Phenotypic characterization kit

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Contents and type of traits:

General data about the phenotyping assay

- Number of plants per accession
- Cultivation type (Open field/Greenhouse/Mesh tunnel)
- Temperature °C (mean, maximum, minimum values over the cropping period)
- Type of soil (OPTIONAL; if performed, minimum descriptors are organic matter content, USDA soil texture classification, pH, EC)
- Type of water (OPTIONAL)
- Watering (flooding, local irrigation)
- Fertilization (indicate the overall splitting of macronutrients: kg ha^{-1} of N, P_2O_5 and K_2O)
- Mode of cultivation (brief description)
- UTM coordinates
- Take note of the accession with low or lacking viability

Qualitative traits (one measure per accession)

1. Growth habit (1. determinate/ 2. semi-determinate/ 3. indeterminate).
2. Leaf shape (1. regular leaf/ 2. potato leaf/ 3. double feathered)
3. Leaf attitude (1. semierect/ 2. horizontal/ 3. horizontal dropping/ 4. dropping)
4. Leaf border (1. entire/ 2. undulate/ 3. serrated/ 4. strong serrated)
5. Inflorescence (1. uniparous/ 2. fishbone/ 3. forked/ 4. irregular (not as divided as compound)/ 5. compound)
6. Leafy inflorescence (1. non-leafy/ 2. leafy/ 3. leafy with shoots)
7. Style position (1. inserted/ 2. same level as stamen/ 3. slightly exserted/ 4. highly exserted)
8. Jointless pedicel (0. absence/ 1. presence)
9. External fruit color (1. yellow/ 2. orange/ 3. pink/ 4. red/ 5. purple/ 6. brown/ 7. green)
10. Green shoulder (0. uniform/ 1. light green/ 2. medium green/ 3. dark green)

11. Skin color (1. colourless/ 2. yellow)
12. Fruit load (on a scale from 1 to 9: 1. scarce/ 3. medium-scarce/ 5. medium/ 7. medium-high/9. high)
13. Fruit predominant shape, using the fruit shape categories provided by Rodriguez *et al.* 2011 (Plant Physiol 156:275-285) (1. flat/ 2. rectangular/ 3. ellipsoid/ 4. obovoid/ 5. round/ 6. oxheart/ 7. long/ 8. heart)
14. Fruit shoulder shape (1. Flat/ 2. Slightly depressed/ 3. Moderately depressed/ 4. Strongly depressed)
15. Shape of pistil scar (1. dot/ 2. stellate/ 3. linear/4. irregular)
16. Fruit fasciation (1. not present/ 2. low/ 3.intermediate/ 4. severe)
17. Puffiness appearance (presence of cavities inside fruit) (1. not present/ 2. low/ 3. intermediate/ 4. severe)
18. Ribbing at calyx end (1. very weak/ 2. weak/ 3. Intermediate/ 4. strong)

Quantitative traits related to plant structure and agronomic performance (measured in each plant)

(a) Plant structure (one measure per plant; Time of characterization: approximately when fruits from the third truss start to ripen)

19. Height until the first inflorescence (cm)
20. Height until the last inflorescence (cm)
21. Total number of inflorescences (*n*).
22. Total plant height (cm)

(b) Yield

23. Yield in the 25 controls (kg/plant, in a per plant basis).

Fruit quantitative traits (8 fruits per accession)

4 fruits will be sliced in the longitudinal section and the remaining 4 in the transversal section to record in a individual fruit basis the following variables:

1. Locule number (fruits from the transversal section)
2. Fruit weight (all the fruits)
3. °Brix: minimum of 2 measures per accession (mean value of the pool of 4 fruits from each section (tranversal/longitudinal)), maximum of 8 measures (individual fruit measurement)
4. Tomato Analyzer longitudinal (minimum of 4 fruits) and transversal (minimum 4 fruits) section morphometrics. This data will allow to calculate variables such:
    1. Fruit fasciation degree. diameter. length. pericarp area. etc. These variables are directly provided by Tomato Analyzer software.
    2. Fruit shape: fruit shape will be classified using the methodology proposed by Rodriguez et al. 2011. Plant Physiol 156:275-285.
    3. Fruit internal color: *L. a. b* are calculated by the software (remember that for this function scanner must be adjusted using a color pallet).
5. Optional traits for fruit characterization:
    1. Fruit firmness (measured with a penetrometer (Type used in Valencia: Fruit pressure tester mod. FT 0.11 Lbs.) (Descriptor optional)
    2. Colour (L,a,b parameters) Measured in Valencia with Chroma Meter CR-400/410. Konica Minolta (Descriptor optional)

