

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

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15

16 **Abstract**

17

18 The influence of irrigation doses on standard and high lycopene tomato varieties has
19 been analyzed during two years in one of the main processing tomato growing areas of
20 Spain. Deficit irrigation (75% ET_c) implied a mean reduction in water use of 28.2%,
21 while it caused a significant reduction in the marketable production of 16.4% and
22 increase in soluble solids (8.4%) and Hunter a/b ratio (2.4%). The effect on lycopene
23 content was not significant. Increasing irrigation dose over the recommended 100% ET_c
24 had no significant effect on the agronomical performance, while it provoked a dilution
25 effect reducing total soluble solids and lycopene content. The effects on 33 tomato
26 volatiles were also analyzed, 11 of them related to main aroma notes and 22 to the
27 background volatile profile. The effect of deficit irrigation on aroma was dependant on
28 climatic conditions and it may either not have a significant effect on the aroma profile
29 or it may lead to higher logodor units in main aroma volatiles. High lycopene cultivars
30 showed higher contents in most volatiles, including some volatiles originated in
31 pathways that have not been related with carotenoid degradation processes. In both the

32 fresh and processing tomato market the improvement of organoleptic and functional
33 quality and the reduction of the impact of agriculture on environment represent main
34 goals. The use of high lycopene cultivars and restricted irrigation would enhance the
35 aroma of materials targeted to quality markets, contributing to increase the efficiency of
36 water use in agriculture.

37

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

39

40 **Introduction**

41 Tomato is one of the most important vegetable crops in the world. Its consumption
42 either fresh or processed has increased continuously for the last decade. In both forms of
43 consumption there is an increasing demand for high quality products, including
44 organoleptic and functional quality, the latter being considered as the capacity of its
45 consumption to prevent certain diseases. In fact, the high level of consumption of
46 tomato makes of this vegetable an excellent source of health promoting compounds
47 such as vitamins C and E, lycopene, β -carotene, lutein and flavonoids (Dorais et al.,
48 2008).

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

54 Water deficit irrigation would not only reduce the use of this input but would also result
55 in an increased organoleptic quality of the fruit, though it may reduce fruit weight and

56 yield. Nevertheless, a compromise between quality and marketable yield is still
57 possible. The results obtained by Mitchell et al. (1991) supported that deficit irrigation
58 and saline irrigation may be feasible crop water management options to produce high
59 quality field-grown processing tomatoes without major yield reductions. Similarly,
60 Veit-Köhler et al. (1999) found that small reductions in water supply resulted in higher
61 quality of tomatoes due to higher contents of sugars, titratable acids, vitamin C and
62 aroma volatiles (particularly C6-aldehydes hexanal, (*Z*)-3-hexenal and (*E*)-2-hexenal),
63 while the proportion of marketable fruits remained high. In any case, little is known
64 regarding the effect of deficit irrigation on the aroma profile of tomatoes, as well as the
65 differences in the performance of standard and high-lycopene cultivars.

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

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

91 **MATERIALS AND METHODS**

92 *Plant material*

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

100 *Cultivation and experimental design*

101 Cultivation was carried out in the facilities of Instituto Navarro de Tecnología e
102 Infraestructuras Agroalimentarias S.A. (INTIA S.A.) at Cadreita (42°12’34.75’’N;
103 1°43’1.08’’W; 267m) in Navarre (Spain). Plants were grown on plastic mulch and drip
104 irrigated. The effect of irrigation dose on the aroma profile of tomato varieties was

105 studied with three watering doses that satisfied 75%, 100% and 125% of crop
106 evapotranspiration (ET_c). The higher irrigation dose was selected in order to explore the
107 effects of excess of irrigation sometimes observed in certain farms. Hydric requirements
108 were calculated as a function of crop evapotranspiration following FAO56 methodology
109 (Allen et al., 1998). 75% ET_c corresponded to 252.48 Lm^{-2} , 100% to 351.38 Lm^{-2} and
110 125% to 438.20 Lm^{-2} during the year 2009 and to 295.80 Lm^{-2} , 412.30 Lm^{-2} and 530.10
111 Lm^{-2} respectively during the year 2010. For each condition three plots of 200 plants per
112 variety were established using a randomized complete block design. Plantation density
113 was 35,700 plants/ha.

114 In the year 2009 plants were sown on 26th of March and transplanted on the 14th of May.
115 The varieties were harvested between 27th of August and 23rd of September. ‘Kalvert’
116 and ‘Loralie’ showed the shortest growing cycle and ‘H-9036’ the longest. In the year
117 2010 plants were sown on 26th of March and transplanted on 18th of May. The harvest
118 period ranged between 21st of September for early varieties and 13th of October for the
119 late one.

120 *Agronomical determinations*

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

125 *Sample preparation and basic determinations*

126 A bulk sample was obtained in a per block basis, with at least 40 representative fruits
127 obtained from different plants. All the fruits of the sample were ground and
128 homogenized. With this juice, total soluble solids (TSS) and pH were determined. A

129 digital refractometer (ATAGO PR-1, Tokyo, Japan) with 0.1° Brix precision was used
130 to evaluate TSS (results expressed as °Brix at 20°C). Hunter a and b parameters (results
131 expressed as Hunter a/b rate) were determined using a digital colorimeter (CR 300,
132 Minolta, Japan).

133 For the analysis of volatiles, the homogenate was left to stand for 3 minutes and then a
134 saturated solution of CaCl₂ (2.7 ml in 30 g of sample) was added to inactivate volatile
135 degrading enzymes. The efficiency of this system was previously contrasted with the
136 suggested methodology by Buttery et al. (1987). Samples were instantly frozen and
137 maintained at -80°C until analysis. Although sub-zero temperatures might affect the
138 content of some volatiles, all the samples were stored in the same conditions.

139 *Volatile analysis*

140 Thirty three tomato volatiles were monitored in the samples (Supplementary Table 1).
141 Reference aroma compounds and internal standard (i.s.) methyl salicylate-D₄ of 99.5%
142 purity were purchased from Sigma-Aldrich Química S.A. (Madrid, Spain). Stock
143 solutions at 500 mg L⁻¹ were prepared in acetone and stored at -18°C. Working
144 solutions were prepared by volume dilution in diethyl ether-hexane (1:1). Calcium
145 chloride 97% (Riedel de Haen) was purchased from Supelco (Sigma-Aldrich Química
146 S.A., Madrid, Spain). Organic solvents (hexane, ethyl acetate, diethyl ether) of trace
147 residue analysis quality were purchased from Scharlab (Barcelona, Spain). SPE
148 cartridges (Supelco, Sigma-Aldrich Química S.A., Madrid, Spain) were prepared by the
149 manufacturer packing 500 mg of Tenax TA (80-100 mesh) in 6 mL polyethylene tubes
150 (15 mm diameter).

