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Highlights

The cultivar ‘Kalvert’ outstood for the accumulation of most of polyphenols

Genotype has a major effect on the accumulation of studied chemoprotective compounds

Strong environmental interactions were detected for phenolic content in certain cases

Effect of organic farming on L-ascorbic was dependent on cultivar and environment

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Running title: Genotype and organic farming effect on tomato phenolics and L-ascorbic

Polyphenol and L-ascorbic acid content in tomato as influenced by high lycopene genotypes and organic farming at different environments

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Abstract

The accumulation of polyphenols and L-ascorbic acid was evaluated under conventional (integrated pest management, IPM) and organic farming, as means to increase the accumulation of chemoprotective compounds. The effect of genotype was considerably higher than the growing system, in fact it is determining. 'Kalvert', a high-lycopene cultivar, outstood for the accumulation of most polyphenols, though low-carotenoid cultivars with high accumulation were also detected. Organic farming significantly increased the levels of caffeic acid by 20%, but reduced those of ferulic acid and naringenin by 13% and 15% respectively. A strong interaction with the environment was detected: in Navarra the differences were limited, while in Extremadura lower contents of ferulic acid and higher contents of chlorogenic acid and rutin were found in organic farming for certain cultivars. The effect of organic farming on L-ascorbic acid was dependent on cultivar and environment and it only led to an increase in Extremadura by 58%.

Keywords: *Solanum lycopersicum* L.; Organic farming; L-ascorbic acid; Functional quality.

Chemical compounds studied in this article Caffeic acid (PubChem CID: 689043); *p*- coumaric acid (PubChem CID: 637542); *trans*-ferulic acid (PubChem CID: 445858); Chlorogenic acid (PubChem CID: 1794427); Kaempferol (PubChem CID: 5280863); Quercetin (PubChem CID: 5280343); Myricetin (PubChem CID: 5281672); Naringenin (PubChem CID: 932); Rutin (PubChem CID: 5280805); L-Ascorbic acid (PubChem CID: 54670067)

Highlights

- The cultivar ‘Kalvert’ outstood for the accumulation of most of polyphenols
- Genotype has a major effect on the accumulation of studied chemoprotective compounds
- Strong environmental interactions were detected for phenolic content in certain cases
- Effect of organic farming on L-ascorbic was dependent on cultivar and environment

1. Introduction

Consumer awareness on the role of food in the improvement of health and in the prevention of many age-related diseases is becoming increasingly important. In this context, the main goal of aging research in not centered only in increasing lifespan but to improve health during life. Consequently, the development of better foods has become a major goal for the food industry. The fruit and vegetable market is well aware of these demands and tries to supply foods with increased levels of chemoprotective compounds and reduced levels of pesticides and, at the same time, assuring a production with a minimal impact on the environment.

Tomato (*Solanum lycopersicum* L.) is one of the most consumed vegetable worldwide, both fresh and processed. Although tomato does not outstand for its nutritional value nor for the content of chemoprotective compounds, it has become one of the main contributors of healthy components to diet considering the high consumption levels of this product (Chun et al., 2005). Among tomato chemoprotective compounds, carotenoids, polyphenols and vitamin C play an important role in this species.

Carotenoids are responsible for the ripe color of tomatoes. Lycopene is the most abundant carotenoid and its concentration ranges from 18.6 to 65.0 mg kg^{-1} fresh weight (fw) in different tomato varieties (Martínez-Valverde, Periago, Provan, & Chesson, 2002). The second carotenoid of importance is beta-carotene, its concentration is much lower than lycopene, reaching concentrations up to 12 mg kg^{-1} fw (Galpaz, Ronen, Khalfa, Zamir, & Hirschberg, 2006). The intake of tomato carotenoids has been linked with the prevention of certain types of cancer, and especially with prostate cancer (Giovannucci, 1999). In 2007 the US Food and Drug Administration concluded that there was limited evidence supporting an association between tomato consumption and reduced risk of prostate cancer (Kavanaugh, Trumbo, & Ellwood, 2007). But later studies continue pointing out the chemoprotective role of tomato (reviewed by Martí, Roselló, & Cebolla-Cornejo, 2016).

79 The main phenolic compounds found in tomato are the flavonoids rutin, naringenin,
80 naringenin chalcone and quercetin and the hydroxycinnamic acids chlorogenic and caffeic
81 acids (García-Valverde, Navarro-González, García-Alonso, & Periago, 2013; Martínez-Valverde
82 et al., 2002). Kaempferol can be found in tomato, but only in certain materials and low
83 concentrations (Martí et al., 2016). Among them, rutin, a glycoside of quercetin, is the main
84 tomato phenolic compound, with concentrations up to 31.1 mg kg^{-1} fw at the mature red stage
85 (García-Valverde et al., 2013). Polyphenols are gaining importance during the last years as they
86 seem to interfere with the initiation, promotion and progression of cancer. In fact, several
87 studies link the intake of polyphenols and the protection against different types of cancer
88 (reviewed by Martí et al., 2016).
89 Among vitamins, vitamin C is one of the most important in tomato. In this species, vitamin C
90 can be found at concentrations ranging from 85.5 to 560.0 mg kg^{-1} fw in different cultivars
91 (George, Kaur, Khurdiya, & Kapoor, 2004). These contents are low compared to other crops
92 such as *Brassicas*, berries, pepper, kiwi, *Citrus* or strawberry. Nevertheless, tomatoes represent
93 one of the main sources of dietary intake of vitamin C in Mediterranean diets (Garcia-Closas et
94 al., 2004). Apart from being recognized as an important antioxidant, vitamin C has been linked
95 with the protection against cardiovascular diseases (reviewed by Raiola, Rigano, Calafiore,
96 Frusciante, & Barone, 2014).
97 One of the strategies followed to satisfy consumer demands of functional quality in tomato
98 has been led by breeders, who have developed tomato varieties with increased levels of these
99 compounds. Cultivars such as 'Doublerich' with increased vitamin C were commercialized from
100 the mid-20th century. But the success of such cultivars has been limited due to the high
101 dependency on growing conditions and some deleterious effects. Nevertheless, new variants
102 are developed and new sources of variation are continuously described (Leiva-Brondo et al.,
103 2012). The development of cultivars with high lycopene contents has been more successful,
104 especially because it was linked to another important objective of the processing industry:

