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California processing tomatoes: Morphological, physiological and phenological traits associated with crop improvement during the last 80 years

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ABSTRACT

Breeding has greatly increased yields of many crops, but the contributions of particular morphological, phenological and physiological traits to these higher yields are rarely well understood. In the past 50 years, California processing tomato yields per hectare have more than doubled. This study evaluated a group of important processing tomato cultivars released over the past 80 years in California. The objective was to assess how a suite of traits might be associated with genetic improvement for yield gains. A wide array of morphological, physiological and phenological traits and relevant environmental variables was evaluated in the field for a discrete set of eight cultivars originating from a common ancestor. Multivariate statistics were used to analyze the set of 95 variables to understand how cultivars became adapted to a more mechanized agronomic management while also producing higher yields. No single trait seems to have driven yield increases. Instead, distinct assemblies of traits characterize the processing tomato cultivars in different eras. For instance, certain phenological traits (early flowering and concentrated fruit set) were associated with a set of morphological traits (smaller canopies and low vegetative biomass), along with gains in physiological traits (biomass N concentration and photosynthetic rates) in modern varieties. These results provide a platform to examine new suites of traits that could be relevant for future breeding and crop improvement.

1. Introduction

For most crops, breeding has played an important role in increasing yields, but the mechanisms by which particular morphological, phenological and physiological traits contribute to these higher yields are not well understood (Sinclair and Purcell, 2005). Large yield increases are often the result of an interaction between crop improvement and new agronomic practices, *e.g.*, introduction of dwarfing genes and higher nitrogen (N) inputs in wheat, or more determinate growth and mechanized harvest in processing tomatoes (Evans and Fischer, 1999; Stevens and Rick, 1986). More emphasis is needed on how trait associations have contributed to yield gains, in order to overcome future potential environmental constraints, *e.g.*, diminishing water supply (Fischer, 2007; Passioura, 2002; Sadras and Lawson, 2013).

The processing tomato industry in California accounts for >90% of production in the USA, and ~35% worldwide (UCCE, 2008; USDA, 2009). According to the existing USDA (2009) records, crop yields per hectare (ha) have more than doubled in the past 50 years. The evaluation of a suite of traits could provide clues on how genetic improvement has contributed to these yield gains (Grandillo et al., 1999). Deeper understanding of plant phenology and other trait interactions with the environment is becoming more important for increasing yields (Fischer, 2007; Giunta et al., 2007), and trait associations are increasingly recognized as important for improving plant response to agricultural management, and thus for crop breeding in future uncertain climate scenarios.

In processing tomato, crop performance has usually been evaluated based on yield and fruit quality, but little is known about other traits that contribute to gains in these two complex traits. Selecting for a specific trait can inadvertently lead to breeding for trait associations that can have a positive or negative effect on a desired trait (Barrios-Masias et al., 2013; de Meijer and Keizer, 1996). One benchmark in the 1960s was the switch from hand-picked, indeterminate growth habit plants to cultivars with a determinate growth habit for machine-harvest (Stevens and Rick, 1986). Since then, yields have increased from 40 Mg to 90 Mg ha^{-1}, with most of the gain in crop productivity occurring after 1975 (Grandillo et al., 1999; Hanson and May, 2006). New introduction of genes for pest and disease resistance (such as nematode, *Fusarium* and *Verticillium* wilt) has occurred throughout this period (Hajjar and

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Hodgkin, 2007; Thomas, 1980). Between 1977 and 1994, the genetic improvement for California tomatoes was 1.5% per year in yield but without a significant gain in fruit total soluble solids (Grandillo et al., 1999). In addition, <30% of the annual genetic gain was associated with the adoption of hybrid cultivars in the 1990s (Grandillo et al., 1999).

Morphological changes that affect growth habit and biomass allocation have increased yields of major crops (Sinclair, 1998) such as dwarfing genes in wheat (Evans and Fischer, 1999), or in bush-type chickpea (Rubio et al., 2004). Changes in canopy architecture can help improve light use efficiency and provide an advantage for C assimilation when N uptake is optimal (Long et al., 2006; Rascher et al., 2011; Tei et al., 2002). Phenological traits such as the onset of flowering are influenced by plant growth habit, *e.g.*, soybean (Egli, 2005) and can also affect yields, *e.g.*, chickpea (Rubio et al., 2004). Physiological gains in C assimilation by higher photosynthetic rates (P_n) have sometimes been observed in modern cultivars of wheat (Evans, 1993; Fischer et al., 1998; Watanabe et al., 1994). Interestingly, changes in C assimilation potential have been associated with changes in growth habit in wheat (Morgan et al., 1990) and soybean (Tanaka et al., 2008). Gains in P_n are usually accompanied by higher stomatal conductance (g_s), and as a result, intrinsic water use efficiency (WUE_i; P_n/g_s) tends to decrease (Barrios-Masias et al., 2013; Gilbert et al., 2011). Higher P_n is also related to higher leaf-N concentration in particular to investment in Rubisco for higher C assimilation (Poorter and Evans, 1998; Watanabe et al., 1994). When C allocation to shoots is reduced, the number of fruits or grains per plant can increase, *e.g.*, wheat (Fischer and Stockman, 1986). Usually an increase in fruit number has a strong negative correlation with fruit weight, *e.g.*, tomato (Griffing, 1990). Carbon allocation, total plant biomass and fruit biomass can affect the harvest index (HI; yield per total biomass), which has increased in most field crops along with yield gains (Evans, 1993; Higashide and Heuvelink, 2009). Thus, evaluating plant performance for increasing crop yield may most effectively involve a complex interplay of traits and environment through the growing season.

This study evaluated a group of important processing tomato cultivars released over the past 80 years in California. The objective was to assess how a suite of traits might be associated with genetic improvement for yield gains in processing tomatoes. The approach was to evaluate a wide array of morphological, physiological and phenological traits and relevant environmental variables for a discrete set of cultivars originating from a common ancestor, then to use multivariate analyses to understand how changes adapted cultivars for more mechanized agronomic management, following statistical approaches by other studies that focused on germplasm evaluation (de Meijer and Keizer, 1996; Escribano and Lazaro, 2009; Perry and McIntosh, 1991; Yada et al., 2010). These results provide a platform to examine specific traits that could be relevant for future breeding and crop improvement.

2. Materials and methods

2.1. Plant material

A total of eight tomato cultivars representing different eras of the California processing tomato industry in the past 80 years were used in field and greenhouse studies.

- Pearson, mid 1930s release (LA0012)
- VF36, late 1950s release (LA0490)
- VF145 78-79, early 1960s release (LA1222)
- Heinz 1706-BG, early 1970s release (LA4345)
- M82, mid 1970s release (LA3475)
- Apex 1000, early 1980s release (LA3527)
- UC-204C, early 1990s release (LA3130)
- AB2, early 2000s release (AB Seeds)

All seeds but AB2 were obtained from the C. M. Rick Tomato Genetics Resource Center (TGRC) at UC Davis (LA numbered accessions). All cultivars were open pollinated, except AB2 which is a hybrid. Seeds were surface sterilized, germinated in trays, and seedlings maintained under day/night length of 16/8 h in greenhouses at the University of California at Davis (UC Davis). Seedlings were transplanted to either the field or in pots when they had at least 3 true leaves, *i.e.*, 6-week-old plants.

2.2. Field study

A field study was conducted during summer of 2007 at the UC Davis Plant Sciences Research Station in Davis, California. The soil was mapped as a Reiff very fine sandy loam, a coarse-loamy, mixed, nonacid, thermic Mollic Xerofluvent. During the experiment, from June 7 to September 24, the minimum and maximum average temperatures were 12.6 °C and 31.5 °C, respectively, with a minimum of 7.2 °C and a maximum of 40.6 °C, and no rainfall (CIMIS, 2010).

