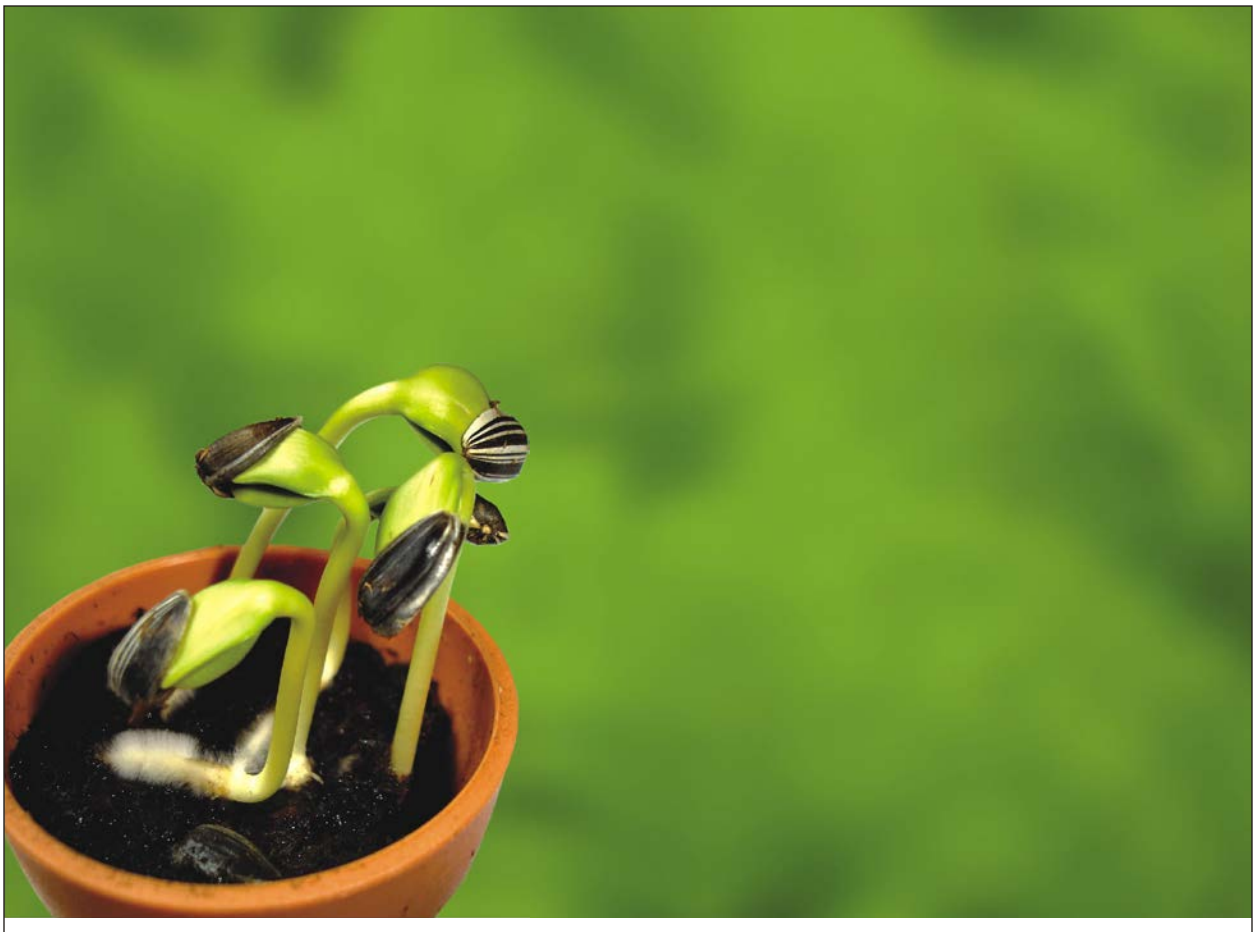

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Research Article
Phenotyping a Tomato Breeding Population by Manual Field Evaluation and Digital Imaging Analysis