105 increasing red color intensity. Several mutants have been used for this purpose, some of them
106 altering specific steps of the biosynthesis pathway, and other altering the regulation of the
107 pathway. Among them, the most efficient rely on the use of *high pigment (hp)* mutations
108 (reviewed by Cebolla-Cornejo, Roselló, & Nuez, 2013).
109 Breeding efforts for enhanced polyphenol content lag behind. Nevertheless, the use of *hp*
110 mutations was initially targeted to increase carotenoid contents but it showed as side effects
111 increased polyphenol and vitamin C contents (Sestari et al., 2014). Consequently, the use of
112 this type of cultivars has gained importance to satisfy the demands of quality markets.
113 Another strategy to improve functional value focuses on the control of the growing
114 environment. In this context, organic farming can both offer tomato fruits with no traces of
115 pesticides nor fertilizers and assure a minimum impact on the environment. Still, consumers of
116 organic food seem to be more interested in the perception of good health, nutrients and taste
117 than in environmental concerns (Hughner, McDonagh, Prothero, Shultsz II, & Stanton, 2007).
118 But, can organic farming really increase the contents of chemoprotective compounds? In the
119 case of carotenoids, contradictory results have been obtained. Caris-Veyrat et al. (2004)
120 obtained higher carotenoid levels under organic farming, Riahi et al. (2009) found no effect of
121 the growing system and Rossi et al. (2008) observed only lower lycopene contents under
122 organic farming. Regarding polyphenols and vitamin C, Hallmann (2012) observed higher
123 contents of these compounds under organic farming. Although in a later study total phenolic
124 acids content was not affected by growing system (only chlorogenic acid content was higher
125 under organic farming), total flavonoid content and quercetin and rutin contents were higher
126 under organic farming (Hallmann, Lipowski, Marszalek, & Rembialkowska, 2013). Vinha,
127 Barreira, Costa, Alves, & Oliveira (2014) also found higher lycopene, vitamin C, total phenolics
128 and flavonoids under organic farming.
129 The existence of uncontrolled factors limits the possibility to extract clear conclusions on the
130 effect of growing system on the accumulation of bioactive compounds. Thus, new experiences

with new environments and farmers are required. Of special interest is the performance of high-lycopene cultivars, which may offer increased accumulation of chemoprotective compounds.

In a recent study, the role of high-lycopene cultivars and organic farming on the yield and quality of processing tomato was evaluated (Lahoz et al., 2016a). We found that the levels of lycopene were not affected by the growing system, while beta-carotene contents were higher under organic farming. The objective of this work is to deepen our knowledge on the accumulation of main tomato polyphenols and L-ascorbic acid, comparing the performance of standard and high-lycopene cultivars under organic farming and conventional Integrated Pest Management (IPM) at different environments.

2. Materials and methods

2.1. Chemicals and reagents

The polyphenols caffeic acid, *p*-coumaric acid, *trans*-ferulic acid, chlorogenic acid, kaempferol, quercetin, myricetin, naringenin and rutin, L-ascorbic acid, methaphosporic acid (MPA), hexadimetrine bromide (HDM), butylated hydroxytoluene (BHT), formic acid, HPLC-grade methanol (MeOH) and HPLC-grade acetonitrile (ACN) were purchased from Sigma-Aldrich (Steinheim, Germany). Boric acid and sodium hydroxide (NaOH) were provided by Panreac (Castellar del Vallés, Spain). Water was purified employing a Milli-Q water system (Millipore, Molsheim, France). Stock solutions of polyphenols were prepared at 500 mg L^{-1} in a MeOH/water (80:20 v/v) mixture and kept stored at -20 °C. The working solutions were prepared by direct dilution in MeOH/water (48:52 v/v). Stock solution of L-ascorbic acid was prepared at 1000 mg L^{-1} in water, stored at 4 °C.

2.2. Plant material and cultivation

156 A total of 6 commercial industrial tomato cvs. were grown in three different environments
157 representing two Spanish growing areas: Extremadura (south-west of Spain), and Navarra
158 (north-east of Spain). In a first step the evaluation was conducted in both locations during
159 2012 and a second evaluation with different climatic conditions was performed during 2013 in
160 Navarra. Cvs. studied were: 'CXD-277' (Campbell's seeds), 'Heinz(H)-9661', 'H-9997', 'H-9036'
161 (Heinz Seed), 'ISI-24424' (Diamond seeds S.L.; Isi Sementi S.P.A.) and 'Kalvert' (Esasem S.P.A.).
162 'H-9036' and 'H-9661' were considered as standard controls because they are extensively used
163 by local producers for their good agronomical performance. The materials were selected
164 considering their accumulation of carotenoids in previous studies. 'H-9661' and 'H-9036' were
165 included as low lycopene cvs. , H-9997' and 'CXD-277' as cvs. with intermediate accumulation
166 and 'ISI-24424' and 'Kalvert' as high-lycopene cvs (Lahoz et al., 2016a).
167 For each growing system, a randomized complete block design with 3 blocks per condition was
168 used, with 25 plants per block and condition. For both growing systems, plants were drip-
169 irrigated. The fertilization doses applied, as well as phytosanitary treatments were those
170 typically employed in each cultivation site and system.
171 In the case of conventional management with IPM, the plantation in Navarra was carried out in
172 the research fields of INTIA in Cadreita (Navarra) on May 10th in 2012 and on 23rd May in 2013.
173 In Extremadura, the plantation was carried out in the fields of the research center Finca "La
174 Orden-Valdesquera" (Badajoz, Extremadura) on April 24th in 2012. For Navarra, a spacing of
175 1.60 m x 0.35 m and two plants per plug (3.57 plants m^{-2}) was applied under a 15 μm
176 polyethylene plastic. In Extremadura, the same growing procedures were applied, however the
177 spacing was 1.50 m x 0.2 m (3.33 plants m^{-2}).
178 In the case of organic management, the plantation in Navarra was carried out in the fields of
179 the local organic farming business GUMENDI in Lodosa (Navarra) on May 4th in 2012 and on
180 May 17th in 2013. The edaphoclimatic conditions of both fields in Navarra (conventional IPM
181 and organic) were similar and close geographically. The spacing employed was the same as in

