

Article

The Profile of Bioactive Compounds in Two Tomato Lines (*hp-2* and *Atv_aft*) When Grown under Low-Input Farming Conditions with or without Mulching and Intercropping

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Abstract: The work analyzed the effect of two types of low-input farming conditions on the yield components and on the bioactive compounds in the pulp and peel of tomato fruits. The first low-input (LI) system involved the application of cow manure and manual weed control; in the second (LI^{MI}), the same system was integrated with mulching (the wood chips of eucalyptus) and intercropping (basil and cabbage plants). The study included the line 392, harboring the *hp-2* gene that increases the pigments of plant and fruit; the line 446 with the *atv* and *Aft* genes which influence the content of polyphenols; and a commercial control (cv. Rio Grande). The experimental design was a split-plot where the farming system (LI and LI^{MI}) was allocated in the main plot and the genotype was in the sub-plot. Within the main plot, each genotype was replicated three times in three randomized blocks. Mulching and intercropping led to a differentiation in the LI^{MI} with respect to the LI system with higher values of the leaf greenness index (61.3 vs. 53.3 Spad units), the number of fruits (70 vs. 46), and the weight of fruits per plant (2716.6 vs. 2195.0 g). However, the LI system showed a higher content of polyphenols (+37.9%) and anthocyanins (+116.7%) in the peel and a higher content of vitamin C (+44.0%) and polyphenols (+11.1) in the pulp. The less complex LI system stimulated the plants to produce natural antioxidant systems to contrast biotic and abiotic offenders, while the introduction of mitigation elements in the LI^{MI} system reduced the need for protective barriers against the environmental stress. The study also revealed that low-input systems can allow for satisfactory yields, minimizing the use of off-farm resources. Growers can combine factors of sustainable agriculture with specific genotypes to maximize the production of healthier foods.

Keywords: sustainable agriculture; tomato mutants; carotenoids; polyphenols



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1. Introduction

The “Farm to Fork” strategy within the European Green Deal is aimed at fostering the production of healthy food with a neutral or positive environmental impact through a strong implementation of sustainable agro-ecological and organic practices [1]. In this context, exploring and encouraging the adoption of sustainable practices and introducing elements of impact mitigation within conventional techniques becomes a priority.

Sustainable agriculture encompasses an array of farming systems which place great emphasis on how food is produced, with a specific concern on fulfilling human needs while respecting natural resources [2]. The systems satisfying the definition are many and some of them were formulated at the beginning of the past century, coexisting with others which have been more recently conceptualized [2–4]. There are no doubts that the adoption of environmentally friendly approaches of food production are by now necessary

to preserve the available natural resources. Even without starting long and complex conversion processes, it is possible to introduce “mitigation” elements in conventional farming that are inspired by sustainable systems, such as organic or synergistic agriculture. Another approach consists of the adoption of low input farming systems, optimizing the farm management and the use of resources while minimizing the use of external resources, such as fertilizers and pesticides [5]. This is particularly true for high-demanding crops such as tomato (*Solanum lycopersicum* L.).

Italy is the greater producer of processing tomato in Europe, with more than 5.1 mil tons collected in 2022 [6]. The species is cultivated on slightly more than 70,000 ha, with an average yield of product close to 70 t ha⁻¹. Owing to the presence of functional compounds as carotenoids, polyphenols, and vitamin C, many studies have highlighted the role of tomato consumption in the prevention of chronic diseases [7,8]. Tomato breeding is providing lines characterized by a variety of fruit pigmentations, from yellow to black [9], determined by the preferential accumulation of such functional compounds. Genes affecting the color of tomato fruit have been known for many years [7,10,11]. Mutations often involve enzymes of specific metabolic pathways [12–14] or transcription factors which control the expression of genes related to the production of specific compounds [15–17]. The availability of these variants can favor the spread of genuine new functional food. An emerging application concerns the use of natural carotenoids and lycopene as a nutritional supplement [8,18].

