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Effect of water deficit on the agronomical performance and quality of processing tomato

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Abstract

The influence of irrigation doses on standard and high lycopene tomato varieties has been analyzed during two years in one of the main processing tomato growing areas of Spain. Deficit irrigation (75% ET_c) implied a mean reduction in water use of 28.2%, while it caused a significant reduction in the marketable production of 16.4% and increase in soluble solids (8.4%) and Hunter a/b ratio (2.4%). The effect on lycopene content was not significant. Increasing irrigation dose over the recommended 100% ET_c had no significant effect on the agronomical performance, while it provoked a dilution effect reducing total soluble solids and lycopene content. The effects on 33 tomato volatiles were also analyzed, 11 of them related to main aroma notes and 22 to the background volatile profile. The effect of deficit irrigation on aroma was dependant on climatic conditions and it may either not have a significant effect on the aroma profile or it may lead to higher logodor units in main aroma volatiles. High lycopene cultivars showed higher contents in most volatiles, including some volatiles originated in pathways that have not been related with carotenoid degradation processes. In both the fresh and processing tomato market the improvement of organoleptic and functional quality and the reduction of the impact of agriculture on environment represent main goals. The use of high lycopene cultivars and restricted irrigation would enhance the aroma of materials targeted to quality markets, contributing to increase the efficiency of water use in agriculture.

Keywords *Solanum lycopersicum* L., quality, environment, lycopene, volatile

Introduction

Tomato is one of the most important vegetable crops in the world. Its consumption either fresh or processed has increased continuously for the last decade. In both forms of consumption there is an increasing demand for high quality products, including organoleptic and functional quality, the latter being considered as the capacity of its consumption to prevent certain diseases. In fact, the high level of consumption of tomato makes of this vegetable an excellent source of health promoting compounds such as vitamins C and E, lycopene, β-carotene, lutein and flavonoids (Dorais et al., 2008).

Consequently, in order to satisfy these demands, it is necessary to develop new varieties with an added value (e.g. high lycopene content) and to identify the optimal growing conditions that maximize their potential. At the same time, to obtain reductions with the minimum impact on the environment is becoming a recurrent consumer demand. In this sense there is growing concern targeted to reduce water use in agriculture.

Water deficit irrigation would not only reduce the use of this input but would also result in an increased organoleptic quality of the fruit, though it may reduce fruit weight and yield. Nevertheless, a compromise between quality and marketable yield is still possible. The results obtained by Mitchell et al. (1991) supported that deficit irrigation and saline irrigation may be feasible crop water management options to produce high quality field-grown processing tomatoes without major yield reductions. Similarly, Veit-Köhler et al. (1999) found that small reductions in water supply resulted in higher quality of tomatoes due to higher contents of sugars, titratable acids, vitamin C and aroma volatiles (particularly C6-aldehydes hexanal, *(Z)*-3-hexenal and *(E)*-2-hexenal), while the proportion of marketable fruits remained high. In any case, little is known regarding the effect of deficit irrigation on the aroma profile of tomatoes, as well as the differences in the performance of standard and high-lycopene cultivars.

In tomato more than 400 volatile compounds have been described (Petro-Turza, 1987), though mainly 20 compounds, and especially hexanal, *(E)*-2-hexenal, *(Z)*-3-hexenal, 1-hexanol, *(Z)*-3-hexen-1-ol, 2-isobutylthiazole, 6-methyl-5-hepten-2-one, geranyl-acetone and β-ionone, have been classically considered as important in the determination of the characteristic tomato flavour (Buttery, 1993). Although recent literature has emphasized the role of other compounds such as 1-penten-3-one, *(E,E)* and *(E,Z)*-2,4-decadienal, 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol), methional, phenylacetaldehyde or 2-phenylethanol (Mayer et al., 2008), and it has questioned the role of some compounds previously found as important (reviewed by Rambla et al., 2014). Apart from these main aroma volatiles, minor volatiles with negative logodor units may still be important to determine specific tomato flavour as background notes (Baldwin et al., 2000). Additionally, the final aroma perception would not only be determined by the concentration and interactions between certain volatiles (either as primary or background notes), but also by the sugars and acids present in the matrix (Baldwin et al., 2008).

In this context, the purpose of this study was to compare the agronomic performance, basic quality attributes, lycopene content and aroma profile of high yielding cultivars with high lycopene cultivars and to analyse the effect of water deficit irrigation in their agronomical performance, basic quality attributes and aroma profiles of these cultivars. The varieties selected for the study are processing varieties that, at least in the area of cultivation studied, are targeted to crushed tomato production. In the industrial process of these varieties, usually no heating phase is included due to the low pH of the raw material and therefore little changes in the aroma profile are expected due to the processing. These results will have a direct application not only for this type of processing, but will also be valuable for fresh tomato production.

MATERIALS AND METHODS

Plant material

Varieties ‘H-9036’ and ‘H-9661’ (Heinz Seed, Stockton) were selected as standard controls by their high level of production in the area of assays and because they are very popular among farmers in the North (‘H-9036’) and East (‘H-9661’) of Spain. Both varieties show standard to low lycopene content (75 to 150 mg kg^{-1}), but high yields (Macua-Gonzalez et al., 2002). Varieties ‘Kalvert’ (Esasem S.V.A., Casaleone) and ‘Loralie’ (Hazera Genetics, Shikmin) were selected for their high lycopene content, usually between 200 and 250 mg kg^{-1} as assessed in previous unpublished trials.

Cultivation and experimental design

Cultivation was carried out in the facilities of Instituto Navarro de Tecnología e Infraestructuras Agroalimentarias S.A. (INTIA S.A.) at Cadreita (42º12’34.75’’N; 1º43’1.08’’W; 267m) in Navarre (Spain). Plants were grown on plastic mulch and drip irrigated. The effect of irrigation dose on the aroma profile of tomato varieties was studied with three watering doses that satisfied 75%, 100% and 125% of crop evapotranspiration (ET_c). The higher irrigation dose was selected in order to explore the effects of excess of irrigation sometimes observed in certain farms. Hydric requirements were calculated as a function of crop evapotranspiration following FAO56 methodology (Allen et al., 1998). 75% ET_c corresponded to 252.48 Lm^{-2}, 100% to 351.38 Lm^{-2} and 125% to 438.20 Lm^{-2} during the year 2009 and to 295.80 Lm^{-2}, 412.30 Lm^{-2} and 530.10 Lm^{-2} respectively during the year 2010. For each condition three plots of 200 plants per variety were established using a randomized complete block design. Plantation density was 35,700 plants/ha.

In the year 2009 plants were sown on 26th of March and transplanted on the 14th of May. The varieties were harvested between 27th of August and 23rd of September. 'Kalvert' and 'Loralie' showed the shortest growing cycle and 'H-9036' the longest. In the year 2010 plants were sown on 26th of March and transplanted on 18th of May. The harvest period ranged between 21st of September for early varieties and 13th of October for the late one.

Agronomical determinations

Each block was harvested following commercial practices in the area: plants were visually inspected and harvested when at least 80% of the fruits had reached the red stage, keeping over-ripened fruits bellow 5%. Percentage of marketable fruits, and marketable production were determined (Table 1).

Sample preparation and basic determinations

A bulk sample was obtained in a per block basis, with at least 40 representative fruits obtained from different plants. All the fruits of the sample were ground and homogenized. With this juice, total soluble solids (TSS) and pH were determined. A digital refractometer (ATAGO PR-1, Tokyo, Japan) with 0.1º Brix precision was used to evaluate TSS (results expressed as ºBrix at 20ºC). Hunter a and b parameters (results expressed as Hunter a/b rate) were determined using a digital colorimeter (CR 300, Minolta, Japan).

For the analysis of volatiles, the homogenate was left to stand for 3 minutes and then a saturated solution of $CaCl_2$ (2.7 ml in 30 g of sample) was added to inactivate volatile degrading enzymes. The efficiency of this system was previously contrasted with the suggested methodology by Buttery et al. (1987). Samples were instantly frozen and maintained at -80ºC until analysis. Although sub-zero temperatures might affect the content of some volatiles, all the samples were stored in the same conditions.

Volatile analysis

Thirty three tomato volatiles were monitored in the samples (Supplementary Table 1). Reference aroma compounds and internal standard (i.s.) methyl salicylate-D_4 of 99.5% purity were purchased from Sigma-Aldrich Química S.A. (Madrid, Spain). Stock solutions at 500 mg L^{-1} were prepared in acetone and stored at -18°C. Working solutions were prepared by volume dilution in diethyl ether-hexane (1:1). Calcium chloride 97% (Riedel de Haen) was purchased from Supelco (Sigma-Aldrich Química S.A., Madrid, Spain). Organic solvents (hexane, ethyl acetate, diethyl ether) of trace residue analysis quality were purchased from Scharlab (Barcelona, Spain). SPE cartridges (Supelco, Sigma-Aldrich Química S.A., Madrid, Spain) were prepared by the manufacturer packing 500 mg of Tenax TA (80-100 mesh) in 6 mL polyethylene tubes (15 mm diameter).

The extraction system set in a previous work (Beltrán et al., 2006) consisted in a 50 mL Erlenmeyer flask attached to a glass cap with two connexion tubes: the inlet connected to a dry N_2 gas supply, and the outlet fitted to the Tenax trap. 30 g of sample and 50 μL of 15 μg mL^{-1} methyl salicylate-D_4 (i.s.) were magnetically stirred (350 rpm) at 35ºC for 120 min with a N_2 flow of 1L·min^{-1} with a new Tenax tube (maintained at room temperature) connected to the flask outlet. The trap was eluted with 3.5 mL of hexane-ether (1:1) mixture and the final extract adjusted to 1 mL by nitrogen evaporation.

Chromatographic determination was carried out using a Varian CP-3800 gas chromatograph coupled to an ion trap mass spectrometry detector (Saturn 4000, Varian Inc. Palo Alto, USA) equipped with a 30 m x 0.25 mm DB-5MS (0.25 μm f. t.) capillary column (carrier gas helium at 1 mL min^{-1} constant flow). Splitless injection of 1 μL (splitless time 1 min) was carried at 200ºC. Oven temperature program: 45ºC (5 min), rate of 3ºC min^{-1} to 96ºC, rate of 6ºC min^{-1} to 150°C, rate of 30ºC min^{-1} to 240ºC (1.5 min) (analysis time of 36 min). Ion trap mass-spectrometer worked in external ionization mode with electron ionization (70 eV) in the positive ion mode and full SCAN acquisition mode. Transfer line and ion source and trap temperatures were established at 250 and 200°C respectively.

Quantitation was performed by internal standard calibration as described by Beltran et al. (2006). Quantitation ion used for the internal standard methyl salicylate-D_4 was 155. This ion corresponded to the molecular mass of the compound after having changed the deuterium in the alcohol group by hydrogen.