182 conventional IPM but a 15 μm biodegradable plastic Mater-Bi® was employed instead
183 polyethylene plastic. In the case of Extremadura, the plantation was carried out on April 24th in
184 2012 in the research center "Finca La Orden-Valdesquera".
185 Conventional production and organic farming were performed following the regulations for
186 Integrated Pest Management and Regulation on Organic Farming of each regional
187 administration (Extremadura and Navarra respectively). Fertilization was calculated
188 considering previous soil analysis (physical and chemical characterization and determination of
189 mineral, nitric and ammoniacal nitrogen, Nmin, in the 0-60 cm soil depth profile) and crop
190 extractions in each growing stage. For this purpose, mean yields of the used varieties in each
191 area of cultivation were considered. In conventional management in Extremadura basal
192 dressing included 50-90-90 kg ha^{-1} using a complex mineral fertilizer 8-15-15. Remaining
193 dressing (until reaching the global 200-140-250 kg ha^{-1} recommendation for the area) was
194 fertirrigated with a complex liquid fertilizer 20-15-15 and a weekly application considering
195 dose/phenology and a mean expected yield in the area of 80 t ha^{-1}. For organic farming, basal
196 dressing included 111-111-37 kg ha^{-1} applied using Agrimartin® FeBiologico (Fertinagro, Teruel,
197 Spain). Remaining dressing was applied at the onset of flowering, maximum crop development
198 and fruit setting. Blood meal, Protesan-15%N (ACP Europe, Ganollers, Spain) 125 kg N ha^{-1} and
199 Patentkali® 30% K_20/10% MgO/42% SO3(K+S Kali Gmbh Kassel, Germany) 700 kg ha^{-1} were
200 used for this purpose. In conventional management in Navarra basal dressing included 54-138-
201 180 kg ha^{-1}, applied using a complex mineral fertilizer 9-23-30. Remaining dressing once
202 considered initial Nmin (146,6 kg N ha^{-1} in 2012 and 152,8 kg N ha^{-1} in 2013) until reaching the
203 recommended 250 kg N ha^{-1} in this area was applied by fertirrigation using complex liquid
204 fertilizer N32 (Herogra, Abolote, Spain). Weekly applications started at the fourth week of
205 cultivation. In the case of organic farming basic dressing included 25 t ha^{-1} of ovine compost
206 with a mean N richness of 15 kg t^{-1}.

Maximum temperature and relative humidity were recorded using a HMP45C probe (Vaisala, Helsinki, Finland) in Navarra and Extremadura, and solar irradiance was recorded using a 110/S pyranometer (Skye, Powys, United Kingdom) in Navarra and a CMP3 pyranometer (Kipp&Zonen, Delft, the Netherlands) in Extremadura.

2.3. Sampling

Considering commercial practices, tomato samples were collected (a single harvest of red-ripe fruits for each cv. and growing system) when the 85% of tomato fruits of the plants were in the commercial-red stage. Two representative red-ripe tomato fruits were taken from each of the 25 plants of the replicates. Later in the laboratory, samples were washed with tap water, and a biological mean of each replicate was obtained blending longitudinal wedges of equivalent weight from each tomato until a completely homogeneous sample was obtained. Then, it was stored at -80 °C until analysis.

2.4. Analysis of polyphenols

Phenolic extraction was performed following the procedure described by Martí, Valcárcel, Herrero-Martínez, Cebolla-Cornejo, & Roselló (2015). Briefly, 1 g of homogenized sample was weighted and 5 mL of MeOH/water (48:52 v/v) containing 1 g kg^{-1} BHT were added. An ultrasonic extraction was done by immersing the samples in an ultrasonic bath Elmasonic S30H (Elma Electronics AG, Wetzikon, Switzerland) at a frequency of 60 Hz during 177 min. All extraction procedure was done in absence of light to avoid the oxidation of target compounds. The resulting extracts were centrifuged at 4000 rpm (2361g) during 5 min in an Eppendorf 5804R refrigerated centrifuge at 4 °C (Hauppauge, NY, USA). Supernatants were filtered through a 0.2 µm pore size polytetrafluoroethylene (PTFE) filter before their analysis by High Performance Liquid Chromatography (HPLC).

232 The separation and quantification of polyphenols was performed using an 1100 Series HPLC
233 system (Agilent Technologies, Waldbronn, Germany), equipped with a quaternary pump, a
234 degasser, an auto-sampler, and a diode array detector (DAD). The chromatographic column
235 used was a fused-core Kinetex-XB C18 column (150 mm x 4.6 mm internal diameter; particle
236 size, 2.6 μm) from Phenomenex (Torrance, CA, USA). The chromatographic analysis was
237 performed following the procedure described by Martí et al. (2015) with some modifications.
238 The flow rate was kept at 0.8 mL min^{-1} and the sample injection volume was set at 10μL, all the
239 separation procedure was performed at room temperature. The mobile phase solvents
240 employed were water, ACN and MeOH, acidified with formic acid 1 mL L^{-1}. A multi-segmented
241 gradient was performed varying MeOH and ACN concentrations from 30% and 0% to 24% and
242 18%, respectively, until minute 12, followed by a rise of MeOH concentration up to 30% at
243 minute 13 maintaining ACN concentration at 18%, finally MeOH concentration was decreased
244 from 30% to 20% meanwhile ACN concentration was raised from 18% to 30% until minute 20,
245 finally the initial conditions were recovered for the next sample injection. Detection and
246 quantification of polyphenols was performed using the DAD detector at different wavelengths
247 depending on each polyphenol. Thereby, 255 nm was used for rutin, 290 nm for naringenin,
248 320 nm for caffeic, *p*-coumaric, ferulic and chlorogenic acids, and 365 nm for kaempferol,
249 quercetin and myricetin. Absorption spectra were recorded for further identification of
250 compounds. Peak identification was done by comparing the elution times and the recorded
251 spectra, and when required, samples were spiked to support the identification. Samples were
252 analyzed twice.
253
254 **2.5. Analysis of L-ascorbic acid**
255 The extraction of L-ascorbic acid was performed following the method described by Galiana-
256 Balaguer, Roselló, Herrero-Martínez, Maquieira, & Nuez (2001) with some modifications.
257 Homogenized samples were centrifuged at 12000 rpm (10483g) during 5 min at 4 °C using a

5415R centrifuge (Eppendorf, Hauppauge, NY, USA). Resulting supernatants were diluted 1/10 with a 20 g L^{-1} MPA solution and filtered through a 0.2 µm pore size cellulose acetate (CA) filter before analysis.

The quantitation of L-ascorbic acid was performed using an Agilent Technologies 7100 capillary electrophoresis system (Waldbronn, Germany) equipped with a diode array detector. Uncoated fused silica capillaries (32cm total length, 24cm effective length, 375 µm outside diameter, 50 µm internal diameter) from Polymicro Technologies (Phoenix, AZ, USA) were rinsed at 50 °C with NaOH 1M during 5 min, followed by 5 min of NaOH 0.1M and 10 min of water prior its first utilization. Before each working session, the capillary was flushed at 25 °C with running buffer during 30 min. Between runs, capillary was flushed with running buffer during 3 min. Running buffer was prepared daily in an 400mM boric acid solution containing 1 g L^{-1} HDM adjusted to pH 8. All solutions and buffers were filtered prior injection through a 0.2 µm pore diameter CA filter. Injection was performed hydrodynamically at 3400Pa for 5s. A voltage of -15kV at 25 °C was applied. Detection and quantification was performed at 254 nm.

