypes

All traits presented in Table 1 were measured manually on nuts samples. Weight of whole nut (WNW), shell weight (SW) and kernel weight (KW) had average of 6.63 gram, 3.80 gram and 2.85 gram and standard deviations (SD) of 1.77, 1.18 and 0.79, respectively. Nut sample achieved a mean of kernel recovery (KR) at 43.06% with standard deviation at 7.55%. Nut length from hilum to micropyle (NLHM) has slightly smaller mean and standard deviation compared to nut width at equator (NEW). Shell thickness at hilum and micropyle had considerably higher means than shell thickness in equator. Shell thickness at hilum side 1 (STHH1) had the highest mean at 3.97 mm while shell thickness at equatorial region side 2 (STHE2) has the smallest mean at 1.92 mm. Standard variations of all shell thickness measurements ranged from 0.68 to 0.84. Means of shell thickness at different positions recorded in samples were consistent with the range of shell thickness 1mm-4mm presented in literature [3]. There was inconstancy in weight measurements of sample nuts. This was because some nuts had little, or no kernel resulted from infection of nut borders.

Table 1. Extent of variations of trait across genotypes.

Traits	Maximum	Minimum	Mean	Standard Deviation
WNW (g)	2.03	13.40	6.63	1.77
SW (g)	1.25	8.77	3.80	1.18
KW (g)	0.28	5.89	2.85	0.79
KR (%)	8.17	67.14	43.06	7.55
NLHM (mm)	15.67	34.80	23.77	2.13
NEW (mm)	16.95	36.11	24.58	2.45
STHM1 (mm)	1.08	5.19	3.23	0.77
STHM2(mm)	1.11	5.37	3.17	0.78
STHH1 (mm)	0.83	6.61	3.97	0.78
STHH2 (mm)	0.91	6.46	3.82	0.84
STHE1(mm)	0.80	4.09	1.98	0.68
STHE2 (mm)	0.83	4.62	1.92	0.70

3.2. Extent of Variation within Genotypes

SD of WNW in most of genotypes was lower than SD of WNW across genotypes except for genotypes TDN-2013-14 and TDN-2014-7. Genotype TDN-2013-5 had a slightly higher SD of SW than that of all genotypes. Higher SD of KW in comparison with SD of

KW across genotypes was shown in 9 genotypes, including TDN-2013-8, TDN-2013-10, TDN-2013-15, TDN-2013-25, TDN-2014-7, TDN-2014-8, TDN-2014-11, TDN-2014-12 and TDN-2015-6. Among these 9 genotypes, 5 genotypes had higher SD of KR compared to SD of KR across genotypes, namely TDN-2013-10, TDN-2013-25, TDN-2014-7, TDN-2014-8 and TDN-2014-12. Only 2 genotypes had higher SD of NLHM than that of all genotypes while 8 genotypes shown higher SD of NEW compared with that of all genotypes. The majority of genotypes presented smaller SD of shell thickness in comparison to the range of SD of shell thickness across genotypes except for 4 genotypes, including TDN-2013-2, TDN-2013-8, TDN-2013-14 and TDN-2015-2.

Significant variations in nut traits were observed in some genotypes, for instance, TDN-2013-14. This can be related to xenia effect because of practices of cross pollinations in macadamia trials and orchards. Pollen-parents can affect not only embryo (kernel) but also tissues of or maternal origins such as the husk and shell [12]. Moreover, because sample nuts were dropped throughout the season and collected together at the same time, nutritional partition can affect nut traits, even from the same genotype. Nitrogen label in *M. integrifolia* was traced after injection into branches and soil application and found that the xylem sap N concentration changed throughout seasons Fletcher, et al. [13], which affect the physiology and fruit setting capacity of macadamia trees.

3.3. Trait Corelations

NLHM and NEW had strongly positive correlations with SW, KR and WNW, ranging from 0.67-0.82 but had an insignificant correlation with KR (Table 2). The highest correlation coefficient in these two variables were with WNM, recorded at 0.82 and 0.80 respectively. Shell thickness measurements across different positions had a relatively positive correlations with SW, ranging 0.63-0.71. These traits had inconsiderably positive correlations with KW, with the highest and lowest coefficient recorded at 0.24 and 0.05. Relatively positive correlations were shown in shell thickness variables and WNW, ranging from 0.45-0.58. In contrast, negative correlations were recorded between shell thickness and KR. STHE1 had the highest correlation coefficient against KR, namely −0.59. Negative correlations between shell thicknesses with kernel recovery indicated the possibility of using shell thicknesses as a predictor for KR.

Table 2. Correlations between manually measured traits of macadamia nuts.