Lycopene analysis

Determination of lycopene content was carried out by Centro Nacional de Tecnología y Seguridad Alimentaria (San Adrián, Spain). A bulk sample of 40 fruits was obtained per repetition (3 biological replicates) following the ISO 874 UNE-34-117-81 guidelines. Fruits were ground and homogenized in dark conditions. Carotenoids were extracted with a mixture of hexane:acetone:ethanol, 20:25:25 (Sadler et al., 1990). The determination of lycopene in the hexane phase was based on spectrophotometric analysis using a JASCO V-530 spectrophotometer (Jasco Analítica Spain, Madrid, Spain) at 501 nm (Rao et al., 1998). Two analytical replicates per sample were made.

Statistical analysis

Agronomical and basic quality parameters were evaluated jointly using multivariate analysis. Following the approach of previous works (Cebolla-Cornejo et al., 2011) the volatiles quantified were arranged in two sets of variables. The first one included main aroma notes, with 11 volatiles that have been previously described as important in tomato aroma (Baldwin et al., 2000) and showed concentrations higher than their odor threshold. In this case, the concentrations were expressed as logodor units, a variable more closely related to aroma perception. Odor thresholds (ng g^{-1}) used for calculations were the following: *(E)*-2-hexenal: 17, 2-isobutylthiazole: 3.5, 6-methyl-5-hepten-2-one: 50, hexanal: 4.5, *(Z)*-3-hexen-1-ol: 70, β-ionone: 0.007, *(Z)*-3-hexenal: 0.25, geranylacetone: 60, methyl salicylate: 40, *(E)*-2-heptenal: 13 (Buttery et al., 1987) and *(E,E)*-2,4-decadienal: 0.07 (Buttery et al., 1971). The second set included the remaining 22 compounds: a representation of volatiles included in the aroma background, expressed as concentrations. Some of these compounds (phenylethanol, β-cyclocitral, phenylacetaldehyde, 1-hexanol) had previously described as important but showed negative logodor units. For this set, logodor units were not used as the variable loses its purpose.

The significance of the factors year, cultivar, irrigation dose and double and triple interactions were calculated for each set of variables using a multivariate analysis of variance (MANOVA) with the S-Plus 8.0 software (Insightful Corp., Seattle, USA). In order to provide a better understanding of the effect of cultivar and irrigation dose, two-way MANOVA biplots were calculated for each year (Amaro et al., 2003). In the

MANOVA biplot subspace, the similarity between groups (cultivars, irrigation doses or combinations of cultivar x irrigation dose) can be measured as an inverse function of its distance on the graph. The angle between variables can be interpreted as an approximation of its correlation. The inner product $g_k' h_j$ of a group marker g_k by a variable marker h_j approximates the mean $\bar{x}_{kj}$ of the k^{th} group on the j^{th} variable allowing for the characterization for the differences between groups. Univariate Bonferroni confidence circles are added to the group markers in such a way that the projections of the circles onto the direction representing a variable approximate a confidence interval. The significance of the difference between groups over a particular variable can be established checking the overlapping of their projections. The procedure is conservative in the sense that if no overlap is found it can be concluded that there is a significant difference, but if there is an overlap a significant difference can be found along another direction for the multidimensional space. All MANOVA biplot calculations and graphs were made with MultBiplot, a software free-licensed by Prof. Vicente-Villardon (2014).

In order to study the relation between lycopene content and total volatile composition (expressed as concentrations) a Partial Least Square (PLS) regression was used. Prior to the PLS regression, the data were autoscaled with mean-centering and dividing by the standard deviation of the variable (Martens and Naes, 1989) to avoid the distortion caused by different variable scaling. The PLS regression model was calculated using full cross-validation resampling method. The goodness of the model fit was tested using the Root Mean Square Error of Calibration (RMSEC) and the Root Mean Square Error of Cross Validation (RMSECV).

Two criteria were used to select the number of latent variables of the PLS model: an additional latent variable was only chosen when the RMSECV was improved by at least

2% and the number of new variables was minimized as possible. Model precision was improved by selection of volatiles, using the method of interval PLS (iPLS) variable selection which performs a hierarchical, sequential and exhaustive search for the best combinations of variables. Interval PLS was performed in reverse mode, with intervals successively removed from the analysis (Wise et al., 2006).

The calculations of PLS regressions were made using PLS_Toolbox v 6.7 (Eigenvector Research Inc, Wenatchee, USA) for Matlab v 7.6.0 (Mathworks Inc, Natick, USA).

Results

Climatic conditions

The 2009 season was excellent for cultivation of processing tomato, with high temperatures and absence of important rains during most part of the growing cycle. In the 2010 season there were worse climatic conditions during the first phase of the growing cycle. In June, one month after transplantation, when the established plants should have showed a boom in vegetative growth, the temperatures and radiation were much lower than usual (Fig. 1, dotted line), causing a considerable delay in the vegetative development of the plantations. In fact, 2010 harvest was delayed nearly one month (vertical lines in Fig. 1). This fact implied that the last phases of fruit ripening occurred later in time compared with 2009, with lower temperatures, radiation and ET_0 (Fig. 1). Irrigation with 75% ET_c resulted in a reduction of water use compared to standard irrigation practices (100% ET_c) of 28.1% in 2009 and 28.3% in 2010.

Agronomical performance and basic quality parameters

The year effect had minor significant effects on % marketable fruits, pH and Hunter a/b (Table 1), while the effects on marketable production, TSS and lycopene were not significant. Therefore, the trends for the latter variables seemed rather stable independently of the environment analysed. Irrigation dose had a significant effect on agronomical variables, TSS, pH, Hunter a/b and lycopene content. Considering the global results for both years, deficit irrigation (75% ET_c) compared to the standard irrigation dose (100% ET_0) implied a clear reduction (16.4%) in marketable production (Table 1). Regarding basic quality parameters, deficit irrigation caused an increase in TSS (8.4%) and the Hunter a/b ratio related to fruit redness (2.4%), while the increase observed in lycopene content (6.9%) was not significant (as well as for pH). This effect was confirmed for each year independently with the MANOVA biplots (Fig. 2 and 3).

The cultivar x year interaction was significant for all the variables but for TSS and lycopene. In fact, the MAVOVA biplot for the complete model including interactions in both years showed a different impact of water deficit irrigation on the year and cultivar considered (Supplementary Fig. 1). In 2009 'Kalvert' was highly affected by deficit irrigation, 'H-9661' experienced an intermediate effect and a rather small effect was detected in 'Loralie' and 'H-9036'. In 2010 the highest effect was detected in 'H-9036', followed by 'Loralie', 'H-9661' and 'Kalvert'.

Increasing irrigation dose over the recommended 100% ET_c had no significant effect on the agronomical performance, nor on TSS and the Hunter a/b ratio (Table 1), though it had a significant dilution effect on the contents of lycopene (10.5% reduction) and reduced the pH.

High lycopene varieties offered considerably lower productions compared to 'H-9036', which showed the highest yield. Specifically, 'Kalvert' showed a 42.1% lower production and 'Loralie' a 24.2% (Table 1). As expected, high lycopene varieties presented high lycopene contents, that probably caused higher Hunter a/b ratios. Fruit pH, and TSS were also higher in the high lycopene cultivars. These trends were confirmed in the MANOVA biplots for each year.

Figure 1. Climatic data registered from transplantation till the end of harvest during 2009 (continuous line) and 2010 (dotted line). Vertical lines indicate the initiation of harvest.

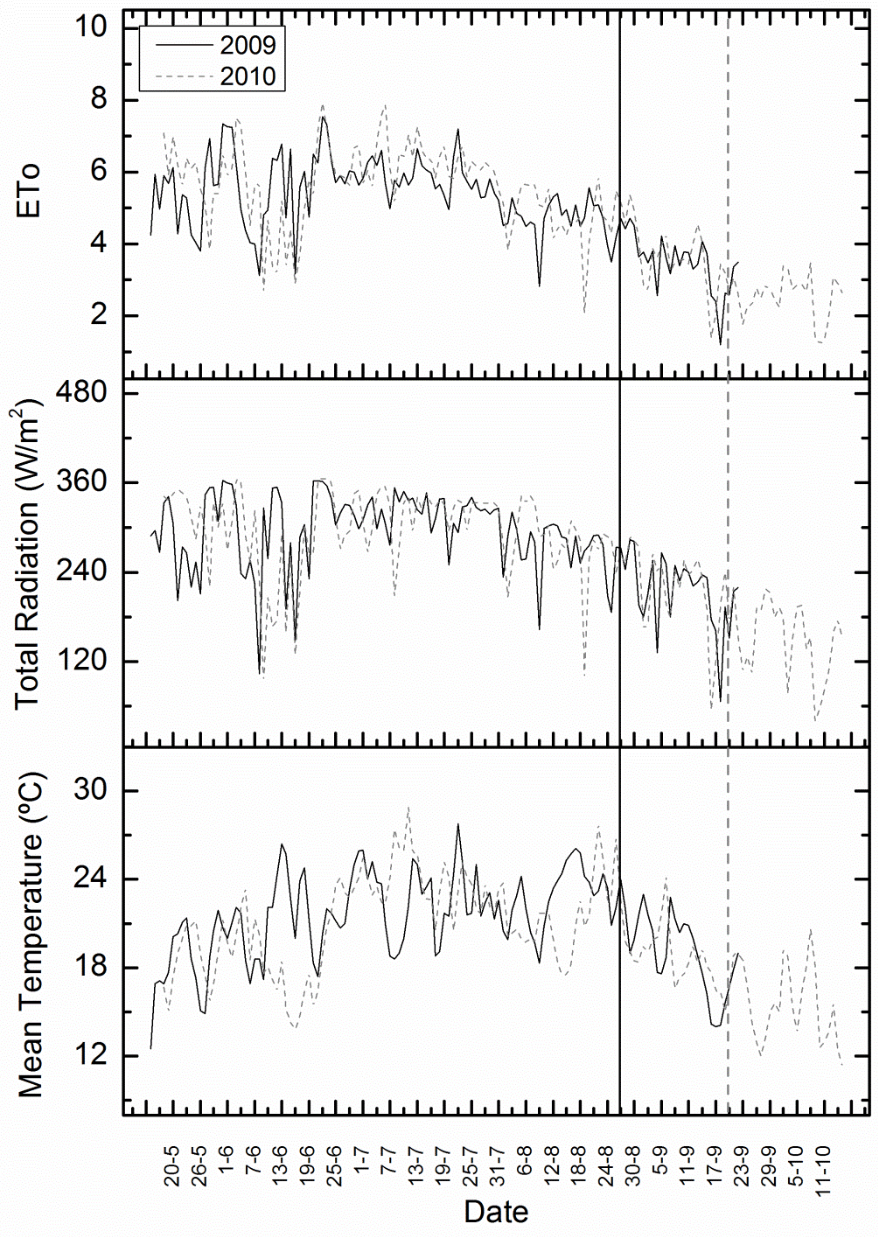


Table 1. Effect of environment (year and site), cultivation system and cultivar on marketable production, basic quality aspects and carotenoid content.