The field had been in wheat during the winter, and beds were prepared (1.52 m from furrow to furrow) for transplanting in spring of 2007. A total of 16 beds were divided in three blocks of four beds and one buffer bed on each side. Plots were 7.2-m long, and each block had a total of eight plots assigned to the eight cultivars in a randomized complete block design (RCBD; 24 plots total in the experiment). On June 7, 6-week-old seedlings were hand-transplanted in the center of the bed at a spacing of 0.6 m between plants (total of 12 plants per plot-length per bed and 48 plants per plot). In order to evaluate plant performance under minimal resource competition between neighboring plants, plant density was about half of what is currently used in modern commercial production. This density was intended to provide similar access to resources (fertilizer, water and light) even among cultivars with bigger canopies. Plants were sprinkler-irrigated after transplanting and two more times within the first 2 weeks to assure good establishment. Seven furrow irrigations followed until September 7 (92 days after transplanting, DAP) at intervals of ~11 days, and a total estimate of 620 mm of water applied for the entire growing season.

To monitor soil moisture content with a neutron probe, two PVC tubes of 5.4 cm in diameter were placed in each plot (one 2-m long and one 3-m long tube per plot; 48 tubes total). Tubes were placed in bed 2 and 3 between the edge and center of the bed, *i.e.*, 25 cm away from bed center, and toward the shared furrow. A Giddings machine was used to core the soil and place the tubes (Giddings Machine Company, Colorado, USA). Measurements were taken at every 30 cm interval with a count duration of no less than 16 s. Measurements were taken immediately before an irrigation event, and three days after to prevent furrow damage and soil compaction. Changes in soil moisture indicated the amount of water evapotranspired, but the first furrow irrigation (25 DAP) was not included because the disturbed soil was still settling around the tubes, assuring good contact for better neutron probe measurements later on.

A total of 95 variables were collected in the 109 days of the field experiment (Table 1). These variables included one-time measurements such as number of flowers per inflorescence; frequent measurements of the same variable, *e.g.*, soil canopy cover; and indexes derived from other variables, *e.g.*, HI (harvestable fruit/total aboveground biomass). Variables were grouped in three trait categories: morphological, physiological and phenological for which 51, 31 and 13 variables were obtained, respectively. These three categories were used based on traits associated with yield improvement in other crops (Fischer and Stockman, 1986; Gilbert et al.,

Table 1
Description of variables and evaluation days during the tomato growing season. Variables are classified according to three trait groups: morphological, physiological and phenological. The total number of variables is 95 when considering variables at several dates after planting.

Description	Abbreviation	Days after planting
Morphological traits		
Number of flowers per inflorescence	FlwrInf	48
Photosynthetically active radiation intercepted (% PAR intercepted bed^{-1})	PAR	40, 62, 91
Vertical PAR reaching 10–20 cm above the soil in the canopy (%)	PARv2	78
Vertical PAR reaching 20–30 cm above the soil in the canopy (%)	PARv3	78
Vertical PAR reaching 30–40 cm above the soil in the canopy (%)	PARv4	78
Vertical PAR reaching 40–50 cm above the soil in the canopy (%)	PARv5	78
Soil canopy cover (% cover bed^{-1})	Cover	12, 25, 40, 54, 68, 89, 104
Leaf biomass (g plant^{-1})	Leaf	41, 69, 97
Stem biomass (g plant^{-1})	Stem	41, 69, 97
Green, unripe fruit biomass (g plant^{-1})	Green	69, 97
Red, harvestable fruit biomass (g plant^{-1})	Red	97
Decay, non-harvestable fruit biomass (g plant^{-1})	Spoiled	97
Total fruit biomass (green + red + spoiled; g plant^{-1})	Fruit	97
Total aboveground biomass (g plant^{-1})	Total	41, 69, 97
Leaf area (cm^2 leaf^{-1})	Larea	41, 69, 97
Leaf weight (g leaf^{-1})	Lwt	41, 69, 97
Stem count	Scnt	41, 69, 97
Harvest index (harvestable-fruit total-plant-biomass^{-1})	HI	97
Proportion of green fruit under a 4.0 cm diameter (%)	SmallGreen	97
Proportion of red fruit under a 4.0 cm diameter (%)	SmallRed	97
Proportion of green fruit between 4.0 and 5.5 cm diameter (%)	MedGreen	97
Proportion of red fruit between 4.0 and 5.5 cm diameter (%)	MedRed	97
Proportion of green fruit over a 5.5 cm diameter (%)	BigGreen	97
Proportion of red fruit over a 5.5 cm diameter (%)	BigRed	97
Total soil moisture depleted between irrigations for depth 30 cm (mg cm^{-3})	Soildepth_30	106
Total soil moisture depleted between irrigations for depth 60 cm (mg cm^{-3})	Soildepth_60	106
Total soil moisture depleted between irrigations for depth 90 cm (mg cm^{-3})	Soildepth_90	106
Total soil moisture depleted between irrigations for depth 120 cm (mg cm^{-3})	Soildepth_120	106
Total soil moisture depleted between irrigations for depth 150 cm (mg cm^{-3})	Soildepth_150	106
Total soil moisture depleted between irrigations for depth 180 cm (mg cm^{-3})	Soildepth_180	106
Physiological traits		
N concentration in leaves (%)	LeafN	41, 69, 97
N concentration in stems (%)	StemN	41, 69, 97
N concentration in green fruit (%)	GreenN	69, 97
N concentration in red fruit (%)	RedN	97
N concentration in leaflets used for leaf gas exchange measurements (%)	LfletN	75,76
^{13}C discrimination from leaflets used for leaf gas exchange measurements (Δ)	D13 C	75, 76
Photosynthetic rate (P_n; μmol m^{-2} s^{-1})	Photo	75, 76, 77
Stomatal conductance (g_s; mol m^{-2} s^{-1})	Cond	75, 76, 77
Intrinsic water use efficiency (WUE$_i$; P_n/g_s)	WUE	75, 76, 77
Vapor pressure deficit of leaf (kPa)	VpdL	75, 76, 77
Soil moisture depleted between 36 and 42 DAP (mg cm^{-3})	SoilMoist_1	42
Soil moisture depleted between 46 and 55 DAP (mg cm^{-3})	SoilMoist_2	55
Soil moisture depleted between 59 and 70 DAP (mg cm^{-3})	SoilMoist_3	70
Soil moisture depleted between 74 and 82 DAP (mg cm^{-3})	SoilMoist_4	82
Soil moisture depleted between 86 and 91 DAP (mg cm^{-3})	SoilMoist_5	91
Soil moisture depleted between 95 and 106 DAP (mg cm^{-3})	SoilMoist_6	106
Phenological traits		
Plants with flowers (%)	Flwr	22, 29, 34
Number of inflorescences per plant	Infl	41, 69
Relative PAR intercepted from maximum reached at 91 DAP (%)	PrcntPAR	40, 62
Green fruit proportion from total fruit biomass (%)	GreenProp	97, 109
Red fruit proportion from total fruit biomass (%)	RedProp	97, 109
Spoiled fruit proportion from total fruit biomass (%)	SpoiledProp	97, 109

2011; Perry and McIntosh, 1991; Sadras and Lawson, 2013; Sekloka et al., 2008). Traits and indicators were classified based on the predominant effect on plant growth and development although some overlap between categories is possible.

2.2.1. Morphological traits

Canopy cover was measured using an infrared digital camera (Dycam, Woodland Hills, California, USA) mounted on an inverted 'L'-shaped pole to capture an image directly above the bed. Images were taken at 2-week intervals starting on 19 DAP. An area of 1.52 m (bed width) by 2.40 m length (4 plants) was processed with Briv32 Version 1.27 software to obtain the fraction of the bed surface covered by the canopy (% cover bed^{-1}). Canopy light interception was measured at 40, 62 and 91 DAP using a portable-tube solarimeter with sensors for photosynthetically active radiation (PAR; AccuPAR-80, v. 4.5, Decagon Devices, Inc. Washington, USA). Measurements were taken at 30 cm intervals over a similar area as for canopy cover (1.52 m × 2.40 m). At every interval a PAR reading was taken above and below the canopy. Data are expressed as percent light intercepted per bed (% PAR intercepted bed^{-1}). On 78 DAP, vertical PAR measurements were taken to account for light dissipation inside the canopy (% intercepted PAR at segment). The PAR bar was divided into 10-cm segments, placed vertically into the middle of the canopy, and a measurement taken for four plants per plot (96 plants total). A total of four segments were used for data analysis (10–20 cm, 20–30 cm, 30–40 cm and 40–50 cm). The 50–60 cm segment was used as the 100% PAR incidence above the canopy.

