Quantitative potato tuber phenotyping by 3D imaging

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Abstract

The accurate phenotyping of the external quality attributes of potato tubers is important in potato breeding. Currently, the assessment of potato tuber shape, together with eye density and depth, are based on subjective naked eye visual evaluation. However, such a manual visual assessment makes it very difficult to reliably phenotype these and other important, more complicated, geometrical traits, such as shape uniformity.

In this study, a 3D image analysis method has been developed for counting potato eyes and estimating eye depth based on an evaluation of the curvature of an acquired 3D point cloud. Six shape uniformity-related traits, together with their shape indices (SI), were measured for six potato varieties. These were collected from three field experiments designed initially to study the effects of variation in nitrogen (N), potassium (K) and compound fertilisers along with tuber mass, on all investigated external traits. We demonstrate that a 3D image analysis technique can estimate the number of potato eyes and their depth with a high degree of accuracy. In addition, three shape uniformity traits were identified as offering a better power discrimination between varieties. The preliminary experiment found potato tuber mass to significantly affect both the shape uniformity and eye count, while fertiliser treatments showed no effect on all traits except SI. However, further investigation with a larger sample size is required for confirmation.

Keywords: 3D image analysis; phenotyping; curvature estimation; shape uniformity; potato eye; potato shape

Nomenclature

Abbreviations		SI	Shape index
ABA	Abscisic acid	T	Triangle
ANOVA	Analysis of variance	TP	True positive
CF	Compound fertiliser	Z5	Zhongshu5
CM	Compound microorganism	Z10	Zhongshu10
CV	Coefficient of variation	Z18	Zhongshu18
DBSCAN	density-based spatial clustering of applications with noise	Z19	Zhongshu19
FAO	Food and Agriculture Organisation		
FN	False negative	**Parameters**	
FP	False positive	A	Area of the convex hull
GA	Gibberellin acid	A_m	Area of the neighbouring incident triangles
HSV	Hue, Saturation and value	D	Euclidean distance between the top and bottom slice images
K	Potassium	d_i	Euclidean distance between neighbouring slice images
L	Large	K (x_i)	Mean curvature normal operator at vertex x_i
M	Medium	$K_H(x_i)$	The mean curvature at x_i
MAS	Marker-assisted selection	minPts	Minimum number of points required to form a dense region
N	Nitrogen	N(i)	The 1-ring neighbours of the ith vertex
OIF	Organic-inorganic fertiliser	p	Perimeter of convex hull

PCA	Principal component analysis	r^2	Coefficient of determination
RGB	Red, green and blue	x_i	The ith vertex
RMSE	Root mean squared error	α_{ij}, β_{ij}	Angles between the two adjacent triangles sharing and subtending the edge of $x_i x_j$
S	Small	ε	Maximum distance between two vertices
SCF	Soil conservation fertiliser		
SfM-MVS	Structure from Motion Multiview Stereo		

1. Introduction

Potato (*Solanum Tuberosum L.*) is the fourth most important staple food in the world after wheat, maize and rice. The Food and Agriculture Organisation (FAO) estimated global potato production at over 368 million metric tons in 2013, substantially increased from 334 million tons in 2010 (FAOSTAT, 2013). Potato varieties with shallow eyes and regular round or oblong shapes are most often preferred by customers, including domestic consumers and processors. These attributes readily facilitate further processing to produce chips and French fries (Van Eck et al., 1994), and serve to reduce losses in peeling. With rapid advances in genomic technologies, marker-assisted selection (MAS) is now commonly being used in breeding programmes. Quantitative trait loci (QTLs) can be identified, and genes of interest cloned from the identified QTLs (Tester and Langridge, 2010). Potato varieties have shown genetic differences in potato shape, shape uniformity, number of eyes and eye depth, and a number of candidate genes underlying the main QTL have been identified in tuber shape and eye depths (Lindqvist-Kreuze et al., 2015). Although with appropriate genomic selection, the breeding process and genetic gain can be faster and greater respectively, high-throughput phenotyping is still a major bottleneck in real-world applications. Currently, phenotyping is often highly subjective, labour-intensive and sometimes destructive. A large number of studies on high-throughput phenotyping or 'phenomics' have been published in recent years, with the majority focused on various forms of imaging-based technologies (Samal and Choudhury, 2020; Yang et al., 2020). For instance, 2D image analysis has been shown to have good potential in quantifying external fruit quality and has been widely applied to measure basic shape characteristics, including aspect ratio and volume (AKodagali and Balaji, 2012; Beyer et al., 2002; He et al., 2017; Ishikawa et al., 2018). More recently, a reduction in hardware and computational cost has seen 3D imaging techniques being increasingly explored. For example, a 3D model reconstructed using the Structure from Motion Multiview Stereo (SfM-MVS) technique has been successfully deployed to estimate more sophisticated shape characteristics of strawberry, including eight significant uniformity-related traits (Li et al., 2020). In addition, a structured light-based technique has been successfully applied to the 3D reconstruction of the whole plant (Nguyen et al., 2015), where a commercialised device with integrated software was able to perform both data collection and 3D reconstruction (Morena et al., 2019). Another 3D reconstruction technique, in the form of laser scanning, has been applied for fruit size measurement, although the scanning stage is comparatively slower than the other two methods (Scarmana et al., 2020).