		% Marketable fruits	Marketable production (10^3 kg ha^{-1})	Total soluble solids (ºBrix)	pH	Hunter a/b	Lycopene mg kg^{-1}
Year (Y)	*p value*	<0.001	0.341	0.079	0.005	0.012	0.297
	2009	84.66	151.90	4.81	4.43	2.54	188.16
	2010	88.38	148.04	4.68	4.49	2.48	180.60
Irrigation dose (D)	*p value*	0.003	<0.001	<0.001	0.016	0.008	0.002
	75% ETc	84.92^{a}	132.49^{a}	5.02^{b}	4.48^{b}	2.56^{b}	199.52^{b}
	100% ETc	86.53^{ab}	158.40^{b}	4.63^{a}	4.49^{b}	2.50^{a}	186.61^{b}
	125% ETc	88.11^{b}	159.02^{b}	4.59^{a}	4.42^{a}	2.47^{a}	167.01^{a}
Cultivar (C)	*p value*	0.362	<0.001	<0.001	<0.001	<0.001	<0.001
	'H-9661'	86.79^{a}	158.01^{c}	4.55^{a}	4.37^{a}	2.39^{a}	128.57^{a}
	'H-9036'	87.36^{a}	189.02^{d}	4.42^{a}	4.40^{a}	2.34^{a}	121.72^{a}
	'Kalvert'	85.53^{a}	109.53^{a}	5.03^{b}	4.48^{b}	2.73^{b}	247.40^{b}
	'Loralie'	86.39^{a}	143.31^{b}	4.98^{b}	4.61^{c}	2.59^{b}	239.84^{b}
YxD	*p value*	0.433	0.070	0.849	0.638	0.300	0.253
YxC	*p value*	<0.001	<0.001	0.110	0.009	0.004	0.720
DxC	*p value*	0.058	0.081	<0.001	0.438	<0.001	0.170

Different letters in irrigation dose and cultivar indicate significant differences (Tukey test)

Fig. 2. MANOVA biplots of agronomical and basic quality parameters in the year 2009 considering the factors irrigation (a) and cultivar (b). For each data point the cultivar code (K: Kalvert, L: Loralie, H61: H-9661, H36: H-9036) and irrigation dose (75% ET_c, 100% ET_c, 125% ET_c) are included. Circles represent Bonferroni confidence intervals. Bold and higher font sizes represent significant effects in individual ANOVAs.

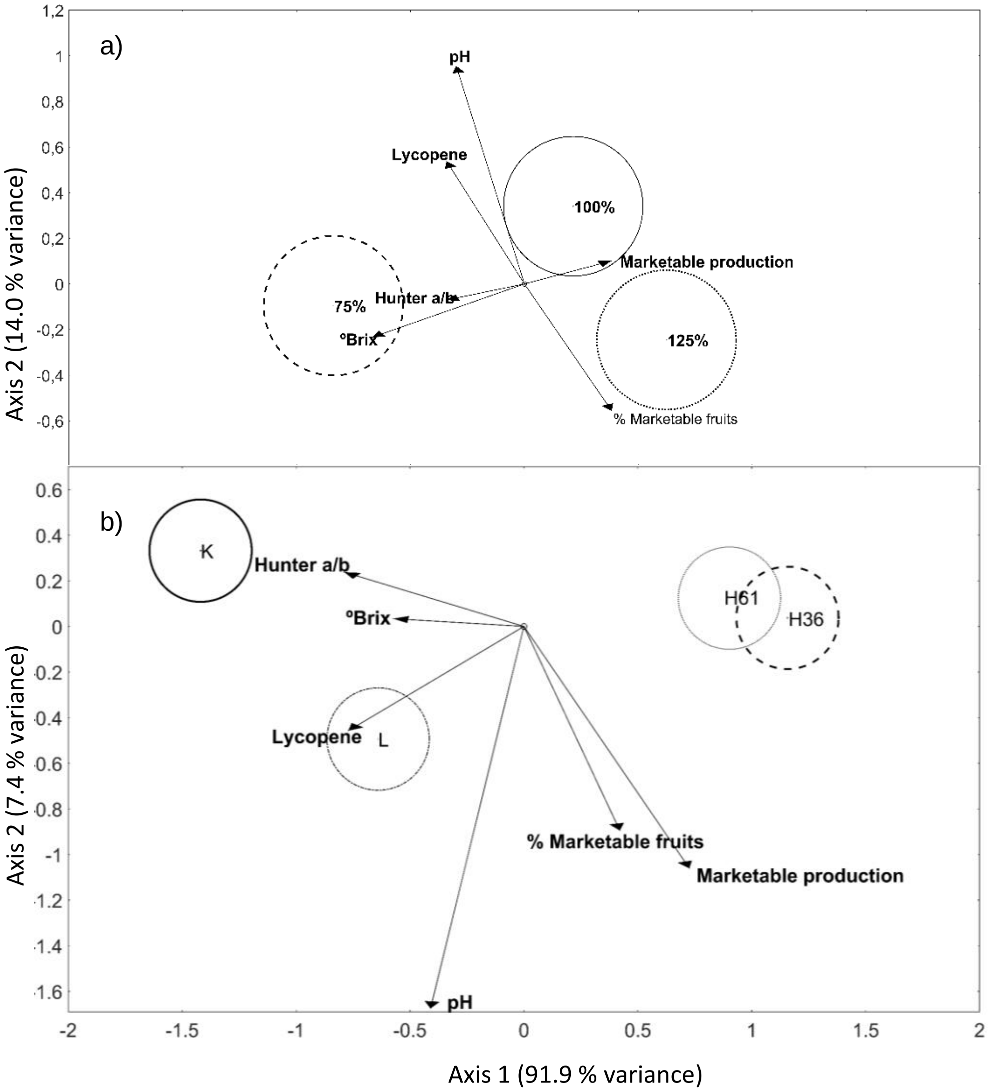
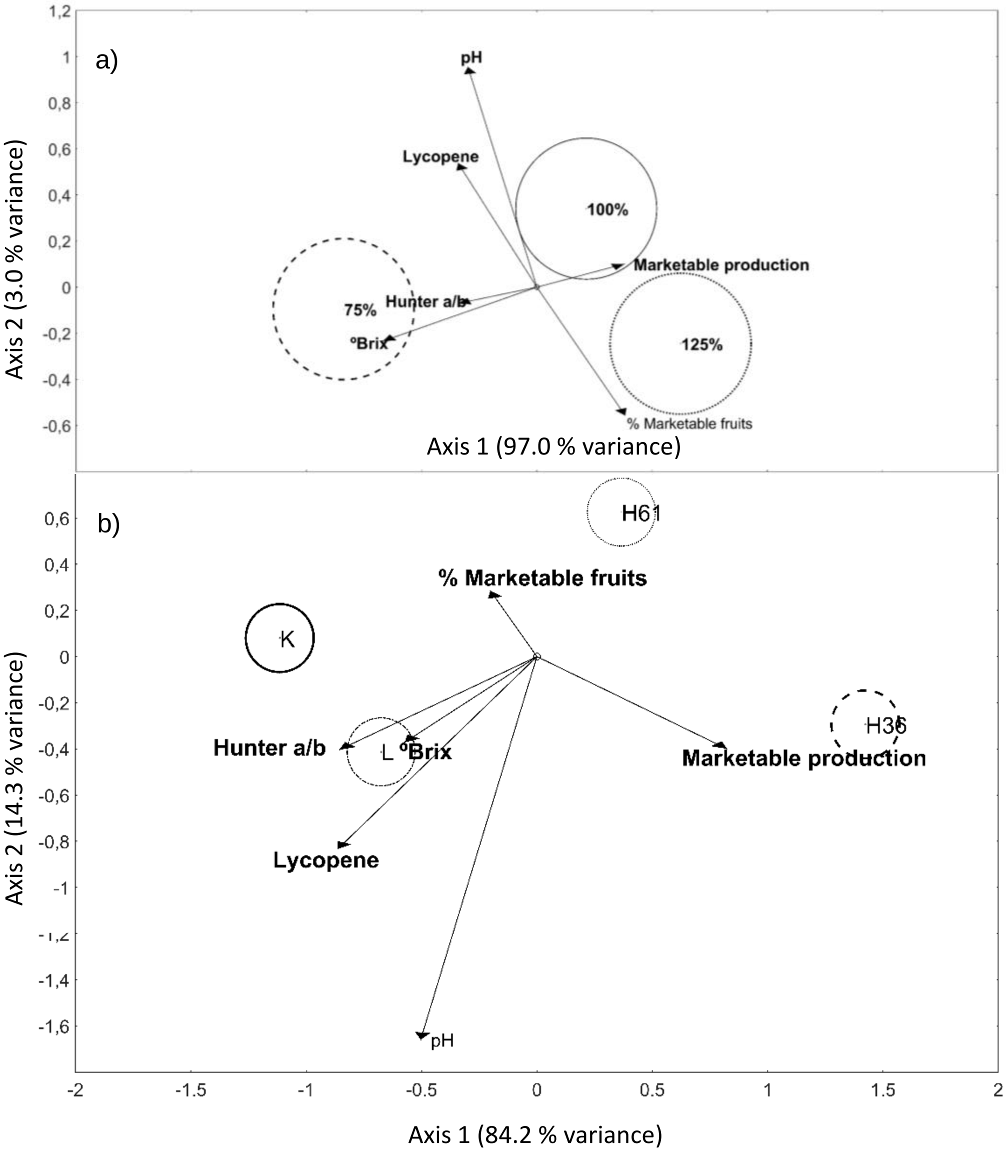


Fig. 3. MANOVA biplots of agronomical and basic quality parameters in the year 2010 considering the factors irrigation (a) and cultivar (b). For each data point the cultivar code (K: Kalvert, L: Loralie, H61: H-9661, H36: H-9036) and irrigation dose (75% ET_c, 100% ET_c, 125% ET_c) are included. Circles represent Bonferroni confidence intervals. Bold and higher font sizes represent significant effects in individual ANOVAs.



Main aroma volatiles

The complexity of the data regarding aroma composition (Supplementary Tables 1 and 2) demanded a holistic approach based on multivariate analysis. The MANOVA analysis showed that all the factors studied: year, irrigation dose, cultivar and their double and triple interaction had a significant effect on the logodor values of the main aroma volatiles analysed (Table 2). In order to get a better understanding of the influence of controlled agronomic factors (irrigation and cultivar) on main aroma volatiles the analysis was repeated for each year separately. This approach was justified by the existence of significant uncontrolled effects in year factor and its interactions (year x irrigation and year x cultivar). In the year 2009 irrigation dose, cultivar and their interaction had a significant effect, but in the year 2010 the irrigation effect was not significant.