Pictures

Take pictures of plant, inflorescences, flowers, fruits and unusual characteristics of each accession as indicated in pictures (Annex images). Add always a ruler for size reference.

Heterogeneous accessions

- If the accession is clearly a mixture of two types separate the seeds from selfing and characterize the two types
- If the accession is segregating in more than two types discard the accession

Observations.

Write down unusual characteristics no considered in the descriptors used.

Annex 1. Tomato Analyzer procedure

Fruit sampling

Eight fruits from each accession will be sampled from 2nd to 4th truss. Fruits will be selected visually to represent mean fruit shape of each accession (abnormal fruits will be discarded for the analysis). Fruits will be harvested at the RR stage.

Longitudinal and transversal sections

According to Tomato Analyzer user's manual (http://www.oardc.ohio-state.edu/vanderknaap/files/Tomato_Analyzer_3.0_Manual.pdf) all the variables will be recorded in the longitudinal section, except the following that will be recorded in the transversal section:

1. Pericarp area
2. Pericarp thickness
3. Lobedness degree
4. Rectangular
5. Circular
6. Ellipsoid

For this reason we need to capture morphological data from both sections.

Color measurement using Tomato Analyzer: pre-adjustements

In order to measure internal color of fruits, scanner must be adjusted previously using a color palette as described in Tomato Analyzer user's manual. First of all check that a high-resolution scanner is used. Commercial scanners are enough to perform the analysis (for further information: Darrigues et al. (2008) J. Am. Soc. Hort. Sci. 133(4):579-586), although some ancient scanners could deform excessively the color. Secondly scan a palette color, enter the correct values of L*, a*, b* provided by the color palette in the color test dialog box and calculate the calibration equation values. Perform the calibration of the scanner enough time before starting the phenotyping process (1-2 months) in order to avoid problems when the fruits start to arrive at the laboratory.

For further information please read: Tomato Analyzer Color Test User Manual (http://www.oardc.ohio-state.edu/vanderknaap/files/Color_Test_3.0_Manual.pdf)

Figure. Color palette.

Tomato Analyzer pre-adjustements

Using the scanner:

(a) Check that the scanner software will obtain a TIFF format image.

(b) Check that scanner DPI is calibrated to 300 pixels.

Using the Tomato Analyzer software (Settings):

(a) SELECT ATTRIBUTES: check that you have selected the correct attributes for the transversal or longitudinal sections (see below)

(b) SCANNER DPI AND UNITS: adjust the Scanner DPI to **300** and select the units **cm**.

(c) BLOCKINESS POSITION: adjust Blockiness position to the following numbers: *Upper position: **0.1**; Lower position: **0.9***.
(d) PROXIMAL AND DISTAL ANGLES: adjust Macro Distance to **20%** and Micro Distance to **3%**.
(e) NUMBER OF MORPHOMETRIC POINTS: adjust to **10**.
(f) DEFAULT ECCENTRICITY ELLIPSE SIZE: adjust to **90**.

Fruit processing with Tomato Analyzer

Transversal section
(1) List the 4 fruits with an indelible marker.
(2) Create an Excel file with the following columns:

Genotype	Fruit number	Section	Fruit weight (g)	Locule number	º Brix
TR_TH_001	1	Transversal			
TR_TH_001	2	Transversal			
TR_TH_001	3	Transversal			
TR_TH_001	4	Transversal			

3) Record the fruit weight of each fruit

Genotype	Fruit number	Section	Fruit weight (g)	Locule number	º Brix
TR_TH_001	1	Transversal	68		
TR_TH_001	2	Transversal	70		
TR_TH_001	3	Transversal	52		
TR_TH_001	4	Transversal	66		

4) Cut the 4 fruits in the transversal section
5) Record the locule number of each fruit

Genotype	Fruit number	Section	Fruit weight (g)	Locule number	º Brix
TR_TH_001	1	Transversal	68	4	
TR_TH_001	2	Transversal	70	3	
TR_TH_001	3	Transversal	52	4	
TR_TH_001	4	Transversal	66	4	

6) Record the °Brix with a refractometer (if it's preferred, a mean value can be obtained from the 4 fruits by blending them and recording the °Brix value from the pool).