2.6. Statistical analysis

The effects of environment (site of cultivation and year), genotype, growing system, and their interactions on polyphenol content were evaluated with a MANOVA test using SPSS 22.0 software (NYSE: IBM, Armonk, USA). Pillai trace test was used to calculate p-value. Individual ANOVAs and Tukey B multiple range tests were performed to complement the analysis. Additionally, to ease a comprehensive study of the effect of genotype and growing system on polyphenols, a graphical MANOVA Biplot representation was obtained for the three environments. Bonferroni circles were used to represent the confidence intervals (α = 0.05), and their projection on each variable enable the identification of significant differences between groups. For the compounds in which the MANOVA biplot did not detect significant effects of the type of cultivation the vectors were marked in dashed lines. Multibiplot, a

284 freeware licensed software by Vicente-Villardón was used to perform the Biplot analysis.
285 Effects on L-ascorbic acid contents were analyzed separately with an ANOVA and Tukey B test,
286 as the biosynthesis pathway is different.
287
288 **3. Results and discussion**
289 **3.1. Effects on polyphenol profile: main effects**
290 The MANOVA test showed a significant effect ($p<0.01$) on the phenolic profile for all the
291 studied factors (environment, genotype and growing system), as well as their double and triple
292 interactions involving the environment. The specific effects on each compound were then
293 independently analyzed with ANOVAs (Table 1). In the case of kaempferol, contents were
294 below the limit of quantitation in the samples analyzed, and it was not included in the tables.
295 The environment affected polyphenol content, with higher contents of chlorogenic and ferulic
296 acids and lower levels of rutin in Extremadura than in Navarra in 2012 (Table 1). For the rest of
297 polyphenols similar concentrations were found in both sites. In Navarra in the conditions of
298 2013 higher contents of caffeic acid and myricetin and lower contents of quercetin were
299 observed.
300 The environment affected polyphenol content, with higher contents of chlorogenic and ferulic
301 acids and lower levels of rutin in Extremadura than in Navarra in 2012 (Table 1). For the rest of
302 polyphenols similar concentrations were found in both sites. In Navarra in the conditions of
303 2013 higher contents of caffeic acid and myricetin and lower contents of quercetin were
304 observed. The contents of rutin in Navarra 2013 were lower compared to Navarra 2012.
305 The effect of environment within a year involves changes in the site of cultivation. This effect is
306 extremely complex, as it implies differences in climate, soils, plant densities and even farmers.
307 Regarding climate, Extremadura is usually characterized by higher solar irradiance and higher
308 temperatures than Navarra, which is located in a higher latitude. Accordingly, plantation and
309 harvest in Extremadura are traditionally earlier. In this particular year, the differences were

not dramatic, though a higher number of days with maximum temperatures over 35 °C, as well as higher accumulated solar radiation and maximum temperatures, were recorded in this site of cultivation (Fig. 1).

It has been described that a dramatic increase of soluble phenolic compounds can be found as a response of plants to the stress produced by high temperatures (Rivero et al., 2001), although this study was performed under continuous stressing temperatures. Later, in a more detailed study Gautier et al. (2008) found higher levels of rutin in tomatoes exposed to high temperatures (32 °C) but only in higher irradiance conditions, while chlorogenic acid contents were not affected by temperature nor irradiance. Our results seem to indicate that for the accumulation rutin (a major polyphenol in tomato) intermediate conditions would be optimum, as it happens for lycopene accumulation (reviewed by Cebolla-Cornejo et al., 2013). Higher temperatures and irradiance levels such as those of Extremadura would limit the accumulation of rutin while the accumulation of hydroxycinnamic acids would not be affected. Intermediate conditions would favor the accumulation of rutin, at the expense of hydroxycinnamic acids, as in Navarra 2012. On the other hand, a further reduction in both temperature an radiation (Navarra 2013) would limit again the accumulation of major flavonols due to a limitation of photosynthesis, as carbohydrates are the substrates for flavonoid biosynthesis via the shikimic acid and phenylpropanoid pathways (Dorais, Ehret, & Papadopoulos, 2008).

Nevertheless, other explanations cannot be ruled out. Raffo, La Malfa, Fogliano, Maiani, & Quaglia (2006) could not find a correlation between different polyphenols and climatic parameters in cherry tomato, and in fact the accumulation of rutin and chlorogenic acid was not correlated. It cannot be discarded that in our case, as Raffo et al. (2006) also suggested, other uncontrolled factors (fertirrigation, plant density…) may have exerted a higher influence than the temperature and irradiance.

335 As expected, genotype had an important effect on polyphenol accumulation, with the
336 exceptions of ferulic and *p*-coumaric acids (Table 1). 'Kalvert' clearly outstood for the
337 accumulation of most polyphenols, including chlorogenic acid (13.80 mg kg^{-1} fw), caffeic acid
338 (2.19 mg kg^{-1} fw), rutin (36.33 mg kg^{-1} fw), myricetin (2.86 mg kg^{-1} fw), quercetin (1.41 mg kg^{-1}
339 fw) and naringenin (11.89 mg kg^{-1} fw). Although other genotypes with lower polyphenol
340 accumulation, in some cases attained similar contents in certain compounds. For example, 'H-
341 9997' also presented high concentrations of quercetin and naringenin and intermediate of
342 rutin, and 'H-9661' also outstood for rutin.
343 In previous works, high accumulation of carotenoids in 'Kalvert' and intermediate levels in 'H-
344 9997' were reported (Lahoz et al., 2016a). The presence in these cvs. of a *hp* gene could
345 explain the concomitant high levels of polyphenols, as higher amounts of these metabolites
346 have been found in materials carrying *hp-1* or *hp-2* mutations (reviewed by Martí et al., 2016).
347 Nevertheless, in that study 'ISI-24424' also presented intermediate carotenoid accumulation
348 and, accordingly, higher contents of polyphenols would also have been expected. Still, it is
349 difficult to establish whether a cv. is *hp* or not, since the genes used in the development of
350 each cv. are not revealed by breeding companies. On the other hand, the identification of high
351 contents of rutin in 'H-9661', a cv. with low carotenoid content (Lahoz et al., 2016a), opens the
352 door to find alternative genes, other than *hp*, targeted to improve polyphenol content. Even
353 more, both strategies could be joined to further increase these contents.
354 The effect of growing system was more limited and it was significant only for caffeic and ferulic
355 acids and naringenin (Table 1). The accumulation of caffeic acid was higher (by 20%) under
356 organic farming, while the accumulation of ferulic acid and naringenin was higher (by 13% and
357 by 15%) in conventional IPM farming. Hallmann (2012) found higher amounts of phenolic
358 compounds in organic tomatoes. In their work, organic fruits accumulated higher amounts of
359 rutin, myricetin and quercetin in comparison with conventional IPM ones. Mitchell et al. (2007)
360 reported higher levels of quercetin and kaempferol under organic farming, suggesting that