151 The extraction system set in a previous work (Beltrán et al., 2006) consisted in a 50 mL
152 Erlenmeyer flask attached to a glass cap with two connexion tubes: the inlet connected
153 to a dry N₂ gas supply, and the outlet fitted to the Tenax trap. 30 g of sample and 50 µL

154 of 15 $\mu\text{g mL}^{-1}$ methyl salicylate- D_4 (i.s.) were magnetically stirred (350 rpm) at 35°C for
155 120 min with a N_2 flow of $1\text{L}\cdot\text{min}^{-1}$ with a new Tenax tube (maintained at room
156 temperature) connected to the flask outlet. The trap was eluted with 3.5 mL of hexane-
157 ether (1:1) mixture and the final extract adjusted to 1 mL by nitrogen evaporation.

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

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

172 *Lycopene analysis*

173 Determination of lycopene content was carried out by Centro Nacional de Tecnología y
174 Seguridad Alimentaria (San Adrián, Spain). A bulk sample of 40 fruits was obtained per
175 repetition (3 biological replicates) following the ISO 874 UNE-34-117-81 guidelines.
176 Fruits were ground and homogenized in dark conditions. Carotenoids were extracted
177 with a mixture of hexane:acetone:ethanol, 20:25:25 (Sadler et al., 1990). The

178 determination of lycopene in the hexane phase was based on spectrophotometric
179 analysis using a JASCO V-530 spectrophotometer (Jasco Analítica Spain, Madrid,
180 Spain) at 501 nm (Rao et al., 1998). Two analytical replicates per sample were made.

181 *Statistical analysis*

182 Agronomical and basic quality parameters were evaluated jointly using multivariate
183 analysis. Following the approach of previous works (Cebolla-Cornejo et al., 2011) the
184 volatiles quantified were arranged in two sets of variables. The first one included main
185 aroma notes, with 11 volatiles that have been previously described as important in
186 tomato aroma (Baldwin et al., 2000) and showed concentrations higher than their odor
187 threshold. In this case, the concentrations were expressed as logodor units, a variable
188 more closely related to aroma perception. Odor thresholds (ng g^{-1}) used for calculations
189 were the following: (*E*)-2-hexenal: 17, 2-isobutylthiazole: 3.5, 6-methyl-5-hepten-2-
190 one: 50, hexanal: 4.5, (*Z*)-3-hexen-1-ol: 70, β -ionone: 0.007, (*Z*)-3-hexenal: 0.25,
191 geranylacetone: 60, methyl salicylate: 40, (*E*)-2-heptenal: 13 (Buttery et al., 1987) and
192 (*E,E*)-2,4-decadienal: 0.07 (Buttery et al., 1971). The second set included the remaining
193 22 compounds: a representation of volatiles included in the aroma background,
194 expressed as concentrations. Some of these compounds (phenylethanol, β -cyclocitral,
195 phenylacetaldehyde, 1-hexanol) had previously described as important but showed
196 negative logodor units. For this set, logodor units were not used as the variable loses its
197 purpose.

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

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

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

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

228 2% and the number of new variables was minimized as possible. Model precision was
229 improved by selection of volatiles, using the method of interval PLS (iPLS) variable
230 selection which performs a hierarchical, sequential and exhaustive search for the best
231 combinations of variables. Interval PLS was performed in reverse mode, with intervals
232 successively removed from the analysis (Wise et al., 2006).

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

235

236 **Results**

237 *Climatic conditions*

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

249 *Agronomical performance and basic quality parameters*

250 The year effect had minor significant effects on % marketable fruits, pH and Hunter a/b
251 (Table 1), while the effects on marketable production, TSS and lycopene were not

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

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

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

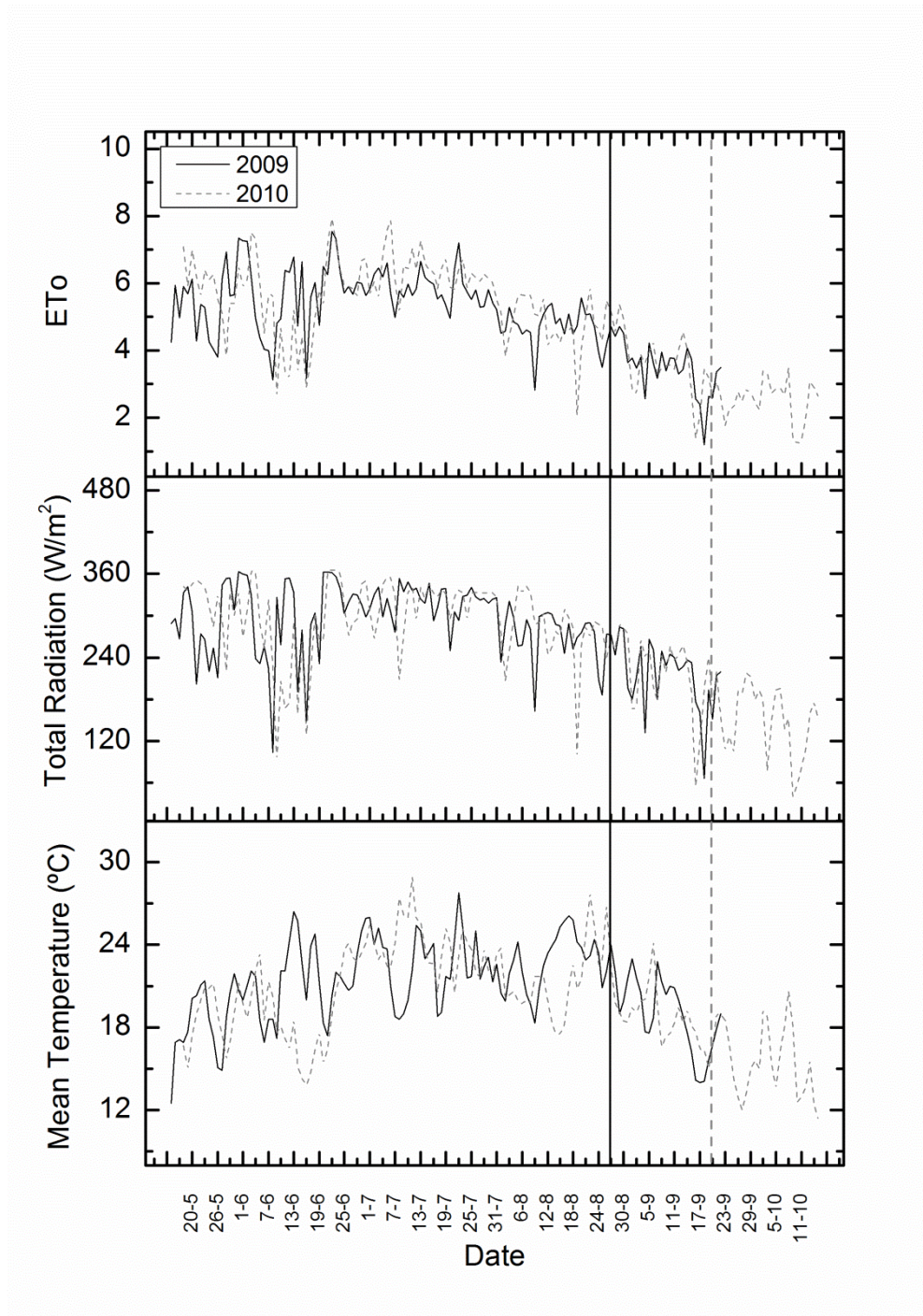
272 High lycopene varieties offered considerably lower productions compared to ‘H-9036’,
273 which showed the highest yield. Specifically, ‘Kalvert’ showed a 42.1% lower
274 production and ‘Loralie’ a 24.2% (Table 1). As expected, high lycopene varieties
275 presented high lycopene contents, that probably caused higher Hunter a/b ratios. Fruit