Biomass sampling was done on two plants per plot at 41 and 69 DAP (48 plants total per sampling time), and on three plants at 97 DAP (72 plants total). Plants were clipped at the base of the stem, sorted into leaves, stems and fruits, and number of inflorescences and stems (main + lateral stems) counted. At 97 DAP, fruits were sorted into three groups: greens, harvestable and spoiled. Green and harvestable fruits were sorted by diameter into three categories: small = <4.0 cm, medium = 4.0–5.5 cm and large = >5.5 cm. Seven leaves were subsampled for leaf area and weight. Leaf area was measured with a LI-3100 Leaf Area Meter (LI-COR Inc., Nebraska, USA). Biomass subsamples were oven dried at 60 °C and weighed to determine total biomass. The HI was calculated as harvestable fruits divided by total aboveground biomass at 97 DAP, on a dry weight basis.

The number of flowers per inflorescence were obtained at 48 DAP when all cultivars were flowering and had more than ten inflorescence per plant. In every plot, the number of flowers was counted in ten inflorescences. The total sum of the soil moisture changes between wet and dry cycles for each 30-cm soil depth increment (down to 180 cm) was considered to be indicators of the total water lost (g-H_2O cm^{-3}) and of the depth of root water extraction.

2.2.2. Physiological traits

Leaf, stem and fruit biomass from samplings at 41, 69 and 97 DAP were finely ground and analyzed for N concentration with an ECS 4010 CHNSO Analyzer (Costech Analytical Technologies, Inc., California, USA).

Leaf gas exchange measurements were taken on three consecutive days (75, 76 and 77 DAP) on 24 plants per day (one plant per plot per day). A mature, fully expanded leaf from the top of the canopy was measured with a field portable open flow infrared gas analyzer (IRGA) (Model 6400, LI-COR Inc., Nebraska, USA). For each leaf, one of the most distant pair of leaflets from the petiole base was used. Measurements were taken between 10:00 and 13:00 h with a 6 cm^2 chamber, with the CO_2 reference set at 400 μmol m^{-2} s^{-1}, and with saturating light using a LED source (PAR in: 2000 μmol m^{-2} s^{-1}). At the end of each measurement on 75 and 76 DAP, each leaflet was sampled, oven dried at 60 °C and analyzed for $\delta^{13}C$ and total N on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the UC Davis Stable Isotope Facility (http://stableisotopefacility.ucdavis.edu/). Data used for the analyses were: P_n, g_s, WUE_i and vapor pressure deficit of the leaf (VpdL as kPa). The WUE_i was calculated as P_n/g_s, and $\Delta^{13}C$ was calculated from the $\delta^{13}C$ values using an air $\delta^{13}C$ value of −8‰ as described by Comstock et al. (2005).

The change in soil moisture between irrigations in the top 180 cm was considered to be an indicator of plant water demand at different physiological stages. A total of six such 'periods' or events were included for analysis.

2.2.3. Phenological traits

As soon as flowering began, three counts of the number of plants with open flowers were conducted on 22, 29 and 34 DAP for all plants in the two middle beds (total of 24 plants per plot). The number of inflorescences per plant was counted on biomass sampling days (41 and 69 DAP) as described above. As an indirect measure of plant developmental stage to a full size canopy, the relative PAR intercepted from its maximum at 91 DAP was calculated for PAR intercepted at 40 and 62 DAP (*e.g.*, 40-DAP PAR/91-DAP PAR). Concentrated fruit ripening, which is desirable for a one-time machine harvest, was evaluated as the proportion of green, harvestable and spoiled fruit from the total fruit biomass at 97 and 109 DAP. Both dates were used because fruit ripening was not synchronous among cultivars.

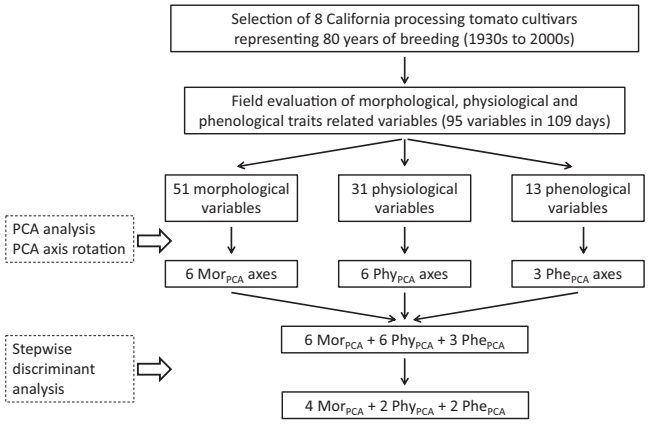

Fig. 1. Diagram of steps taken to compare eight processing tomato cultivars as a suite of morphological, physiological and phenological traits through extensive field-data collection and use of principal component analysis (PCA) to condense the total number of variables and followed by a stepwise discriminant analysis to determine the suite of traits that maximized differences among cultivars.

2.3. Greenhouse study

A study under controlled greenhouse conditions was conducted between March 25 and May 9, 2008 (0 and 45 DAP, respectively) at the UC Davis Plant Sciences facility. Forty 11-liter pots were filled with a mixture of 40% loamy soil, 40% perlite and 20% sand. Six-week-old seedlings were transplanted and randomly arranged in five blocks along two benches in a RCBD with eight cultivars and 40 plants total. Supplemental light was provided under a day/night length cycle of 16/8 h. Fertilization was done four times with a 15-5-15 liquid fertilizer (0.5 g N per event per pot). Watering occurred at least three times per week to reach a pre-set pot weight corresponding to just below pot water holding capacity.

The number of inflorescence and flowers open per plant were counted four times between 35 and 45 DAP. The number of fruits per plant was counted at 45 DAP, and the percent of fruit set per total opened flowers calculated. At 45 DAP, leaflets from fully mature and expanded leaves from the top of the plant were sampled, and the plant was cut at the base, and both the leaflets and the rest of the plant were dried at 60 °C and analyzed for $\delta^{13}C$ and total N (see above).

2.4. Statistical analysis

A series of multivariate analyses were performed using JMP 9 (SAS Institute Inc., North Carolina, USA) (Fig. 1). Principal component analysis (PCA) was used to reduce the total number of variables in each trait category, *i.e.*, morphological, physiological and phenological traits. Each trait category was analyzed for multivariate outliers using the Mahalanobis distance procedure (McCune and Grace, 2002). The total number of axes retained for each analysis depended on their eigenvalues being greater than one, and the cumulative percent of variance explained ≥70%. The axes retained in each PCA were rotated by the 'varimax' procedure to facilitate interpretation of results (McCune and Grace, 2002). These rotated axes were later used in a descriptive, stepwise discriminant analysis (DA) to find the discriminant functions that maximized the between-cultivar variation (Somersalo et al., 1998; Yada et al., 2010). A total of 15 PCA axes were included in the stepwise DA; six morphological, six physiological and three phenological (see Section 3). The stepwise procedure was used to further reduce the number of axes included in the discriminant analysis and reduce potential correlations among rotated axes originating from different trait groups. The discriminant functions from the analysis

Table 2
Retained, rotated PCA axes for each of the trait groups with a description based on the variables most associated with it (see Appendixes). These variables are then classified based on whether ANOVA differences among cultivars were found or not. Variables in parenthesis had negative loadings. Refer to Table 1 for variable descriptions of abbreviation + days after planting.

