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Semi-determinate growth habit adjusts the vegetative-to-reproductive balance and increases productivity and water-use efficiency in tomato (*Solanum lycopersicum*)

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ABSTRACT

Tomato (*Solanum lycopersicum*) shows three growth habits: determinate, indeterminate and semi-determinate. These are controlled mainly by allelic variation in the *SELF-PRUNING* (*SP*) gene family, which also includes the "florigen" gene *SINGLE FLOWER TRUSS* (*SFT*). Determinate cultivars have synchronized flower and fruit production, which allows mechanical harvesting in the tomato processing industry, whereas indeterminate ones have more vegetative growth with continuous flower and fruit formation, being thus preferred for fresh market tomato production. The semi-determinate growth habit is poorly understood, although there are indications that it combines advantages of determinate and indeterminate growth. Here, we used near-isogenic lines (NILs) in the cultivar Micro-Tom (MT) with different growth habit to characterize semi-determinate growth and to determine its impact on developmental and productivity traits. We show that semi-determinate genotypes are equivalent to determinate ones with extended vegetative growth, which in turn impacts shoot height, number of leaves and either stem diameter or internode length. Semi-determinate plants also tend to increase the highly relevant agronomic parameter Brix × ripe yield (BRY). Water-use efficiency (WUE), evaluated either directly as dry mass produced per amount of water transpired or indirectly through C isotope discrimination, was higher in semi-determinate genotypes. We also provide evidence that the increases in BRY in semi-determinate genotypes are a consequence of an improved balance between vegetative and reproductive growth, a mechanism analogous to the conversion of the overly vegetative tall cereal varieties into well-balanced semi-dwarf ones used in the Green Revolution.

Introduction

The Green Revolution brought about a great improvement in productivity of cereal crops through the manipulation of plant architecture (Peng et al., 1999; Spielmeyer et al., 2002). Plant architecture can influence yield by altering the relative growth and position of vegetative and reproductive organs in the plant canopy, also affecting fruit harvest. In most Solanaceae there is an alternation between vegetative and reproductive phases, which present themselves as modular units called sympodia. In tomato (*Solanum lycopersicum*), the vegetative apical meristem converts into floral after a series of 6–12 internodes with leaves (Samach and Lotan, 2007). Vegetative growth, however, continues through the topmost axillary meristem, which grows vigorously displacing the inflorescence to the side and producing a new sympodium with three leaves and an inflorescence (Lifschitz and Eshed, 2006). This process is repeated indefinitely in plants of indeterminate growth habit.

Determinate growth in tomato is a consequence of a recessive mutation in the *SELF-PRUNING* (*SP*), which belongs to the *CETS* (*CENTRORADIALIS/TERMINAL FLOWER 1/SELF-PRUNING*) gene family (McGarry and Ayre, 2012). The loss-of-function *sp* allele reduces the number of leaves of successive sympodial units, resulting in

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Abbreviations: Δ^{13}C, Carbon isotope discrimination; BRY, Brix × ripe yield; dag, days after germination; LS, lateral shoot; MS, main shoot; MT, Micro-Tom; PS, primary shoot; QTL, quantitative trait locus; *sft*, *single flower truss*; *Sp*, *Self-pruning*; SU, sympodial unit; *WELL*, *Water economy locus in Lycopersicon*; WUE, water-use efficiency.

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two consecutive inflorescences that top vertical growth, producing a compact plant (Pnueli et al., 1998). In tomato, the *CETS* family has 13 members (Tomato Genome Consortium, 2012) among which the best characterized are *SP2I* (*SP2G* as per its revised map position), *SP3D*, *SP5G* (*SP5E*), *SP6A* (*SP5G*) and *SP9D* (Carmel-Goren et al., 2003; Chitwood et al., 2013). *Solanum pennellii* alleles of *SP5G* (*SP5E*) or *SP9D* in the *sp*/*sp* background lead to a variation in determinate growth habit characterized by elongated internodes, greater number of nodes between successive inflorescences, and delayed termination of the top-most sympodium (Fridman et al., 2002; Jones et al., 2007). This growth habit has been termed "semi-determinate" (Fridman et al., 2002; Carmel-Goren et al., 2003), but it has never been fully characterized. *SP3D* corresponds to the *SINGLE FLOWER TRUSS* (*SFT*) gene, whose *Arabidopsis* ortholog is *FLOWERING LOCUS T* (*FT*) (Lifschitz et al., 2006). Determinate tomato plants (*sp*/*sp*) heterozygous for the loss-of-function allele of *SFT* (*sft* mutation) produce a greater number of leaves, inflorescences, and flowers per inflorescence, which, coupled to a higher individual fruit weight, lead to up to 60% increase in yield, when compared to isogenic *sp*/*sp* plants (Krieger et al., 2010; Jiang et al., 2013). If homozygous in a *sp*/*sp* background, the *sft* mutation leads to the production of an indeterminate vegetative inflorescence, resulting in a plant with reduced fruit production (Molinero-Rosales et al., 2004; Lifschitz et al., 2006; Shalit et al., 2009).

Determinate growth in *sp*/*sp* tomatoes causes almost simultaneous fruit ripening, which, in addition to the compact growth habit, allows large-scale mechanical harvesting (Stevens and Rick, 1986). The drawback is that determinate growth produces fruits with reduced content of total soluble solids when compared to isogenic indeterminate lines (Emery and Munger, 1970; Rousseaux et al., 2005). Plants with semi-determinate growth habit, however, tend to show increases in both yield and in total soluble solids in fruit (Fridman et al., 2002; Carmel-Goren et al., 2003; Krieger et al., 2010). We hypothesized that semi-determinate growth would balance both the excessive reproductive or vegetative growth of determinate and indeterminate plants, respectively. We previously created an introgression line from *S. pennellii* with increased water-use efficiency named "*Water Economy Locus in Lycopersicon*" (*Well*) (Zsögön, 2011). As *Well* is semi-determinate we also tested whether the water-use efficiency (WUE) could be another improvement intrinsic to the semi-determinate growth habit. We assessed this by comparing productivity parameters (e.g. yield, total soluble solids) and WUE in near-isogenic tomato lines with different growth habit in a single genetic background, cv. Micro-Tom (MT). We present a better characterization of the semi-determinate growth habit and suggest that the adjustment of vegetative-to-reproductive growth could act as a key factor determining crop yield. Moreover, we provide evidence that WUE can be affected by growth habit.