The *high pigment-2* (*hp-2*) gene increases all the pigments in plant and fruit, with particular reference to carotenoids [10,15], but it is able to affect also the content of anthocyanins [19]. A more pronounced effect on the polyphenols and anthocyanin content is caused by the presence of the variants *atv* (*atrovioleaceum*) and *Aft* (*Anthocyanin fruit*). The first one (*atv*) determines the accumulation of anthocyanins in the vegetative parts, while the dominant allele *Aft* allows for the build-up of anthocyanins in the epidermal layer of the fruit in the presence of a sufficient light level [11]. The breeding activity led to the selection of a line (*Aft_atv*) of fruits with purple peel and a red-colored pulp [17,20].

The objective of this work was to evaluate the behavior of tomato lines selected for their high content of bioactive substances when grown with two different low-input farming systems. In detail, we analyzed the yield components and the content of bioactive compounds in the pulp and peels of two tomato lines harboring the *hp-2* or the *Atv_apt* genes. The first low-input system (LI) involved just the application of cow manure as the fertilizer, manual weed control, and the use of products authorized in organic farming to limit the biotic constraints; in the second (LI^{MI}), the same system was integrated with mitigation measures as the application of mulching with on-farm resources and the presence of intercropping. The approach presented in this study aims to introduce solutions for novel cultivation methods based on “natural horticulture” (NH), whose objective is to transform short-term monoculture rotations to long-term biodiverse rotations. In this regard, intercropping is a strategy that increases the biodiversity of the cultivations, which are otherwise too simplified, and in turn leads to an increase in soil micro-organisms and the diversity of mesofauna and macrofauna, with a positive effect on the soil quality. As reported by [2] for the agroecology concept, biological complementarities occurring with mixed crops in a single plot, such as intercropping, improve the nutrient and input efficiency, the use of space, and the regulation of pests, with the final result being the enhanced stability of the crop yield. To achieve the highest biodiversity, the principles of NH require that plants must belong to at least three botanical families, chosen among species able to promote the maximum synergic effect between vegetables or to exploit the repellent or allelopathic effects against pests [21]. On the other side, permanent mulching replaces the tillage in soil management by means of the creation of a homogenous topsoil layer, the amelioration of the structure stability, and the control of weeds and edaphic pests due to its suppressive effect [21]. Intercropping and mulching are, in this case, coupled to the growth of tomato lines of interest for their capacity of producing antioxidant compounds in the fruit. The work wished to test the following hypotheses: (i) low-input

systems can allow satisfactory yields, minimizing the use of external resources, and (ii) sustainable agro-ecologic systems may enhance the production of bioactive compounds.

2. Materials and Methods

2.1. Plant Material

The study compared two true (not isogenic) breeding lines selected by the Dept. of Agriculture and Forest Sciences (DAFNE) of the University of Tuscia (Viterbo, Italy), plus a commercial processing variety as a control (Table 1).

Table 1. Description of the tomato genotypes used in the study.

Line/Variety	Mutant Genes	Modified Bioactive Compound Class	Fruit Type	Code	Ref.
V711392 (Breeding line)	<i>hp-2</i>	All	Round, flesh and peel red	392	[15]
V710446 (Solenero®)	<i>Aft atv</i>	Polyphenols (Plant and fruits)	Round, red flesh, black peel	446	[20]
Rio Grande (Processing variety)	-	None	Oval, flesh and peel red	RG	

The lines and the variety had determined the growth habit. The breeding lines brought the *hp-2* gene (V711392) and the combination *Aft atv* with purple fruits (V710446): the presence of mutant genes influencing the pathway of carotenoids and/or polyphenols caused a modification of the bio-compounds content in the plant, the peel, or the pulp of the fruits (Figure 1).

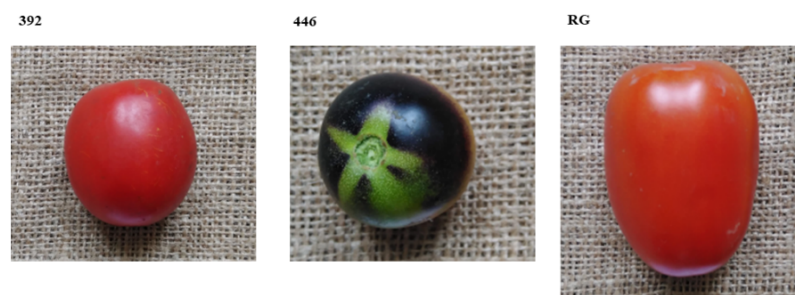


Figure 1. Fruit type of the genotypes used in the study.