Trait group and axis	Axis description	Top five variables with loadings > 0.5 associated with the rotated PCA axes and grouped based on ANOVA results showing cultivar differences or not	
		$p < 0.05$	n.s.
Morphological traits			
$Mor1_{PCA}$	Canopy architecture	Leaf_69, Total_97, PAR_62, Total_69, Cover_54	
$Mor2_{PCA}$	Harvest index	BigGreen, (HI), BigRed, (Red_97), Spoiled_97	
$Mor3_{PCA}$	Leaf area and weight	Lwt_69, Larea_69, Lwt_41	Larea_97, Lwt_97
$Mor4_{PCA}$	Fruit size and Inflorescence morphology	SmallRed, (MedGreen), FlwrInfl_48, SmallGreen, (MedRed)	
$Mor5_{PCA}$	Water uptake by depth		Soildepth _30, _60, _90 and _180
$Mor6_{PCA}$	Early aboveground biomass		Leaf_41, Total_41, Soildepth_120, Lwt_41, Stem_41
Physiological traits			
$Phy1_{PCA}$	WUE_i when VpdL >1.3 kPa		VpdL_76, VpdL_77, WUE_77 (Photo_77), (Cond_77)
$Phy2_{PCA}$	N concentration late in season	RedN_97, GreenN_97, LfletN_76	LeafN_97, (SoilMoist_6)
$Phy3_{PCA}$	Early season water uptake	(LfletN_75)	SoilMoist _1, _2 and _3
$Phy4_{PCA}$	WUE_i when VpdL <1.0 kPa		(Cond_75), WUE_75, VpdL_75
$Phy5_{PCA}$	Stem N and ^{13}C discrimination	StemN_69, StemN_97, StemN_41	D13C_75, D13C_76
$Phy6_{PCA}$	Early season leaf and fruit N concentration	LeafN_41	GreenN_69
Phenological traits			
$Phe1_{PCA}$	Flowering, fruit set and holding (no decay)	(SpoiledProp_109), RedProp_109, (SpoiledProp_97)	Flwr_29, Flwr_34
$Phe2_{PCA}$	Concentrated fruit ripening	(GreenProp_109), RedProp_97, (GreenProp_97)	Infl_41
$Phe3_{PCA}$	Earliness to full canopy	PrcntPAR_62, PrcntPAR_40	Infl_69, Flwr_34

served to identify which PCA axes were most influential in differentiating among the eight cultivars.

Certain measured variables were highly associated with each retained PCA axis as evidenced by loading values above 0.5. The top five, highly correlated variables (loading value > 0.5) from each axis were then individually analyzed by ANOVA using the GLM procedure of SAS 9.1 (SAS Institute Inc., North Carolina, USA). When continuous measurements for the same variable were performed, a repeated-measure analysis under a split-plot treatment structure was followed, *e.g.*, number of flowers that were open or leaf area (cm^2 $leaf^{-1}$). Each day was considered as a 'subplot', and the error term for the cultivars, *i.e.*, main plot, was specified as '*e* = cultivar × block'. Because all cultivars were compared under similar conditions with minimal resource limitation (*i.e.*, no treatment applied), it was assumed that variance and its correlation structure between any two time point measurements were the same within a cultivar. The Shapiro–Wilk *W* test for normal distribution and Levene's test for homogeneity of variance were used to test that data fulfilled the ANOVA assumptions. Data was transformed as necessary when assumptions were not met. The Tukey–Kramer HSD test was used to determine significant differences among cultivars ($p < 0.05$).

3. Results

3.1. Field study

3.1.1. Morphological traits

Six rotated axes ($Mor1_{PCA}$ to $Mor6_{PCA}$; Table 2 and Appendix A) explained ≥70% of the variance in morphological traits, and were retained from the PCA analysis performed on the initial 51 raw morphological variables. $Mor1_{PCA}$ accounted for 28% of the total variance, and it was mainly defined by canopy architecture traits, *e.g.*, soil canopy cover, PAR intercepted, and mid- and late season total aboveground biomass. $Mor2_{PCA}$ accounted for 11% of the total variance, and it was mostly related to high HI and harvestable fruit associated with less spoiled or oversized fruit (>5.5 cm diameter). $Mor3_{PCA}$ accounted for 10% of the total variance and was mainly associated with leaf area and weight. $Mor4_{PCA}$ accounted for 10% of the total variance, and it was mostly related to a reduction in medium-size fruit biomass associated with more flowers per inflorescence and an increase in the total small fruit biomass. $Mor5_{PCA}$ accounted for 8% of the total variance and it was mainly associated with total water extracted by roots from the top 90 cm of soil. $Mor6_{PCA}$ accounted for 8% of total variance and was mostly associated with early aboveground biomass.

The univariate ANOVAs performed on the most important variables, *i.e.*, top five variables with loadings > 0.5 on the retained and rotated Mor_{PCA} axes, showed cultivar differences for many of the morphological traits (Tables 2 and 3). On $Mor1_{PCA}$, canopy architecture described by the percent of soil canopy cover and PAR intercepted showed a reduction in canopy size from older to newer cultivars with the exception of AB2, which had values more similar to the older indeterminate Pearson and VF36 cultivars with indeterminate growth habit (Table 3). This reduction in canopy size was accompanied by a decrease in aboveground biomass as well (with the exception of AB2). On $Mor2_{PCA}$, no clear pattern in HI was observed among cultivars although HI was the lowest for VF36 and the highest for M82. At 97 DAP, the total harvestable fruit was generally similar among cultivars, but VF36 had the lowest mainly due to late ripening (*i.e.*, green fruit over a 5.5 cm diameter; Table 3).

On $Mor3_{PCA}$, leaf area (cm^2 $leaf^{-1}$) and weight (g $leaf^{-1}$) varied among cultivars without a clear pattern with the cultivar time of release (Table 3; data analyzed as repeated measures). Leaf area was highest in cultivar AB2 and lowest in Apex1000. Leaf weight was also highest in AB2, but it differed less among cultivars than leaf area. On $Mor4_{PCA}$, cultivars released since the mid 1970s had more than 70% of fruit biomass in the medium-size category (4.0–5.5 cm diameter), which was greater than some older cultivars. Cultivar Heinz 1706 (early 1970s release) had the lowest biomass in

Table 3
Results from the univariate ANOVA on the variables most associated with each retained, rotated PCA axis within each of the trait groups (refer to Table 2 for eight processing tomato cultivars released from 1930s to 2000s (from left to right). Variables with more than one sampling date (DAP; days after planting) were analyzed by ANOVA with a repeated measures structure. Values are mean ± standard error. Means followed by different letters are significantly different at $p < 0.05$ using the Tukey–Kramer HSD test. Mean comparisons for each variable *x* DAP are done within each row.