Deficit irrigation (75% ET_c) increased the logodor units of almost all the main aroma volatiles in 2009 (Fig. 4a). It should be considered though, that individual ANOVAs for each compound for this factor revealed significance, $p<0.05$, only for geranylacetone, *(Z)*-3-hexen-1-ol and *(EE)*-2,4-decadienal (variables with significant effects are written in bold in the figure). The significance of the concentration effect of the deficit irrigation was also confirmed as the projections of the Bonferroni confidence circles for this level over the vector of each compound did not overlap on the projection of the other factor levels (Fig. 4a). The 100% ET_c tended to increase the level of *(E,E)*-2,4-decadienal, while the 125% had a dilution effect, with most vectors heading to the opposite direction.

Regarding the cultivar effect, the MANOVA biplot (Fig. 4b), clearly differentiated cultivars ‘Kalvert’ and ‘Loralie’ and the group including ‘H-9661’ and ‘H-9036’. The overlap of the Bonferroni confidence intervals of the last two cultivars entailed little differences between both materials in their aroma profiles. In general ‘Kalvert’ and ‘Loralie’ held a position favouring higher values of logodor units. In this case a higher number of compounds were significantly affected by this effect.

For a complete interpretation of the main effects and interaction a MANOVA biplot for the complete model was obtained (Fig. 4c). For both ‘Kalvert’ and ‘Loralie’ it seemed that there was a dilution effect of higher irrigation doses on logodor units, though the differences between 75% and 100% ET_c were limited. A higher separation between the Bonferroni confidence circles of both cultivars than between the confidence circles of different irrigation levels of each cultivar indicated that the cultivar effect was more important than the irrigation dose. The position of Bonferroni confidence circles of ‘H-9661’ and ‘H-9036’ confirmed that differences in the aroma profile, both at the cultivar and irrigation level would be small.

The results obtained in 2010 confirmed a clear differentiation between ‘Kalvert’ and ‘Loralie’ and the group formed by ‘H-9661’ and ‘H-9036’ (Fig. 5a). Again ‘Kalvert’ and ‘Loralie’ showed a position in the biplot favouring higher values of logodor units, especially for cultivar ‘Kalvert’. The complete model for 2010 confirmed the reduced effect of irrigation dose on logodor units, and the higher importance of the genotype over environmental effect (Fig. 4c).

Table 2. Significance of factor effects obtained in the multivariate analysis of variance (p-value) of the logodor values of main and background tomato aroma volatiles

	Year	Year (Y) Effect	Irrigation Dose (D) Effect	Cultivar (C) Effect	Y x D Interaction	Y x C Interaction	D x C Interaction	Y x D x C Interaction
Main aroma volátiles (logdor)	2009 and 2010	$<10^{-3}$	0.008	$<10^{-3}$	0.011	$<10^{-3}$	$<10^{-3}$	0.003
	2009		0.001	$<10^{-3}$			$<10^{-3}$	
	2010		0.224	$<10^{-3}$			0.019	
Background aroma volátiles (concentration)	2009 and 2010	$<10^{-3}$	0.006	$<10^{-3}$	0.009	$<10^{-3}$	$<10^{-3}$	$<10^{-3}$
	2009		0.133	0.002			$<10^{-3}$	
	2010		0.004	$<10^{-3}$			$<10^{-3}$	

Background volatiles

Year, irrigation and cultivar effects and their interactions were highly significant in the concentration of background volatiles (Table 2). When the results were analysed separately to evaluate the year effect, irrigation, cultivar and their interaction were significant in 2010. In 2009 the cultivar x irrigation interaction was significant but the main factor irrigation was not significant.

In the case of background volatiles, the cultivar had a lower prominence in the background profile (Supplementary Fig. 2a and 2b). In both complete models it could also be observed that in this case irrigation dose involved a change in profile affected by a strong cultivar x irrigation interaction but the dilution effect observed for main compounds, was not detected.

Lycopene and volatiles

Reverse-iPLS analysis relating the lycopene content and the volatiles concentrations were performed for each year to identify the relations between this carotenoid and the aroma profiles. Even though the year effect was not significant for lycopene (Table 1), separate year analyses were performed as the year effect was significant for the volatile concentration data (Table 2).

In 2009, the best model relating both sets of variables included all analysed compounds with the exception of 1-hexanol, β-cyclocitral and *(E,E)*-2,4-heptadienal. The performed PLS model with the selected variables required only one latent variable (LV1) to explain a 50.32% of variation of the aroma matrix. The representation of scores on LV1 and measured lycopene content for the year 2009 showed a clear differentiation between groups of cultivars. 'H-9661' and 'H-9036' obtained negative scores on the LV1, whereas 'Kalvert' and 'Loralie' obtained positive values (Fig. 6a). Little differentiation was found between the cultivars in each group, only 'H-9661' may show a slight trend towards lower values in LV1 than 'H-9036' while 'Kalvert' showed higher scores on LV1 over 'Loralie'.

This genotypic differentiation was also observed in the lycopene content, with 'Kalvert' and 'Loralie' obtaining, as expected, higher values regardless of the irrigation dose or cultivation year. In the high lycopene cultivars, the higher irrigation dose generally led to lower lycopene contents. In this group, the range of variation for lycopene content was higher. In the low lycopene cultivars, a similar trend could be observed.

The distribution observed (Fig. 6a) showed a moderately positive correlation between lycopene content and LV1 (R2=0.726). The LV1 had the highest positive correlations with camphor, α-pinene, 6-methyl-5-hepten-2-ol, 2-carene, (E,E)-2,4-hexadienal, (Z)-citral, α-terpineol, (E)-3-hexenal, β-ionone and nonanal and the lowest negative correlations with benzaldehyde, linalool, 2-hydroxybenzaldehyde and diphenyl ether.

The reverse iPLS model for the 2010 data selected all the volatiles with the exception of (Z)-3-hexenal, phenylacetaldehyde, methyl salicylate, β-ionone, (E)-2-octenal, α-pinene, linalool and (Z)-citral. The PLS model with the selected volatiles only required one latent variable to explain 67.15% of variation. This latent variable showed the highest correlations with (E,E)-2,4-heptadienal, camphor, 6-methyl-5-hepten-2-ol, 2-

carene, and diphenyl ether and the lowest correlations with (E)-2-heptenal, β-cyclocitral, (E,E)-2,4-decadienal and benzaldehyde.

The representation of LV1 scores over lycopene content showed a higher correlation between both variables (R2=0.875). The same differentiation between the aroma profile of high and low lycopene cultivars was obtained (Fig. 6b). This year ‘Loralie’ and ‘H-9961’ had slightly higher scores on LV1 than ‘Kalvert’ and ‘H-9036’, respectively.

Fig. 4. MANOVA biplots of main aroma logodor units in the year 2009 considering the factors irrigation (a), cultivar (b) and the complete model with main factors and interaction (c). For each data point the cultivar code (K: Kalvert, L: Loralie, H61: H-9661, H36: H-9036) and irrigation dose (75% ET$_c$, 100% ET$_c$, 125% ET$_c$) are included. Circles represent Bonferroni confidence intervals. Bold and higher font sizes represent significant effects in individual ANOVAs.

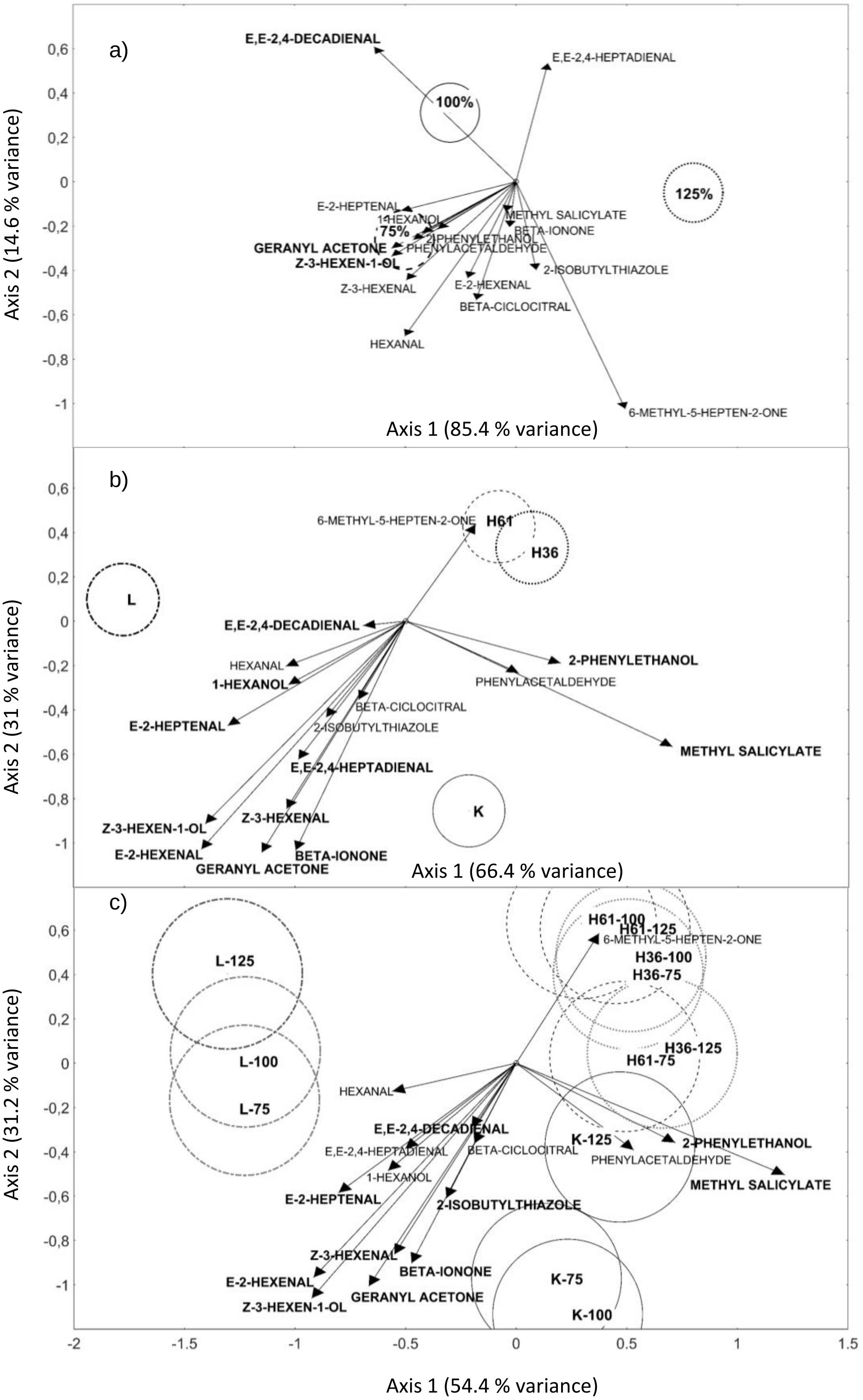


Fig. 5. MANOVA biplots of main aroma logodor units in the year 2010 considering the factor cultivar (a) and the complete model with main factors and interaction (b). The MANOVA biplot of the irrigation factor is not shown as the factor was not significant for this year. For each data point the cultivar code (K: Kalvert, L: Loralie, H61: H-9661, H36: H-9036) and irrigation dose (75% ET_c, 100% ET_c, 125% ET_c) are included. Circles represent Bonferroni confidence intervals. Bold and higher font sizes represent significant effects in individual ANOVAs.