276 pH, and TSS were also higher in the high lycopene cultivars. These trends were
277 confirmed in the MANOVA biplots for each year.

278

279 **Figure 1.** Climatic data registered from transplantation till the end of harvest during
280 2009 (continuous line) and 2010 (dotted line). Vertical lines indicate the initiation of
281 harvest.

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

		% Marketable fruits	Marketable production (10 ³ kg ha ⁻¹)	Total soluble solids (°Brix)	pH	Hunter a/b	Lycopene mg kg ⁻¹
Year (Y)	<i>p value</i>	<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)	<i>p value</i>	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)	<i>p value</i>	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	<i>p value</i>	0.433	0.070	0.849	0.638	0.300	0.253
YxC	<i>p value</i>	<0.001	<0.001	0.110	0.009	0.004	0.720
DxC	<i>p value</i>	0.058	0.081	<0.001	0.438	<0.001	0.170

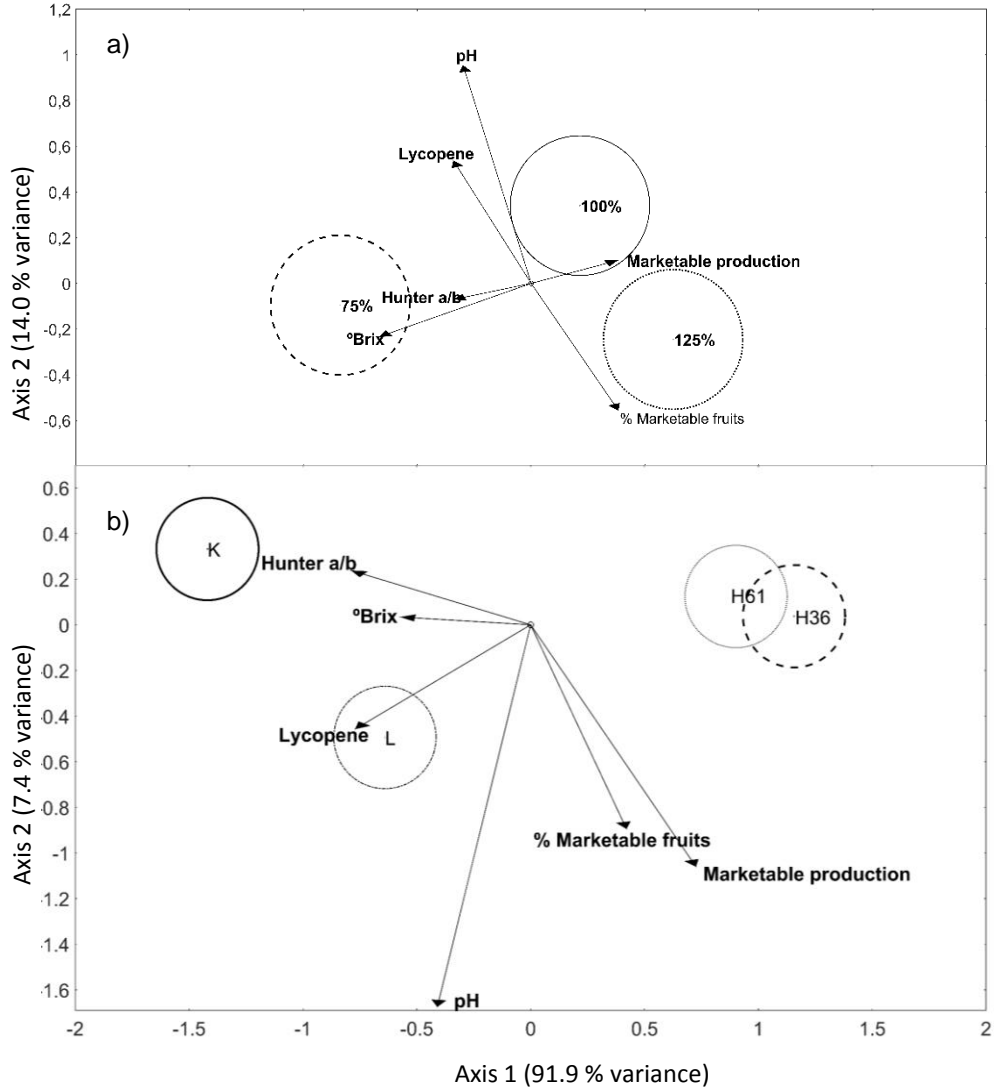
288 *Different letters in irrigation dose and cultivar indicate significant differences (Tukey*
 289 *test)*