Variable	DAP[a]	Cultivar							
		Pearson	VF36	VF145	Heinz 1706	M82	Apex1000	UC-204C	AB2
Morphological traits									
Leaf biomass (g plant^{-1})	41	71 ± 15a	65 ± 0a	49 ± 10a	56 ± 2a	53 ± 7a	52 ± 2a	53 ± 9a	68 ± 7a
Stem biomass (g plant^{-1})	41	35 ± 6a	25 ± 1a	21 ± 4a	29 ± 2a	21 ± 3a	21 ± 1a	20 ± 3a	22 ± 2a
Total aboveground biomass (g plant^{-1})	41	106 ± 21a	89 ± 0a	70 ± 14a	86 ± 4a	75 ± 11a	73 ± 2a	74 ± 12a	91 ± 9a
Leaf area (cm^2)	41, 69, 97	128 ± 10bc	178 ± 16ab	134 ± 11bc	126 ± 7bc	162 ± 16abc	117 ± 6c	144 ± 9abc	196 ± 11a
Leaf weight (g leaf^{-1})	41, 69, 97	1.0 ± 0.1b	1.3 ± 0.1ab	1.1 ± 0.1b	1.0 ± 0.0b	1.4 ± 0.1ab	1.1 ± 0.1b	1.4 ± 0.1ab	1.6 ± 0.1a
Flowers per inflorescence	48	4 ± 0c	5 ± 0c	7 ± 1bc	14 ± 1a	9 ± 1b	6 ± 1c	6 ± 0c	10 ± 0b
Canopy cover (% cover bed^{-1})	54	82 ± 3a	73 ± 8ab	55 ± 1bc	69 ± 5ab	47 ± 2c	43 ± 3c	49 ± 2c	70 ± 4ab
Light interception (% PAR intercepted bed^{-1})	62	70 ± 4a	63 ± 5ab	50 ± 3bcd	50 ± 3bcd	37 ± 3d	38 ± 1d	41 ± 1cd	53 ± 3bc
Leaf biomass (g plant^{-1})	69	239 ± 29a	216 ± 10ab	158 ± 7abc	144 ± 14bc	119 ± 6c	147 ± 13bc	146 ± 2bc	207 ± 20ab
Total aboveground biomass (g plant^{-1})	69	594 ± 81a	436 ± 29ab	363 ± 30abc	357 ± 27bc	264 ± 12c	328 ± 32bc	290 ± 12c	453 ± 36ab
Harvestable fruit biomass (g plant^{-1})	97	221 ± 15a	123 ± 17b	172 ± 29ab	174 ± 20ab	174 ± 13ab	142 ± 17ab	202 ± 4ab	228 ± 57a
Spoiled fruit biomass (g plant^{-1})	97	32 ± 3ab	42 ± 11ab	8 ± 2bc	24 ± 4abc	3 ± 2c	8 ± 3bc	10 ± 6bc	13 ± 2abc
Total aboveground biomass (g plant^{-1})	97	765 ± 46a	684 ± 9ab	595 ± 42abc	535 ± 48bcd	418 ± 23d	472 ± 25cd	540 ± 43bcd	642 ± 62abc
Harvest index	97	0.29 ± 0.02ab	0.18 ± 0.02b	0.28 ± 0.03ab	0.32 ± 0.01ab	0.42 ± 0.05a	0.30 ± 0.02ab	0.38 ± 0.04a	0.35 ± 0.05a
Small fruit (<4.0 cm diameter; %)[b]	97	20 ± 8b	2 ± 0c	28 ± 10b	94 ± 2a	21 ± 2b	23 ± 7b	10 ± 0b	10 ± 3b
Medium fruit (4.0–5.5 cm diameter; %)[b]	97	57 ± 0b	21 ± 7c	65 ± 7ab	6 ± 2c	77 ± 1ab	71 ± 4ab	83 ± 4a	81 ± 3a
Large fruit (>5.5 cm diameter; %)[b]	97	22 ± 8b	78 ± 7a	7 ± 4bc	0 ± 0c	2 ± 1c	6 ± 3bc	7 ± 3bc	8 ± 3bc
Soildepth_30 (mg cm^{-3})[c]	106	203 ± 28a	177 ± 29a	193 ± 18a	180 ± 66a	124 ± 66a	181 ± 13a	161 ± 51a	203 ± 42
Soildepth_60 (mg cm^{-3})[c]	106	156 ± 14a	146 ± 22a	151 ± 13a	156 ± 29a	116 ± 26a	174 ± 6a	119 ± 17a	138 ± 17a
Soildepth_90 (mg cm^{-3})[c]	106	154 ± 20a	150 ± 23a	135 ± 16a	162 ± 16a	117 ± 19a	125 ± 14a	118 ± 22a	131 ± 12a
Soildepth_120 (mg cm^{-3})[c]	106	135 ± 4a	129 ± 12a	127 ± 26a	144 ± 6a	117 ± 6a	126 ± 20a	120 ± 25a	142 ± 23a
Soildepth_180 (mg cm^{-3})[c]	106	70 ± 17a	72 ± 22a	72 ± 18a	77 ± 26a	53 ± 10a	65 ± 4a	75 ± 13a	61 ± 7a
Physiological traits									
Leaf N (%)	41	4.4 ± 0.1bc	4.5 ± 0.1ab	4.3 ± 0.1bc	4.4 ± 0.1bc	4.4 ± 0.1bc	4.6 ± 0.1a	4.4 ± 0.0bc	4.3 ± 0.1c
Stem N (%)	41	3.1 ± 0.1bc	3.4 ± 0.1ab	3.6 ± 0.1a	3.6 ± 0.0a	2.7 ± 0.1c	2.8 ± 0.1c	2.9 ± 0.2c	2.8 ± 0.1c
SoilMoist_1 between 36 and 42 DAP (mg cm^{-3})[c]	42	128 ± 43a	101 ± 46a	104 ± 21a	117 ± 46a	75 ± 42a	82 ± 33a	106 ± 44a	105 ± 22a
SoilMoist_2 between 46 and 55 DAP (mg cm^{-3})[c]	55	234 ± 2a	235 ± 27a	175 ± 10a	212 ± 23a	155 ± 32a	158 ± 2a	162 ± 21a	184 ± 8a
SoilMoist_3 between 59 and 70 DAP (mg cm^{-3})[c]	70	229 ± 21a	243 ± 7a	210 ± 2a	235 ± 16a	184 ± 37a	216 ± 26a	188 ± 25a	221 ± 23a
Stem N (%)	69	1.9 ± 0.1ab	1.9 ± 0.2ab	2.0 ± 0.2ab	2.4 ± 0.1a	1.6 ± 0.1b	2.0 ± 0.1ab	1.9 ± 0.1ab	1.8 ± 0.1ab
Green fruit N (%)	69	3.6 ± 0.2a	3.9 ± 0.1a	3.8 ± 0.1a	3.8 ± 0.1a	3.5 ± 0.2a	3.8 ± 0.1a	3.8 ± 0.1a	3.5 ± 0.0a
Photosynthetic rate (P_n; μmol m^{-2} s^{-1})	75, 76, 77	24.3 ± 2.3bc	21.9 ± 1.2c	25.4 ± 1.7abc	28.2 ± 1.4ab	30.8 ± 1.0a	26.1 ± 1.2abc	25.8 ± 1.3abc	28.3 ± 1.6ab
Stomatal conductance (g_s; mol m^{-2} s^{-1})	75, 76, 77	0.8 ± 0.1a	0.8 ± 0.1a	0.8 ± 0.1a	1.1 ± 0.1a	1.2 ± 0.1a	1.0 ± 0.1a	0.9 ± 0.2a	1.0 ± 0.1a
WUE$_i$ (P_n/g_s)	75, 76, 77	40 ± 7a	33 ± 4a	35 ± 3a	29 ± 5a	27 ± 2a	30 ± 4a	34 ± 4a	31 ± 4a
Vapor pressure deficit of leaf (VpdL; kPa)	75, 76, 77	1.6 ± 0.3a	1.7 ± 0.3a	1.6 ± 0.2a	1.3 ± 0.3a	1.2 ± 0.2a	1.4 ± 0.2a	1.6 ± 0.2a	1.4 ± 0.2a
^{13}C discrimination (Δ)	75, 76	21.4 ± 0a	21.7 ± 0a	21.2 ± 0a	21.1 ± 0a	21.7 ± 0a	21.8 ± 0a	21.7 ± 0a	21.4 ± 0a
Leaflet N (%)	75, 76	3.7 ± 0.2b	3.5 ± 0.2b	4.2 ± 0.2ab	3.8 ± 0.3ab	4.2 ± 0.1ab	4.7 ± 0.1a	4.1 ± 0.1ab	4.2 ± 0.2ab
Leaf N (%)	97	2.2 ± 0.2a	2.3 ± 0.0a	2.2 ± 0.1a	2.1 ± 0.0a	2.3 ± 0.1a	2.5 ± 0.0a	2.1 ± 0.1a	2.1 ± 0.1a
Stem N (%)	97	1.4 ± 0.1ab	1.2 ± 0.0ab	1.4 ± 0.2ab	1.6 ± 0.1a	1.2 ± 0.1b	1.5 ± 0.0ab	1.5 ± 0.0ab	1.4 ± 0.1ab
Green fruit N (%)	97	3.2 ± 0.1b	3.7 ± 0.3ab	3.8 ± 0.2ab	3.5 ± 0.1ab	3.3 ± 0.1ab	4.0 ± 0.1a	3.6 ± 0.2ab	3.3 ± 0.4ab
Harvestable fruit N (%)	97	2.9 ± 0.3b	3.3 ± 0.3ab	3.7 ± 0.5ab	3.4 ± 0.1ab	3.4 ± 0.4ab	4.1 ± 0.0a	3.7 ± 0.3ab	3.1 ± 0.5ab
Phenological traits									
Open flowers per plant (%)	22, 29, 34	62 ± 12abc	34 ± 13c	40 ± 13c	90 ± 5a	80 ± 7ab	72 ± 9ab	57 ± 12bc	73 ± 12ab
Relative PAR intercepted (% of maximum at 91 DAP)	40	45 ± 3abc	38 ± 3bcd	31 ± 2d	47 ± 4abc	54 ± 8a	35 ± 3cd	29 ± 1d	47 ± 3ab
Number of inflorescences per plant	41	27 ± 5a	12 ± 2a	14 ± 3a	25 ± 1a	19 ± 5a	17 ± 5a	28 ± 3a	14 ± 1a
Relative PAR intercepted (% of maximum at 91 DAP)	62	102 ± 2a	93 ± 8a	83 ± 4ab	96 ± 9a	88 ± 6ab	75 ± 3b	75 ± 2b	86 ± 0ab
Number of inflorescences per plant	69	45 ± 6ab	42 ± 7ab	32 ± 7ab	67 ± 10a	38 ± 17ab	22 ± 1ab	20 ± 4b	36 ± 2ab
Green fruit (% of total fruit biomass)	97	32 ± 1ab	46 ± 8a	41 ± 4ab	26 ± 3ab	19 ± 5b	36 ± 3ab	24 ± 6ab	33 ± 7ab
Harvestable fruit (% of total fruit biomass)	97	60 ± 1ab	41 ± 5b	56 ± 5ab	65 ± 2ab	79 ± 6a	60 ± 4ab	73 ± 8a	63 ± 8ab
Spoiled fruit (% of total fruit biomass)	97	9 ± 1ab	14 ± 4a	3 ± 1ab	9 ± 3ab	1 ± 1b	4 ± 2ab	3 ± 2ab	4 ± 1ab
Green fruit (% of total fruit biomass)	109	12 ± 2ab	20 ± 3a	14 ± 2ab	6 ± 2b	4 ± 1b	14 ± 4ab	10 ± 3ab	8 ± 2b
Harvestable fruit (% of total fruit biomass)	109	64 ± 1b	50 ± 3c	73 ± 1ab	85 ± 2a	83 ± 5a	77 ± 6a	81 ± 3a	83 ± 3a
Spoiled fruit (% of total fruit biomass)	109	24 ± 2a	30 ± 0a	13 ± 3b	9 ± 0b	12 ± 5b	9 ± 4b	9 ± 1b	8 ± 1b