361 plants with limited N accumulate more flavonoids than those that are well-supplied. Caris-
362 Veyrat et al. (2004) also found higher concentrations of rutin and naringenin in organic
363 tomatoes, but chlorogenic acid contents were higher in conventionally grown tomatoes. As an
364 explanation, Oliveira et al. (2013) suggested that the higher concentrations of sugars, vitamin C
365 and polyphenols that they found under organic production would be related to stressing
366 conditions resulting in oxidative stress, as phenylalanine ammonia lyase, cell membrane lipid
367 oxidation and superoxide dismutase activities were higher in fruits grown under organic
368 farming. In our case, the differences are limited, probably suggesting that other factors (i.e.
369 farmers) might be more important. On the other hand, Anton et al. (2014) found, as in our
370 case, a limited effect of growing system, and they reported that polyphenols were more
371 dependent on year and genotype effects. Nevertheless, the existence of strong environmental
372 effects and interactions implied the necessity to evaluate the effect of growing system
373 specifically for each environment.
374 We determined polyphenol content in raw samples, but it should be considered that some
375 authors have found no effect of processing on total phenolics (Dewanto, Wu, Adom, & Liu,
376 2002), while others such as Gahler, Otto, & Böhm (2003) obtained increased levels (they
377 suggested that a possible release of phenolics from the matrix might explain it). In any case, it
378 seems clear that the higher contents observed in raw material would be useful to increase the
379 functional value of processed tomato.
380
381 **3.2. Effects on polyphenol profile: interactions**
382 As the MANOVA revealed strong interactions, it was necessary to perform separate MANOVA
383 biplot analysis for each environment, in order to determine the performance of each genotype
384 and growing system in each one. The analysis of the differences between MANOVA biplots
385 reveals the interaction effects. These biplots revealed the superior performance of 'Kalvert' in
386 the accumulation of most polyphenols, independently of the site of cultivation (Fig. 2),

387 revealing a high stability in the trait. For the rest of the cvs., the performance was strongly
388 dependent on the environmental conditions.
389 Under milder climate conditions, as those in Navarra, minor differences were found for the
390 majority of the cvs. between conventional IPM and organic farming. Even among cvs. only
391 'Kalvert' outstood and in this case, the differences between both growing systems were
392 limited, as the projections of the Bonferroni confidence circles overlapped for most vectors
393 (Fig. 2). It should be mentioned, though, that in Navarra 2013, 'ISI-24424' under organic
394 farming showed a different performance with higher caffeic acid and lower naringenin
395 contents, but this response was not reproduced in the other environments.
396 In conditions with higher irradiance and temperature, as in Extremadura, the specific
397 performance of each cv. would be more clear. In fact, in the biplot for Extremadura a clear
398 differentiation can be observed among cvs. The performance of intermediate content
399 genotypes such as 'ISI-24424' and 'H-9661' is more similar to 'Kalvert', while 'H-9997' outstood
400 for the accumulation of naringenin and caffeic acid. Low content genotypes, 'CXD-277' and 'H-
401 9036' showed a much lower accumulation than the rest. In this site, the difference between
402 organic and convention cultivation is mainly due to the higher accumulation of ferulic acid, as
403 for most cvs. the differentiation depends on the second component, which is parallel to the
404 vector of ferulic acid. For the rest of the compounds with significant effects of the type of
405 cultivation (solid lines in Fig.2) the differences are limited. Only in certain cvs., such as 'H-
406 9661', the projections on chlorogenic or rutin revealed slightly higher accumulation under
407 organic farming. Again, the effect of the growing system seems limited compared to the effect
408 of the genotype.
409
410 **3.3. Effects on L-ascorbic acid accumulation**
411 The environment and genotype had a significant effect on L-ascorbic acid concentration (Table
412 2). Higher accumulation was obtained in the conditions of Navarra 2012. Although other

413 explanations cannot be ruled out, it seems that the environment may justify this difference. As
414 reviewed by Dumas, Dadomo, Di Lucca, & Grolier (2003), light exposure is favorable to vitamin
415 C accumulation, and Liptay, Papadopoulos, Bryan, & Gull (1986) concluded that higher
416 temperatures (24°C vs. 31°C) enhanced ascorbic acid content. Therefore, the conditions of
417 Extremadura would favor accumulation. But, it has also been described that for similar
418 irradiance conditions, ascorbic acid concentration is lower at higher temperatures when the
419 range of temperatures is shorter (27°C vs. 32°C) and maximum temperatures are higher
420 (Gautier et al., 2008), as in our case. It seems that this may be the explanation for the higher
421 contents detected in Navarra, with less stressing temperatures. Regarding the differences
422 between Navarra 2012 and 2013, the lower contents of the latter would be caused by the
423 lower radiation and temperatures registered during this year (Fig. 1).
424 Regarding the genotype effect, L-ascorbic acid accumulation ranged from 107.54 to 136.37 mg
425 kg^{-1} fw. 'CXD-277' again was the cv. with the lowest accumulation. In this case, the low
426 accumulation of polyphenols and L-ascorbic acid of this cv. contrasts with the relatively high
427 accumulation of carotenoids and, especially, of sugar and acids observed in previous studies
428 (Lahoz et al., 2016a). On the other hand, the highest levels corresponded to 'H-9661' and
429 'Kalvert'. The high accumulation observed in 'Kalvert' might be related to the presence of a *hp*
430 mutation, as these mutations have been linked with increased levels of carotenoids,
431 polyphenols and ascorbic acid (reviewed by Martí et al., 2016) This cv. also offers a great
432 accumulation of sugars, acids and even aroma compounds (Lahoz et al., 2016a; Lahoz et al.,
433 2016b), thus proving to be an ideal cv. targeted to high quality markets. Nevertheless, the
434 price premium paid should compensate its lower yield compared to conventional cvs. such as
435 'H-9036' or 'H-9661') (Lahoz et al., 2016a).
436 Growing system did not affect L-ascorbic acid accumulation (Table 2). Nevertheless, a strong
437 environmental effect and interactions were detected. In fact, in 2013 an outbreak of *Alternaria*
438 was detected, and consequently the results had to be analyzed independently for each

environment (Fig. 3). As a result, the effect of growing system in Extremadura (with higher radiation and temperature) was significant, increasing mean cultivar L-ascorbic contents by 58%. In the milder conditions of Navarra 2012 the effect was not significant, while in Navarra 2013 it was negative and contents under organic farming were reduced by 45% (Fig. 3).