The V711392 genotype was obtained by selecting, within the segregation of a processing F₁ commercial hybrid and fixed in the F₅ generation for the *hp-2*, self-pruning (*sp*) and uniform (*u*) traits. The V710446 genotype was obtained after crossing an *Anthocyanin fruit* (LA1996) variant genotype and the *atrovioleaceum* mutant (LA0797), two introgression lines obtained from the C.M. Rick Tomato Genetics Resource Center (University of California, Davis, CA, USA). The V710446 *AftAftatvatv* combination was fixed in the F₅ generation, as described in the literature [20].

2.2. Field Experiment Description

Plantlets at the 4–5th true leaf stage were transplanted on May 2019 at the experimental field of the CREA Research Centre for Engineering and Agro-Food Processing, Monterotondo, Italy (42° N 05'56.86", 12° E 37'26.23"). The soil characteristics are shown in Table 2. For at least three years, the soil was fertilized with cow manure and no chemicals were used for the control of any pathogen or pest. The preceding crop was broccoli (*Brassica oleracea* var. *italica*).

Table 2. Soil physical and chemical properties.

Soil Properties	U.M. *	
Sand	%	25
Silt	%	38
Clay	%	37
pH		7.9
Total nitrogen (N)	%	0.16
Assimilable phosphorous (P)	mg kg ⁻¹	14.1
Exchangeable potassium (K)	mg kg ⁻¹	365.3

* Unit of measurement.

The experimental design was a split-plot where the farming systems (LI or LI^{MI}) were the main plot and the genotype (RG, 392, and 446) was the sub-plot. Within the main plot (farming system), each genotype was replicated three times in three randomized blocks.

The elemental plot of each genotype was composed by ten plants arranged in twin rows: the twins were spaced 100 cm (center-to-center), and the distance between the rows and between the plants within the rows was 40 cm. The plot size in the LI area was 2.8 m², corresponding to a plant density of approx. 3.5 plants m⁻² (35,000 p ha⁻¹). The whole LI^{MI} surface was mulched with the dried wood chips of eucalyptus produced in the same year. At both sides of the twin of the elemental plot, tomato was intercropped with one row of basil and one row of cabbage plants. Basil and cabbage were transplanted 40 cm apart from the tomato in the space left between two adjacent twin rows. Therefore, the plot was larger (2.2 m × 2 m) in the LI^{MI} system but the plant density for tomato remained approx. 3.5 m² (35,000 p ha⁻¹). Mulching and intercropping were introduced to favor the exploitation of the water supply, the improvement in the soil's structure, the increase in the organic matter, as well as to take advantage of the positive effect of increased biodiversity for reducing the chemical input.

During the cultivation, plants were threatened by common pathogens (late blight) and parasites (southern green shield bug, tomato pinworm) that challenged the defense systems and required their control. In both areas (LI and LI^{MI}), the weeding was manual, and the pathogens or pests were controlled using the products authorized in organic farming (Table 3).

Table 3. Treatments, compounds, and doses used in the experiment as related to farming system.

Product	Farming System	Dose	N. of Treatments
Copper sulphate	LI and LI ^{MI}	3 g L ⁻¹	1
NeemAzal [®]	LI	2 mL L ⁻¹	1
Rapax [®] (<i>Bacillus thuringiensis</i>)	LI and LI ^{MI}	2 mL L ⁻¹	3
Tioflor	LI	5 g L ⁻¹	2

The plants were drip irrigated with lines placed between the rows. The water supply complied the indications provided by the 2019 Integrated Production Regulations for processing tomato (<http://agricoltura.regione.emilia-romagna.it/produzioni-agroalimentari/temi/bio-agro-climambiente/agricoltura-integrata/disciplinari-produzione-integrata-vegetale/Collezione-dpi/2019/orticole-2019>, accessed on 12 January 2022).

Meteorological data were collected by the Arsiat control unit of Monterotondo (RM), location: Grotta Marozza (92 m asl) (https://www.siarl-lazio.it/E1_2.asp, accessed on 12 January 2022).