In potato breeding, accurate and fast tuber shape assessment is still a bottleneck in our effort to understand the association between the genetic variant and phenotype. Great efforts have been devoted to measuring potato shape from a 2D image analysis. Potato shape is most often divided into five categories based on the length to width ratio as: round, round-oval, oval, long-oval and very long. Prior work has included an image analysis algorithm for automated segmentation and aspect ratio estimation in video frames developed to assess this characteristic (Razmjooy et al., 2012; Si et al., 2018). A more sophisticated method was developed by using six parameters from a spherical-harmonic model (Torppa et al., 2007). Potato shape uniformity is an important trait due to its direct influence on customer satisfaction and benefits in further processing, including the production of chips or French fries. Afshin et al. (2016) developed a classification model with extracted size-shape features including roundness, elongation, eccentricity and extent, that involved the use of Fourier-shape features. Their total correct classification rate was 98% although this was only allocated to two shape categories ('regular' and 'irregular'). To improve size classification accuracy and take thickness information into account, a low-cost 3D stereo camera was applied to generate accurate potato tuber volume estimation, and showed promising results as an online size grading system (Smith et al., 2018; Su et al., 2017; Torppa et al., 2007). An oval difference degree and a grid calculation method were developed for surface area shape detection and subsequent classification of tubers into categories of normal, bump, bent shape, and hollow (Su et al., 2018). It is clear that as a high dimensional feature, shape uniformity cannot be objectively measured by manual assessment. Also, the number of potato eyes and their depth are usually evaluated manually, where eye depth is allocated into four categories of: superficial, slightly deep, deep and very deep. Manual assessment for potato eye categorisation can be fast, but given that each potato tuber has multiple eyes with varied depths, their determination can be extremely rater dependent (Lindqvist-Kreuze et al., 2015). Tuber formation and development is a complex process, which is affected by environmental, biochemical and genetic factors, for which Nitrogen (N), potassium (K) and phosphorus (P) all play important roles (Rens et al. 2015, Grzebisz et al. 2018). However, the mechanisms concerning the phenotypic plasticity of shape uniformity of potato tubers under differing nutrient regimes together with the role of size remains unknown.

This study aimed to develop automated 3D image analysis software to: (1) assess algorithm performance for counting potato eyes together with their depth estimation; (2) analyse the tuber shape uniformity of six potato varieties; (3) evaluate the influence of genotype, tuber mass and different nutrient regimes on tuber shape uniformity, eye number and depth.

2. Material and methods

2.1. Potato samples

In order to understand the nutrient influence on the external quality traits of potato tubers, potato samples were collected from three large field experiments at Chabei Research Station (41°27'N, 115°3'E, Elevation 1358 m) in Zhangjiakou, Hebei province, China. The soil type is chestnut, typical in the Hebei Bashang plateau. The soil organic matter and total nitrogen content were 2.70% and 0.09%, respectively; the soil potassium level was 142 mg kg^{-1}.