Materials and methods

Plant material

Plant material is described in Table 1. The *sft* mutation was introgressed into tomato (*Solanum lycopersicum* L.) cv Micro-Tom (MT) from its original background (LA2460, possibly cv. Ailsa Craig). Since MT harbours the *sp* mutation (Martí et al., 2006), the functional *Sp* allele was introgressed from cv. Moneymaker. The introgression (from *Solanum pennellii* LA716) and characterization of Water economy locus in Lycopersicon (Well) allele are described in Zsögön (2011). Introgression of mutations into the MT cultivar was carried out as described previously (Carvalho et al., 2011). Briefly, pollen was collected from parent plants and used to fertilize emasculated MT flowers. The resulting F_1 hybrids were self-fertilized to obtain recombinant F_2 populations, which were visually screened for the compact size of MT and the phenotype of the mutation of interest. Selected plants were backcrossed with MT up to the sixth generation (BC_6), self-fertilizing every second generation to screen for homozygous mutants. After BC_6F_2 the resulting genotypes can be considered near-isogenic lines (Stam and Zeven, 1981). All experiments were conducted on BC_6F_3 plants or subsequent generations.

Growth conditions

Plants were grown in a glasshouse at the Laboratory of Hormonal Control of Plant Development, ESALQ-USP (Piracicaba, SP, Brazil). The growth conditions were mean temperature of 28 °C, 11.5 h/13 h (winter/summer) photoperiod, 250–350 μmol photons $m^{-2}\,s^{-1}$ PAR irradiance, attained by a reflecting mesh (Aluminet, Polysack Indústrias Ltda, Leme, Brazil), and automatic irrigation to field capacity four times a day. Seeds were germinated in 350 mL pots with a 1:1 mixture of commercial potting mix Basaplant® (Base Agro, Artur Nogueira, SP, Brazil) and expanded vermiculite supplemented with $1\,g\,L^{-1}$ 10:10:10 NPK and $4\,g\,L^{-1}$ dolomite limestone ($MgCO_3 + CaCO_3$). Upon appearance of the first true leaf, seedlings of each genotype were transplanted to pots containing the soil mix described above, except for NPK supplementation, which was increased to $8\,g\,L^{-1}$. After transplanting, plants were sprayed twice at 14-d intervals with $1\,g\,L^{-1}$ Peters 20–20–20 fertilizer.

Characterization of growth habit and productivity traits

Growth habit measurements were performed 50 days after germination (dag). Leaf insertion angle on the fourth leaf (from the base), length of third, fourth and fifth internode, number of leaves up to the first inflorescence, and on the main shoot (MS) (leaves

Table 1
Description of the genotypes in the Micro-Tom background used in this work.

Genotype	Effect/gene function	Origin	Reference
MicroTom (MT)	This tomato cultivar presents the recessive allele *self-pruning* (*sp*), which leads to a determination of growth and a uniform ripening of fruits. Defective in a CETS transcription factor that acts as flowering repressor. The locus is on chromosome *6*		Yeager (1927), Meissner et al. (1997)
Self-pruning (*Sp*)	Plants with the wild type allele *Sp* resume vegetative growth after each flower induction. Thus, maintaining the vegetative plant cyclic	cv. Moneymaker	Pnueli et al. (1998)
Single flower truss (*sft*)	Plants with the mutated allele have a low flowering induction. Defective in a CETS transcription factor that acts as a floral inductor. The locus is on chromosome *3*	LA2460	Molinero-Rosales et al. (2004)
sft Heterozygous (*sft*/+)	Heterozygous *sft* plants in the *sp* background present changes in growth habit	Cross between MT and *sft*	Krieger et al. (2010)
Water economy locus in Lycopersicon(*Well*)	Plant with the natural allelic variation presents change in growth habit and longer internodes. Water-use efficiency is increased. Unknown gene function. The locus is on chromosome *1*	LA 716	Zsögön (2011)
*Well*heterozygous (*Well*/+)	Heterozygous *Well* plants, in the background *sp* present changes in growth habit and longer internodes	Cross between MT and *Well*	

of primary shoot plus leaves on sympodial units following the first inflorescence); height of the plant on the primary shoot, MS, and lateral shoot (LS) were measured. Leaf insertion angle was determined on photographs of 10 plants per genotype analysed with AutoCad 2010 (Autodesk, Inc., San Rafael, CA, USA). The angle with respect to the shoot was determined from the top.

Productivity performance of plants was assessed 90 dag. The following parameters were determined: number of fruits per plant; total fruit weight per plant (yield); average weight per fruit; total soluble solids content in fruits (Brix); and frequency of green and ripe fruits. Total soluble solids content was assessed using a digital refractometer (PR-101α, Atago, Tokyo, Japan) in fruits from 10 different plants per genotype. Shoot diameter was determined at the height of the sixth, seventh and eighth internode in 10 plants per genotype 65 dag, using a digital pachymeter (Western Ferramentas, São Paulo, SP, Brazil).

To assess the balance between vegetative and reproductive growth, plant organs were classified in vegetative (roots, shoots, leaves) or reproductive (flowers and fruits). Ten plants per genotype were harvested 100 dag and oven-dried at 70 °C for 72 h. Dry mass was determined using electronic scales (Tecnal, Piracicaba, SP, Brazil).

Gravimetric measurement of water-use efficiency (WUE)

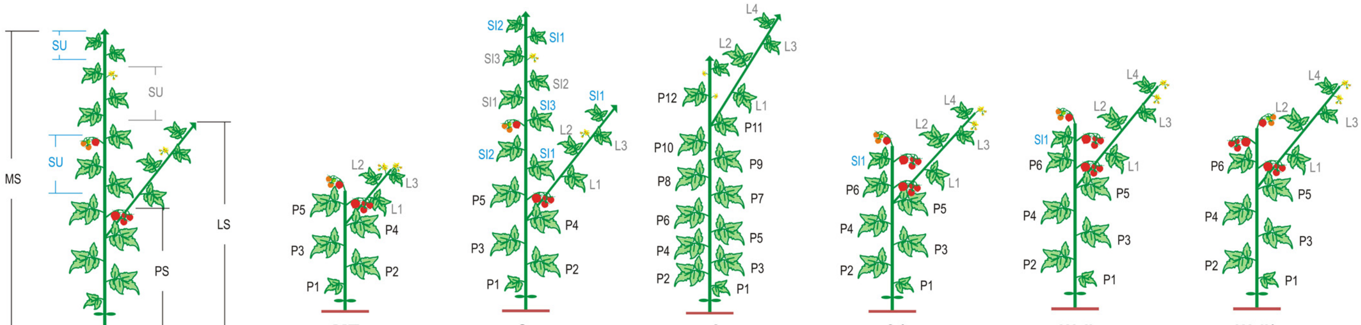
Seeds from all genotypes were germinated as described above and seedlings were transplanted to "self-watering" pots (a pot connected to a reservoir through a wick, wrapped in aluminium foil to prevent evaporation). The reservoir was filled with 600 ml water and weight of the whole pot recorded. Three control pots without plants were included in order to correct water use for water lost directly from the soil by evaporation. Water loss was assessed by re-weighing every second day. Twelve replicates were done per genotype. After 32 d, plants (roots, leaves and stems) were harvested and oven-dried at 65 °C for 48 h. Leaf area was determined before drying using a LI-3000A planimeter (Li-Cor, Lincoln, NE, USA). Specific leaf area (SLA) was calculated from representative leaflets after determination of individual leaf dry weights as: SLA = leaflet area (cm^2)/leaflet dry mass (g). water-use efficiency (WUE) was calculated for each plant as the ratio of whole plant dry biomass accumulated between transplanting (when seedling biomass can be considered negligible) and harvest 32 d later, to amount of water transpired during the same period. The setup described above was used to determine WUE of fruit yield in another experiment. Ten plants per genotype were harvested 107 d after transplanting, and their fruits oven-dried. WUE_{fruit} was calculated as the amount of fruit dry mass produced per unit water transpired.