2.3. Field Measurements

The flowering date (DFW) was taken at the opening of flowers at least in four plants of the single plot and was expressed in the days after transplant (DAT). The plant height (PLH) was measured before the collection of the first fruits at the beginning of August,

80 days after transplanting (DAT). The leaf greenness index (LGI) was checked on the imparipinnate leaflet and on the two underlying leaves of the composed leaf at 30, 60, and 80 DAT using a SPAD-502 Chlorophyll meter (Minolta Inc., Osaka, Japan). The PLH and the LGI were measured on the four central plants of each plot.

Soon after the transplant, three plants in the middle of the plot were tagged and used as a sample to examine the productive traits. The fruits produced by each line were collected at full ripening (red ripe). To ensure the harvesting at the same ripening stage, the collection of the fruits required three harvesting dates in the following periods: the first and third decade of August and the first decade of September. The final harvest also involved the species associated with tomatoes in the LI^{MI} system (cabbage and basil). The specific observations concerned:

- (1) Fruit set (FS%). The ratio (%) between the number of fruits (TNF) and the number of flowers (NFL) observed before the first harvesting on the first and second truss of the tagged plants;
- (2) The total fruit number (TNF) and total weight of fruits (TWF) per plant. The weight was measured by a precision balance Kern (Stuttgart, Germany) mod. KB 10000-1N (d = 0.1 g);
- (3) The total soluble solid content (°Brix) was measured with an optical refractometer. The reading was made by placing one–two droplets of the juice extracted from five red ripe fruits randomly chosen in each single plot;
- (4) The pH value was measured on the juice (pHmeter Crison mod. 50 50 T, Barcelona, Spain).

2.4. Analysis of Bioactive Substances

About 1.5 kg of fruits of each genotype grown in the LI and LI^{MI} system were used for the determination of the content of vitamin C, polyphenols, and carotenoids. The fruits were carefully washed in tap water and then boiled for 15 min. The tomato pomace (peels and seeds) was separated from the pulp by an electric tomato squeezer (Bialetti, Italy). For each tomato line and farming system, three pulp and pomace samples were prepared: about 40 mL of pulp extract were collected in 50 mL Falcon tubes; the corresponding pomace (about 100 g each) were put in food bags. Both types of samples were stored at −20 °C until their use. At the LAMeT (Laboratory of Territorial and Products Analysis) of the University of Cassino, the samples were analyzed for the content of vitamin C, carotenoids, and polyphenols.

The anthocyanins, carotenoids, and polyphenols were determined spectrophotometrically using the UV–Vis spectrophotometer (Lambda 25 Perkin-Elmer, Waltham, MA, USA). All the determinations were carried out in triplicate.

The total anthocyanins were measured spectrophotometrically as described by [22]. Briefly, the frozen sample (200 mg) was extracted in acidified methanol (1.5% HCl, *v/v*) overnight at room temperature and then centrifuged at 10,000 rpm per 10 min. The absorbance of the supernatant was measured spectrophotometrically at 535 nm using as blank methanol:HCl (98.5:1.5 *v/v*). The pigment content was calculated in mg 100 g^{−1} FW.

The carotenoids were quantified according to [23]. Lycopene and β-carotene were extracted with acetone-*n*-hexane (4:6) and then centrifuged at 3000 × *g* for 5 min at 4 °C. The absorbance of the supernatants was measured spectrophotometrically at 663, 645, 505, and 453 nm using acetone-*n*-hexane (4:6) as blank. The lycopene and β-carotene concentrations were quantified using the equations proposed by [24] as follows:

$$[\text{lycopene}](\mu\text{g ml}^{-1}) = -0.0458 A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$$

$$[\beta\text{-carotene}](\mu\text{g ml}^{-1}) = 0.216A_{663} - 1.220A_{645} - 0.304A_{505} + 0.452A_{453}$$

where A₆₆₃, A₆₄₅, A₅₀₅, and A₄₅₃ are the absorbances at 663, 645, 505, and 453 nm, respectively. This method allows for the simultaneous determination of lycopene and β-carotene in the presence of chlorophyll.