A total of three large field experiments were laid out in a randomised block design, planted on 1st May 2019. Experiment 1 consisted of four sub-experiments, each of which contained three blocks. There were five plots for each block, randomly allocated to one of the five levels of nitrogen (N) fertiliser input (0, 100, 200, 300 and 400 kg ha^{-1}) with fixed P and K rates at 240 and 300 kg ha^{-1}; each sub-experiment area was allocated to one of the four varieties: Favorita, Zhongshu10 (Z10), Zhongshu18 (Z18) and Zhongshu19 (Z19). Experiment 2 consisted of three blocks, with twelve plots per block. Four levels of potassium (K) fertiliser input (0, 150, 300 and 450 kg ha^{-1}) with fixed N and P rates at 300 and 240 kg ha^{-1} were applied to each of the three varieties: Zhongshu5 (Z5), Z18 and Shepody. Experiment 3 contained three blocks, each with fifteen plots; five compound fertilisers (Table 1) were applied to each block for two varieties, including Z5 and Z18. There were in total 126 plots in this experiment and three potato samples, including the large, medium and small sizes, which were subjectively collected from each plot to increase the variability of size and shape (Table 2).

Table 1. Details of the five compound fertilizer treatments used in the new fertiliser experiment to study the effects of these treatments on potato growth and yield

	F1	**F2**	**F3**	**F4**	**F5**
Treatment*	Compound fertiliser (CF, kg ha^{-1}) ($N:P_2O_5:K_2O$ =15:15:15)	F1+25% F1 (CF, kg ha^{-1})	F1+Organic-inorganic fertiliser (OIF, kg ha^{-1})	F1+Soil Conservation fertiliser (SCF, kg ha^{-1})	F1+Compound microorganism (CM, kg ha^{-1})
Base Fertiliser	CF480	CF780	CF480+OIF300	CF480+OIF300	CF480+OIF600

*CF: Sino-Arab Chemical Fertilizers Co.,Ltd (SACF), N:P2O5:K2O = 15:15:15;

SCF: Guizhou Bao Tu ecological recycling agriculture technology Co., Ltd., N:P:K = 6:4:10;

OIF: Yunnan Tumama Fertilizers Co.,Ltd, $N:P_2O_5:K_2O$ = 8:8:14, Organic matter ≥12%;

CM: Bacillus subtilis / Bacillus licheniformis, complex fermentation, microbial content ≥ 0.2 billion per gram.

2.2. Manual assessment

In order to evaluate the performance of the 3D image analysis for potato eye evaluation, manual assessment was conducted for both the number and depth of the eyes. The number of eyes was counted manually for 122 randomly selected tuber samples. 78 eyes were selected from 15 tubers for eye depth measurement. Modelling clay was molded into each potato eye depression, where the resulting positive molding defined the eye shape after peeling off. The eye depth was then obtained by measuring the height of the positive clay model of the eye. All eyes were individually labelled using a marker pen so that they could later be correctly located in acquired point clouds.

Table 2. Mean and standard deviation potato tuber mass (g) for each variety in the three experiments.

	Favorita	**Z5**	**Z10**	**Z18**	**Z19**	***Shepody***
1	267.9±121.0		222.3±96.7	221.7±132.7	251.0±151.6	
2		260.4±114.4		224.9±96.4		204.6±72.5
3		183.1±93.9		162.0±58.7		

2.3. Image acquisition system

A 3D imaging platform (Greenpheno Ltd, Wuhan, China), placed in a darkroom, was used to reconstruct a complete 3D model of the potato tubers. The imaging platform consisted of a Basler camera (acA2040-25gc, Germany) mounted on a tripod with a resolution of

2046 x 2046 pixels and exposure time of 10 ms, a turntable (MERA300, Hongxingyang Technology Ltd, China), a white LED lighting panel (1200 x 600 mm) behind the camera, and a blue cuboid holder in the middle of the turntable (29 x 29 x 58 mm) on which potato tubers were pinned. The schematic of the measurement configuration is shown as figure 1a. With a viewing angle of 35° in the horizontal, the distance between the lens and sample was approximately 80 cm. The camera trigger and turntable rotation were controlled and synchronised via custom-built software, and images were captured evenly at 37 viewing angles at a speed of 56 s per complete rotation.

The image capture and processing were conducted using a high-performance computer with Intel Core i9 9900k processor, 32 GB of memory, and a NVIDIA GeForce 2080ti graphics processing unit (GPU).

Figure 1. Schematic showing configuration of the measurement system (a) and the resulting point cloud of the potato tuber and holder (b).