[a] Days after transplanting.
[b] Category includes green + red fruits.
[c] Refer to Table 1.

medium-size fruit, but the highest in small fruit biomass and number of flowers per inflorescence. On $Mor5_{PCA}$, differences in rooting depth, as inferred by soil water extraction, showed no significant differences among cultivars, despite high loading scores for water in the soil profile. Likewise, on $Mor6_{PCA}$ early biomass variables (41 DAP) had high loading scores but were not different among cultivars.

3.1.2. Physiological traits

Six rotated factors ($Phy1_{PCA}$ to $Phy6_{PCA}$; Table 2 and Appendix B) explained ≥70% of the variance from the PCA analysis performed on the initial 31 raw physiological variables. $Phy1_{PCA}$ accounted for 25% of the total variance, and it was mostly related to high WUE_i associated with lower P_n and g_s when VpdL was >1.3 kPa (Table 3; data analyzed as repeated measures). $Phy2_{PCA}$ accounted for 12% of the total variance, and it was mainly associated with N concentration in the aboveground biomass at 97 DAP. $Phy3_{PCA}$ accounted for 11% of the total variance, and was mainly associated with early soil water uptake. $Phy4_{PCA}$ accounted for 10% of the total variance, and it was mostly defined by low WUE_i associated with high g_s when VpdL was <1.0 kPa. $Phy5_{PCA}$ accounted for 10% of the total variance, and was mainly associated with stem N concentration and leaflet $\Delta^{13}C$. $Phy6_{PCA}$ accounted for 7% of the total variance, and was associated with N concentration in early season biomass.

The univariate ANOVAs performed on the most important variables, *i.e.*, top five variables with loadings > 0.5 on the retained and rotated Phy_{PCA} axes, showed few cultivar differences for each physiological trait (Tables 2 and 3). On $Phy1_{PCA}$, WUE_i was similar among cultivars, despite a wide range in their mean WUE_i, which was mainly driven by higher g_s. Overall, newer cultivars tended to have higher P_n and g_s. On $Phy2_{PCA}$, the N concentration in leaflets (76 DAP) and fruits (97 DAP) was highest in Apex 1000 and lowest in Pearson, but there was no clear pattern related to cultivar time of release (Table 3). On the other PCA axes, no clear differences were observed with the exception of stem N concentration ($Phy5_{PCA}$), which was lower in newer cultivars (Table 3).

3.1.3. Phenological traits

Three rotated factors ($Phe1_{PCA}$ to $Phe3_{PCA}$; Table 2 and Appendix C) explained ≥70% of the variance from the PCA analysis performed on the initial 13 raw phenological variables. $Phe1_{PCA}$ accounted for 29% of the total variance, and it was mostly related to early flowering, fruit set and fruit holding capacity without decay. $Phe2_{PCA}$ accounted for 25% of the total variance, and it was related to even fruit ripening associated with a lower proportion of green fruit by harvest (97 DAP). $Phe3_{PCA}$ accounted for 20% of the total variance, and it was mainly associated with earliness in reaching maximum canopy cover, *i.e.*, higher relative PAR intercepted earlier in the season in relation to each cultivar's maximum observed value.

The univariate ANOVAs performed on the most important variables, *i.e.*, top five variables with loadings > 0.5 on the retained and rotated Phe_{PCA} axes, showed cultivar differences for some phenological traits (Tables 2 and 3). On $Phe1_{PCA}$, earliness of flowering (the percentage of plants with open flowers at ~30 DAP) ranged between 34% and 90% plants with open flowers for VF36 and Heinz 1706, respectively. Most newer cultivars were on the higher end of the range (Table 3). The spoiled fruit category was lower in newer cultivars, which increased the proportion of their harvestable fruits by 109 DAP. On $Phe2_{PCA}$, the proportion of harvestable fruit at 97 DAP ranged from 41% to 79%, while green fruit was 46% and 19% for VF36 and M82, respectively (Table 3). On $Phe3_{PCA}$, the relative PAR intercepted in relation to a cultivar's seasonal maximum value, though significantly different among cultivars, was not related to the cultivar's time of release (Table 3). The main differences were early in the season, with M82 intercepting more PAR relative to its maximum, and cultivars VF145 and UC-204C intercepting the least.

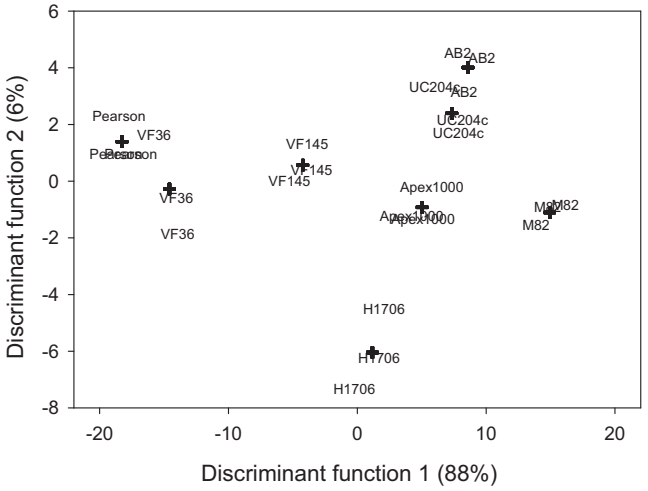

Fig. 2. Discriminant analysis plot based on eight retained, rotated-PCA axes for maximizing differences among 8 processing tomato cultivars (3 replicates each) released from 1930s to 2000s. Year of release represents the centroid for each cultivar. Variance explained by the first two discriminant functions is presented in parentheses.