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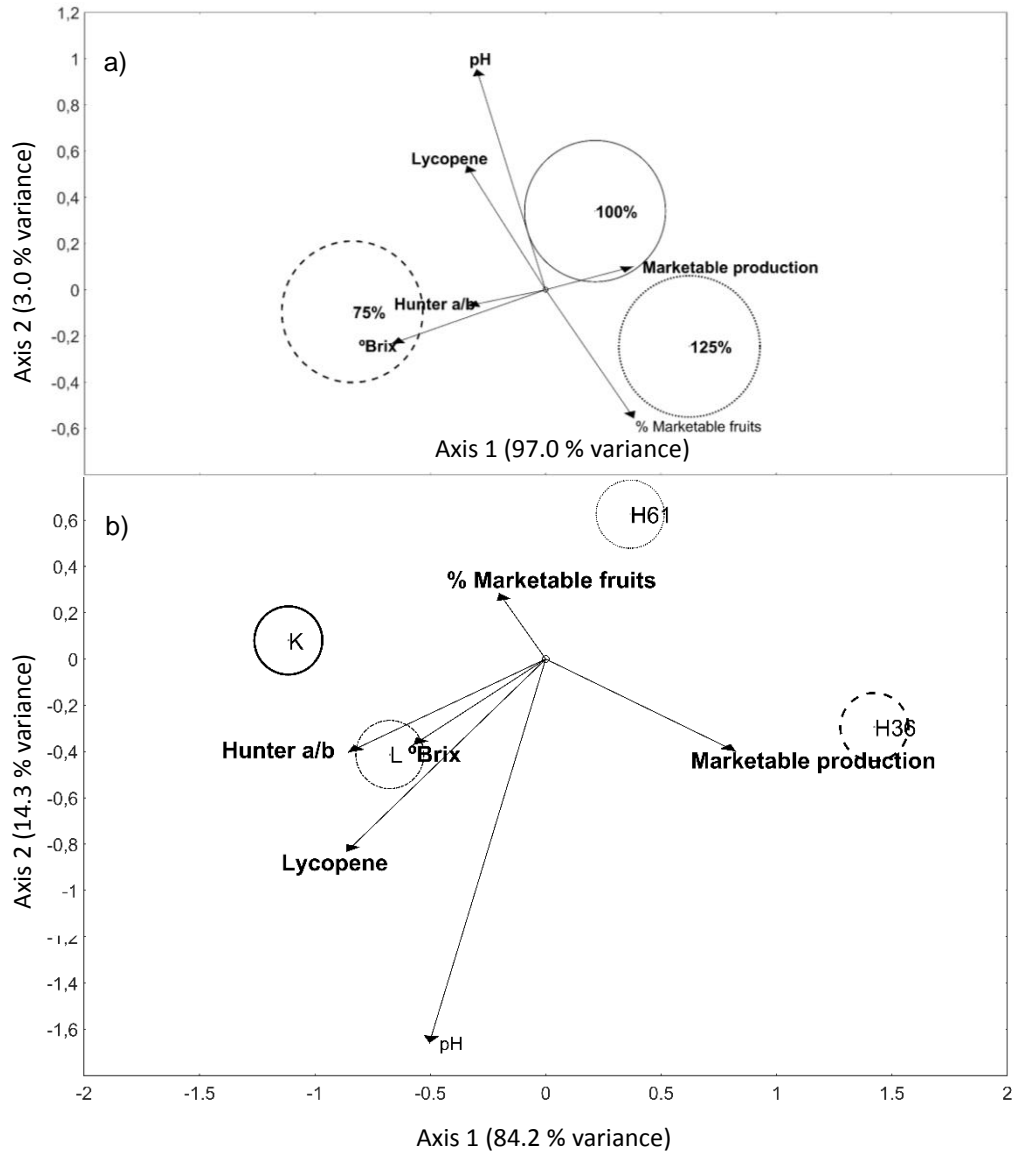
292 **Fig. 2.** MANOVA biplots of agronomical and basic quality parameters in the year 2009
 293 considering the factors irrigation (a) and cultivar (b). For each data point the cultivar code (K:
 294 Kalvert, L: Loralie, H61: H-9661, H36: H-9036) and irrigation dose (75% ET_c, 100% ET_c, 125%
 295 ET_c)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

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 298



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

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306 *Main aroma volatiles*

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

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

328 Regarding the cultivar effect, the MANOVA biplot (Fig. 4b), clearly differentiated
329 cultivars 'Kalvert' and 'Loralie' and the group including 'H-9661' and 'H-9036'. The
330 overlap of the Bonferroni confidence intervals of the last two cultivars entailed little

331 differences between both materials in their aroma profiles. In general ‘Kalvert’ and
332 ‘Loralie’ held a position favouring higher values of logodor units. In this case a higher
333 number of compounds were significantly affected by this effect.

334 For a complete interpretation of the main effects and interaction a MANOVA biplot for
335 the complete model was obtained (Fig. 4c). For both ‘Kalvert’ and ‘Loralie’ it seemed
336 that there was a dilution effect of higher irrigation doses on logodor units, though the
337 differences between 75% and 100% ET_c were limited. A higher separation between the
338 Bonferroni confidence circles of both cultivars than between the confidence circles of
339 different irrigation levels of each cultivar indicated that the cultivar effect was more
340 important than the irrigation dose. The position of Bonferroni confidence circles of ‘H-
341 9661’ and ‘H-9036’ confirmed that differences in the aroma profile, both at the cultivar
342 and irrigation level would be small.

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

349 **Table 2.** Significance of factor effects obtained in the multivariate analysis of variance (p-value)
 350 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	