2.4. 3D model reconstruction

In order to optimise processing speed and reconstruction accuracy, the original 2D image was first cropped to a lower resolution (1957 x 1401 pixels), which was high enough to accommodate potato tubers of all sizes in this study. The cropped image was then converted from RGB (red, green and blue) to a grayscale image. Because the background intensity was lower than the potato tuber and its holder, an arbitrary thresholding was then applied to remove the background - setting the background pixel values to zero. Commercial software Agisoft Photoscan (Agisoft, LLC, St. Petersburg, Russia) was used to reconstruct the 3D point cloud model by implementing the SfM-MVS algorithm. This first generates a sparse point cloud to identify overlapping images based on SfM using input images calibrated for the camera locations. This is followed by applying MVS to produce a dense point cloud (Fig. 1b) based on the calibrated images (Westoby et al., 2012).

2.5. Point cloud pre-processing

Due to the arbitrary 3D coordinate system generated by SfM, which leads to inconsistent orientations for samples, pre-processing was conducted to calculate the coordinates obtained by the moment and translation of the raw point cloud to the origin of the 3D coordinate system. Principal component analysis (PCA) was applied to the coordinates of all cloud points to obtain the eigenvector corresponding to the largest eigenvalue. This gave the major principal axis and so the main orientation along the potato tuber and holder. A rotation matrix was then derived and used to rotate the point cloud to align it with the z axis of the system coordinate frame. The resulting point cloud was converted from RGB (red, green and blue) to HSV (hue, saturation and value). Exploiting the colour difference between potato tuber and its holder, arbitrary thresholding was applied on the hue channel so that the potato tuber could be separated from the whole point cloud. In order to facilitate curvature analysis, a mesh was generated from the pre-processed 3D point cloud, as shown in figure 2a, by Poisson mesh reconstruction (Kazhdan et al., 2006).

2.6. Accuracy of the 3D reconstruction

The accuracy of the 3D model reconstruction was evaluated by comparing the tuber volumes measured by image analysis with the water displacement method for 30 sample tubers (Li et al., 2017), where the volume of the tested tubers ranged from 150 to 718 cm^3. A QuickHull algorithm was performed to obtain the convex hull of the point cloud. This consisted of many triangles together with the cloud centroid. The volume of the convex hull was calculated by integrating the volumes of the individual tetrahedrons (Yamamoto et al., 2018). As the blue holder could be segmented from the point cloud and the volume was known, the absolute estimated tuber volume could be derived.

2.7. Curvature-based potato eye counting and depth estimation

The mean curvature describes locally how the curvature of an embedded surface in an ambient space, such as Euclidean space, changes from one place to another. It can thus be used to detect and describe the eye traits of a potato tuber. The mean curvature values of the vertices were calculated using the method described in Meyer et al. (2003), where the potato tuber is represented as a triangular mesh. Then for each vertex x_i, its 1-ring neighbours N(i) can be determined. The mean curvature normal operator K (x_i) at vertex x_i was calculated as:

$$K(x_i) = \frac{1}{2A_m} \sum_{j \in N(i)} (\cot \alpha_{ij} + \cot \beta_{ij})(x_i - x_j)$$

where A_m is the area of the neighbouring incident triangles. This is calculated using the following iterative procedure, irrespective of whether the triangle T incident to x_i is obtuse or not: (a) initialize A_m=0, (b) for each T, if it is non-obtuse, then A_m += A_v; otherwise, if the angle at x_i in T is obtuse, then A_m += area(T)/2; otherwiseA_m += area(T)/4, where $A_v = \frac{1}{8} \sum_{j \in N(i)} (\cot \alpha_{ij} + \cot \beta_{ij}) ||x_i - x_j||^2$, α_{ij} and β_{ij} are the angles between the two adjacent triangles sharing and subtending the edge of $x_i x_j$. The mean curvature K_H at x_i is finally estimated as half the magnitude of K(x_i): $K_H(x_i)$=0.5||K(x_i)||.

A surface region with a negative value was deemed as a downward concavity and hence potentially an eye. An arbitrary threshold (-1.8) was applied to remove both convex and shallow concave vertices. To cluster together the vertices corresponding to the same eye, density-based spatial clustering of applications with noise (DBSCAN) was applied. Finding DBSCAN groups points that are close to each other was based on the measurement of Euclidean distance (Ngo and Macabebe, 2016). Two parameters including ε (eps) and the minimum number of points required to form a dense region (minPts) were required to implement DBSCAN. Eps determines the maximum distance between two vertices for which one can be considered as within the neighborhood of the other, and minPts defines the minimum number of points for each cluster (Fig. 2b). Here, the value of 0.1 (ε) and 20 (minPts) were selected for the best agreement with ground-truth data. The number of eyes (Eye_N) for each potato tuber was determined by the number of clusters and the average curvature of each cluster was used to correlate with actual eye depth. In this study, the average of all eye depths for each sample (Eye_D) was used for data analysis.