The polyphenols were determined as described by [25]. Briefly, 65–70 mg of the sample was weighed and extracted with 1 mL of hydrophilic (ethanol: HCl 0.06 N ratio 1:1, *v/v*) extractant. After centrifugation, the extract was used for a colorimetric reaction with Folin–Ciocalteu reagent and 10% sodium carbonate solution. After two hours in the dark, the samples were read at 730 nm. The polyphenol content was determined from a calibration curve prepared with gallic acid at known concentrations and was diluted with both extracts. The results were expressed as gallic acid $\text{mg } 100 \text{ g}^{-1} \text{ FW}$. The ascorbic acid content was determined using a 2,6-dichlorophenol (DIP) titrimetric method adapted from [26]. The results were expressed as the mean value in $\text{mg } 100 \text{ g}^{-1}$ of tomato for the three replicates.

2.5. Statistical Analysis

The data were checked for normality and then subjected to an analysis of variance with the MSTATC software (Michigan State University, East Lansing, MI, USA, <http://web.archive.org/web/20111012082739/http://www.msu.edu/~freed/disks.htm>, accessed on 12 January 2022), using the model number 2 (Completely Randomized Design for the farming system, while the genotype was a split-plot) within the Factor submenu. The means were separated by Duncan's multiple range test.

Principal component analysis (PCA) was performed using the PAST software, version 3.22 [27]. The PCA was performed to explore and visualize the difference among the farming systems and for the content of bioactive substances, the fruit components (pomace or pulp), and the farming systems.

3. Results

3.1. Field Measurements

The growing period matched the cultivation season for tomatoes at Italian latitudes, with temperatures increasing from the transplant to ripening (Figure 2).

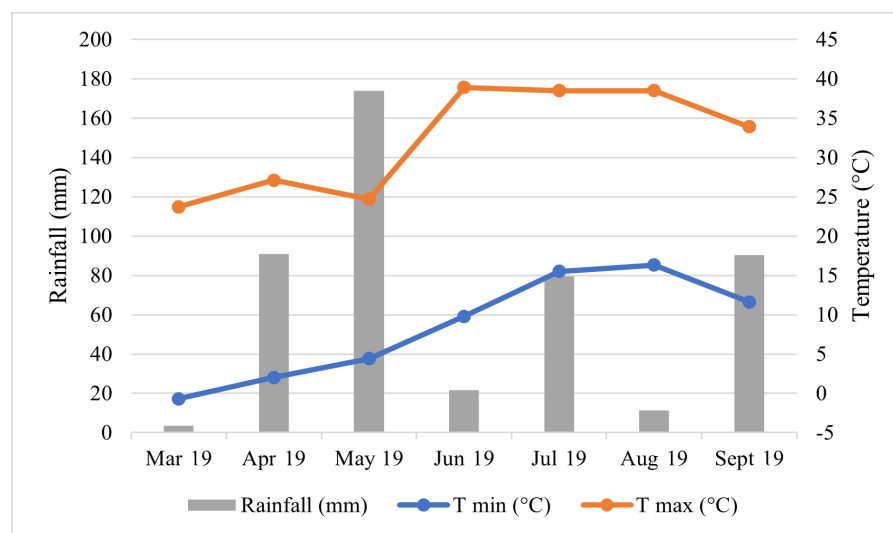


Figure 2. Minimum and maximum temperatures registered in the period January–June 2019 and monthly rainfall.

However, contrary to what was observed in the previous years, the rainfall in the period was high and in May, the maximum temperature was about 7 °C lower than in the previous five years. The farming system did not influence the plant height, the flowering date, and the number of flowers per plant (Table 4).

Table 4. Morphological and phenological traits. For each factor, values within a column followed by the different letter are statistically different at the level of $p \leq 0.01$ (capital) and $p \leq 0.05$ (lowercase) according to Duncan's test. * $p \leq 0.05$, ** $p \leq 0.01$, ns, not significant.