Carbon isotope discrimination

Leaves of five random plants per genotype were harvested and ground to fine powder. Samples were sent to the Laboratory of Stable Isotopes (CENA, USP, Piracicaba, Brazil), where they were analysed for $^{13}C/^{12}C$ ratio using a mass spectrometer coupled to a Dumas elemental analyser ANCA-SL (Europa Scientific, Crewe, UK). Carbon isotope ratios were obtained in δ-notation, where $\delta = R/R_{standard} - 1$ and R and $R_{standard}$ are the isotope ratios of the plant sample and the Vienna Pee Dee Belemnite (VPDB) standard, respectively. $\delta^{13}C$ of atmospheric CO_2 was assumed to be -8 per mil. The $\delta^{13}C$ values for the samples were then converted to carbon isotopic discrimination values, $\Delta^{13}C = (\delta_a - \delta_p)/(1 + \delta_p)$, where δ_a is the $\delta^{13}C$ of atmospheric CO_2 and δ_p the $\delta^{13}C$ of the plant material(Farquhar and Sharkey, 1982). $\Delta^{13}C$ is highly correlated with water-use efficiency in a large number of species (Farquhar et al., 1989), including tomato (Martin and Thorstenson, 1988).

Stomatal density determinations

Stomatal density on both adaxial and abaxial surfaces of leaves were determined using the imprinting technique (Weyers and Johansen, 1985; Campos et al., 2009). Dental resin (Xantopren VL Plus + Optosil Xantopren Universal Activator, Heraeus Kulzer South America Ltda, São Paulo, SP, Brazil) was spread on well-exposed leaflets of full-expanded leaves (leaves 4–6 counting from the base of the shoot) and removed immediately upon drying. The imprints were coated with nail polish (Revlon, NYC, USA) and the resulting film was mounted on a 50% glycerine solution



	MT	*Sp*	*sft*	*sft/+*	*Well*	*Well/+*
Number of leaves on the primary shoot (PS)	5.30 ± 0.15 c	5.20 ± 0.13 c	12.20 ± 0.13 a	6.10 ± 0.10 b	5.90 ± 0.10 b	6.10 ± 0.17 b
Number of leaves on the main shoot (MS)	5.30 ± 0.15 c	12.80 ± 0.25 a	12.40 ± 0.27 a	7.20 ± 0.48 b	6.70 ± 0.21 b	6.20 ± 0.20 bc
Height of the PS	6.19 ± 0.22 d	6.76 ± 0.22 cd	15.26 ± 0.28 a	7.70 ± 0.25 c	9.50 ± 0.36 b	9.74 ± 0.30 b
Height of the MS	10.36 ± 0.48 c	18.15 ± 0.41 a	17.38 ± 0.44 a	13.78 ± 0.66 b	14.59 ± 0.57 b	14.30 ± 0.28 b
Height of the lateral shoot (LS)	10.73 ± 0.23 c	15.43 ± 0.57 b	18.56 ± 0.58 a	15.48 ± 0.42 b	16.29 ± 0.85 ab	17.37 ± 0.86 ab
Internode length	1.35 ± 0.70 b	1.29 ± 0.06 b	1.17 ± 0.05 b	1.42 ± 0.01 b	2.04 ± 0.10 a	2.07 ± 0.09 a
	Determinate	**Indeterminate**		**Semi-determinate**		

Fig. 1. Schematic representation of growth habit in tomato cv. Micro-Tom (MT): determinate (MT), indeterminate (*Sp* and *sft*) and semi-determinate (*sft/+*, *Well* and *Well/+*). Parameters that define growth habit are: (i) number of leaves on the primary shoot (PS) (i.e. number of leaves up to the first inflorescence); (ii) number of leaves on the main shoot (MS) (i.e. number of leaves of PS plus leaves on sympodial units (SU) following the first inflorescence); (iii) height of PS, MS and lateral shoot (LS) and iv) internode length (cm). Measurements performed 50 dag. Data are mean ± s.e.m. (*n* = 10 plants). *Sp* = *Self-pruning*, *sft* = *single flower truss*, *Well* = *Water economy locus in Lycopersicon*. *sft/+* and *Well/+* mean heterozygote *sft* and *Well*, respectively. The genotypes MT, *sft*, *sft/+*, *Well* and *Well/+* are homozygous for the recessive allele *self-pruning* (*sp*).

and observed and photographed at magnification under light microscopy (Eclipse E200, Nikon Instruments Inc., Tokyo, Japan). Stomatal density was determined on the photographs using ImageJ (http://rsbweb.nih.gov/ij/). Imprints were taken for leaflets of five plants and 10 photographs were analysed per side of the leaflet, covering an area of 0.32 mm^2.

Data analysis

ANOVA and Tukey HSD tests were performed using Assistat 7.6 beta (http://assistat.com). Percentage data were converted to inverse function (1/*X*) before analysis. MANOVA and canonical discriminant analysis were carried out using SAS (SAS Institute Inc., Cary, NC, USA).