Genotype	Fruit number	Section	Fruit weight (g)	Locule number	º Brix
TR_TH_001	1	Transversal	68	4	6.4
TR_TH_001	2	Transversal	70	3	6
TR_TH_001	3	Transversal	52	4	5.4
TR_TH_001	4	Transversal	66	4	7.8

7) Put the fruits in the correct order in the scanner

(make some practices before in order to know the correspondence between the disposition of the fruits in the scanner and the order of Tomato Analyzer output data file)

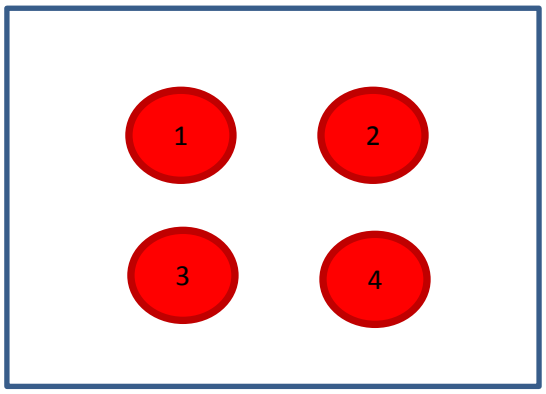


Figure. A possible fruit order in the scanner.

8) Analyze the image with TA software and obtain solely quantitative data for the following attributes:

1. Pericarp area
2. Pericarp thickness
3. Lobedness degree
4. Rectangular
5. Circular
6. Ellipsoid

9) Export data to the Excel file

Genotype	Fruit number	Section	Fruit weight (g)	Locule number	º Brix	Pericarp area	Pericarp thickness	Lobedness degree
TR_TH_001	1	Transversal	68	4	6.4	32.9331	6.5955	6.6633
TR_TH_001	2	Transversal	70	3	6	31.573	6.3669	6.3669
TR_TH_001	3	Transversal	52	4	5.4	27.9943	6.6294	6.731
TR_TH_001	4	Transversal	66	4	7.8	28.7595	6.6125	6.6209

Longitudinal section:

(1) List the 4 fruits with an indelible marker

(2) Create an Excel file with the following columns:

Genotype	Fruit number	Section	Fruit weight (g)	º Brix
TR_TH_001	1	Longitudinal		
TR_TH_001	2	Longitudinal		
TR_TH_001	3	Longitudinal		
TR_TH_001	4	Longitudinal		

3) Record the fruit weight of each fruit

Genotype	Fruit number	Section	Fruit weight (g)	º Brix
TR_TH_001	1	Longitudinal	68	
TR_TH_001	2	Longitudinal	70	

TR_TH_001	3	Longitudinal	52	
TR_TH_001	4	Longitudinal	66	

4) Cut the 4 fruits in the longitudinal section

5) Record the °Brix with a refractometer (if it's preferred, a mean value can be obtained from the 4 fruits by blending them and recording the °Brix value from the pool).

Genotype	Fruit number	Section	Fruit weight (g)	º Brix
TR_TH_001	1	Longitudinal	68	6.4
TR_TH_001	2	Longitudinal	70	6
TR_TH_001	3	Longitudinal	52	5.4
TR_TH_001	4	Longitudinal	66	7.8

6) Put the fruits in the correct order in the scanner
(make some practices before in order to know the correspondence between the disposition of the fruits in the scanner and the order of Tomato Analyzer output data file)

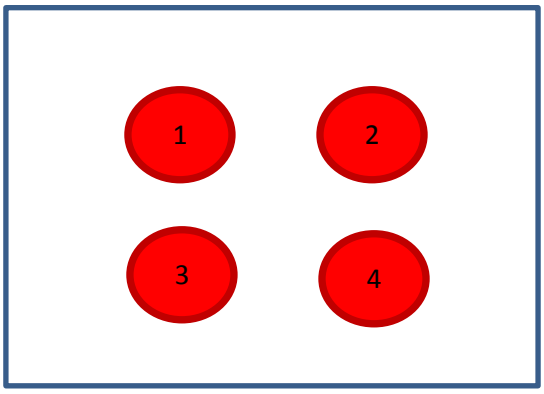


Figure. A possible fruit order in the scanner.