In Extremadura, the lower contents detected in conventional IPM growing might be related to the fertilization applied. Dumas et al. (2003) described that high rates of nitrogen fertilizers tend to decrease vitamin C content in tomato. In the same sense, Toor, Savage, & Heeb (2006) found higher levels of ascorbic acid in tomatoes grown with organic fertilization than with mineral nutrient solutions. But again, as stated above, the effect of organic farming may be also a response towards stressing conditions under this management (Oliveira et al., 2013).

Several authors have observed a similar trend. Caris-Veyrat et al. (2004), Chassy, Bui, Renaud, Van Horn, & Mitchell (2006) and Vinha et al. (2014) found considerably higher levels of ascorbic acid under organic farming (higher than 30%, 23%, 30%, respectively). Other authors such as Hallmann (2012) have also observed this effect, but much more limited (in this case the difference was limited to a 1% difference). Similarly, Juroszek, Lumpkin, Yang, Ledesma, & Ma (2009) found no significant differences in the content of ascorbic acid between conventional and organic farming management. Maybe these conditions resemble those of Navarra 2012, where the increase in 5.8% of L-ascorbic under organic farming was not significant.

On the other hand, Rossi et al. (2008) observed lower levels of vitamin C content under organic farming (almost one half). This behavior is similar to that found in Navarra in 2013. Although the reduction observed in ascorbic acid contents in Navarra 2013 should be carefully handled, as following an *Alternaria* pathogen infection an oxidative burst reaction characterized by the rapid production of reactive oxygen species (ROS) occurs, and defense mechanisms including ascorbate peroxidase activities are triggered to scavenge the excess of H_2O_2 (Meena et al., 2016). Consequently, a reduction in the pool of ascorbate would be expected when oxidative

465 damage occurs (Ding et al., 2009). It is impossible to determine if the reduction observed in
466 this environment was due to the growing system or to the infection. Nevertheless, it seems
467 clear that organic farming with reduced preventive and curative measures will be exposed to
468 reductions in L-ascorbic contents.
469 Apart from the obvious great differences between both cultivations systems and their
470 application by different research groups or farmers, the specific selection of genotypes for
471 each study may play an important role to justify these differences, as Caris-Veyrat et al. (2004)
472 found that some varieties may increase ascorbic acid levels under organic farming, while
473 others remained with similar levels. This trend was also observed in our study, with a higher
474 impact of organic farming on the contents of 'CXD-277' than in the rest of cultivars (Fig. 3).
475 The benefits of genotype selection and growing environment on processing tomato may be
476 useful only for certain products. Vitamin C is unstable at high temperatures, therefore
477 processing tends to reduce its contents. Dewanto et al. (2002) and Gahler et al. (2003)
478 confirmed the decline in vitamin C with increasing heating time and processing steps in
479 different tomato products. Nevertheless, Gahler et al. (2003) observed that in some products
480 this decline can be compensated by the loss of water and an increase in dry matter. In any
481 case, the higher contents observed in raw material would, again, be useful to increase the
482 functional value of processed tomato.
483
484 **4. Conclusions**
485 The use of high lycopene cvs. such as 'Kalvert' can offer increased levels of polyphenols and L-
486 ascorbic acid, that joined to the high levels of carotenoids, sugars and acids and aroma
487 volatiles, positions this type of materials as ideal to satisfy the demands of high-quality
488 markets. Nevertheless, considerably amounts of polyphenols and L-ascorbic acid can be also
489 detected in conventional cvs. with higher yields. The genotype effect has a considerably higher
490 impact on the accumulation of these chemoprotective compounds than the growing system

491 selected. Organic farming has a limited effect on the accumulation of polyphenols, which is
492 highly dependent on the site of cultivation. On the other hand, organic farming increases L-
493 ascorbic acid contents, though this increase again depends on the cultivar and site of
494 cultivation considered.
495
496 **Conflict of interest**
497 The authors declare no conflict of interest.
498
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630

631

Figure 1. Climate conditions in Navarra (Nav 2012 and Nav 2013) and Extremadura (Ext 2012) during the cultivation periods. Vertical lines indicate plantation (P) and harvest (H) dates in Navarra (N) or Extremadura (E) during the year 2012 (12) or 2013 (13) and under conventional (C) or organic farming (O) management. When starting (s) end ending dates (e) were different, it is indicated with lower case letters.

Figure 2. MANOVA biplot for polyphenol accumulation in Extremadura 2012, Navarra 2012 and Navarra 2013 under conventional (Conv-cv. name) or organic farming (Org-cv. name). Polyphenol abbreviations: chlorogenic acid (Chlor), caffeic acid (Caff), *p*-coumaric acid (*p*-Cou), ferulic acid (Fer), rutin (Rut), myricetin (Myr), quercetin (Quer), naringenin (Naring). Circles represent Bonferroni confidence intervals. Circles with solid lines correspond to organic farming and circles with dashed lines correspond to conventional farming. Significance of differences is inferred when the projections of confidence circles on each vector do not overlap. Solid vector lines represent significant effects of the type of cultivation and dashed lines represent no significant effects of this factor.

Figure 3. Mean increase (%) of L-ascorbic acid content when the plants were cultivated under organic farming compared to conventional management. Left: Mean effect of the cultivar in each environments. Right: Mean effect of the environment (Extremadura 2012, Navarra 2012 and Navarra 2013) considering all the cultivars.**ANOVA *p*-value<0.001; ns not significant.