Factor	PLH [§] (cm)	DFW (days)	NFL (n.)	LGI (Spad Unit)
System				
LI	56.1	27	6.1	53.3 ^b
LI ^{MI}	55.3	28	7.1	61.3 ^a
Genotype				
392	49.3	29 ^B	7.5 ^a	58.1
446	61.0	33 ^A	7.1 ^a	59.0
RG	56.8	20 ^C	5.3 ^b	54.9
Sys × Gen				
392-LI	49.8	29	7.8	53.7
446-LI	62.6	32	6.0	54.2
RG-LI	56.0	20	4.7	52.2
392 LI ^{MI}	48.8	29	7.2	62.5
446 LI ^{MI}	59.4	34	8.1	63.9
RG LI ^{MI}	57.6	20	5.9	57.6
Significance				
System	ns	ns	ns	*
Genotype	ns	**	*	ns
Sys × Gen	ns	ns	ns	ns

[§] PLH (plant height at 75 DAT), DFW (date of flowering), NFL (number of flowers per truss), LGI (leaf greenness index at 75 DAT).

In general, the commercial variety flowered significantly earlier (around ten days before) than the 392 and 446 lines but produced a lower number of flowers per truss (5.3 vs. 7.1 and 7.5, respectively). In terms of the NFL, the LI^{MI} system appeared to have a positive influence for the line 446 and RG increasing the number of flowers per truss, although without a statistical significance.

The LI^{MI} farming system had a significant effect on the leaf greenness index. The value of the LGI (SPAD units) was significantly higher in the leaves of plants grown on the LI^{MI} rather than the LI system (Table 4). The plants in the LI^{MI} system showed a progressive increase in chlorophyll passing from 30 to 80 DAT (Figure 3): the increase was higher for the breeding lines (446 and 392) that resulted in being highly responsive for this trait to the LI^{MI} conditions.

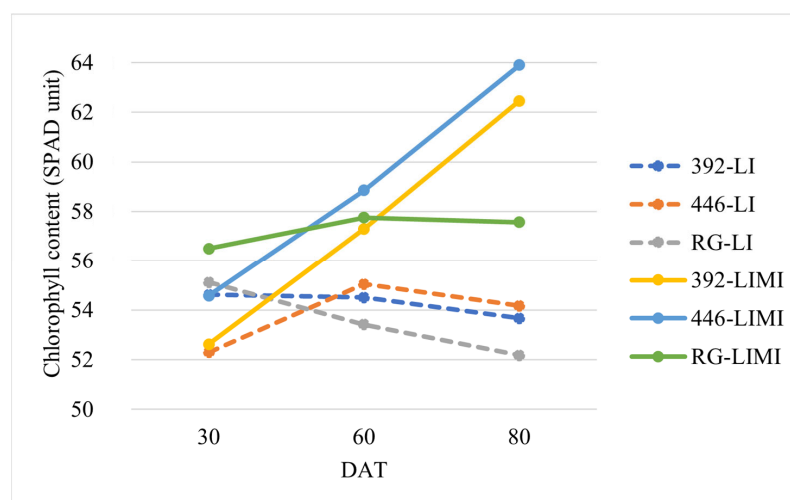


Figure 3. Leaf greenness index of the tomato lines grown on LI (full line) or LI^{MI} (dotted line) conditions.

As shown in Table 5, despite a lower fruit setting, the TNF increased significantly in the LI^{MI} system, leading, in turn, to a higher TWF (+23.7%). The line 392 proved to be particularly fertile, showing a 65.7% fruit set without a significant difference when grown with the LI (66.6%) or LI^{MI} (64.8%) system. In contrast, for RG, the reduction in the fruit set (−39.5%) from the LI to LI^{MI} system was statistically different. The control showed a TWF higher than the breeding lines, but the increment (+15.9%) respects that the line 392 was not significant.

Table 5. Main yield components. For each factor, values within a column followed by the different letter are statistically different at the level of $p \leq 0.01$ (capital) and $p \leq 0.05$ (lowercase) according to Duncan's test. * $p \leq 0.05$, ** $p \leq 0.01$, ns, not significant.