Figure 2. 3D mesh of a potato tuber surface generated from a point cloud (a) and identified eyes labelled with false colours (b).

2.8. Potato tuber external quality traits

The shape index (SI) is a typical shape trait applied to the potato longitudinal section that was measured in 2D space in a previous study (Nankar et al., 2020). However, the results are highly dependent on the viewing angle and shape uniformity. In this study, the SI was defined as the ratio between the tuber length and the maximum width of the largest lateral image slice in parallel with the horizontal plane. Six shape uniformity-related parameters, which showed good correlations with manual assessment for fruit uniformity assessment, were measured in the study. A 3D imaging-based shape uniformity assessment was used to quantify the difference between a series of roundness measures taken from the top view (Fig. 3a) and the side views around the whole tuber, including area and main orientation (Fig. 3b). A more detailed explanation and specific characterisation of all shape uniformity-related traits was provided by Li et al. (2020). All the shape uniformity traits used in this study are listed in Table 3 along with brief descriptions.

2.9. Data analysis

2.9.1 Evaluation of potato eye counting

The evaluation of eye counting was based on the correct recognition of potato eyes present in the 3D model. The region is classified as true positive (TP) if the eye is correctly recognised; as false positive (FP) if there is no eye in the target region; or as false negative (FN) if the eye is present in the 3D model but is not recognised. The performance of the curvature-based potato eye recognition was measured by a precision (Eq. 1) and recall (Eq. 2) analysis (Zhao et al., 2011). A high precision indicates a high TP percentage and low FP percentage, whilst a high recall indicates a high TP percentage.

$$Precision = \frac{TP}{TP + FP} \tag{1}$$

$$Recall = \frac{TP}{TP + FN} \quad (2)$$

Further assessment was by the measurement of the F1 score (Eq. 3), which takes both precision and recall into account and provides an overall measure of the robustness:

$$F1 = \frac{2 * Precision * Recall}{Precision + Recall} \quad (3)$$

2.9.3. Statistical analysis

Minitab 19 (Minitab Inc., USA) was used for the statistical analysis - analysis of variance (ANOVA). For all three experiments, fertiliser treatment and variety were considered as two fixed effects and mass as a covariate in ANOVA.

Table 3. Description of the shape uniformity parameters measured for potato tubers

Shape uniformity parameters	Description
CV_A	Coefficient of variation (CV) of 100 side view areas with 3.6° intervals along the z axis. Only the pixels within the middle 80% of the total height were used for the area calculation.
Max_A/Min_A	Ratio between the maximum and minimum areas within 100 side views.
CV_D	The CV of principal orientations of 100 side view projected images, calculated by principal component analysis (PCA).
L/W	Aspect ratio of the minimum bounding box of the maximum circumference for horizontal slice images.
CIR	Circularity of the convex hull of the maximum circumference calculated by Eq. 4, where A and p are the area and perimeter of the convex hull.

STR	Straightness of the curve passing through the centroid of all horizontal slice images (N=80) calculated by Eq. 5, where d_i is the Euclidean distance between neighbouring slice images, and the D is the Euclidean distance between the top and bottom slice images.

$$CIR = \frac{4\pi A}{p^2} \quad (4)$$

$$STR = \frac{\sum_{i=1}^{N-1} d_i}{D} \quad (5)$$

3. Results

3.1. Performance evaluation of the 3D reconstruction

The accuracy of the 3D reconstruction was evaluated by comparing the actual tuber volumes with the estimated volume measured by image analysis. The comparison is displayed in figure 4, where the small deviation observed in the r^2 and RMSE values of 0.99 and 10.4 cm^3 respectively, shows good performance in the 3D reconstruction.

Figure 4. Regression analysis for tuber volume as measured by both manual assessment and image analysis. Red line is the idealised regression curve.