Results and discussion

Characterization of semi-determinate growth habit in Micro-Tom tomato

The tomato cultivar MT harbours the *sp* allele, a loss-of-function of the *SELF-PRUNING* gene that causes determinate growth (Fig. 1; Martí et al., 2006; Kobayashi et al., 2014). We previously described a line carrying the functional *Sp* allele in MT, which accurately replicates indeterminate growth observed in other tomato backgrounds (Carvalho et al., 2011). The number of leaves on the primary shoot (PS, up to the first inflorescence) was not altered between lines carrying the functional or the mutant allele of the *SP* gene, and neither was internode length or PS height (Fig. 1). This shows that the effect of *SP* gene on growth habit is restricted to the resumption of growth after termination (Pnueli et al., 1998). Plants harbouring the *sft* allele, a loss-of-function of the *SINGLE FLOWER TRUSS* (*SFT*) gene, resemble indeterminate plants (*i.e* plants harbouring the *Sp* allele), although they are structurally different (Shalit et al., 2009; Krieger et al., 2010). The lack of *sft* (florigen) delays flowering, increasing shoot height and the number of leaves on the PS, without affecting internode length. The first inflorescence in *sft* partially maintains a vegetative character, producing a plant with the appearance of indeterminate growth (Shalit et al., 2009).

The main shoot (MS), which is defined as the PS plus the sympodial units (SUs) above it, differs significantly in height between *sp* and *Sp*, and also in *sft*. Unlike other tomato cultivars in which the *sp* mutation leads to a progressive reduction in the number of vegetative nodes in SUs until termination, MT lacks sympodial growth altogether, terminating in two inflorescences (Fig. 1). *Sp* continues vertical growth by the successive production of SUs, which represents the extra contribution to the height and number of leaves on the MS. Increased MS height in *sft*, on the other hand, is not due to sympodial growth but rather to extended vegetative growth on the PS.

Heterozygous *sft*/+ plants showed increases in the number of leaves on the PS. The reappearance of sympodial growth after flowering was also evident, as given by high number of leaves on the MS, yet not to the full extent as in indeterminate plants (Fig. 1).

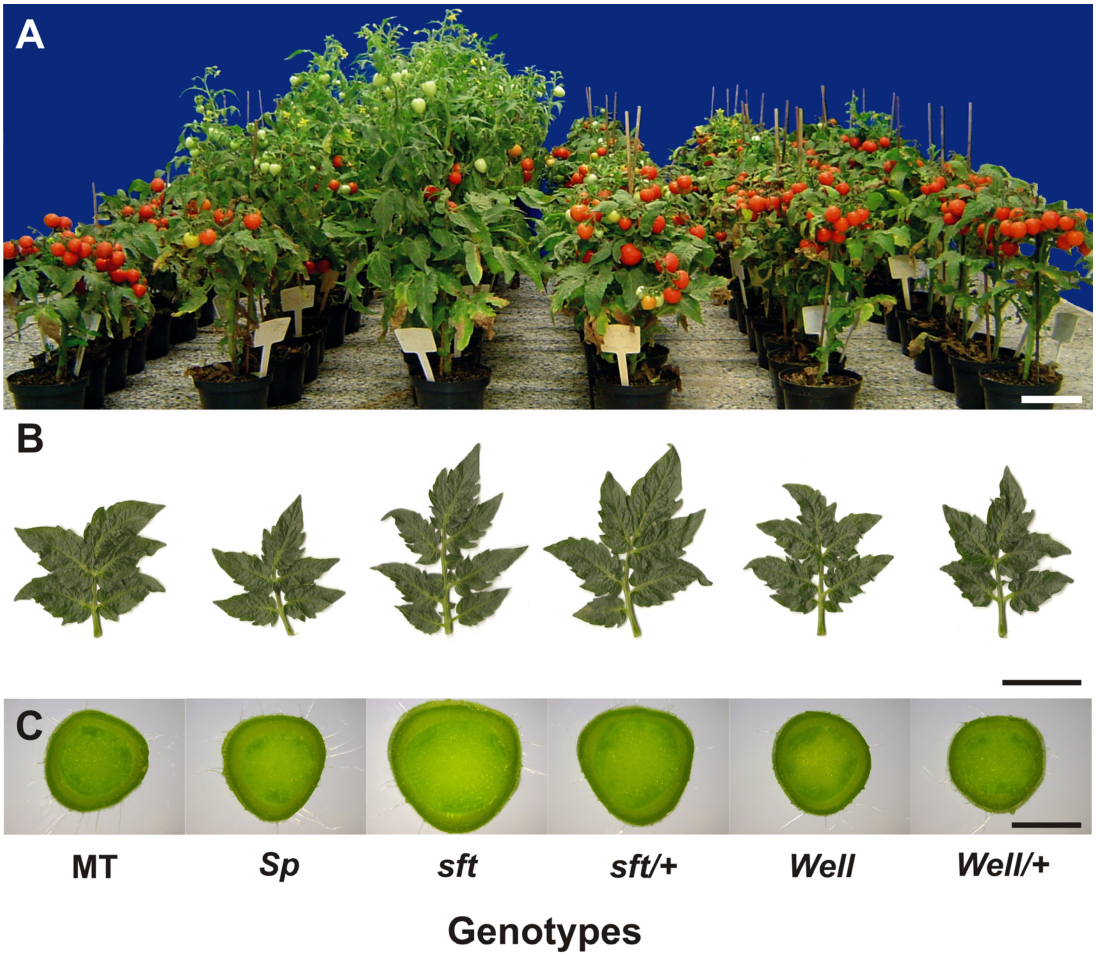


Fig. 2. Representative features of tomato plants with different growth habits in the Micro-Tom background. (A) Fully-grown plants showing different growth habits, 100 dag. Scale bar = 8 cm; (B). Fully-expanded fifth leaf from each genotype, 45 dag. Notice alterations in dissection pattern and leaflet number in *Sp* and *sft* plants, as described by Shalit et al. (2009). Scale bar = 5 cm; (C) Stem cross-sections, 65 dag. Scale bar = 5 mm.

Thus, in spite of carrying the *sp* allele, *sft*/+ plants have a different growth habit which is intermediate between determinate (MT) and indeterminate (*Sp*, *sft*) plants, i.e. semi-determinate. All these effects are mimicked by the *S. pennelli* introgression in homozygous *Well* plants, which also show a longer PS due to increased internode length. Heterozygous *Well* plants display a similar phenotype, but without sympodial growth (Fig. 1).

Taken together, our results suggest that semi-determinate growth is best characterized as an extension of vegetative growth in a determinate background, which is expressed as a higher number of leaves on the MS and extended sympodial growth after termination caused by the *sp* allele.