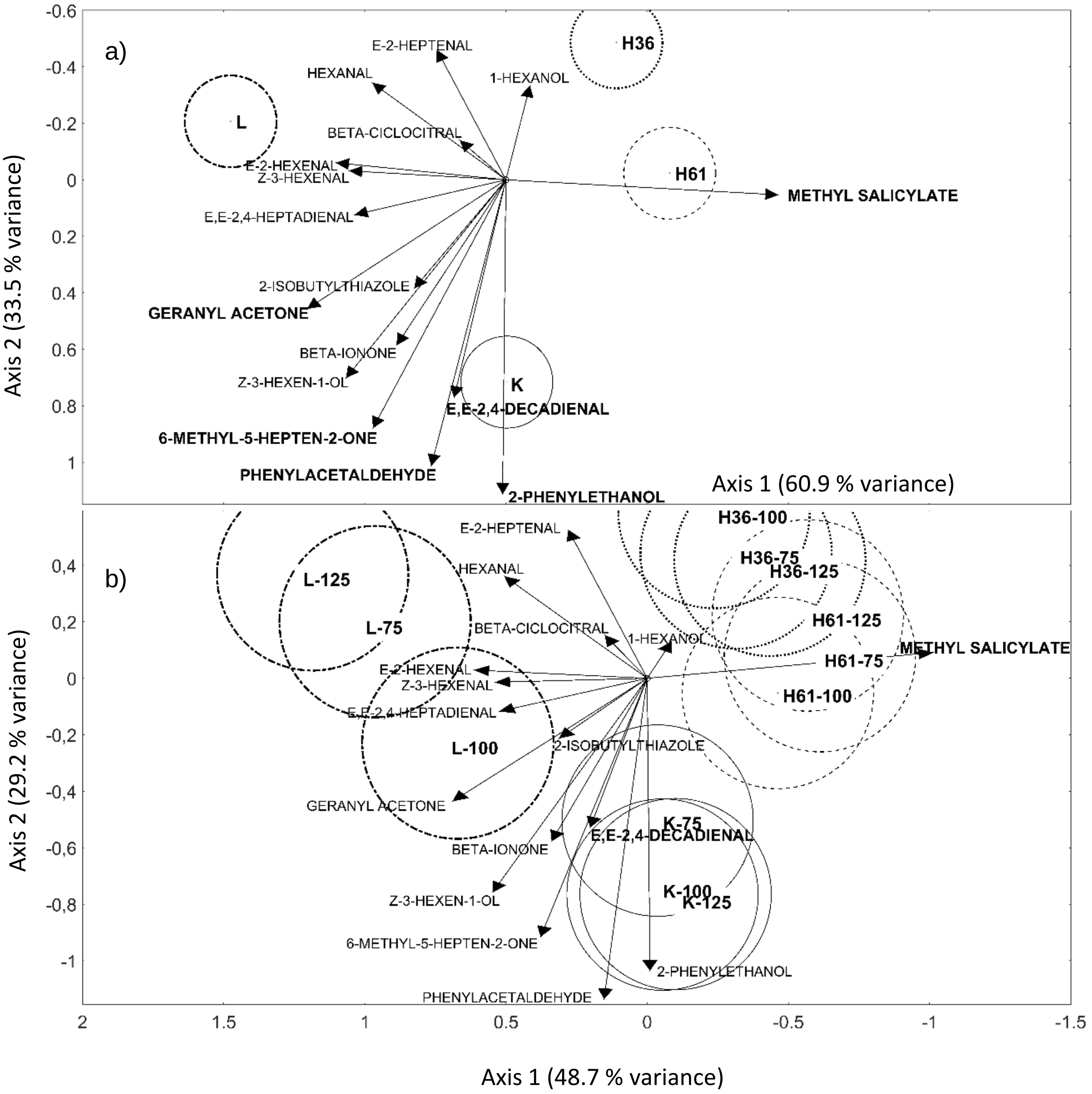
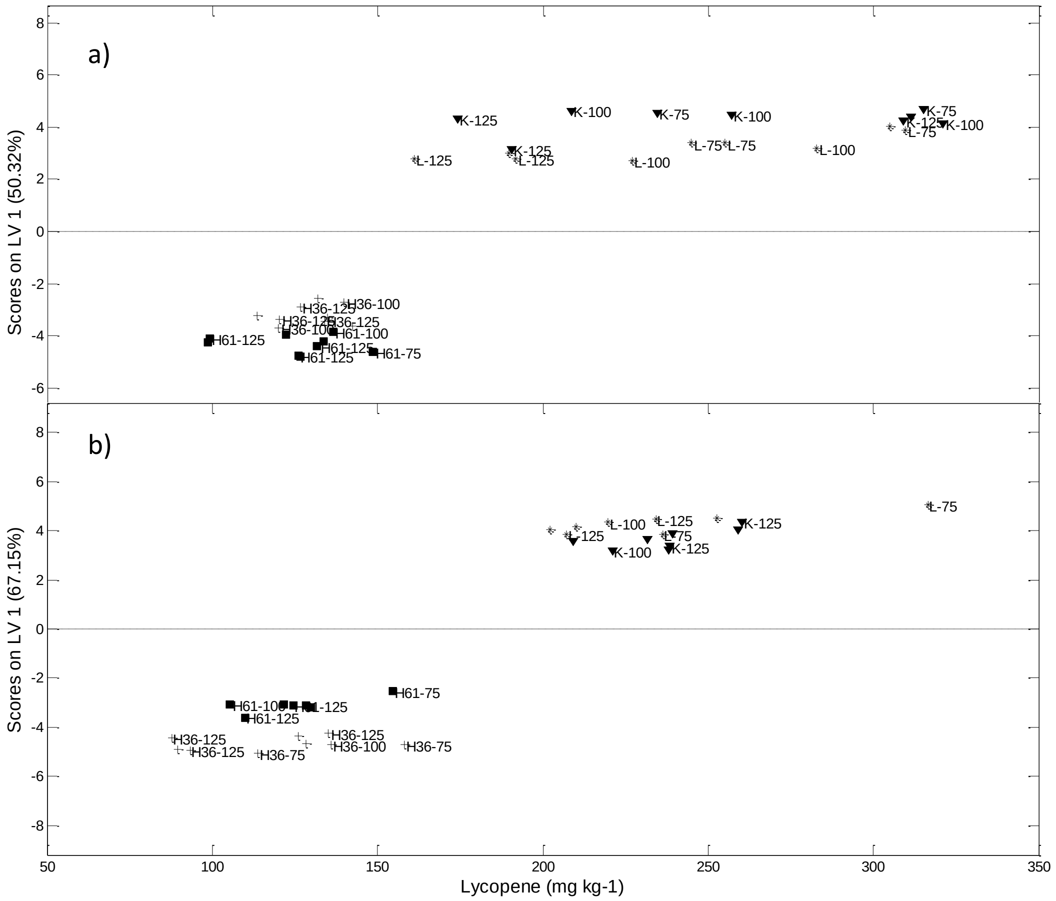


Fig. 6. Representation of lycopene content over the scores of latent variable 1 (LV1) obtained in the PLS model relating main and background volatiles concentrations and lycopene content performed after the selection of variables with a reverse iPLS model for 2009 (a) and 2010 (b). For each data point the cultivar code (K: Kalvert, L: Loralie, H61: H-9661, H36: H-9036) and irrigation dose (75% ET$_c$, 100% ET$_c$, 125% ET$_c$) are included.



Discussion

The growth in water demand by households and industry will eventually reduce the availability of water for irrigation. In this context the future challenge for agriculture will be the production of more food with less water (Cai and Rosegrant, 2006). Deficit irrigation has been widely investigated as a valuable and sustainable production strategy in dry areas (Geerts and Raes, 2009). Our study was targeted to evaluate the possibilities of water deficit irrigation not only to increase the efficiency of water use, but also to offer an added value to agricultural products.

The use of water deficit irrigation to promote the quality of tomato has been extensively studied, but colour, taste-related or nutritional variables have received most part of the attention. Favati et al. (2009), studying the effect of controlling water supply in processing tomato concluded that a limitation of the irrigation volume, especially in the last part of the growing cycle, increased the dry matter and soluble solids in Mediterranean conditions. Cahn et al. (2001) or Hanson and May (2004) also observed an increase in soluble solids with lower irrigation doses. Mitchell et al. (1991) concluded that the increased soluble solids content of tomato fruits in plants stressed with water deficit was due to reduced water intake and maintained synthesis and accumulation of organic solutes.

Our results confirmed this effect not only for soluble solids, but also for lycopene content, resulting in increased fruit redness. But this increased quality levels are obtained at the expense of production. It is not clear if the increase in 0.5ºBrix in high lycopene cultivars would economically compensate the lower productions obtained, even considering the extra price paid by the industry for higher TSS.

The application of deficit irrigation in tomato also would have an effect on the aroma of the raw produce, but it would be dependent on the climatic conditions of cultivation. Hypothetically, in years with an extended growing cycle, with a delayed fruit ripening during months with lower radiation and temperatures, a deficit irrigation regime (75% ET_c) would not have a significant effect on the aroma profile, as it happened in our 2010 growing cycle. On the contrary, in common years with good growing conditions, involving long periods without important rainfalls and high temperatures, like in 2009, water deficit irrigation would have a significant effect on the aroma profile. In this case, a general increase in logodor units would be obtained, implying a more intense aroma. This increase would be especially significant for geranylacetone and *(Z)*-3-hexenol, the first one related with the aroma descriptor fruity and the second with leafy/fresh cut grass (Tandon et al., 2000) and flavour intensity (Tieman et al., 2012). Veit-Köhler et al. (1999) described increased concentrations of C6-aldehydes in the aroma profile of plants with reduced irrigation. In our case, a rough analysis of concentrations in 2009 also highlighted that *(Z)*-3-hexenal and hexanal showed high concentration increases in all the genotypes compared to other volatiles. *(Z)*-3-hexenal has been related with sweet (Krumbein et al., 2004) and tomato/citrus (Tandon et al., 2000) descriptors in sensory evaluation, while hexanal has been related with mouldy (Krumbein et al., 2004) and stale/grassy/green (Tandon et al., 2000) descriptors. It should be considered though, that Tieman et al (2012), using transgenic tomatoes with altered 13-lipoxygenase activity and thus, altered concentrations of C6-volatiles, revealed no significant impact of these volatiles on consumer liking.