Table(s)

Table 1. Effect of the site of cultivation, genotype and growing system on polyphenol content expressed in mg kg^{-1} of fresh weight

		Chlorogenic acid	Caffeic acid	*p*-Coumaric acid	Ferulic acid	Rutin	Myricetin	Quercetin	Naringenin
Environment	*p value*	<0.001	<0.001	0.036	<0.001	0.001	<0.001	<0.001	0.196
(S)	Extremadura 2012	13.90^{b}*	0.98^{a}	0.32^{b}	0.61^{b}	20.36^{a}	0.85^{a}	1.08^{b}	9.86^{a}
	Navarra 2012	6.91^{a}	0.94^{a}	0.30^{ab}	0.46^{a}	25.86^{b}	0.81^{a}	0.94^{b}	8.83^{a}
	Navarra 2013	8.10^{a}	1.82^{b}	0.22^{a}	0.48^{a}	21.21^{a}	1.82^{b}	0.49^{a}	8.86^{a}
Genotype	*p value*	<0.001	<0.001	0.510	0.025	<0.001	<0.001	<0.001	<0.001
(G)	'CXD-277'	7.53^{a}	0.82^{a}	0.26^{a}	0.48^{ab}	14.74^{a}	0.86^{ab}	0.48^{a}	7.53^{a}
	'H-9661'	9.66^{a}	0.97^{ab}	0.29^{a}	0.53^{ab}	29.72^{c}	0.64^{ab}	0.64^{a}	8.72^{a}
	'H-9997'	8.85^{a}	1.15^{b}	0.28^{a}	0.51^{ab}	20.63^{b}	1.12^{b}	1.14^{bc}	11.75^{b}
	'H-9036'	8.81^{a}	0.97^{ab}	0.35^{a}	0.61^{b}	12.39^{a}	0.17^{a}	0.52^{a}	6.49^{a}
	'ISI-24424'	9.15^{a}	1.37^{c}	0.25^{a}	0.42^{a}	21.06^{b}	1.30^{b}	0.81^{ab}	8.71^{a}
	'Kalvert'	13.80^{b}	2.19^{d}	0.26^{a}	0.54^{ab}	36.33^{d}	2.86^{c}	1.41^{c}	11.89^{b}
Growing system	*p value*	0.155	<0.001	0.054	0.031	0.749	0.509	0.656	0.008
(C)	Conventional	9.31	1.13	0.25	0.55	22.29	1.21	0.86	9.90
	Organic	9.96	1.36	0.31	0.48	22.67	1.11	0.81	8.46
SxG	*p value*	0.12	<0.001	<0.001	0.001	<0.001	<0.001	0.068	0.011
SxC	*p value*	<0.001	<0.001	0.124	0.021	<0.001	0.115	0.050	0.284
GxC	*p value*	0.075	<0.001	0.358	0.015	0.012	0.050	0.169	0.436

*Different letters indicate significant differences at p <0.05 (Tukey B test).

Table(s)

Table 2. Effect of the site of cultivation, genotype and growing system on L-ascorbic acid content expressed in mg kg^{-1} of fresh weight

	p value		L-ascorbic acid
Environment (E)	<0.001	Extremadura 2012	113.94[a]*
		Navarra 2012	155.82[b]
		Navarra 2013	100.56[a]
Genotype (G)	0.007	'CXD-277'	107.54[a]
		'H-9661'	136.37[b]
		'H-9997'	117.19[ab]
		'H-9036'	132.11[ab]
		'ISI-24424'	112.40[ab]
		'Kalvert'	135.02[b]
Growing system (C)	0.933	Conventional	123.21
		Organic	123.67
ExC	<0.001		
ExG	0.002		
CxG	0.106		
ExGxC	0.318		

*Different letters indicate significant differences at p <0.05 (Tukey B test).

Figure(s)

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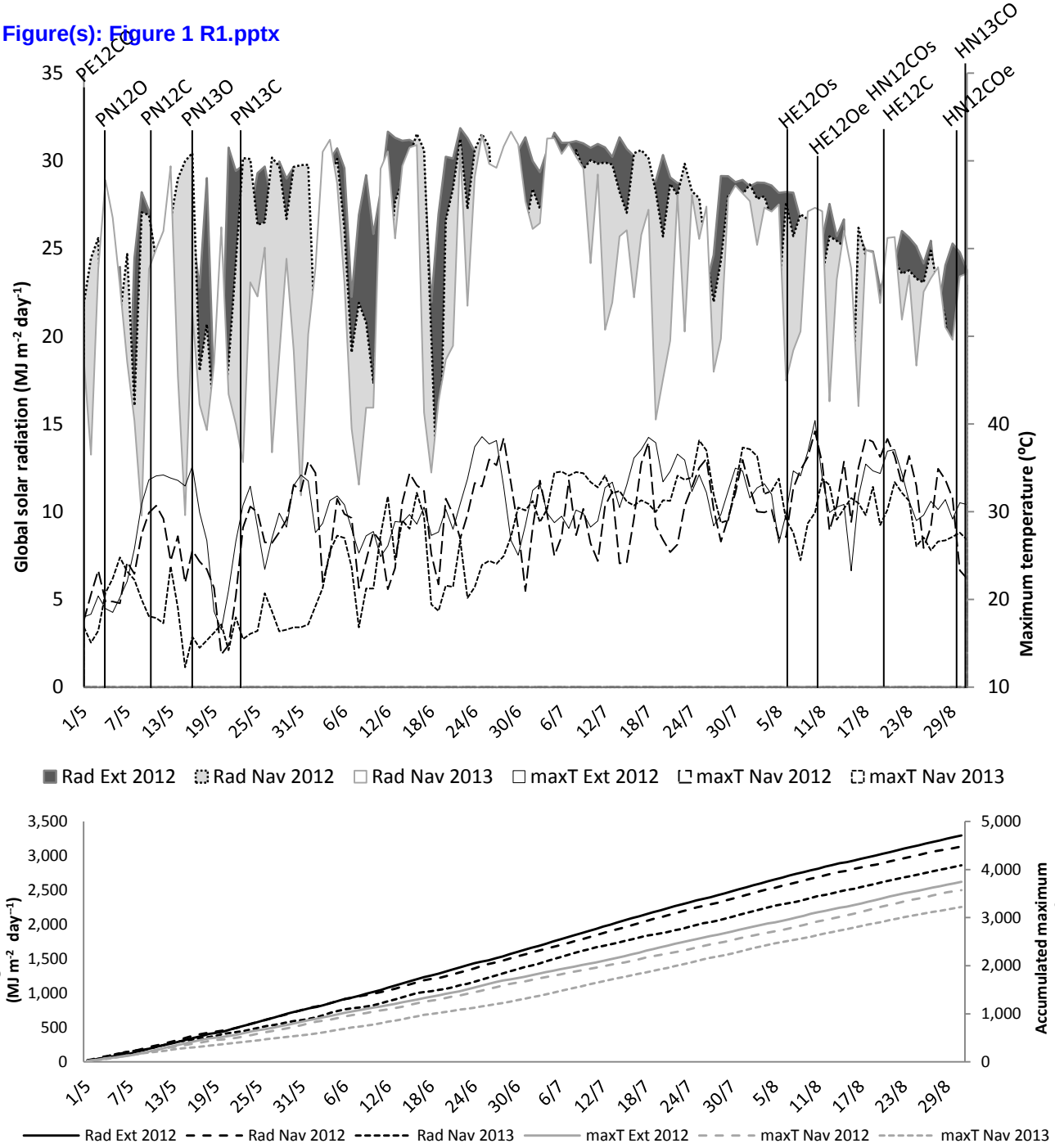