Correlation Coefficient	SW	KW	WNW	KR
NLHM	0.75	0.67	0.82	−0.17
NEW	0.69	0.75	0.80	−0.03
STHM1	0.69	0.24	0.57	−0.54
STHM2	0.71	024	0.58	−0.55
STHH1	0.63	0.12	0.49	−0.56
STHH2	0.66	0.14	0.52	−0.56
STHE1	0.64	0.05	0.45	−0.59
STHE2	0.66	0.06	0.46	−0.58

3.4. Image-Based Measurements

Table 3 represented the correlations between measurements extracted from imageJ software and yields indicators, previously measured manually. Image-based measurements of area, perimeter, width and length in both outershell and innershell had strongly positive correlations with WNW, SW and KW. Among outer-shell parameters, the highest range of correlation coefficient was recorded with WNW, namely 0.94–0.95, followed by SW and KW, 0.90–0.93 and 0.76–0.80 respectively. The highest range of correlation coefficient among innershell parameters was recorded with KW, namely 0.80–0.88, followed by WNW and SW, 0.70–0.76 and 0.56–0.61 respectively. Outershell parameters had negative correlations with KR, ranging from −0.54 to −0.56 whereas there was insignificant correlation recorded between innershell parameters and KR. Both circularity and roundness measurements of outershell and innershell showed no correlations with manual measurements.

NSA has strong positive correlations with WNW and SW, 0.88 and 0.96, respectively. Similarly, ANT had positive correlations with both WNW and SW, 0.77 and 0.89, respectively. Both parameters had considerably negative correlation with KR, recorded at −0.82 for NSA, and −0.87 for ANT. Insignificant correlations with KW were recorded in both NSA and ANT.

Table 3. Correlations between image-measured traits and manually measured traits of macadamia nuts.

Correlation Coefficient	SW	KW	WNW	KR
OA	0.93	0.80	0.97	−0.54
OP	0.93	0.79	0.97	−0.56
OW	0.91	0.79	0.95	−0.55
OL	0.90	0.76	0.94	−0.56
OC	−0.05	0.12	0.01	0.16
OR	−0.06	−0.02	−0.05	0.07
IA	0.58	0.88	0.73	−0.06
IP	0.61	0.88	0.76	−0.11
IW	0.60	0.82	0.73	−0.16
IL	0.56	0.80	0.70	−0.07
IC	−0.18	0.19	−0.07	0.37
IR	−0.08	0.06	−0.04	0.22
NSA	0.96	0.47	0.88	−0.82
ANT	0.89	0.30	0.77	−0.87

Higher correlation coefficient with KR was shown in average nutshell thickness extracted from image-based approach in comparison with shell thicknesses at particular positions. Thus, average nutshell thickness was a better predictor for KR. This also suggested image-based approach offered higher accuracy than manual measurements. The struggles faced by manual approach can be explained by un-uniform curve and shape of nuts, difficulty to recognise the equatorial regions and degrading quality of calipers over time.

Nevertheless, the amount time taken for manual measurements was less than that for image-based measurements. It took approximately 80 seconds each nut to manually measure nutshell length, width and thicknesses at hilum, micropyle and equatorial regions. For image-based approach, the amount of time used to take image and process images was about 150–180 seconds for each nut. This indicated a need to develop faster image processing techniques.

One potential pathway is to develop automatic segmentation of nutshell and kernel. This approach was developed for phenotyping blueberry fruit [14] and leaf stomata [15,16]. Detection and segmentation of blueberry from 2D images was performed on a deep learning model built on Mask R-Convolutional Neural Network (R-CNN) [14]. Information about berry count, maturity and compactness was extracted from the model and had a high correlation with manually measured values, namely R square at 0.886. An automatic stomata detection algorithm based on mask CNN was developed to identify and count individual stomata [16]. Similar approach can be developed to segment nutshell and kernel from images and measure nut traits, particularly nutshell thickness.

4. Conclusions

This study confirmed the correlation between nutshell thickness and kernel recovery, indicating the utilisation of nutshell thickness as a predictor of KR. This study presented the first report on image-based phenotyping in macadamia. The image-based approach was robust for the measurement of average nutshell thickness, which has the highest correlation coefficient with KR among all phenotyping traits. Future development can employ automatic segmentation and R-CNN to improve the speed and efficiency.

Supplementary Materials: The table of traits measurement of genotypes are available upon request.

Author Contributions: Conceptualization, T.D.N., B.T. and M.A..; methodology, T.D.N.; writing—original draft preparation, T.D.N.; writing—review and editing, M.A., B.T.; supervision, M.A., B.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Queensland Alliance for Agriculture and Food Innovation.

Acknowledgments: This study acknowledges the support from Elise Elliott for conducting manual measurements of all nut sample.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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