The impact of semi-determinate growth habit on vegetative-to-reproductive balance

We next assessed how the three different growth habits (Fig. 2A) affect the vegetative-to-reproductive balance through measurement of different growth parameters. Many vegetative traits, e.g. leaf area, leaf architecture, leaf insertion angle and stem diameter, have well-known effects on source-sink relationships, which ultimately influence agronomic performance (Turnbull, 2005). Leaf architecture showed relatively little variation between genotypes, except for *sft*, which showed more indented leaves and increased leaflet number (Fig. 2B). *sft* also showed the greatest leaf area and the thickest stems (Fig. 2C, Supplementary Table S1), as reported before for other genetic backgrounds (Shalit et al., 2009). Leaf insertion angle with respect to the stem was significantly lower in *sft* and the *Well* introgression (Supplementary Table S1). This trait is agronomically relevant and deserves further scrutiny, although under our growth conditions (a single plant per pot, diffuse irradiance in glasshouse) its effect was probably negligible. Total dry mass determined before the onset of fruit growth (45 days after germination (dag)) was not significantly correlated with growth habit (Supplementary Table S1). We then assessed the partitioning of dry mass between different organs (stem, roots, leaves, fruits, Fig. 3).

Indeterminate genotypes showed a trend to increase dry mass partitioning to vegetative organs (stem, roots and leaves), which was particularly evident in *sft* (Fig. 3A). The differences for individual organ dry mass values were not statistically significant to discriminate between determinate (MT) and semi-determinate (*sft*/+, *Well* and *Well*/+) genotypes. The proportional partition of dry mass of vegetative versus reproductive organs in semi-determinate lines, however, was intermediate between determinate and indeterminate lines (Fig. 3B). The determinate MT showed a higher proportion of reproductive in detriment of vegetative growth. Conversely, in *sft* a higher vegetative growth proportionally reduced reproductive growth. This suggests that semi-determinate genotypes, although not significantly more reproductive or vegetative than determinate and indeterminate ones, fine-tune the vegetative-to-reproductive growth balance. Given that reproductive growth translates into yield and that, conversely, vegetative growth is also important as the source of photosynthate to increase productivity, we next determined the impact of this altered partitioning in the plants' productivity.

The impact of the semi-determinate growth habit on productivity

Industrial tomato cultivars are almost invariably determinate, thus making MT a suitable model for this commercial segment. A contingency of processing tomato culture is that the fruits are harvested only once. We thus assessed total fruit yield of each genotype at a single harvest, which took place at a point where the largest possible number of fruits were ripe but without visible overripening (which leads to fruit rot on the plant and yield losses). The highest total fruit yield was observed in *sft*/+, as described before for different commercial cultivars (Krieger et al., 2010), whereas the homozygous *sft* showed the lowest yield (Fig. 4A). The latter is in agreement with the low partitioning of dry matter to reproductive organs in this genotype (Fig. 3B). Comparing total and ripe fruit yield, the discrepancy between these two parameters became evident for indeterminate genotypes (*Sp* and *sft*). This is expected for lines with continuous vegetative growth, where fruit ripening is an ongoing process. In MT, a greater proportion of the total fruit was ripe, which was also the case for semi-determinate lines (Fig. 4A). We next assessed the quality of the ripe fruit through comparison of total soluble solids content.

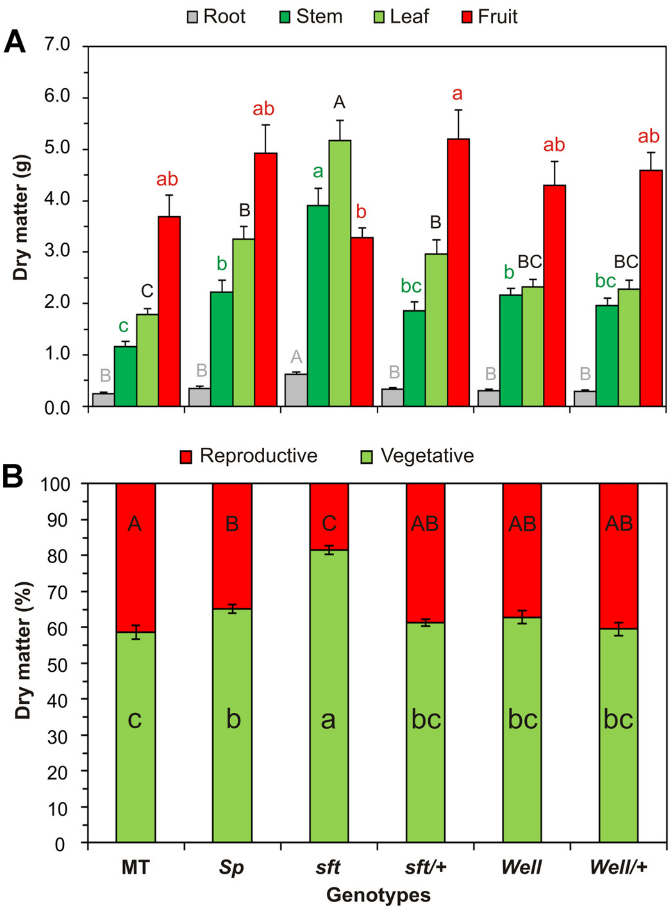


Fig. 3. Dry matter partitioning is altered in lines with different growth habit. (A) Dry matter partition between roots (grey bars), stems (dark green bars), leaves (light green bars) and fruits (red bars) for each genotype; (B) Vegetative-to-reproductive balance calculated as the relative contribution of vegetative (roots, stems, leaves) and reproductive (fruits) components of the plant to the plants' total dry mass. All measurements performed 100 dag. Data are mean ± s.e.m. ($n=10$ plants). Bars of the same colour with different letters indicate significant differences with Tukey's test at 1% probability. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Tomato growth habit is known to affect soluble solids in the fruit (Emery and Munger, 1970). Our results show that, as described previously (Eshed and Zamir, 1995), indeterminate lines (*Sp*, *sft*) have higher Brix than the determinate MT. Semi-determinate lines (*sft*/+, *Well* and *Well*/+) also showed significantly higher Brix than MT (Fig. 4B). The heterozygous *sft* mutant has also been shown to increase Brix in the tomato M82 background (Krieger et al., 2010). Two linked quantitative trait locus (QTLs) on chromosome 9 have been reported to increase Brix in M82 (Fridman et al., 2002). The line carrying both QTLs was described as semi-determinate due to the presence of the *S. pennellii* allele for the *CETS* family gene *SP9D* in the introgression. IL5-4, which harbours the *SP5G* allele (*SP5E*, after Chitwood et al., 2013) from *S. pennellii*, has also been