7) Analyze the image with TA software. Select all the attributes except the attributes listed before for the transversal section.

9) Export data to the Excel file

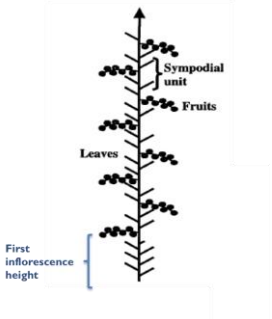
Genotype	Fruit number	Section	Fruit weight (g)	º Brix	Width	Length	...
TR_TH_001	1	Longitudinal	68	6.4	32.9331	6.5955	6.6633
TR_TH_001	2	Longitudinal	70	6	31.573	6.3669	6.3669
TR_TH_001	3	Longitudinal	52	5.4	27.9943	6.6294	6.731
TR_TH_001	4	Longitudinal	66	7.8	28.7595	6.6125	6.6209

Annex 2. Plant structure

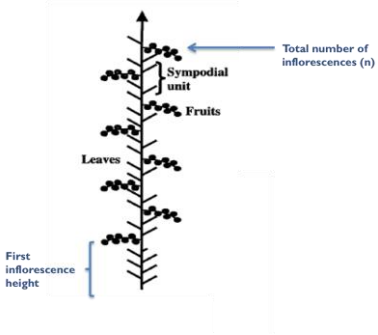
Objective: collect data to calculate the height of the first inflorescence and the mean distance between inflorescences within the plant.
Time of characterization: approximately when fruits from the third truss start to ripen.

Procedure:

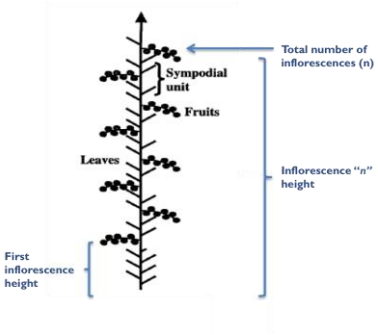
1) Measure the first inflorescence height



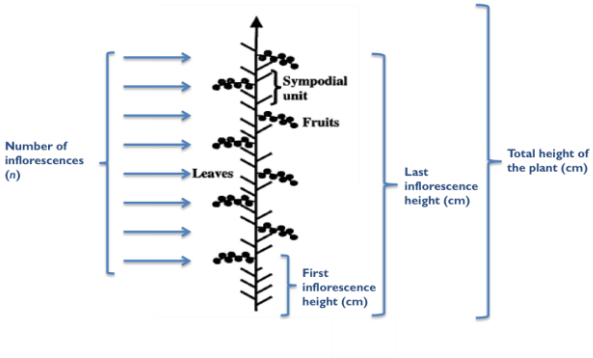
2) Count the total number of inflorescences within the plant (last inflorescence should show the first flower position as a fruit)



3) Measure the height of the last inflorescence



4) Finally, measure the total height of the plant at that moment. After taking the measure, the main stem of the plant can be cut



5) Calculate the following variables

Genotype	Plant	Height of the first inflorescence (cm)	Total number of inflorescences	Height of the last inflorescence (cm)	Total height of the plant (cm)	Mean distance between inflorescences
TR_TH_001	1	x	n	y	**z**	=(y-x)/(n-1)

Source of images in Annex 2, 1-4: Mateus Henrique Vicente et al. (2015): Semi-determinate growth habit adjusts the vegetative-to-reproductive balance and increases productivity and water-use efficiency in tomato (Solanum lycopersicum). Journal of Plant Physiology, 177:11-19.

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