3.2. Potato eye counting and depth estimation

With the current PC configuration, the processing time for the eye traits generation was around 2.5 minutes per tuber. To evaluate the performance of curvature-based potato eye counting, the results from image analysis methods were compared with manual assessments (Fig. 5a). Linear regression indicated the image analysis-based potato eye counting was highly consistent and comparable with manual counting ($r^2 = 0.90$, RMSE

= 0.95). The image analysis method was also superior in recall (0.95) and precision (0.96), showing a high F1 measure (0.95).

For eye depth, a linear relationship was found between the curvature values (Fig. 5b) and depth from manual assessment ($r^2 = 0.81$, RMSE = 0.51 mm). The slope (-0.902) and intercept (0.111) of this linear relationship were used to estimate the eye depth of other potato samples collected from the main experiments.

Figure 5. Comparison of the estimated potato eye number by a 3D image analysis software with manual counting (a). Correlation between the estimated curvature values and actual potato eye depth assessed manually (b). Red lines are idealised regression curves.

3.3. The effects of genotypes, fertiliser treatment and tuber mass on shape traits

With the current PC configuration, the processing time for the generation of tuber shape traits was between 6 and 10 seconds per tuber. Significant differences were found in SI, eye traits and all shape uniformity related traits except STR (Table 4) among the tested varieties in all three experiments. Genotypes differed significantly in STR for both experiments 1 and 3, approaching significantly in experiment 2 (*p-value* = 0.051). Fertiliser treatments did not significantly affect any trait, except N fertiliser on SI: where increasing N led to decreasing SI. The analysis of mass effects showed consistent results for SI and all shape uniformity related traits except CV_A, CV_D and STR.

According to the statistical analysis for experiments 1 and 2, L/W, Max_A/Min_A and CIR showed better discriminative power among varieties than the other three shape uniformity-related traits. According to the national potato quality standard in China, potato tubers can be categorised into small (< 100 g), medium (100 – 300 g) and large (> 300 g) size groups. Due to the limited number of tubers in the small group for all experiments, and large group for experiment 3, further analysis was only conducted on these three shape uniformity-related traits and the eye traits in experiment 1 and 2 by selecting the samples of each variety from the medium and large groups. Most of the varieties showed higher shape uniformity for the medium sized tubers than for the large ones (*p-value* < 0.05). The only exception was Z18, which did not show significant differences among sizing groups in both experiments (Fig. 6).

A significant difference was found for the number of eyes (Eye_N) among varieties. Z5, Z19 and *Favorita* all had higher counts than other varieties (Fig. 7). The effect of tuber mass on Eye_N was significant for all three experiments. More detailed analysis was conducted for each individual variety (Fig. 7a and c), and generally the Eye_N showed an increasing trend from medium to large sized tubers in both experiments. Consistent with previous knowledge, Z5 (Fig. 7c) and *Favorita* (Fig. 7a) are varieties with high Eye_N. Z19 had significantly higher Eye_D than either Z10 and Z18 (*p-value* < 0.05) in experiment 1 (Fig. 7b), and Z5 showed the highest Eye_D in experiment 2 (Fig. 7d). The influence of tuber mass on Eye_D was inconsistent between experiments, but no significant difference was observed between large and medium size groups for both experiments.

Table 4. ANOVA of potato tuber and eye traits in dependency of genotype, fertiliser treatment and mass.