Consequently, the application of a water deficit irrigation regime would have either a neutral or positive effect on the aroma profile of raw materials depending on the climatology of the year. Therefore, a limitation of water use would lead not only to save an increasingly scarce resource but also to an improved organoleptic quality, via soluble solids concentration and aroma profile and intensity.

The strong environment x irrigation (year in our case) interaction detected for aroma volatiles has also been reported in other quality variables. Several authors have observed higher differences in soluble solids contents between sites of cultivation than within the same site at different deficit irrigation regimes (Rodriguez et al., 1994; Patanè and Cosentino, 2010). In our case, year x irrigation dose interaction was not significant in basic agronomical and quality parameters, and the year effect had limited consequences, while Garcia and Barrett (2006) concluded that the impact of growing season might be more important than any other factor, including the genetic background in terms of peelability and other processing important quality attributes.

The results obtained in this study emphasize the importance of the genotypic effect on the aroma profile of tomato varieties. Despite the considerable year x cultivar and irrigation x cultivar interactions, specific aroma profiles could be identified. For example, the cultivars ‘H-9661’ and ‘H-9036’ selected considering their good agronomical performance by farmers have shown a profile characterized by lower logodor units for the main aroma volatiles. On the contrary high lycopene cultivars ‘Kalvert’ and ‘Loralie’ stood out by high levels of almost all compounds (including background volatiles), but with a different tinge. The fact that in both years the cultivar ‘Kalvert’ presented higher levels of methyl salicylate may result in a worse acceptance compared with ‘Loralie’, as methyl salicylate has been described with plastic/pesticide (Tandon et al., 2001) descriptors. Additionally, low levels of this compound have been recommended to improve tomato flavour (Vogel et al. 2010). Nevertheless, it should be noted that both cultivars accumulated *(E,E)*-2,4-decadienal, and this compound has been recently related with a positive influence on tomato acceptance (Mayer et al., 2008) and its role in flavour intensity has also been acknowledged (Tieman et al., 2012). This considerably superior effect of genotype over environment, including cultivation year and irrigation dose, has also been described for different growing conditions: open-air or in protected cultivation (Cebolla-Cornejo et al., 2011).

It is difficult to compare the quantitative volatile profile of the varieties tested in this work with previous literature. As Ruiz et al. (2005) have previously reported, data available in the scientific literature is relatively scarce and shows values over a great range, probably due not only to the use of different varieties but also of different analytical methods. In our opinion, the differences in the exact moment of analysis and differences in growing conditions should also be accounted for. In any case, the concentrations obtained for hexanal (i.e.) were on the range described by other authors for fresh tomato varieties (Mayer et al., 2008; Birtic et al., 2009).

The lycopene content was also strongly influenced by genotype, with minor modifications by the irrigation and year effects. In general, higher irrigation doses over 100% ET_c may lead to lower lycopene contents, though the differences between standard and deficit irrigation at 75% ET_c were not significant. The effects of water availability in carotenoid content in tomato seem to be sometimes contradictory (see review by Dumas et al., 2003). Nevertheless, recent studies tend to support the use of a reduction in irrigation doses. Pernice et al. (2010) concluded that reduced irrigation or none irrigation increased the level of lycopene in fresh and processed tomato. Favati et al. (2009) also reported that a limitation of water supply during the whole cycle or part of it increased lycopene and β-carotene contents. Wang et al. (2011) reported that the best compromise between yield and quality, including lycopene content, would be reached restricting irrigation during the flowering and fruit development stage. According to Dorais et al. (2001) low water supply would lead to an increase in abscisic acid that may influence ethylene production and hence the concentration of carotenoids. In other crops such as wine grapes, it has been stated that water deficit in Chardonnay activates parts of the phenylpropanoid, energy, carotenoid and isoprenoid metabolic pathways that contributed to increased concentrations of antheraxanthin, flavonols and aroma volatiles (Deluc et al., 2009). Dabbou et al. (2011) found that the aroma profile in olive oil was strongly determined by the genotype, and the effects of water deficit irrigation would be limited.

Regarding the relation of high lycopene content and the aroma profile, an unexpected result was obtained. Evidence has been provided that carotenoid accumulation patterns have deep effects in the norisoprene and monoterpene aroma volatile composition (Lewinsohn et al., 2005). Geranyl acetone would be derived from phytofluene, phytoene, ζ-carotene and neurosporene, geranial (*(E)*-citral) and 6-methyl-5-hepten-2-one from neurosporene, pro-lycopene, lycopene or δ-carotene and β-cyclocitral and β-ionone from β-carotene (Lewinsohn et al., 2005). In our study, most of these compounds were selected by the reverse-iPLS analysis as important compounds related to lycopene content variation. But other compounds arising from the lipid oxidation of unsaturated fatty acids, aminoacids, shikimate pathway or others were also selected. Similarly, Vogel et al. (2010) analysing carotenoid mutants with blockage of the carotenoid synthesis pathway at different levels observed that there were a few significant differences in non apocarotenoid volatiles in each growing season, but this effect was not analysed in depth.

Conclusions

Our results indicate that high lycopene cultivars outstand not only for the accumulation of this carotenoid but also for high soluble solids content and enhanced aroma profiles. The use of this type of cultivars with low water deficit irrigation (75% ET_c) will improve the organoleptic quality of raw materials. This approach would result in products that could be targeted to quality niche markets and at the same time contribute to the preservation of an increasingly scarce resource: the water.

Acknowledgements

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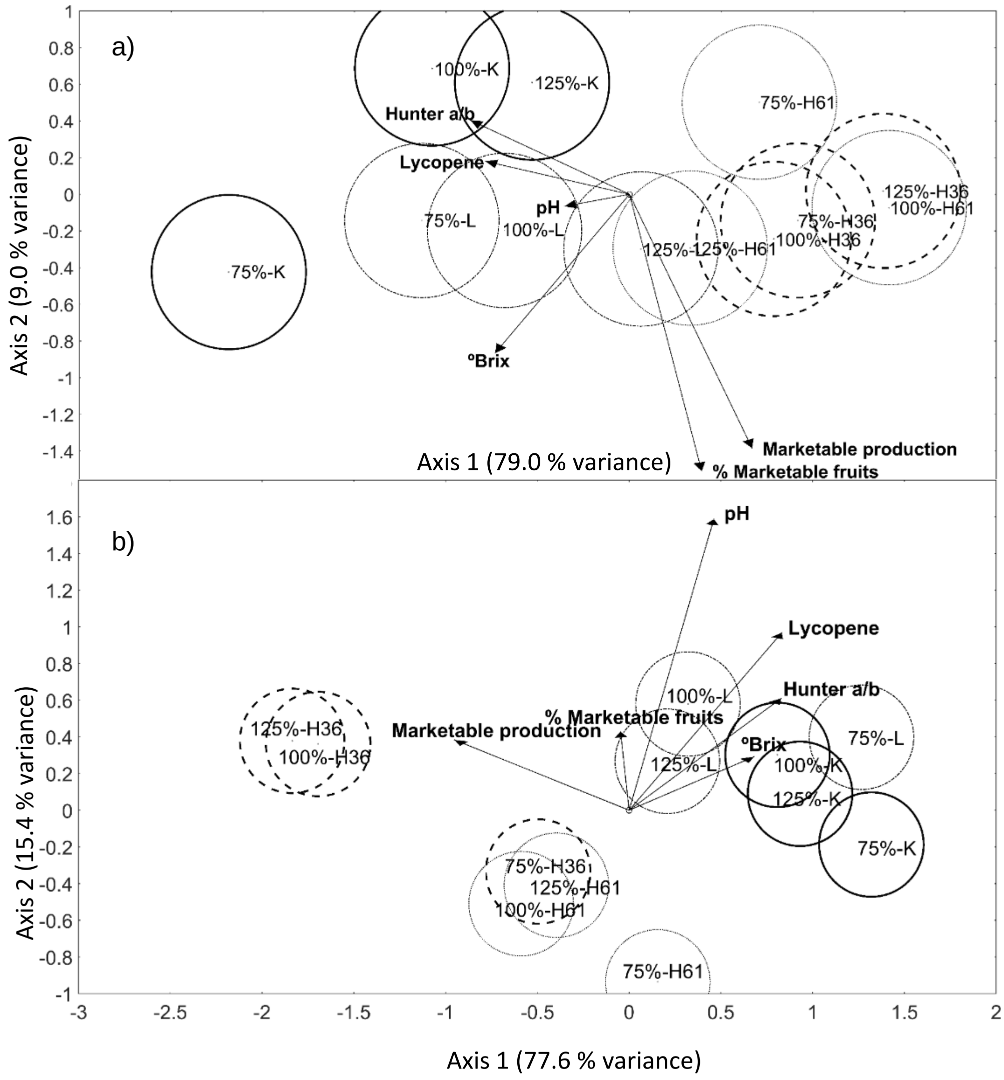
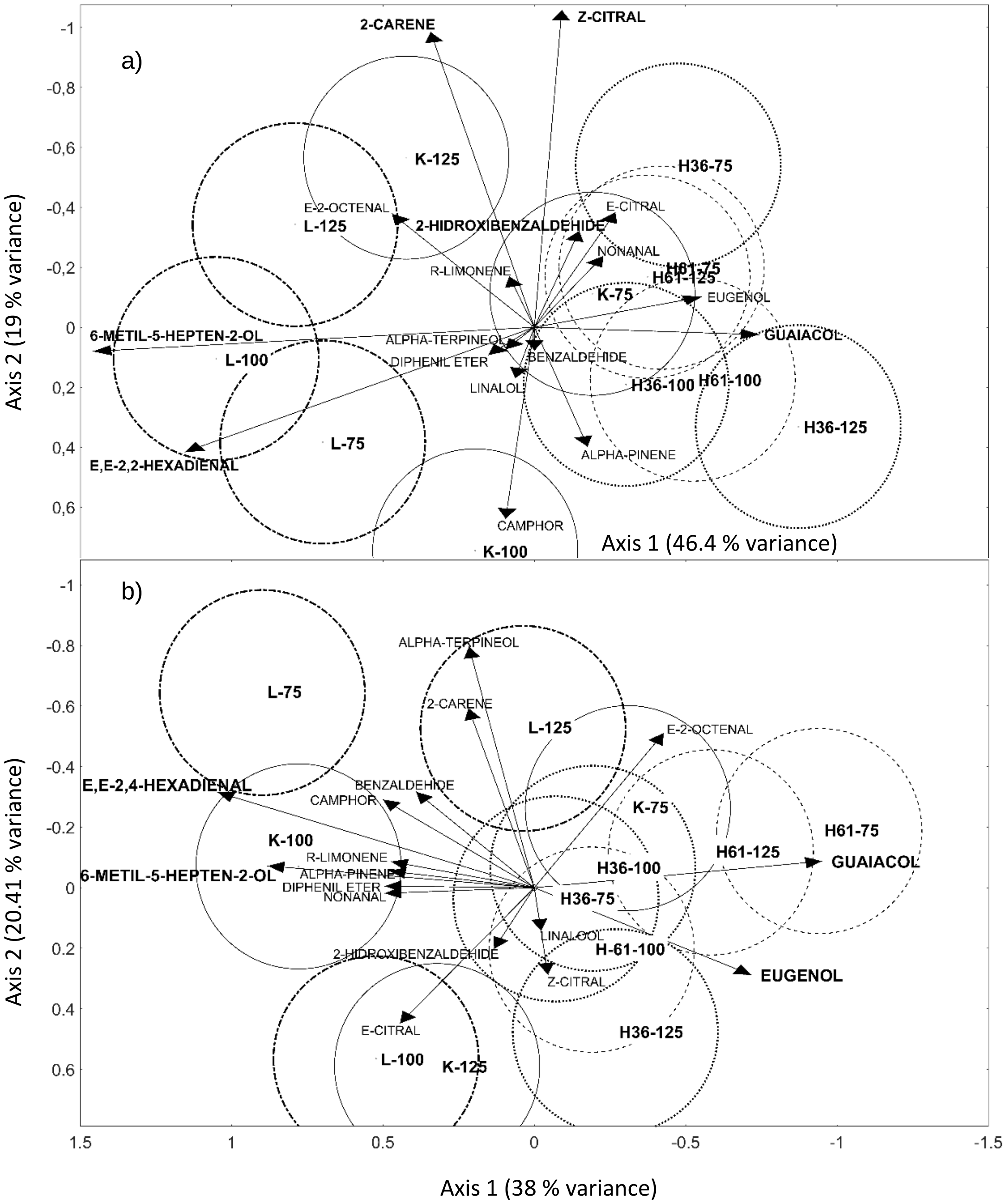
SUPPLEMENTARY TABLES AND FIGURES