3.1.4. Discriminant analysis

For the purpose of identifying the PCA axes that maximized the differences among cultivars, the stepwise DA on the total 15 retained-PCA axes (six morphological, six physiological and three phenological rotated axes; see methods for selection criteria) kept eight rotated-PCA axes for running the DA: $Mor1_{PCA}$, $Mor3_{PCA}$, $Mor4_{PCA}$, $Mor6_{PCA}$, $Phy1_{PCA}$, $Phy2_{PCA}$, $Phe1_{PCA}$ and $Phe2_{PCA}$. The first discriminant function explained most of the variance, 88% (Fig. 2). In order of importance, this function had the following standardized scoring coefficients for each rotated axis: $Phe1_{PCA}$ (3.33), $Mor6_{PCA}$ (3.04), $Phy1_{PCA}$ (−2.34), $Mor1_{PCA}$ (−2.22), $Phy2_{PCA}$ (2.11), $Phe2_{PCA}$ (2.02), $Mor3_{PCA}$ (1.59), $Mor4_{PCA}$ (−0.53). Thus, this function was mostly related to a combination of traits including early flowering, low vegetative biomass, concentrated fruit set and ripening, and high N concentration in the aboveground biomass late in the season, as well as lower WUE_i and smaller canopies (Table 2). The second discriminant function only explained 6% of the total variance, mainly affected by $Mor4_{PCA}$, and thus was related to many flowers per inflorescence and small fruit size (Fig. 2 and Table 3). The discriminant functions did not misclassify observations into any cultivar group.

3.2. Greenhouse study

The aboveground biomass (classified as a morphological trait) of plants grown in the greenhouse followed a similar pattern as in the field study. There were no differences among most cultivars, but VF36 had higher biomass than Apex 1000 (Table 4). The $\Delta^{13}C$ was low for cultivar AB2 and high for VF145, indicating a trend similar to the field leaf gas exchange data. Newer cultivars started flowering earlier than older cultivars, and produced more flowers during the experiment (data not shown). The number of inflorescences per plant was higher in cultivars Heinz 1706, M82 and AB2, while percent fruit set was higher in M82, UC-204C and AB2, the newer cultivars. Thus, flowering traits (phenological traits) had a similar response in the greenhouse as in the field.

Table 4
Results from the univariate ANOVA on plant biomass, flowering traits, fruit set and leaf gas exchange of eight processing tomato cultivars evaluated in a greenhouse experiment. Variables with more than one sampling date (DAP; days after planting) were analyzed by ANOVA with a repeated measures structure. Values are mean ± standard error. Means followed by different letters are significantly different at $p < 0.05$ using the Tukey–Kramer HSD test.

Variable	DAP[a]	Cultivar							
		Pearson	VF36	VF145	Heinz 1706	M82	Apex1000	UC-204C	AB2
Total biomass (g plant^{-1})	41	30.7 ± 3.6ab	32.7 ± 1.6a	31.1 ± 0.3ab	30.7 ± 1.3ab	26.4 ± 1.4ab	24.6 ± 2.4b	24.9 ± 0.4ab	30.7 ± 0.9ab
Open flowers per plant	35	1 ± 0c	2 ± 0bc	1 ± 0c	6 ± 1a	6 ± 1a	2 ± 1bc	2 ± 1bc	4 ± 1ab
Open flowers per plant	41	5 ± 1b	5 ± 1ab	6 ± 2ab	12 ± 3a	9 ± 1ab	6 ± 1ab	5 ± 1ab	8 ± 1ab
Inflorescences per plant	35	0.6 ± 0.2bc	1.0 ± 0.0abc	0.4 ± 0.2c	2.0 ± 0.3a	1.8 ± 0.2a	0.6 ± 0.2bc	0.6 ± 0.2bc	1.6 ± 0.2ab
Inflorescences per plant	41	2.6 ± 0.7bc	1.8 ± 0.2c	2.2 ± 0.6c	5.0 ± 0.9a	4.6 ± 0.2ab	2.2 ± 0.4c	2.4 ± 0.2bc	2.8 ± 0.4abc
Fruit set per plant (% of total flowers)	41	0.0 ± 0.0c	5.2 ± 2.4bc	2.4 ± 1.6bc	13.5 ± 1.5ab	27.3 ± 4.5a	6.7 ± 3.5bc	21.9 ± 6.0a	24.0 ± 5.2a
^{13}C discrimination (Δ)	31, 32, 43, 44	20.0 ± 0.3b	20.3 ± 0.2ab	20.9 ± 0.2a	20.1 ± 0.2b	19.9 ± 0.1b	19.8 ± 0.2b	20.4 ± 0.2ab	19.0 ± 0.2c

[a] Days after transplanting.

4. Discussion

This study shows that phenological and physiological changes in processing tomato cultivars accompanied the canopy changes driven by the switch to a determinate growth habit in the 1960s. The multivariate analyses showed that no single trait seems to have driven yield increases in processing tomato cultivars. Instead, a suite of traits are interrelated to produce a plant phenotype that contributed to higher crop yields (Sinclair and Purcell, 2005). The selection for plants with earlier flowering may have prompted shifts in sink demand from the shoot (and possibly roots) to fruit. In addition, a higher number of flowers and fruit set early on may have increased sink demand. Assimilate supply can become a limiting factor in the fruit filling stage (Fischer, 2007), suggesting a tradeoff with the new cultivars' shorter vegetative stage and reduced canopy size. As will be discussed below, higher demand and potentially lower supply of assimilate may have been partly compensated for, in the newer cultivars, by an increase in P_n and N utilization efficiency (Evans and Fischer, 1999). Increased C assimilation in a number of other species has resulted in lower WUE_i, because higher stomatal conductance is needed to increase CO_2 concentrations at the site of carboxylation (Condon et al., 2004; Gilbert et al., 2011). Purposeful selection for higher yields, both before and after the transition to determinate growth habit, appears to have been made possible by a combination of plant traits, and by inadvertently minimizing the tradeoffs among them. For example, in the latest cultivars, decreased total aboveground biomass and increased fruit set (morphological traits), along with reduced stem N concentration and increased C assimilation rate (physiological traits) are associated with early flowering and synchronous ripening (phenological traits). If biomass reduction had not accompanied earlier flowering, then yield increases might not have been achieved.

4.1. Changes in suites of traits

Earliness is a key factor in the evolution of cultivars for mechanized production, since time available for flowering and fruit development increases (Evans, 1993). Newer cultivars tended to have earlier onset of flowering and less shoot biomass than older cultivars. Tomato plants with early flowering can reduce the number of leaves before the first inflorescence appears and decrease the number of leaves between subsequent inflorescences (Jones et al., 2007). Thus, phenological traits such as an early onset of flowering affect canopy development and architecture. With the exception of the only hybrid cultivar in this study, AB2, newer cultivars had less aboveground biomass than older cultivars. Farmers now choose to grow hybrids (such as AB2) because their higher vigor is believed to be the reason for higher yields. In cotton, a more compact plant with reduced vegetative branches and early flowering also resulted in higher yields under short season plantings (Sekloka et al., 2008). The mechanistic explanation may be related to the fact that a shorter vegetative stage and early onset of flowering may favor a stronger and early sink demand from fruits instead of roots and stems (Campbell et al., 1986), and even decrease vegetative growth on plants with higher vigor by favoring fruit filling.

The PCA results showed that patterns of plant water extraction from the soil were mostly associated with the retained $Phy3_{PCA}$ and $Mor5_{PCA}$ axes. Several positive correlations were observed between aboveground biomass and soil water extraction (*e.g.*, water extraction at 55 DAP and stem biomass at harvest; $p = 0.02$). It might be possible that inadvertent selection for cultivars with smaller root systems has happened in California's Central Valley, where irrigation water has not been much of a concern even under dry summers (Jackson, 1995). If there had only been changes in plant morphology (smaller canopies and possibly smaller root systems) without gains

in C assimilation per unit leaf area, then earlier flowering may not have been such a beneficial phenological trait in newer cultivars, given that higher C demand by fruits occurs earlier in the season.