351

352 *Background volatiles*

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

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

363 *Lycopene and volatiles*

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

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

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

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

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

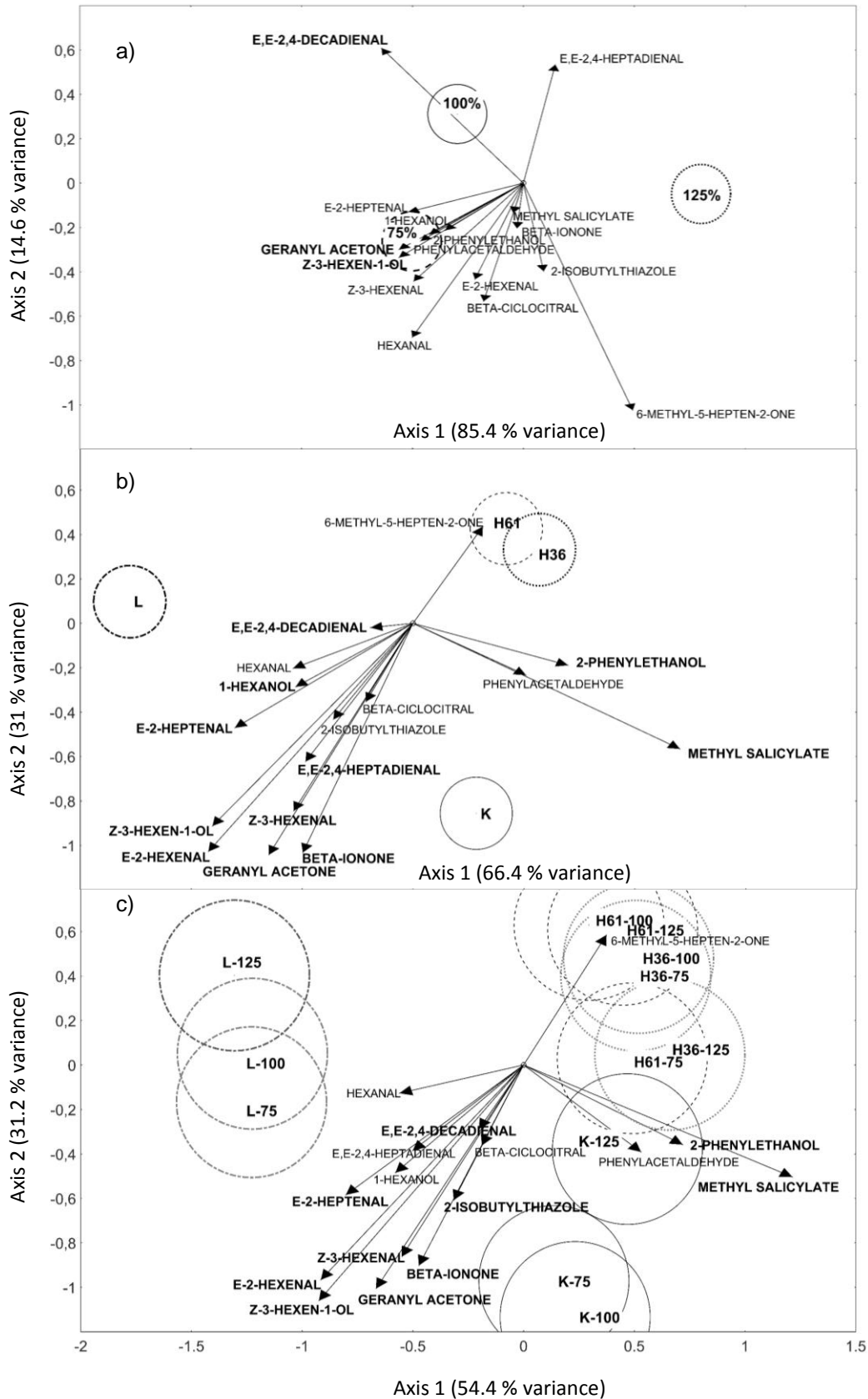
394 carene, and diphenyl ether and the lowest correlations with (E)-2-heptenal, β -
395 cyclocitral, (E,E)-2,4-decadienal and benzaldehyde.

396 The representation of LV1 scores over lycopene content showed a higher correlation
397 between both variables ($R^2=0.875$). The same differentiation between the aroma profile
398 of high and low lycopene cultivars was obtained (Fig. 6b). This year 'Loralie' and 'H-
399 9961' had slightly higher scores on LV1 than 'Kalvert' and 'H-9036', respectively.

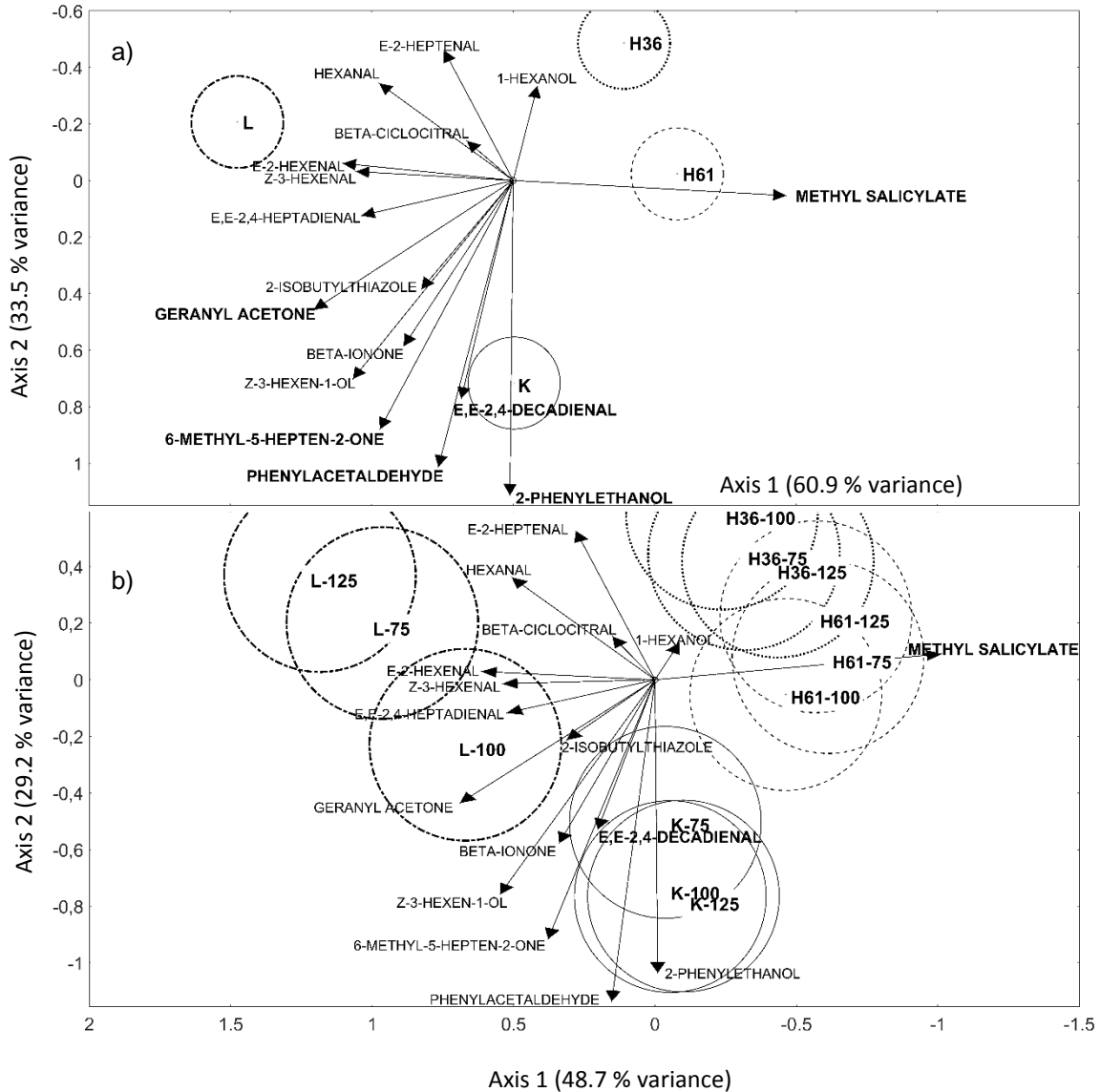
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401 **Fig. 4.** MANOVA biplots of main aroma logodor units in the year 2009 considering the factors
 402 irrigation (a), cultivar (b) and the complete model with main factors and interaction (c). For
 403 each data point the cultivar code (K: Kalvert,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