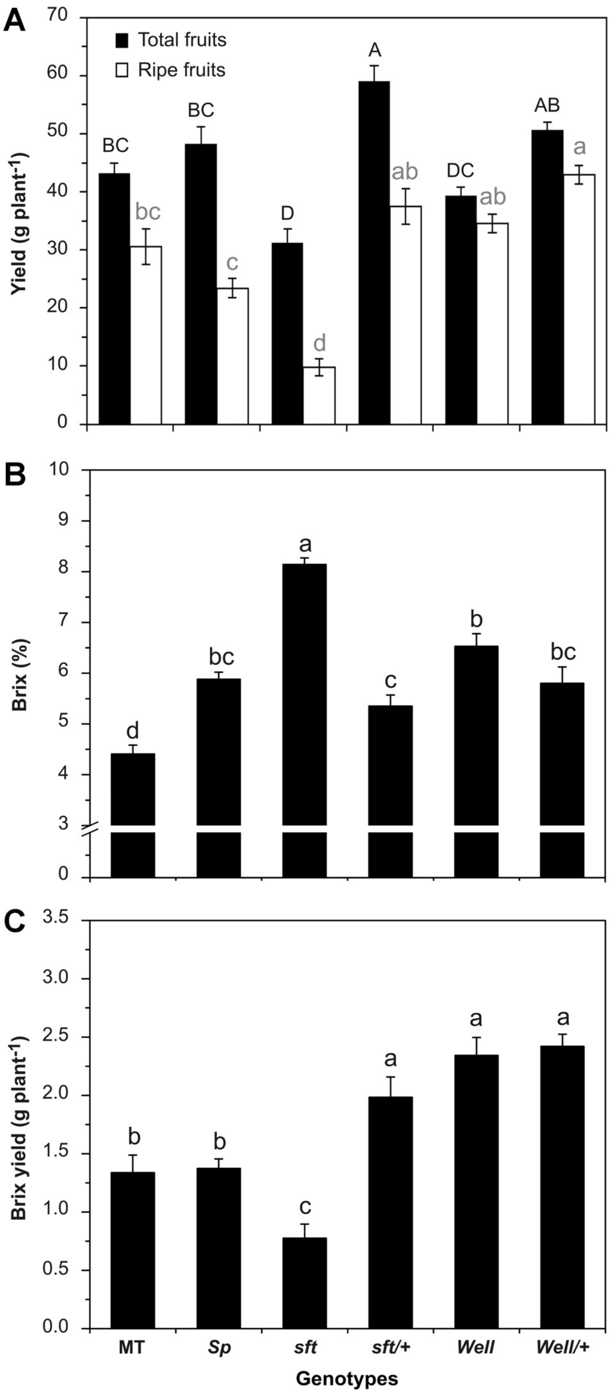


Fig. 4. Semi-determinate lines in the Micro-Tom (MT) background show improved productive parameters. (A) Average fruit yield per plant of each genotype, broken down in total yield (unripe + ripe fruits, closed bars) and ripe yield (open bars); (B) Average total soluble solids content in ripe fruits (Brix); (C) Average values of Brix × yield of mature fruits. All measurements were performed 90 dag. Data are mean ± s.e.m. (n = 10 plants). Bars with different letters indicate significant differences with Tukey's test at 1% probability.

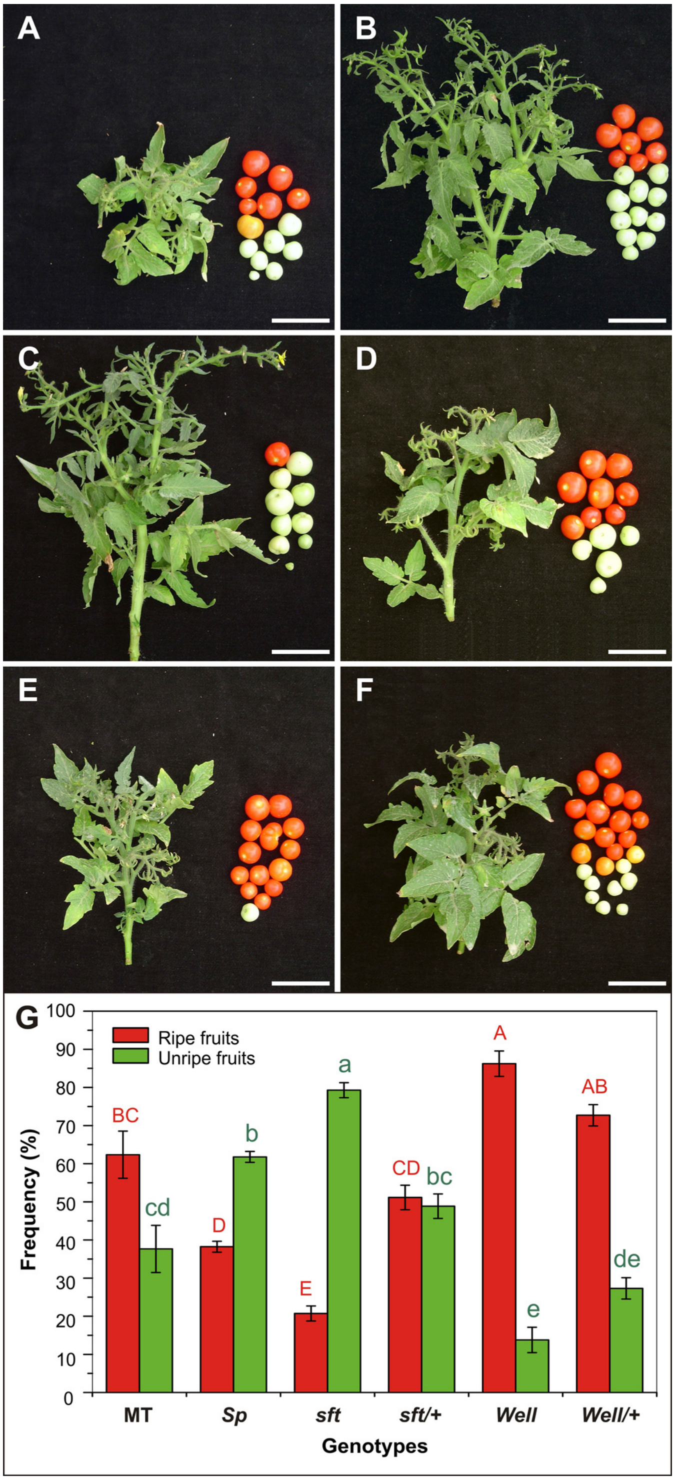


Fig. 5. Fruit ripening is concentrated in semi-determinate lines. Representative plants of (A) MT, (B)*Sp*, (C) *sft*, (D) *sft/+*, (E) *Well* and (F) *Well/+*, harvested 90 dag. Scale bar = 5 cm. (G) Average frequency of ripe and unripe fruits per plant. Data are mean ± s.e.m. (n = 10 plants). Bars with different letters indicate significant differences with Tukey's test at 1% probability.

described as semi-determinate (Jones et al., 2007) and shown to increase Brix in both homozygous and heterozygous dose (Eshed and Zamir, 1995).