The increase in P_n observed for some of the newer tomato cultivars may be the result of breeding under high light intensities, high temperature, and well irrigated and fertilized field conditions in California (Bolaños and Hsiao, 1991). *Avena barbata* populations originating from regions with high light intensity have higher photosynthetic rates (Somersalo et al., 1998). Gains in P_n have also been reported for greenhouse tomato cultivars released in the past 50 years (Higashide and Heuvelink, 2009). In these field-grown tomatoes, the trend for higher P_n for newer cultivars was accompanied by a trend for higher g_s. A positive correlation between g_s and P_n was observed ($r^2 = 0.6$; $p < 0.0001$) here. Higher P_n and g_s have been shown to be positively correlated with a gain in yield in wheat cultivars (Fischer et al., 1998) and tomatoes (Barrios-Masias et al., 2013). Higher stomatal conductance is thought to be the most common driver for lower WUE_i, but if accompanied by increases in photosynthetic capacity, could be advantageous for C assimilation (Gilbert et al., 2011). Several studies have shown that the relative increase in g_s tends to be higher, however, than the relative gains in P_n, and as a result WUE_i decreases (Barrios-Masias et al., 2013; Fischer et al., 1998 Tanaka et al., 2008). Although no significant differences in WUE_i were found among cultivars, g_s and WUE_i were negatively correlated ($r^2 = 0.74$; $p < 0.0001$), indicating that in tomatoes the gain in C assimilation has occurred at the cost of lower WUE_i. Regardless of the decrease in WUE_i, gains in field-level crop WUE (yield/applied water) has increased 50% since the 1970s (Hanson and May, 2006). The suite of traits selected for mechanical harvest such as earlier flowering, smaller canopies and concentrated fruit set may have contributed to this gain by a curtailed vegetative stage and initiation of leaf senescence soon after fruit filling.

It is generally difficult to directly relate gains in P_n to higher yields (Sinclair et al., 2004; Fischer et al., 1998), but it could be hypothesized that it is a desirable trait especially under higher density plantings (Jackson and Koch, 1997). In some of the newer cultivars, higher leaf area and weight may favor new, fully mature leaves to capture more light near the top of the canopy, contributing an important role in C assimilation when demand is highest (Evans and Fischer, 1999; Fischer, 2007; Tei et al., 2002). This is especially true considering that P_n decreases in aging leaves and that light distribution within the canopy is often sub-optimal (Bolaños and Hsiao, 1991). In soybean, the contribution to higher C assimilation was more dependent on increased photosynthetic potential rather than to higher leaf area index (Rascher et al., 2010). In addition, tomato leaves have the capacity to assimilate C even at irradiances of >2000 μmol-PAR m^{-2} s^{-1}, which are typical of average light intensities for field conditions in California (Bolaños and Hsiao, 1991).

Along with gains in P_n, a trend for higher N concentration in tomato leaflets was observed for newer cultivars. Modern cultivars tend to have higher N accumulation capacity, which is related to an increase in P_n and often to higher soil N availability to the plant (Bolaños and Hsiao, 1991; Giunta et al., 2007; Poorter and Evans, 1998; Sinclair et al., 2004). Processing tomatoes have been shown to have a high recovery rate (>50%) of soil N under low and optimal fertilization rates (Benincasa et al., 2011). Of course, a cultivar must be able to utilize available N for processes that favor biomass allocation to the harvestable organ (Sadras and Lawson, 2013; Sinclair, 1998; Tei et al., 2002). For processing tomatoes, higher C assimilation capacity, *e.g.*, from increased Rubisco, could favor vegetative growth (Campbell et al., 1986), as well as C allocation to reproductive organs from an early flowering cultivar with a strong sink demand. Our results show lower N concentrations early in the season and a reduction in total stem biomass by harvest in newer cultivars (data not shown). This supports the idea that changes in allocation and growth habit accompanied the reduction in biomass, permitting early N and C allocation to other organs, *e.g.*, reproductive organs.

4.2. Traits for higher crop yield

Breeders of processing tomatoes were purposefully selecting for concentrated fruit set and ripening as means to increase crop yields after the introduction of the mechanical harvest in the 1960s, and growers now expect to have >90% of harvestable fruit by the time of harvest (UCCE, 2008). In doing so, a suite of other morphological, physiological and phenological traits that contribute to this gain have been selected for such as medium fruit size, lower stem N and reduced fruit spoilage. But the gains in yields in California have not increased fruit quality (especially total soluble solids; TSS), which is a main breeding objective (Grandillo et al., 1999; Thomas, 1980). In Israel, fruit quality has increased but with little simultaneous gains in yield. Long-term studies have shown a negative relationship between genetic gains in yield and total soluble solids (Grandillo et al., 1999). The continual integration of new germplasm especially for pest and disease resistance undoubtedly made for complexity in backcrossing to a desirable harvestable phenotype with the desired yield and quality characteristics (Hajjar and Hodgkin, 2007).

There are still opportunities for increasing yield based on the findings of this study. Increased HI may be one option, by further reducing total plant biomass along with associated suites of traits that have been described above (*e.g.*, early flowering and increased C assimilation capacity; not just biomass allocation). Under the low planting densities of this study, only a slight gain in HI has occurred in the past 80 years, much less than compared to other major crops (Fischer, 2007; Giunta et al., 2007; Sinclair, 1998). For tomatoes, an increase in HI would also depend on maximizing the percent of harvestable fruit *vs.* green and spoiled fruit, a trend seen here for the newer cultivars. Along with disease tolerance and fruit holding capacity, greater flowering synchrony and successful fruit formation may be a strategy for achieving a higher proportion of harvestable fruit. In fact, a higher percentage of fruit set per flowers produced was found in many of the newer cultivars in the greenhouse study.

Most of the cultivars with higher fruit set had a greater percentage of fruit classified in the medium-size, rather than the large- or small-size categories. Increasing fruit size can also be another option for higher yields if tradeoffs between fruit size, plant biomass and fruit number are minimized. In one greenhouse tomato study, gains in yields were associated with bigger fruits and higher plant biomass without a gain in HI (van der Ploeg et al., 2007). In wheat, gains in yield have been achieved by increasing the number of kernels and a big effort was made to not lose kernel weight (Fischer, 2007); an exception is in Italian durum wheat in which an increase in yields has been possible due to gains in kernel number and size in modern cultivars (Giunta et al., 2007). In contrast, in greenhouse tomatoes, the yield gains observed in the past 50 years are more related to increased plant biomass with larger fruit rather than to more fruits per plant (van der Ploeg et al., 2007), suggesting that selection in the field *vs.* greenhouse favors a different suite of canopy traits. In field-grown processing tomatoes, not only have smaller plants helped to increase yields, they have allowed growers to add to the yield potential by using densities between 17,300 and 22,200 plants ha^{-1} depending on the site, local environment, management constraints and cultivar (Smith et al., 1992; UCCE, 2008).

Looking ahead to selection strategies for increasing yield, we envision that the use of smaller plants is advantageous along with better selection for suites of traits that increase HI, fruit size, and photosynthetic capacity. Given future concerns about summer drought and climate change in California, maintaining yields with

lower water inputs may best be achieved by a coordinated effort among breeders, physiologists and ecologists, to directly evaluate suites of traits in the selection process.

5. Conclusion

This study focused on a suite of traits that contributed to the increase in yields of more than 100% in the last 80 years. Since the switch from indeterminate to determinate growth habit in the 1960s several other traits have contributed to increase yields. A set of phenological traits (early flowering and fruit set) was associated with morphological traits (smaller canopies) and gains in physiological traits (N use efficiency and P_n) that were important in differentiating among cultivars. Among the cultivars released after determinate growth habit was incorporated in processing tomatoes, cultivars H1706, M82 and AB2 show a distinct assembly of traits, such as flowering pattern, canopy development, N concentration and biomass accumulation. Future crop improvement could be directed to incorporate traits for smaller plants and increased HI, fruit size and photosynthetic capacity, along with traits that increase resource use efficiency, such as N uptake and partitioning, and root growth pattern, for adapting crop management to new irrigation and fertilization strategies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eja.2013.11.007.

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