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Abstract

Background and Objective: High precision phenotype data improve efficiency during selections within breeding populations. The objectives of this study were to evaluate precision of plant phenotyping methods within a tomato breeding population assembled at the University of Agriculture, Abeokuta, Nigeria. The precision of three phenotyping methods namely field evaluation of morphological characteristics (FM), laboratory digital imaging analysis of fruits (FDI) and seeds (SDI) were tested on 10 tomato accessions within the breeding population. **Materials and methods:** The FM phenotyping involved a randomized complete block design field experiment with three replications to get data on shoot length, branch number, leaf number, node number, flower number and fruit number. The FDI phenotyping was carried out on randomly selected fruits from each of the replicates with the aid of Veho™ software to obtain digital data on fruit length, fruit width, fruit radius, fruit circumference and fruit surface area. The SDI phenotyping was done on three replicates of seeds of each tomato accession, scanned with the Winseedle™ equipment which estimates seed length, curve length, seed width, curve width, curvature, volume circle, surface area and width-length ratio. Principal Component Analysis (PCA) was done on the data sets. **Results:** In PCA 2, cumulative eigen values were 68.02% for FM descriptors, 96.17% for FDI descriptors and 84.45% for SDI descriptors, indicating that digital imaging data of fruit descriptors would best distinguish this tomato breeding population. Among the FDI descriptors, fruit width and associated traits like fruit surface area, radius and circumference had the highest eigen vector loading of the PCA (0.43) and so was adjudged the best distinguishing trait. **Conclusion:** It was concluded that digitalized phenotyping offer more precision than manual phenotyping of the tomato breeding population. Laboratory digitalized fruit descriptors constitute the most precise phenotyping dataset and thus recommended for rapid discrimination and parental selections within the population.

Key words: Tomato breeding, precision phenotyping, digital descriptors, principal component analysis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tomatoes are fruit vegetables being consumed globally, so breeding projects are undertaken globally to improve them primarily against local productivity challenges and secondarily for market quality. By FAO statistics, approximately 223 million t of tomatoes are produced annually on 6 million h globally[1]. In Africa, wide variations exist in germplasm collections including cultivated and wild genotypes constituting potential sources of genes for developing new varieties[2]. Modern breeding methods make it possible to precisely identify genes and gene functions through Genomics Assisted Breeding (GAB) methods. For tomatoes, GAB had been used to map and select for various genes associated with agronomic traits and diseases resistance[3,4]. Thus, the success of modern breeding programmes depends on precision of measurements of phenotyped traits and their correlation with expression of genes or Quantitative Trait Loci (QTLs).

Complementary to crop improvements using genomic selection techniques are precision phenotype data sets[5,6]. Traditionally, tomato breeders characterize germplasm materials by growing the lines in nurseries and fields in randomized experimental trials and manually collect data on crop agronomic characteristics. This method of characterization is labor and time intensive and largely lacks precision in distinguishing genotypes because of human errors and biases. Hence, it is important to explore new methods of phenotyping that can distinguish genotypes with considerably improved precision in a timely fashion. Digital imaging analysis had been explored as high precision phenotyping tools for this purpose in many crops[7-17]. Precision phenotyping is a term coined from precision agriculture, which is commonly used to describe the use of digitalized technologies for crop management purposes[6]. It basically involves the use of software to capture digital data with scalable technologies ranging from simple laboratory digital image capturing platforms[7-11,13] to aerial[15,16] and real-time satellite imaging platforms[17]. Recent advances in precision phenotyping covers the use of these technologies to acquire specific crop data on agronomic traits, crop nutrition and water regime, soil fertility and pests and diseases status[6].

This study was set up to explore the use of digital images as a tool for phenotyping a tomato population in a breeding programme for resistance to fusarium and bacterial wilt at Federal University of Agriculture, Abeokuta under a DelPHE, UK supported project[18]. The germplasm assembled includes local landraces and improved genotypes from Asian Vegetable Research and Development Center (AVRDC). Molecular genotyping of the population was done using various markers in the lifetime of the funding support[19]. Several crosses had also been done and the breeding population is expanding. In this study it compared the precision of two laboratory digital phenotyping tools with manual field phenotyping for identification of genotypes within this population. The specific objectives of this study were to: (i) To explore simple digital precision alternatives to manual phenotyping and (ii) To identify best descriptor traits for digital precision phenotyping in the tomato breeding population.