A hurdle for tomato breeding is that soluble solids content is inversely correlated to yield, making the product of Brix × ripe yield (BRY) the single most relevant agronomic parameter for processing tomato (Tanksley et al., 1996). Evaluation of this parameter in our lines showed that semi-determinate plants had significantly higher BRY than either determinate or indeterminate lines (Fig. 4C).

A trait that further contributes to the improved BRY of the semi-determinate lines is concentrated fruit ripening (Fig. 5). This, again, is not exclusive to semi-determinate lines, but a hallmark of all determinate lines (Stevens and Rick, 1986). Concentrated ripening in determinate lines is a condition for a single harvest, whereas for indeterminate lines (*Sp* and *sft*) the proportion of unripe fruit is greater than that of ripe fruits at any given time (Fig. 5G).

The impact of the semi-determinate growth habit on water-use efficiency

The *Well* line used here was generated screening *S. pennellii* introgressions in MT for improved drought resistance and WUE (Zsögön, 2011). The introgression was mapped to a pericentric segment of chromosome *1* and the locus called *WELL* for "*Water Economy Locus in Lycopersicon*". Besides consistently lower values of carbon isotope discrimination (Δ^{13}C), which is negatively correlated with leaf-level WUE (Farquhar and Sharkey, 1982; Farquhar et al., 1989), the line harbouring the *Well* allele from *S. pennellii* displayed an altered growth habit, which we characterized as semi-determinate (Zsögön, 2011; Fig. 1). Here, we found significantly lower Δ^{13}C values (more than 1.0‰ difference) in all semi-determinate lines compared to determinate and indeterminate ones (Fig. 6A). Lower Δ^{13}C values have been previously reported for an introgression line harbouring a chromosomal segment from *S. pennellii* that contains the *SP5G* (*SP5E*) locus (Xu et al., 2008). Said line (IL5-4) is also semi-determinate (Jones et al., 2007), besides harbouring a functional *OBSCURAVENOSA* (*OBV*) allele (Jones et al., 2007; Barrios-Masias et al., 2014), which controls leaf hydraulic conductivity (Zsögön et al., 2015). Differences in Δ^{13}C, however, do not always necessarily scale up from leaf to whole-plant WUE (Seibt et al., 2008). We expect this to be particularly true when comparing genotypes with such marked differences in biomass allocation between leaves and other organs (Fig. 3A). Thus, in the same set of plants we determined dry matter gain versus transpired water, i.e. long-term WUE also called as $\mathrm{WUE_{biomass}}$. There was a trend to higher $\mathrm{WUE_{biomass}}$ in semi-determinate plants compared to both determinate and indeterminate ones (Fig. 6B). The different proportions of vegetative to reproductive mass between lines with divergent growth habit (Fig. 3), however, obscure potential differences in the more relevant parameter $\mathrm{WUE_{fruit}}$, i.e. the amount of fruit dry mass produced per unit water transpired, which we determined next. In this case, the results highlighted the superior performance and the agronomic relevance of semi-determinate lines, which, besides the improvements in BRY described before, had a higher $\mathrm{WUE_{fruit}}$ ("more fruit per drop", Fig. 6C).

What are the main phenotypic components determining growth habit?

As discussed above, the three growth habits present differences in the vegetative/reproductive balance. Still, this balance is difficult to describe using univariate analysis because all related characteristics are biologically correlated. We therefore evaluated the growth habits' performances under a multivariate analysis, using "canonical discriminant analysis" (CDA). This analysis allowed a better characterization of the growth habits and helps to understand how the vegetative characteristics can impact productivity traits.

The pairwise squared Mahalanobis' distances and the probability of a significant effect of contrasts, by the *F*-test (*P*, 0.05), between treatments are presented in Table 2. All treatments were significantly dissimilar, according to the squared Mahalanobis' distances (Table 2).

For analysis of vegetative and reproductive traits, the first (Can 1) and second (Can 2) canonical variables explained 78.03% and 12.13% of total variation (Table 3). Thus, from the six original traits, only two canonical variables were necessary to explain 90.16% of total variation. The reduction of the number of traits in two canonical variables facilitated the evaluation of the performance of growth habits types.