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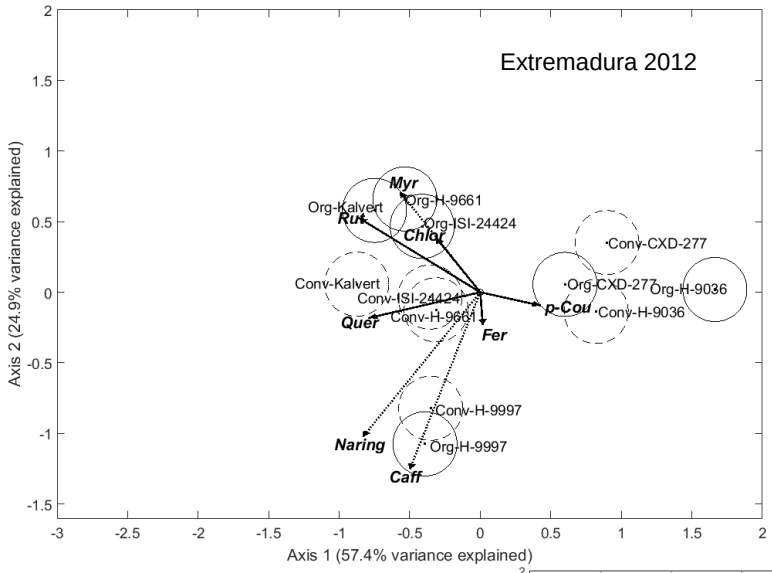

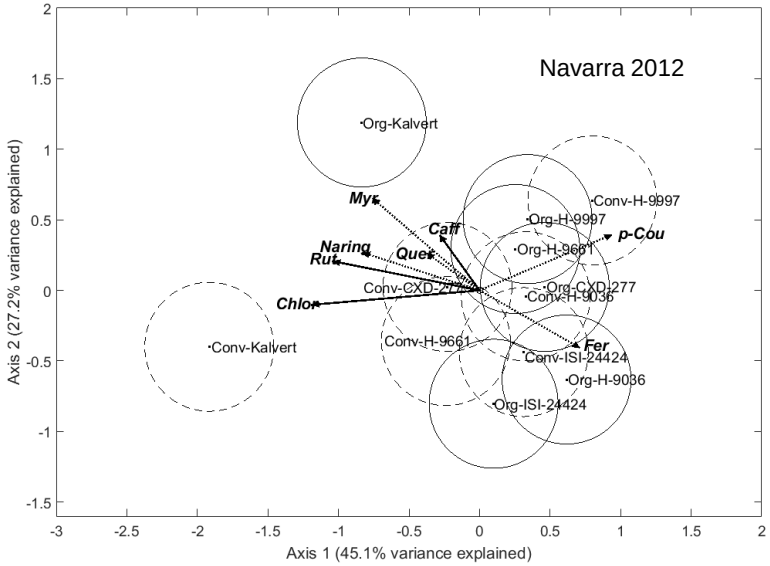

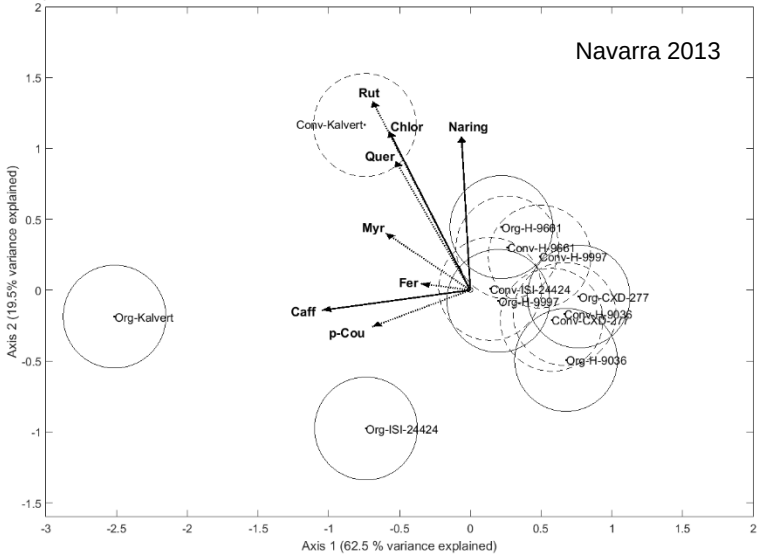

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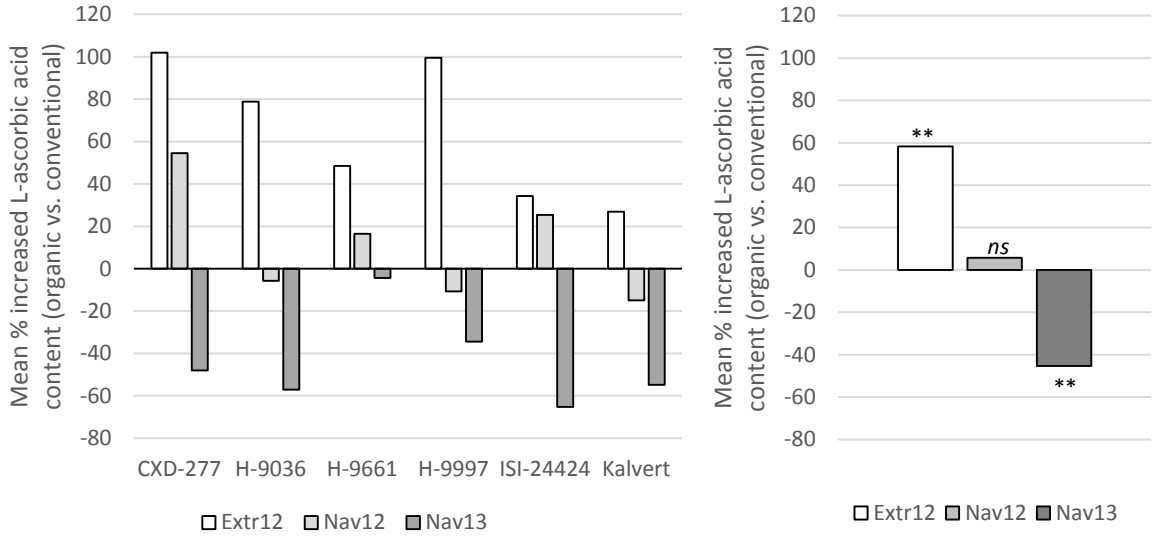
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