	Treatment		SI	L/W	CV_A	Max_A/Min_A	CV_D	CIR	STR	Eye_N	Eye_D
Variety x N x Weight	**Variety**										
	Favorita		1.97 a	1.21 b	0.20 b	1.18 b	1.56 b	0.98 b	1.05 b	10.34 a	3.56 ab
	Z10		1.66 b	1.20 b	0.17 b	1.16 bc	1.57 b	0.98 b	1.06 b	6.28 c	3.02 c
	Z18		1.89 a	1.17 b	0.18 b	1.13 c	1.59 b	0.98 b	1.05 b	7.08 bc	3.44 bc
	Z19		1.50 c	1.33 a	0.21 a	1.27 a	1.76 a	0.96 a	1.08 a	8.02 b	4.06 a
		F[a]	52.7***	22.6***	16.1***	45.0***	20.4***	35.4***	6.97***	18.51***	9.31***
	N										
	N0		1.83 a	1.24	0.19	1.19	1.64	0.97	1.05	7.96	3.51
	N100		1.76 ab	1.24	0.19	1.20	1.62	0.97	1.06	8.09	3.41
	N200		1.83 a	1.24	0.20	1.19	1.63	0.98	1.06	7.44	3.47
	N300		1.68 b	1.22	0.19	1.18	1.59	0.98	1.06	7.63	3.53
	N400		1.68 b	1.20	0.18	1.17	1.62	0.98	1.07	8.56	3.67
		F	4.82**	1.20 ns	0.49 ns	1.29 ns	0.69 ns	1.05 ns	2.08 ns	0.90 ns	0.39 ns
	Weight										
		F	112.9***	11.09***	1.79 ns	8.12**	0.18 ns	18.85***	2.34 ns	36.52***	0.82 ns
Variety x K x Weight	**Variety**										
	Shepody		2.22 a	1.27 a	0.21 a	1.23 a	1.59a	0.97 a	1.05	6.08 a	3.34 a
	Z18		1.97 b	1.18 b	0.18 b	1.14 b	1.59a	0.98 b	1.04	6.94 a	3.62 a
	Z5		1.63 c	1.45 c	0.23 a	1.37 c	1.73b	0.95 c	1.06	10.21 b	5.14 b
		F	72.8***	54.3***	17.8***	105.0***	20.4***	29.85***	3.05 (*p*=0.051)	31.77***	44.3***
	K										
	K0		1.94	1.29	0.20	1.23	1.63	0.96	1.06	7.83	3.91
	K150		1.97	1.31	0.21	1.25	1.65	0.97	1.04	7.06	4.02
	K300		1.91	1.31	0.20	1.24	1.64	0.97	1.06	8.40	4.02
	K450		1.94	1.30	0.21	1.26	1.62	0.96	1.05	7.68	4.18
		F	0.34 ns	0.15 ns	0.51 ns	0.60 ns	0.49 ns	0.44 ns	0.72 ns	1.84 ns	0.50 ns
	Weight										
		F	17.28***	5.38*	2.86 ns	8.62**	0.79 ns	6.39*	0.05 ns	31.77***	4.36*
Variety x CF x Weight	**Variety**										
	Z18		1.91 a	1.17 a	0.18 a	1.13 a	1.59 a	0.98 a	1.04 a	6.10 a	3.50 a
	Z5		1.53 b	1.38 b	0.22 b	1.32 b	1.79 b	0.95 b	1.07 b	9.49 b	5.14 b
		F	124.2***	88.5***	24.5***	212.2***	61.5***	100.4***	16.0***	81.5***	102.0***
	CF										
	F1		1.76	1.29	0.21	1.24	1.65	0.97	1.04	7.34	4.35
	F2		1.71	1.28	0.20	1.23	1.67	0.97	1.06	7.21	4.73
	F3		1.75	1.29	0.19	1.22	1.70	0.96	1.07	8.05	3.96
	F4		1.71	1.28	0.19	1.22	1.71	0.97	1.05	7.87	4.28
	F5		1.68	1.25	0.21	1.22	1.72	0.97	1.06	7.74	4.28
		F	0.67 ns	0.59 ns	0.93 ns	0.50 ns	1.10 ns	0.76 ns	2.27 ns	0.60 ns	2.37 ns
	Weight										
		F	6.88**	10.97**	0.11 ns	12.14**	0.47 ns	13.84***	0.00 ns	87.65***	12.88**

*Significant at 0.05 level; ** Significant at 0.01 level; *** Significant at 0.001 level

[a]F: F-value

Figure 6. Analysis of the effect of potato size groups: large (L) and medium (M) on L/W, Max_A/Min_A and CIR in experiment 1 (a-c) and 2 (d-f).

Figure 7. The effect of potato size group large (L) and medium (M) on Eye_N and Eye_D in experiment 1 (a, b) and 2 (c, d).

4. Discussion

The present study is the first to use 3D imaging technology for counting potato eyes and assessing qualitative traits, including potato Eye_N and Eye_D, using an imaging-based analysis. Generally, large differences in Eye_N were found between large and medium sized tubers for Z18 and Z19. For relatively small potatoes, eyes might not have fully developed and hence the image analysis software might not be sensitive enough to detect the extremely shallow eye depressions, which may result in the underestimation of Eye_N. Consequently, phenotyping Eye_N in a practical breeding programme requires a relatively large potato tuber for more robust results; thus, future studies are necessary to establish a mass threshold for each variety to better assess eye count reliably.