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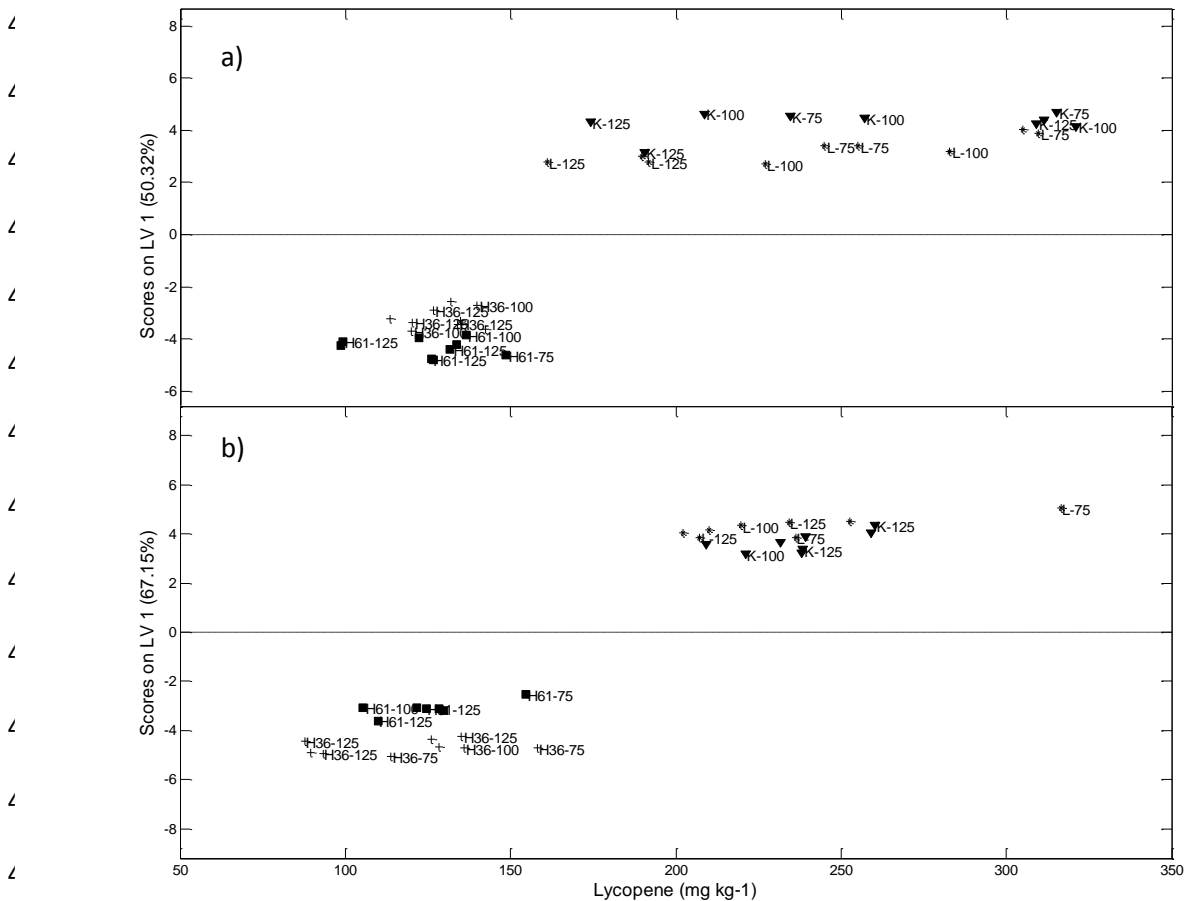


411 **Fig. 5.** MANOVA biplots of main aroma logodor units in the year 2010 considering the factor
 412 cultivar (a) and the complete model with main factors and interaction (b). The MANOVA biplot
 413 of the irrigation factor is not shown as the factor was not significant for this year. For each data
 414 point the cultivar code (K: Kalvert, L: Loralie, H61: H-9661, H36: H-9036) and irrigation dose
 415 (75% ET_c, 100% ET_c, 125% ET_c) are included. Circles represent Bonferroni confidence intervals.
 416 Bold and higher font sizes represent significant effects in individual ANOVAs.



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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.



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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

479 in dry areas (Geerts and Raes, 2009). Our study was targeted to evaluate the
480 possibilities of water deficit irrigation not only to increase the efficiency of water use,
481 but also to offer an added value to agricultural products.