Supplementary Fig. 1. MANOVA biplots of agronomical and basic quality parameters in the years 2009 (a) and 2010 (b) considering the complete models. For each data point the cultivar code (K: Kalvert, L: Loralie, H61: H-9661, H36: H-9036) and irrigation dose (75% ET_c, 100% ET_c, 125% ET_c) are included. Circles represent Bonferroni confidence intervals. Bold and higher font sizes represent significant effects in individual ANOVAs.



Supplementary Fig. 2. MANOVA biplots of background volatiles concentrations obtained with the complete model including main factors and interaction for the years 2009 (a) and 2010 (b). For each data point the cultivar code (K: Kalvert, L: Loralie, H61: H-9661, H36: H-9036) and irrigation dose (75% ET_c, 100% ET_c, 125% ET_c) are included. Circles represent Bonferroni confidence intervals. Bold and higher font sizes represent significant effects in individual ANOVAs.



Supplementary Table 1. Determinations of aroma compounds in tomato cultivars studied in 2009 (mean±s.e.; all compounds expressed in ng g^{-1})

Accession		**H-9036**			H-9661			Kalvert			Loralie		
Irrigation	**t_{RR} (min)[a]**	75% ET_c	100% ET_c	125% ET_c	75% ET_c	100% ET_c	125% ET_c	75% ET_c	100% ET_c	125% ET_c	75% ET_c	100% ET_c	125% ET_c
(Z)-3-Hexenal	0.241	255.2±77.8	176.±24.9	232.5±40.6	429.0±94.9	322.4±64.5	157.8±72.2	697.0±151.8	477.1±136.1	455.2±46.6	635.4±227.9	610.4±166.0	444.7±105.2
Hexanal	0.244	1147.0±554.4	449.7±80.1	350.9±178.8	2101.7±503.8	1129.7±356.3	421.5±237.4	4044.7±899.4	1511.3±872.3	1994.7±848.8	6277.8±1384.2	6028.9±2814.4	3658.4±1452.3
(E)-2-Hexenal	0.336	170.8±42.1	124.0±24.1	213.4±30.2	547.8±95.3	251.1±56.7	141.3±54.0	1824.7±105.4	2125.0±1141.5	1777.6±782.4	3718.8±719.4	3389. 4±907.3	2171.0±729.1
(Z)-3-Hexen-1-ol	0.344	37.2±14.9	26.9±3.5	64.9±20.4	85.8±16.5	51.3±2.4	24.4±10.7	638.6±177.2	358.0±141.8	97.4±17.1	1233.6±238.6	775.9±131.7	199.1±43.5
1-Hexanol	0.37	31.8±2.5	7.8±3.9	51.3±15.7	31.8±22.2	33.0±5.0	8.1±4.2	146.4±36.0	71.9±23.1	12.8±6.4	282.2±85.9	135.9±26.2	56.8±13.6
(E,E)-2-4-Hexadienal	0.446	0.6±0.6	n.d.	0.9±0.9	2.6±0.2	2.3±0.4	0.8±0.8	3.9±1.5	4.0±2.2	5.3±1.5	8.5±0.9	7.2±0.5	3.3±1.7
α-Pinene	0.496	2.2±0.6	1.4±0.4	2.8±0.3	3.2±1.0	1.6±0.2	1.2±0.3	2.8±1.1	2.6±1.6	0.9±0.1	1.9±0.4	2.3±0.8	2.0±0.8
(E)-2-Heptenal	0.541	27.5±2.1	21.0±3.4	28.4±4.3	42.0±4.9	35.7±2.6	17.4±4.3	49.1±4.1	54.3±24.3	42.2±8.6	80.2±12.7	104.0±22.2	43.7±7.5
Benzaldehyde	0.544	9.7±3.8	9.5±0.8	12.0±2.6	13.7±2.6	13.1±1.2	8.2±3.2	14.5±2.0	7.8±2.8	9.6±1.1	15.3±1.5	10.7±2.9	11.4±2.2
6-Methyl-5-hepten-2-one	0.604	895.0±256.4	804.4±216.1	1013.4±292.7	1145.5±139.1	794.1±238.6	935.6±304.8	593.0±119.5	427.3±202.1	1330.6±95.3	502.9±87.4	525.6±12.7	722.2±105.3
6-Methyl-5-hepten-2-ol	0.618	8.6±0.9	12.8±1.9	10.0±2.2	10.4±2.0	10.7±0.6	13.0±4.8	18.5±1.8	21.3±7.2	31.8±7.4	39.2±7.3	45.1±4.6	35.8±3.3
2-Carene	0.632	1.0±0.1	n.d.	0.4±0.1	0.94±0.30	0.6±0.1	0.8±0.0	1.1±0.1	0.3±0.2	0.6±0.2	1.1±0.1	0.9±0.2	0.8±0.2
(E,E)-2-4-Heptadienal	0.651	4.1±0.5	4.1±0.6	6.0±1.0	6.0±3.0	6.4±1.1	5.0±1.8	13.3±1.5	12.2±4.4	12.2±2.3	16.8±0.6	18.0±2.5	12.4±2.4
R-Limonene	0.688	8.8±4.3	4.7±1.1	7.6±0.8	8.4±1.3	6.8±1.5	4.4±0.9	7.4±1.4	5.4±2.3	5.1±1.1	9.0±1.6	7.2±0.4	8.2±2.3
2-Isobutylthiazole	0.693	30.3±5.7	58.3±6.9	97.9±13.3	62.8±3.4	35.6±3.7	48.4±17.0	85.6±21.4	66.9±26.7	69.4±7.4	88.9±7.3	89.4±20.2	56.3±13.7
2-Hydroxybenzaldehyde	0.710	1.5±0.3	2.4±0.7	2.4±0.5	2.7±0.4	2.1±0.2	3.1±1.5	3.0±0.3	3.2±0.8	4.7±0.4	1.6±0.1	1.7±0.4	1.6±0.2
2-Phenylethanol	0.713	8.7±3.1	4.4±0.5	10.4±4.2	11.9±2.7	12.9±2.5	7.2±3.5	8.2±2.4	9.0±.1	9.3±1.7	5.3±0.3	4.2±.0.5	2.3±1.0
(E)-2-Octenal	0.746	20.3±2.6	24.0±1.4	26.8±7.7	34.9±10.0	24.9±0.6	14.5±3.8	30.3±2.6	33.5±14.3	79.1±49.9	36.7±2.3	41.4±4.4	29.8±5.8
Guaiacol	0.805	3.9±0.2	10.4±6.4	10.9±2.9	8.4±3.3	4.8±0.5	6.6±3.1	9.7±3.4	8.3±3.4	11.3±3.1	n.d.	n.d.	n.d.
Linalool	0.829	3.5±1.3	4.1±1.2	5.64±0.2	5.2±1.4	4.9±0.3	3.4±2.3	4.5±1.5	4.5±1.2	4.9±3.3	5.1±0.6	4.5±1.1	5.2±2.1
Nonanal	0.837	25.6±10.3	28.5±5.7	42.4±4.4	29.8±4.8	29.2±4.7	15.5±8.2	30.4±0.4	19.2±5.6	33.5±12.5	24.8±0.6	24.8±6.0	31.7±3.8
Phenylacetaldehyde	0.849	8.6±3.2	4.1±0.5	8.0±1.1	10.9±2.3	11.8±2.2	8.4±3.9	11.8±2.2	9.1±3.2	11.4±1.5	8.6±2.2	4.8±1.3	1.7±0.8
Camphor	0.908	0.96±0.48	1.6±0.1	1.7±0.5	1.1±0.3	1.4±0.1	0.9±0.4	1.2±0.2	1.40±0.4	1.4±0.1	1.5±0.1	1.2±0.3	1.5±0.2
α-Terpineol	0.996	0.8±0.8	1.8±1.0	1.9±1.0	1.6±1.1	1.2±0.6	1.2±1.2	1.4±1.0	1.5±0.8	2.2±1.3	1.9±1.3	1.3±1.3	1.8±1.8
Methyl salicylate	1.001	50.9±3.4	54.0±16.2	69.3±12.3	54.5±15.1	40.7±2.3	42.3±18.9	98.7±25.2	109.2±38.6	107.0±9.6	2.5±0.3	2.0±0.7	1.6±0.3
β -cyclocitral	1.048	2.0±1.4	3.9±2.1	3.5±1.7	4.0±0.9	2.7±1.3	2.3±1.8	4.4±0.8	3.8±1.9	6.3±1.9	4.5±1.1	4.3±0.8	4.2±2.3
(Z)-Citral	1.085	48.3±13.6	56.2±8.4	55.3±11.7	49.9±8.6	48.1±4.3	39.2±21.2	36.0±13.2	31.5±7.3	94.3±14.6	33.1±0.9	34.3±3.5	53.1±7.7
(E)-Citral	1.137	128.9±74.5	117.9±45.8	193.6±80.1	125.1±43.6	131.3±57.7	108.3±63.6	77.6±29.4	67.2±29.2	211.9±108.7	108.8±49.9	85.4±32.6	87.8±26.9
(E,E)-2-4-Decadienal	1.214	6.7±0.8	9.6±4.0	9.1±1.0	5.0±1.0	6.1±0.6	3.2±1.3	6.8±1.4	6.9±1.9	5.0±0.9	9.6±1.0	11.6±4.2	4.0±0.9
Eugenol	1.280	2.7±0.3	7.9±6.4	5.9±1.3	3.2±1.6	1.5±0.1	2.0±1.0	4.3±1.2	2.9±0.9	6.6±2.7	n.d.	n.d.	n.d.
Diphenyl ether	1.348	0.4±0.3	0.8±0.4	0.5±0.1	0.6±0.2	0.5±0.1	0.4±0.3	0.5±0.34	0.7±0.2	0.7±0.2	0.7±0.2	0.4±0.2	0.8±0.2
Geranylacetone	1.431	109.5±5.8	52.0±11.8	63.7±12.6	145.6±44.3	107.2±24.8	46.9±19.5	212.8±28.1	222.5±8.9	175.9±28.5	209.3±41.3	261.8±67.6	168.4±32.2
β -Ionone	1.482	4.0±0.6	3.8±1.3	4.8±0.5	7.0±0.5	5.1±0.1	3.8±1.2	8.0±0.9	8.0±0.8	10.8±2.7	6.8±1.0	8.5±0.6	8.1±1.7

n.d.: not detected

[a] *: Relative retention time referred to internal standard (retention time i.s.: 20.25 min)*