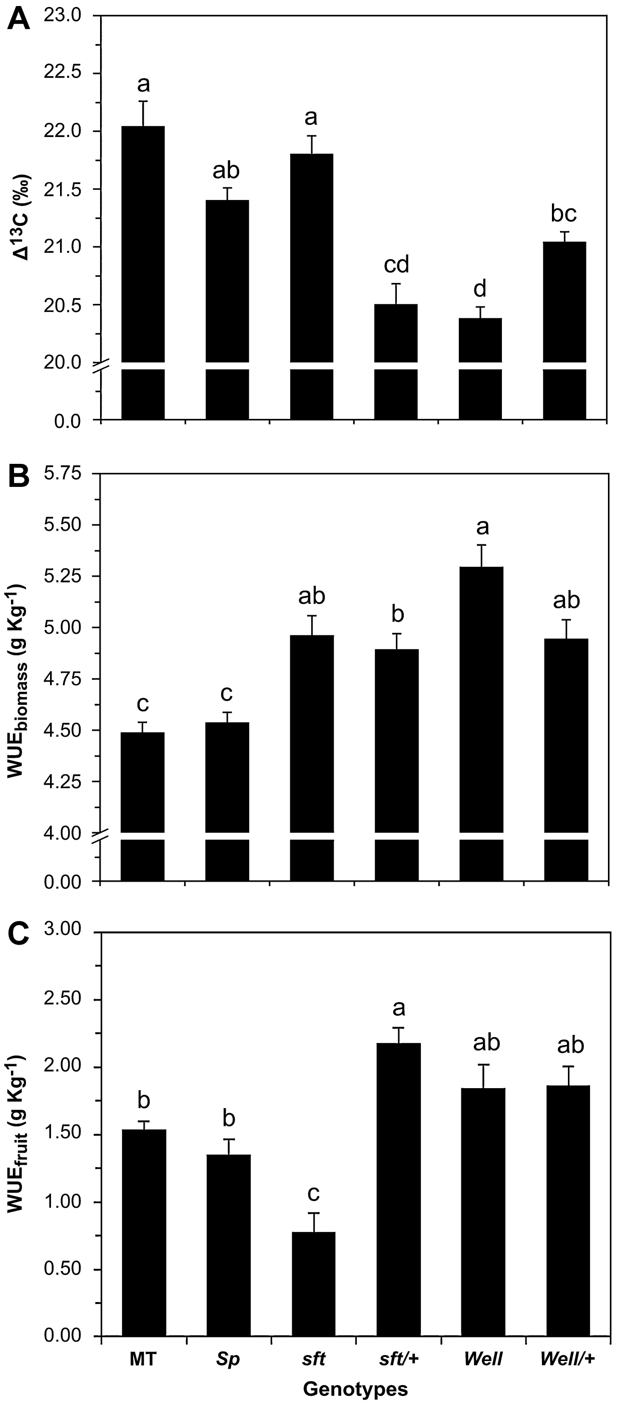


Fig. 6. Water-use efficiency (WUE) is increased in semi-determinate genotypes in the Micro-Tom (MT) cultivar of tomato. (A) Carbone isotope discrimination (Δ^{13}C), a proxy for leaf-level WUE, determined in leaves of 45-d-old plants ($n = 5$ plants); (B) $\mathrm{WUE_{biomass}}$, determined as *g* of dry mass produced per kg of water transpired over a period of 32 d ($n = 12$ plants); (C) $\mathrm{WUE_{fruit}}$, determined as g of fruit dry mass produced per kg of water transpired over a period of 95 d ($n = 10$ plants). Data are mean ± s.e.m. Bars with different letters indicate significant differences with Tukey's test at 1% probability.

The number of the leaves on MS and the vegetative dry weight were the most important traits on the first canonical variable (Table 3). This canonical variable provided a clear distinction between indeterminate genotypes (*Sp* and *sft*) and the other growth habits. These indeterminate genotypes present much more robust vegetative development than the other genetic materials tested,

Table 2
Pairwise squared Mahalanobis' distances¤ and probability values for the contrastsϟ between genotypes.

Genotypes	MT	Sp	sft	sft/+	Well	Well/+
MT		66.70734	97.07064	15.12400	23.42477	16.60850
Sp	***		14.42977	37.42165	53.62919	60.37130
sft	***	***		65.11008	83.90231	97.57951
sft/+	***	***	***		17.47529	9.01787
Well	***	***	***	***		4.56412
Well/+	***	***	***	***	**	

ϟ The probability values for the contrasts by the *F*-test (**P, 0.01 and ***P, 0.001) are below the diagonal line.
¤ The squared Mahalanobis' distances are above the diagonal line.

Table 3
Pooled within-class standardized canonical coefficients and total variation explained by each canonical variable (Can).

Variable	Can1	Can2	Can3	Can4	Can5
Leaf area	−0.0564	−0.5610	0.2141	0.7342	−0.4740
Number of leaves on MS	1.1145	−0.3452	0.0093	−0.3834	−0.0218
Height of the MS	−0.2106	0.9565	0.0964	0.2544	−0.4386
Vegetative dry matter	0.6101	0.4334	−0.2322	0.4146	0.6388
Number of green fruits	−0.1163	−0.0370	0.9225	−0.1400	0.3864
Brix × yield of mature fruits	−0.4661	0.5274	0.5224	0.1286	0.1936
Variation (%)	78.03	12.13	6.00	3.77	0.07

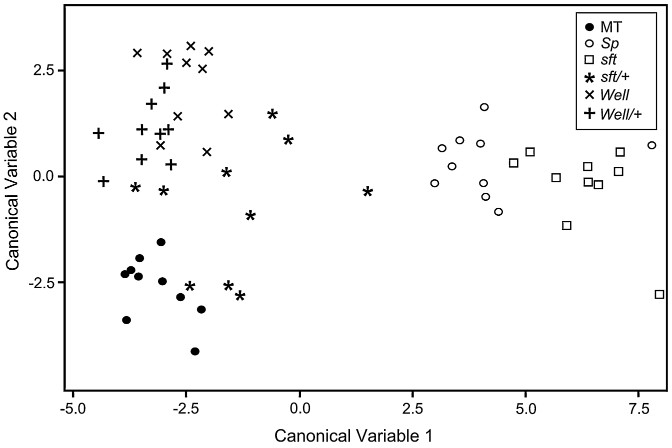


Fig. 7. Representation of canonical discriminant analysis. Six parameters (Table 3) were analysed. Micro-Tom (MT) and genotypes with diverging growth habit were projected onto the 2 main canonical variables. Canonical variable 1 separates indeterminate genotypes (*SP*, *sft*) from the others, whereas canonical variable 2 shows a further separation of the determinate MT from the semi-determinate genotypes (*sft*/+, *Well*, *Well*/+) cluster.

and they are the only genotypes with positive values to Can 1 (Fig. 7).

Vegetative development (Can 1) was, however, not able to discriminate between determinate and semi-determinate growth (Fig. 7). These results agree with univariate analyses of root, stem and dry matter. The vegetative biomass produced by determinate genotype (MT) does not differ from *sft*/+, *Well* and *Well*/+, whose growth habit is semi-determinate. Nevertheless, considering the second canonical variable (Can2), *Well* and *Well*/+ can be separated from the determinate genetic material while *sft*/+ presents a distribution intermediate between the determinate and the others semi-determinate genotypes. Furthermore, the majority of the semi-determinate plants evaluated on this work have a position superior or equal to indeterminate genotypes (Fig. 7). The more weighed coefficients on Can2 are leaf area (−0.5610), height of the MS (+0.9565) and BRY of mature fruits (0.5274) (Table 3).

Considering both canonical variables jointly and how they related to each other, we observe that the semi-determinate growth habit is marked by a larger MS, a smaller leaf area and a higher BRY of mature fruits. This was most likely achieved by a preferential allocation of photoassimilates to stem (height) and fruit growth to the detriment of new leaf development, which here was the main sink. The relationship between these traits could be responsible for the better performance of semi-determinate genotypes.

Conclusions

In this work we have shown that the semi-determinate growth habit in tomato improves important agronomic traits such as fruit total soluble solids × yield (BRY). A possible explanation for this is that semi-determinate growth represents an optimal balance between the 'overly vegetative' indeterminate and the 'overly reproductive' determinate growth habits. If so, this improved vegetative-to-reproductive balance in tomato maybe not solely the consequence of the mutations studied here or their gene dosage (Jiang et al., 2013), but rather the expression of a more general process for crop productivity. This suggests a way to manipulate other crops which have varieties showing different growth habits but not necessarily controlled by the CETS gene family (George, 1970; Ping et al., 2014). An interesting analogy is the Green Revolution, where the yield of cereal crops, with a completely different growth habit, was improved by converting the overly vegetative tall varieties into well-balanced semi-dwarf ones (Peng et al., 1999; Spielmeyer et al., 2002). It remains to be determined whether the associated improvement in WUE in semi-determinate tomatoes can be also ascribed to a better vegetative-to-reproductive balance or should be attributed to other linked trait.

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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.jplph.2015.01.003.

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