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

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

497 The application of deficit irrigation in tomato also would have an effect on the aroma of
498 the raw produce, but it would be dependent on the climatic conditions of cultivation.
499 Hypothetically, in years with an extended growing cycle, with a delayed fruit ripening
500 during months with lower radiation and temperatures, a deficit irrigation regime (75%
501 ET_c) would not have a significant effect on the aroma profile, as it happened in our 2010
502 growing cycle. On the contrary, in common years with good growing conditions,
503 involving long periods without important rainfalls and high temperatures, like in 2009,

504 water deficit irrigation would have a significant effect on the aroma profile. In this case,
505 a general increase in logodor units would be obtained, implying a more intense aroma.
506 This increase would be especially significant for geranylacetone and (*Z*)-3-hexenol, the
507 first one related with the aroma descriptor fruity and the second with leafy/fresh cut
508 grass (Tandon et al., 2000) and flavour intensity (Tieman et al., 2012). Veit-Köhler et
509 al. (1999) described increased concentrations of C6-aldehydes in the aroma profile of
510 plants with reduced irrigation. In our case, a rough analysis of concentrations in 2009
511 also highlighted that (*Z*)-3-hexenal and hexanal showed high concentration increases in
512 all the genotypes compared to other volatiles. (*Z*)-3-hexenal has been related with sweet
513 (Krumbein et al., 2004) and tomato/citrus (Tandon et al., 2000) descriptors in sensory
514 evaluation, while hexanal has been related with mouldy (Krumbein et al., 2004) and
515 stale/grassy/green (Tandon et al., 2000) descriptors. It should be considered though, that
516 Tieman et al (2012), using transgenic tomatoes with altered 13-lipoxygenase activity
517 and thus, altered concentrations of C6-volatiles, revealed no significant impact of these
518 volatiles on consumer liking.

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

524 The strong environment x irrigation (year in our case) interaction detected for aroma
525 volatiles has also been reported in other quality variables. Several authors have
526 observed higher differences in soluble solids contents between sites of cultivation than
527 within the same site at different deficit irrigation regimes (Rodriguez et al., 1994;
528 Patanè and Cosentino, 2010). In our case, year x irrigation dose interaction was not

529 significant in basic agronomical and quality parameters, and the year effect had limited
530 consequences, while Garcia and Barrett (2006) concluded that the impact of growing
531 season might be more important than any other factor, including the genetic background
532 in terms of peelability and other processing important quality attributes.

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

551 It is difficult to compare the quantitative volatile profile of the varieties tested in this
552 work with previous literature. As Ruiz et al. (2005) have previously reported, data
553 available in the scientific literature is relatively scarce and shows values over a great

554 range, probably due not only to the use of different varieties but also of different
555 analytical methods. In our opinion, the differences in the exact moment of analysis and
556 differences in growing conditions should also be accounted for. In any case, the
557 concentrations obtained for hexanal (i.e.) were on the range described by other authors
558 for fresh tomato varieties (Mayer et al., 2008; Birtic et al., 2009).

559 The lycopene content was also strongly influenced by genotype, with minor
560 modifications by the irrigation and year effects. In general, higher irrigation doses over
561 100% ET_c may lead to lower lycopene contents, though the differences between
562 standard and deficit irrigation at 75% ET_c were not significant. The effects of water
563 availability in carotenoid content in tomato seem to be sometimes contradictory (see
564 review by Dumas et al., 2003). Nevertheless, recent studies tend to support the use of a
565 reduction in irrigation doses. Pernice et al. (2010) concluded that reduced irrigation or
566 none irrigation increased the level of lycopene in fresh and processed tomato. Favati et
567 al. (2009) also reported that a limitation of water supply during the whole cycle or part
568 of it increased lycopene and β-carotene contents. Wang et al. (2011) reported that the
569 best compromise between yield and quality, including lycopene content, would be
570 reached restricting irrigation during the flowering and fruit development stage.
571 According to Dorais et al. (2001) low water supply would lead to an increase in abscisic
572 acid that may influence ethylene production and hence the concentration of carotenoids.
573 In other crops such as wine grapes, it has been stated that water deficit in Chardonnay
574 activates parts of the phenylpropanoid, energy, carotenoid and isoprenoid metabolic
575 pathways that contributed to increased concentrations of antheraxanthin, flavonols and
576 aroma volatiles (Deluc et al., 2009). Dabbou et al. (2011) found that the aroma profile in
577 olive oil was strongly determined by the genotype, and the effects of water deficit
578 irrigation would be limited.

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

593 **Conclusions**

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

600 **Acknowledgements**

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605 analysis.

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727 SUPPLEMENTARY TABLES AND FIGURES

728

729

730 **Supplementary Fig. 1.** MANOVA biplots of agronomical and basic quality parameters in the
 731 years 2009 (a) and 2010 (b) considering the complete models. For each data point the cultivar
 732 code (K: Kalvert, L: Loralie, H61: H-9661, H36: H-9036) and irrigation dose (75% ET_c, 100% ET_c,
 733 125% ET_c) are included. Circles represent Bonferroni confidence intervals. Bold and higher font
 734 sizes represent significant effects in individual ANOVAs.