MATERIALS AND METHODS

The field experiments were carried out on the farm of DelPHE-5 project site at Federal University of Agriculture Abeokuta (FUNAAB), Nigeria between June-November, 2014. The laboratory part of the research comprising of digital imaging analyses were done at the Department of Plant Breeding and Seed Technology, FUNAAB. Ten promising parental lines with varying levels of tomato wilt resistance from previous crossing evaluations were selected for this study, including: Delila, Danjos, Tomachiva, Tyre type, AVT09803, Santana, GH41, GH28, Gempride and NG/019 (Table 1).

Field morphological evaluation (FM phenotyping): The FM phenotyping involved the agronomic characterization on each genotype in the field experiment laid out in a Randomized Complete Block Design (RCBD) with 3 replications. Each genotype was planted in 1 m×1 m plots replicated in 3 blocks. Data was collected on the following plant morphological parameters till flowering stage: Shoot length, branch number, leaf number, node number, flower number and fruit number.

Fruit digital imaging analysis (FDI phenotyping): Tomato fruits were harvested from each plot separately into a polythene bag and tagged. Thirty fruits were selected

Table 1: List of genotypes in the tomato wilt breeding population

Genotype	FUNAAB codes	Sources	Plant type
Danjos	FUN/DJ/12/0057	Nigeria	Creeping
Delila	FUN/DL/12/0010	Nigeria	Erect
Tyre type	FUN/TT/12/0019	Nigeria	Creeping
Santana	FUN/ST/12/0017	Nigeria	Creeping
NG/OE/MAR/09/019	FUN/LYC/12/0005	Nigeria	Creeping
Tomachiva	FUN/TC/12/0045	Nigeria	Creeping
GH41	FUN/GH/12/0041	Ghana	Erect
GH28	FUN/GH/12/0028	Ghana	Erect
Gempride	FUN/GP/12/0013	Nigeria	Creeping
AVTO9803	FUN/AV/12/0084	AVRDC	Erect

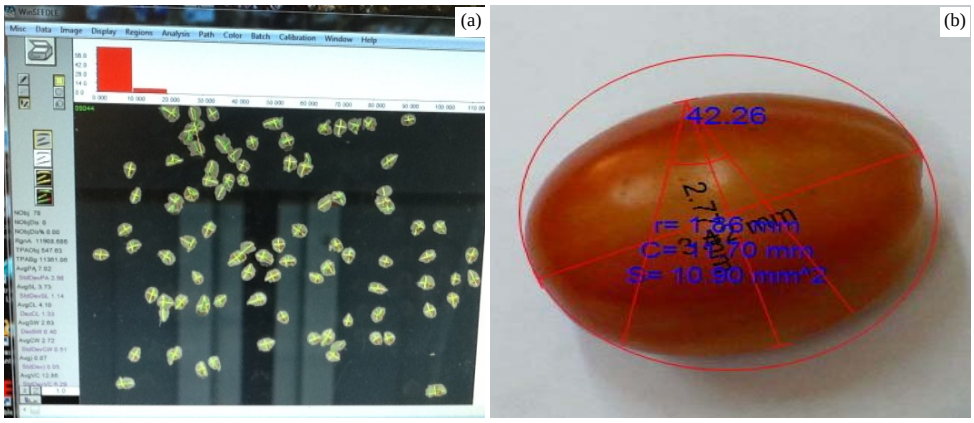

Fig. 1(a-b): (a) Screenshot of Winseedle™ tomato seed images and (b) Screenshot of Veho™ digital measurements of scanned fruits of Tomachiva

randomly from each replicate, the fruits were rinsed with water and wiped to get rid of dirt (soil) on the fruits. The fruits were place on plain sheets of paper in room condition to blot off excess moisture. A digital microscope attached to a computer device, running a digital imaging software-Veho™, UK was used to acquire fruit images and digital fruit morphological data (Fig. 1). The microscope was calibrated to 1 cm×1 cm. A single fruit was sampled at a time on a white background field and focused until high resolution image was visible on the computer screen. The image of each fruit was captured and stored in a folder in the computer system. Data was acquired by clicking on the captured image to transfer them to the measurement window of the software where measurement icon menus were utilized to analyze the fruit images. Trigonometric measurements were taken on captured images of the fruits by drawing the menu icons of the software which automatically generated the dimensions. The data obtained were fruit length, fruit width, fruit radius, fruit circumference, fruit surface area.