It is worth noting that errors in the estimation of Eye_N and Eye_D could be introduced in the imaging setup and 3D reconstruction algorithm as well as in manual assessment. Due to the limitation of the viewing angle for the imaging setup, the bottoms of the potato tubers were partly occluded and could not be fully reconstructed, which led to the underestimation of Eye_N and decreased the accuracy of Eye_D. There is no widely accepted method to accurately measure the potato eye depth as far as we know. A modelling clay method was used in this study but due to the irregular shape of the potato tuber, it was hard to standardise this method, which also may have introduced errors in the eye depth estimation. The effect of genotypes on Eye_N and Eye_D is consistent with previous knowledge and fertiliser input appears not to have had an effect on Eye_N and Eye_D.

Accurate estimation of Eye_D requires a higher quality of 3D model than other traits. For SfM-MVS, high quality 3D reconstruction relies on a large number of viewing angles and sufficient depth variation, which cannot be fully controlled due to the variation of the smoothness of surfaces in the present study, and this was probably the major error source for Eye_D estimation. Inaccurate feature extraction and matching always leads to spikes and holes in reconstructed point clouds/meshes. The density, resolution and quality of the 3D reconstructed point cloud/mesh directly affects the estimation of the mean curvature of the points. The lower the density, resolution and quality of the 3D point cloud/mesh, the less accurate the estimated mean curvature, and thus the less reliable the estimated potato eye depth. To improve the quality of the 3D reconstructed point cloud/mesh, more accurate SfM techniques, such as a fast feature detector (Ghahremani et al., 2021), can be applied. Other 3D scanning techniques can also generate 3D point cloud data that is compatible with the proposed image analysis software. Structured light-based 3D scanning can potentially provide more rapid and automated data acquisition. Structured light-based scanners project a pattern of light onto the sample and calculate the distance of each point based on the shape of the observed pattern of reflected light. Compared with SfM-MVS, structured light requires fewer rotations of the sample and generates high quality point cloud data in a more robust manner. Moreover, commercial structured light scanning systems do not need separate software to implement 3D reconstruction. While it is difficult to apply structured light scanning to shiny specular surfaces, such as strawberry fruit, the rough matt surface of potato tubers may make it suitable in a future study.

Phenotyping potato tuber shape in current potato breeding programmes simply groups shape into six broad shape categories. Any assessment of shape uniformity is still lacking, although this can be a more important trait in both potato retail and processing. Six shape uniformity-related traits, which previously showed good agreement with manual assessment in strawberries, were adopted in this study. Shape uniformity differed significantly among the chosen varieties. Z18 was found to have a better shape-uniformity than other varieties in the three experiments, and Z10 was comparable with Z18 in experiment 1 for all shape uniformity-related traits. It is possible that there is genetic variability in the potato tuber shape uniformity which can potentially be exploited in breeding programmes. In previous studies (Li et al., 2020), CIR, Max_A/Min_A and L/W were considered as more important variables than others, and this was consistent with the present study in that a greater number of varieties could be discriminated against by them. In this study, the potato tubers in the large group showed a trend of lower shape uniformity than the medium sized tubers, except for Z18, which showed a consistently higher shape uniformity than the other varieties. This hypothesis needs to be confirmed in a future study with a larger number of samples. As a high input of N fertiliser can increase the tuber size, a future study can be conducted to understand the optimal N input to balance the tuber size and external quality, including SI and shape uniformity.

Although the effects of fertiliser treatments on shape uniformity and eye traits were initially investigated with a sampling method in this study, further work with a larger number of samples should be conducted based on the automated imaging methodology developed here to confirm the initial finding.

5. Conclusion

Overall, this study demonstrated that the proposed 3D image analysis method is capable of providing quantitative phenotypic data for SI, shape uniformity and eye-related traits in potatoes. L/W, Max_A/Min_A and CIR were found to show the best discriminative power among varieties. The impact of variety, fertiliser and mass on tuber shape uniformity were innovatively assessed based on a 3D image analysis method. In this preliminary study, different fertiliser treatments showed no significant impact on shape uniformity and eye traits, but tuber size significantly affected the selected shape-uniformity traits. Inconsistent results were obtained for the analysis of the effect of tuber mass on the eye depth, probably due to the inadequate quality of the 3D model and limited sample number. By adopting an alternative 3D scanning method, such as a structured light based 3D reconstruction approach, it is believed that high-throughput phenotyping for novel external traits could be obtained in a practical commercial potato breeding programme.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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