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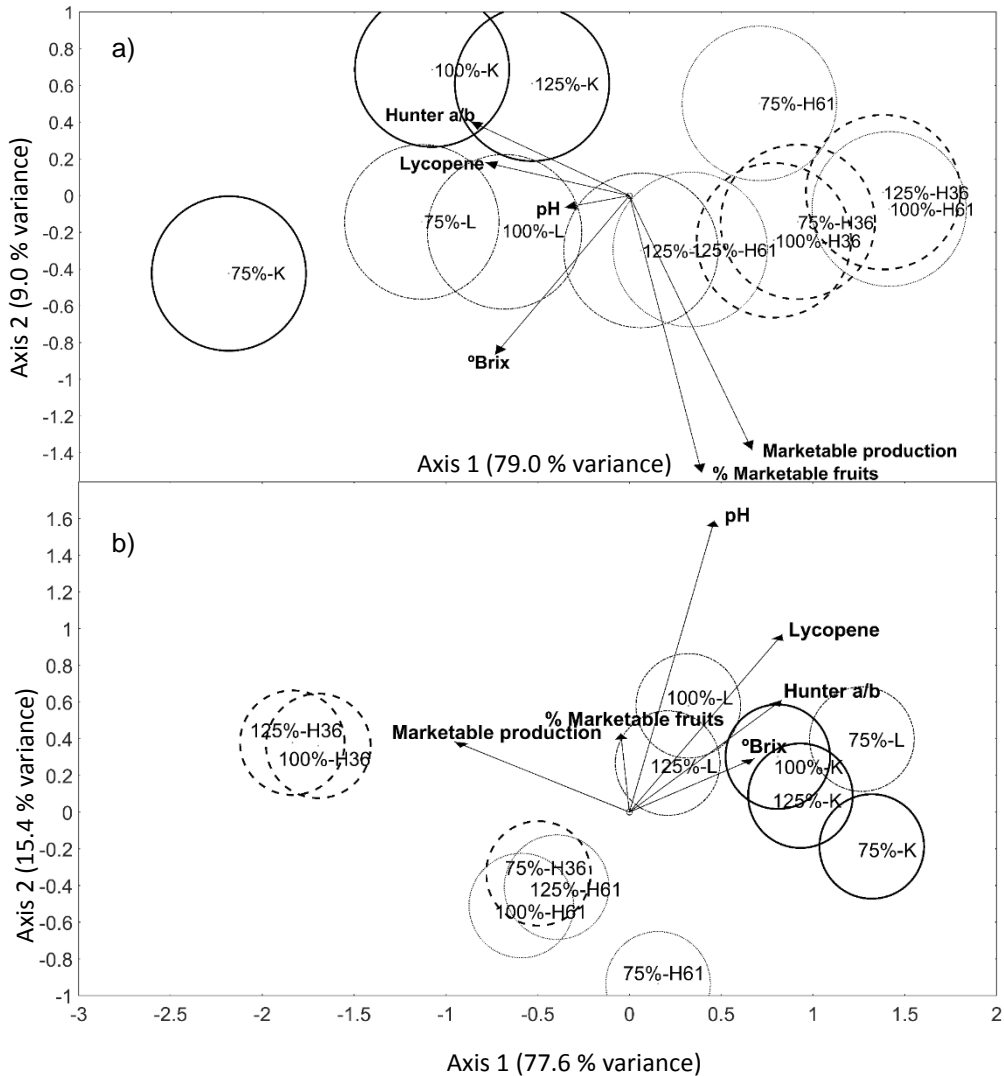
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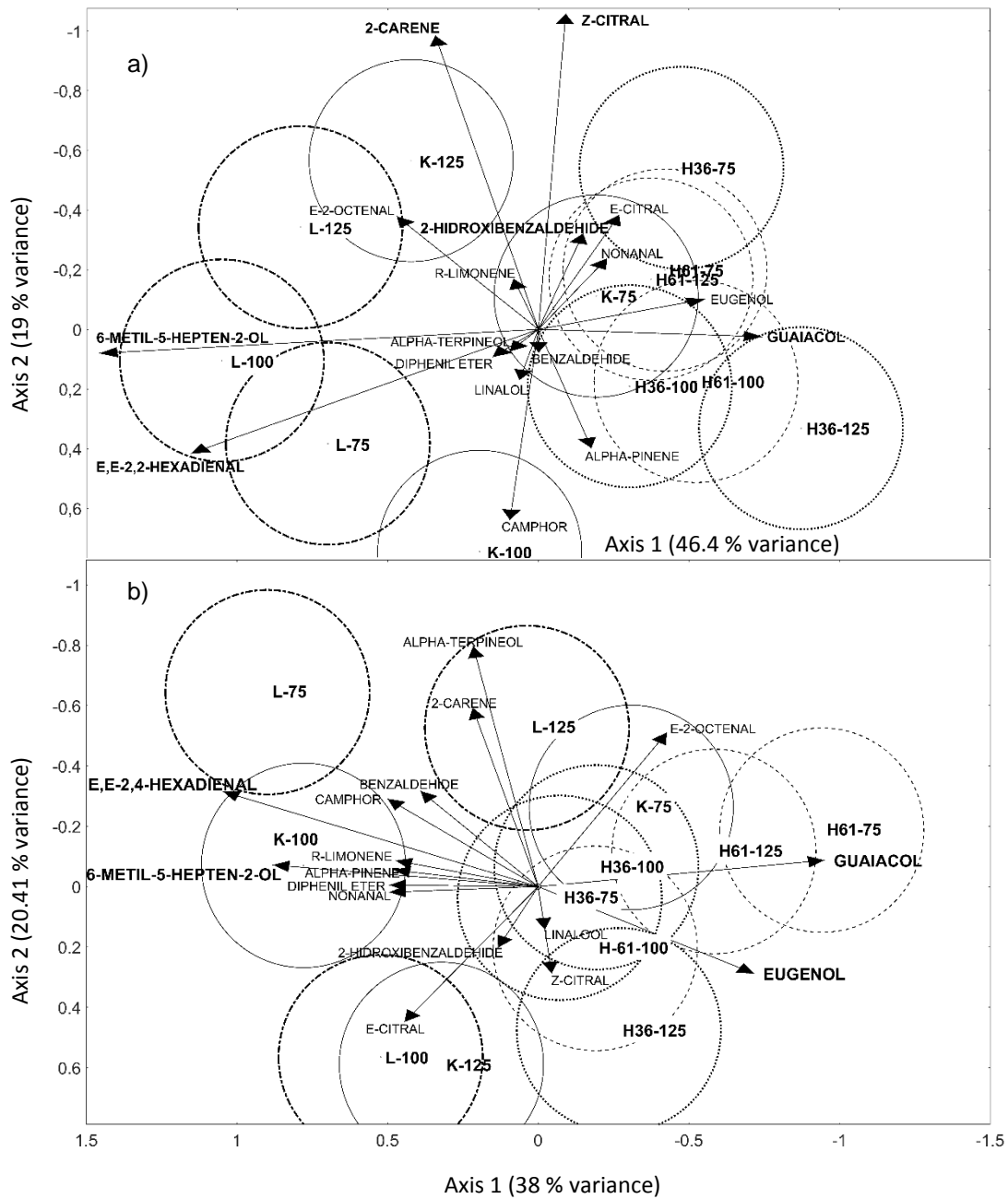
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758 **Supplementary Fig. 2.** MANOVA biplots of background volatiles concentrations obtained with
 759 the complete model including main factors and interaction for the years 2009 (a) and 2010 (b).
 760 For each data point the cultivar code (K: Kalvert, L: Loralie, H61: H-9661, H36: H-9036) and
 761 irrigation dose (75% ET_c, 100% ET_c, 125% ET_c) are included. Circles represent Bonferroni
 762 confidence intervals. Bold and higher font sizes represent significant effects in individual
 763 ANOVAs.
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766 **Supplementary Table 1.** Determinations of aroma compounds in tomato cultivars studied in 2009 (mean±s.e.; all compounds expressed in
767 ng g⁻¹)
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Irrigation	Accession	H-9036			H-9661			Kalvert			Loralie		
		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
(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	10.4±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-Terpeneol	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

769 n.d.: not detected

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

771 **Supplementary Table 2.** Determinations of aroma compounds in tomato cultivars studied in 2010 (mean±s.e.; all compounds expressed in
772 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

773 n.d.: not detected

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

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