Seed digital imaging analysis (SDI phenotyping): Seeds from the harvested tomato fruits were extracted and allowed to dry. The number of seeds per fruit was then scored and recorded. Ten seeds each of three replicates were selected at random and arranged on a plate placed on the scanner bed. Seed images were acquired and analyzed by the Winseedle Pro™, Canada, digital imaging software. The system comprised of an EPSON™ scanner, transparent plates to place seeds on scanner bed and an attached desktop computer device running the software. Figure 1a shows a screenshot of WinSeedle™ software running an analysis on tomato seeds. The acquired images of seed was analysed automatically by Winseedle™ set to generate data based on seed type and background settings. Data generated on the tomato seeds were average projected Pixel area (AvgPA), average seed length (AvgSL), average curved length (AvgCL), average seed width (AvgSW), average curved width (AvgCW), average curvature, average volume circle (AvgVC), average surface area circle (AvgSAC) and width to length ratio (Avg W/L). This experiment was repeated twice.

Data analysis: All the data generated from the three phenotype evaluations were subjected to a multivariate analysis, Principal Component Analysis (PCA) using SAS[20] procedure. The PCA was used to evaluate explained variability within the population by each of the three methods. The precision of the three methods to distinguish the genotypes in the population was based on the eigen values estimated for first 2 PCA axes of each characterization method. The PCA also provided estimates of eigen vectors to identify best descriptor traits that most distinguishes the genotypes for each data set.

RESULTS AND DISCUSSION

The results showed differences in the eigen values and the percentage cumulative variations explained in the PCA by each method, which represents a quantification of precision of each phenotyping method.

Precision differed among the various phenotyping methods of the tomato population based on the eigen values and percentage cumulative variations explained from the PCA analyses of each data set (Table 2). Eigen values at PCA 1 and 2 were highest for FM phenotyping data set followed by the SDI phenotyping and lastly FDI technique (Table 2) indicating that FDI data set most precisely distinguished the

Table 2: Precision of each methods based on the eigen values and percentage cumulative of variability explained by the PCA

	Field morphological parameters		Fruit digital parameters		Seed imaging parameters	
Parameters	Eigen value	Cumulative (%)	Eigen value	Cumulative (%)	Eigen value	Cumulative (%)
PCA 1	15.23	50.78	5.21	86.77	7.64	69.46
PCA 2	5.17	68.02	0.56	96.17	1.65	84.45

Table 3: Eigen vectors of each parameter in principal components 1 and 2 of field morphological data after 4 weeks of transplanting

Traits	PRIN 1	PRIN 2
SL	0.11	-0.19
NoL	0.23	-0.09
NoB	0.24	0.08
NoNOD	0.21	-0.79
NoFLW	0.20	0.01
NoFRT	0.23	0.11

SL: Shoot length, NoL: No. of leaves, NoBr: No. of branches, NoNOD: No. of nodes, NoFLW: No. of flowers, NoFRT: No. of fruits

Table 4: Eigen vectors of each parameter in principal components 1 and 2 of fruit digital images data

Traits	PRIN 1	PRIN 2
FRTL	0.41	-0.31
FRTW	0.43	-0.16
FRTRAD	0.43	-0.13
FRTCIR	0.43	-0.01
FRTSA	0.43	-0.06

FRTL: Fruit length, FRTW: Fruit width, FRTRAD: Fruit radius, FRTCIR: Fruit circumference, FRTSA: Fruit surface area

Table 5: Eigen vectors of each parameter in principal components 1 and 2 of seed digital images parameters

Traits	PRIN 1	PRIN 2
PA	0.35	0.13
SL	-0.34	0.23
CL	0.34	0.13
SW	0.36	-0.02
CW	0.33	-0.25
CUR	-0.03	0.66
VC	0.36	-0.06
SAC	0.35	-0.04
W/L	0.13	-0.62