Supplementary Table 2. Determinations of aroma compounds in tomato cultivars studied in 2010 (mean±s.e.; all compounds expressed in ng g^{-1})

Accession			**H-9036**			H-9661			Kalvert			Loralie	
Irrigation	**t_{RR} (min)[a]**	75% ET_c	100% ET_c	125% ET_c	75% ET_c	100% ET_c	125% ET_c	75% ET_c	100% ET_c	125% ET_c	75% ET_c	100% ET_c	125% ET_c
(Z)-3-Hexenal	0.241	348.1±160.6	250.5±79.0	546.1±255.3	408.7±81.6	488.6±82.6	330.9±32.2	1205.4±26.8	613.1±306.5	914.8±181.8	1497.8±64.6	1287.3±147.7	982.0±211.3
Hexanal	0.244	1889.1±516.7	2766.0±474.1	2434.2±612.0	2628.8±469.5	2094.9±146.7	2096.9±394.3	5159.4±403.8	3129.5±1892.9	4515.3±745.2	8402.3±2051.4	5355.0±832.0	7542.4±542.0
(E)-2-Hexenal	0.336	643.1±271.5	466.3±235.1	465.4±96.3	437.6±59.1	743.0±222.5	513.8±274.8	2511.2±349.3	1812.9±931.8	2488.6±1111.1	4622.6±818.1	2261.6±152.6	2627.0±524.0
(Z)-3-Hexen-1-ol	0.344	86.4±16.8	94.5±5.3	58.8±6.0	88.1±13.3	189.6±88.4	127.1±35.6	152.6±20.4	531.2±374.7	246.3±79.2	289.8±59.9	213.8±105.7	140.6±17.4
1-Hexanol	0.37	175.9±2.5	94.5±17.9	86.9±27.5	110.8±1.0	111.6±40.4	143.0±72.8	38.1±23.5	597.1±291.8	127.2±64.1	114.1±12.2	89.0±14.0	81.1±26.0
(E,E)-2-4-Hexadienal	0.446	1.6±1.0	n.d.	n.d.	n.d.	n.d.	n.d.	1.3±1.3	13.7±7.6	1.5±1.5	5.2±0.8	3.7±0.6	2.2±1.2
α-Pinene	0.496	2.8±0.4	3.0±0.4	4.6±1.1	2.3±0.0	2.1±0.1	1.8±0.0	2.3±1.2	110.3±108.1	2.4±0.4	2.1±0.4	4.2±2.1	2.2±0.1
(E)-2-Heptenal	0.541	34.1±5.2	34.1±7.3	45.2±9.1	64.6±26.9	43.2±7.9	39.3±9.3	75.5±4.2	38.6±26.0	42.1±10.2	338.2±265.8	42.5±7.9	60.2±9.2
Benzaldehyde	0.544	6.7±1.7	6.0±0.2	8.9±2.8	8.4±0.3	8.1±0.4	7.7±0.4	11.9±1.8	8.5±1.5	9.3±1.1	11.1±1.3	9.6±2.3	9.1±0.6
6-Methyl-5-hepten-2-one	0.604	411.1±169.7	287.5±70.2	671.6±274.1	564.2±162.1	490.7±180.1	518.8±49.8	967.2±239.4	562.8±29.9	790.7±21.1	464.9±30.0	813.2±185.1	717.5±141.6
6-Methyl-5-hepten-2-ol	0.618	9.3±1.3	8.4±1.5	13.5±4.6	8.4±0.8	12.9±1.5	11.3±2.4	26.4±4.6	25.6±6.3	24.1±3.4	20.1±1.0	14.1±0.9	14.1±3.2
2-Carene	0.632	0.4±0.2	0.4±0.2	n.d.	0.3±0.2	0.8±0.2	0.5±0.3	n.d.	0.1±0.1	0.4±0.4	0.8±0.3	0.3±0.3	0.5±0.2
(E,E)-2-4-Heptadienal	0.651	2.9±0.5	2.2±0.5	4.1±1.9	7.2±1.8	4.6±1.0	4.1±0.7	13.8±1.5	10.3±5.3	9.2±1.5	14.9±2.6	10.3±1.1	11.0±1.1
R-Limonene	0.688	3.6±0.4	5.8±2.0	5.2±1.8	4.4±1.6	2.8±0.9	3.2±1.3	5.3±3.1	171.9±167.9	4.2±1.2	3.9±1.6	3.5±0.5	5.2±1.9
2-Isobutylthiazole	0.693	152.3±17.1	31.2±11.4	60.9±21.7	61.0±2.4	58.9±12.9	78.8±9.6	114.9±3.5	41.6±16.8	66.1±14.5	102.8±11.0	51.5±4.9	67.9±6.7
2-Hydroxybenzaldehyde	0.710	2.0±1.1	2.6±0.8	2.9±2.0	2.7±1.5	2.6±0.5	3.5±0.7	4.5±0.7	9.1±4.1	2.6±0.5	0.4±0.4	1.8±1.4	0.4±0.4
2-Phenylethanol	0.713	n.d.	n.d.	n.d.	n.d.	29.3±29.3	n.d.	5.3±2.7	5.3±3.4	2.3±2.3	1.3±1.3	n.d.	n.d.
(E)-2-Octenal	0.746	32.9±6.4	27.5±2.9	49.4±16.2	103.5±65.8	32.6±1.4	28.3±0.9	70.1±16.6	28.4±15.0	30.9±5.6	63.6±17.6	36.4±5.4	32.2±3.5
Guaiacol	0.805	4.7±2.6	5.6±0.6	8.2±3.4	21.5±12.2	8.9±1.3	7.8±2.7	16.1±3.6	7.1±4.9	5.7±1.2	n.d.	n.d.	n.d.
Linalool	0.829	3.5±0.4	1.5±0.8	3.8±2.8	3.0±1.5	4.1±0.4	3.6±0.4	3.2±1.6	1.4±1.4	4.2±0.4	4.1±0.1	3.6±0.3	4.2±0.4
Nonanal	0.837	14.2±1.8	10.6±0.1	21.6±8.1	13.4±0.2	12.9±0.2	11.7±0.5	16.5±1.8	52.6±39.2	15.8±2.1	15.4±1.5	14.5±1.3	14.8±0.9
Phenylacetaldehyde	0.849	0.3±0.3	0.2±0.2	n.d.	0.7±0.4	0.5±0.3	0.4±0.2	1.4±0.9	3.2±2.2	0.8±0.4	1.7±1.3	1.0±0.7	0.2±0.2
Camphor	0.908	n.d.	n.d.	n.d.	n.d.	n.d.	0.03±0.03	0.9±0.4	6.9±6.9	n.d.	0.6±0.6	n.d.	1.3±0.7
α-Terpineol	0.996	0.3±0.3	0.3±0.2	n.d.	0.4±0.2	0.3±0.3	0.3±0.3	0.3±0.3	0.4±0.2	0.2±0.2	0.6±0.3	0.2±0.2	0.4±0.2
Methyl salicylate	1.001	58.2±7.3	92.3±12.5	90.8±3.3	88.6±5.6	80.9±12.9	75.0±17.8	141.1±29.7	95.6±70.5	102.9±9.2	2.5±0.9	21.9±19.8	2.1±0.4
β -cyclocitral	1.048	2.4±0.5	1.9±0.3	3.5±1.5	3.2±0.3	2.9±0.2	2.8±0.3	5.7±1.2	3.6±1.8	4.3±0.4	3.2±0.4	4.3±1.0	3.5±0.1
(Z)-Citral	1.085	11.1±4.1	7.5±1.9	17.3±11.4	15.4±5.9	11.9±1.7	15.2±3.8	28.3±9.2	11.6±6.3	18.6±3.2	12.3±0.8	24.9±9.7	18.1±4.8
(E)-Citral	1.137	66.2±16.8	76.3±21.0	74.0±20.6	105.0±34.8	99.3±9.2	78.5±11.9	163.2±43.7	167.9±64.1	142.7±32.1	76.6±10.4	183.2±59.7	110.0±23.2
(E,E)-2-4-Decadienal	1.214	n.d.	1.7±1.7	n.d.	16.7±11.3	2.0±1.0	2.0±1.1	9.1±4.1	102.8±96.8	0.5±0.5	9.2±5.1	1.0±1.0	2.1±1.1
Eugenol	1.280	6.0±3.0	4.3±0.4	6.5±2.5	4.9±2.7	1.9±0.1	2.0±0.5	6.0±1.6	3.1±2.1	2.2±0.4	n.d.	n.d.	n.d.
Diphenyl ether	1.348	0.4±0.1	0.3±0.1	0.6±0.3	0.4±0.1	0.4±0.1	0.4±0.1	0.6±0.1	2.0±1.5	0.5±0.1	0.5±0.2	0.4±0.1	0.4±0.1
Geranylacetone	1.431	65.9±4.9	82.1±14.1	76.8±3.4	142.7±5.0	99.2±34.6	83.8±12.8	182.5±46.2	133.9±60.7	139.3±35.9	164.5±14.4	147.1±12.0	162.4±8.6
β -Ionone	1.482	3.1±0.8	2.6±0.3	4.4±2.5	4.4±0.6	3.7±0.9	3.4±0.1	7.3±0.7	7.1±3.7	5.1±0.8	4.50±0.6	5.9±2.1	4.8±0.5

n.d.: not detected

[a]: Relative retention time referred to internal standard (retention time i.s.: 20.25 min)