PA: Pixel area, SL: Seed length, CL: Curve length, SW: Seed width, CW: Curve width, CUR: Curvature, VC: Volume circle, SAC: Surface area circle, W/L: Width to length ratio

tomato population and the manual field evaluation data sets had minimal precision. All the FM descriptor traits accounted for 50.8 and 68% of the variations in the population in PCA 1 and PCA 2, respectively. The FDI descriptor traits explained 86.8% cumulative variations in the population at PCA 1 and 96% at PCA 2, while SDI descriptors explained 69.5% of variation within the population in PCA 1 and 84.45% in the PCA 2, respectively. Thus digitalized descriptors of fruits explained the highest variation within the population. Low precision of FM data suggests that selections based on field morphological data alone will have little distinguishable capacities resulting in reduced breeding efficiency. For example, lower precision of datasets from traditional field evaluation than digital image data sets means that in plant breeding situations where genes have to be built up for disease resistance, breeding cycles get extended necessitating high precision breeding tools[21]. Moreover, lower precision of field evaluation dataset underscores the need for greater precision phenotype data for the tomato population in agreement with the assertions of Poland and Nelson[12] and Xie *et al*.[15]. Several researchers had reported the use of various digital phenotyping as high precision tools for many crop breeding applications including genotype clustering[11,13], variety identification[14], big data phenotyping for genomic prediction models[6,16,17]. All these studies demonstrated that image analyses provided more precise phenotype data sets than manually acquired measurements or assessor's rating data sets.

The PCA summarizes multivariate data into several principal components and identifies which traits best separates the genotypes[22]. From this study, estimates of eigen vectors from the PCA of the tomato population quantifies the contributions of each trait to the variation explained by the phenotyping methods (Table 3-5) and thus helped to identify most suitable traits that precisely discriminates genotype entries of the breeding population. In accordance with Raji[23], phenotype traits with lower eigen vector values below the thresholds of 0.3 at PCA 1 were adjudged poor descriptors of variations in a given breeding population, which was the case with all the FM traits at PCA 1 from this study (Table 3). However, all the FDI traits had eigen vectors above 0.4 at PCA 1 (Table 4) and in Table 5, most of the SDI traits had eigen vector around 0.36. The results suggested that both digital data of fruit and seed morphological traits better distinguished the population than field evaluation[23]. Of the two digital image data sets, the digital fruit data set had slightly higher precision than digital seed data set in distinguishing this population.

The step of identification of efficient descriptors is essential for application of digital imaging to discriminating lines within breeding populations[8]. From this study, the most efficient laboratory digital descriptors for distinguishing the tomato population were those associated with fruit width including digital fruit width, radius, circumference and surface area (Table 4). Information on efficient descriptors is

important for phenotyping crops for breeding because of differences in morphology of crops. In corn, digital seed length had the highest eigen vectors for sorting and identifying inbred lines as reported by Daniel *et al.*[14], whereas digital fruit descriptors showed better precision than digital seed descriptors from the PCA of the tomato breeding population experimented in this study. The results of this study suggested that identification of efficient descriptors for phenotyping breeding populations of different crops should be determined by experimentation. On this study, It recommended digital fruit width data as best descriptors for this tomato breeding population.

CONCLUSION

This study was to evaluate efficiency of various techniques to discriminate genotypes for rapid identification within the tomato breeding population. The results showed that digital imaging offered phenotype datasets that distinguished the population more precisely than manual evaluation and consequently have potentials to improve breeding efficiency and genetic gains. The three methods used to distinguish the germplasm assembled for breeding fusarium wilt tolerant tomato lines were tested in three experiments namely the field morphological evaluation, digital imaging of fruits and seeds. This study has demonstrated the potential benefits of digitalized phenotyping to increase precision in distinguishing genotypes within breeding populations. Based on PCA analysis, the digital fruit descriptor dataset showed the most genotype distinguishing efficiency and thus adjudged the recommended method for precision phenotyping of the tomato breeding population.

SIGNIFICANCE STATEMENT

Manual field phenotyping of crops is characterized by drudgery, largely lacks precision of measurement of phenotypic traits and prone human errors and biases. However, high precision and high throughput tools are needed to scale up modern plant breeding for increased crop productivity and global food security. While considerable scientific advances had been made in genotyping precision with molecular tools available to breeders nowadays, it becomes necessary to advance the precision of plant phenotyping. This study presents a comparison of plant phenotype data collected by manual measurements in the field with measurements collected by digital imaging. The precisions of the datasets were then evaluated by the Principal Component Analysis. The analysis showed higher precision of the digitalized phenotype data set than the manual field measurements. The distinguishing digital traits were identified from the analysis.

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