Factor	FS (%) §	TNF (n.)	TWF (g)	pH	SS (°Brix)
System					
LI	63.3	46 ^B	2195.0 ^b	4.46 ^a	4.28
LI ^{MI}	52.1	70 ^A	2716.6 ^a	4.36 ^b	4.31
Genotype					
392	65.7 ^A	93 ^A	2995.9 ^A	4.40 ^{AB}	4.18 ^B
446	47.5 ^B	34 ^B	898.4 ^B	4.24 ^B	4.87 ^A
RG	59.8 ^A	46 ^B	3473.1 ^A	4.59 ^A	3.83 ^B
Sys × Gen					
392-LI	66.6 ^A	78	2780.0	4.49	4.40 ^{bc}
446-LI	51.6 ^B	22	763.9	4.21	4.53 ^b
RG-LI	71.7 ^A	38	3041.2	4.68	3.90 ^{bc}
392 LI ^{MI}	64.8 ^A	109	3211.8	4.32	3.97 ^{bc}
446 LI ^{MI}	43.4 ^B	47	1032.9	4.26	5.20 ^a
RG LI ^{MI}	48.0 ^B	55	3905.0	4.50	3.77 ^c
Significance					
System	ns	**	*	*	ns
Genotype	**	**	**	**	**
Sys × Gen	**	ns	ns	ns	*

§ FS% (fruit setting), TNF (total number of fruits), TWF (total weight of the fruits), pH (pH), total soluble solid content (SS °Brix).

The cultivation on the LI^{MI} conditions favored a slight (but significant) lowering of the pH, while no effect was observed for the soluble solids. The genetic effect was instead appreciable for both traits. The line 446 had the lowest pH (4.24) and the highest SS (4.87°Brix), while the opposite behavior was shown by RG. Moreover, for the line 446, we observed a significant difference in the SS between the fruits produced in LI (4.53°Brix) or in LI^{MI} (5.20°Brix) conditions.

The adoption of mitigation options as mulching and intercropping into the LI^{MI} system determined a clear differentiation from the LI system, as emphasized by the displacement of the LI and LI^{MI} areas of PCA analysis (Figure 4).

Both the areas were spread along the PC1, but the LI one was placed mainly on the negative side of PC2 and the LI^{MI} area is shifted on the positive one. Observing the distribution of the variables vector, the loading of the NFL, TNF, and LGI was mainly affected by the LI^{MI} conditions, while the LI system had a greater influence on the PLH.

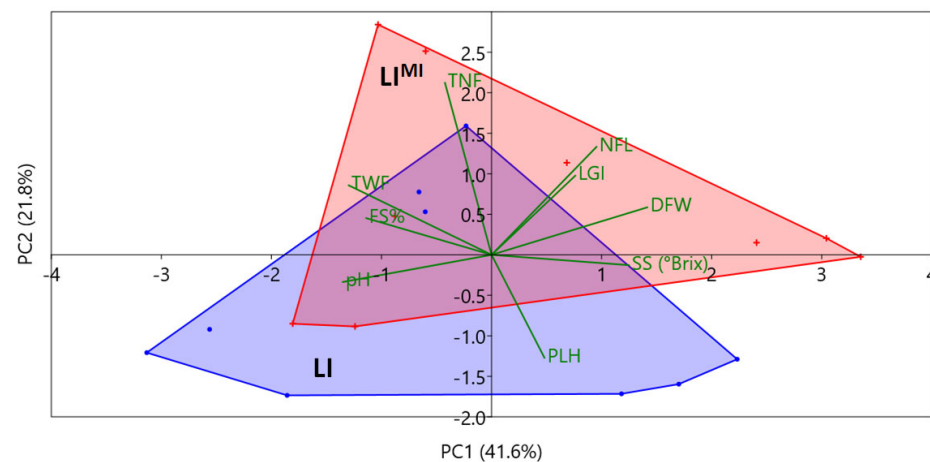


Figure 4. PCA on LI versus LI^{MI} cultivation. PLH (plant height at 75 DAT), LGI (leaf greenness index at 75 DAT), DFW (date of flowering), NFL (number of flowers per truss), FS% (fruit setting), TWF (total weight of the fruits), TNF (total number of fruits), pH (pH), total soluble solid content (SS °Brix).

3.2. Analysis of Bioactive Compounds

The farming system significantly influenced the content in the peels of polyphenols and anthocyanins, but not the content of vitamin C and carotenoids (Table 6). The polyphenols increased by 37.9% and anthocyanins increased by 116.7% in LI compared to LI^{MI}. Neither the farming system nor the genotype influenced the level of carotenoids (Table 6).

Table 6. Content of the main functional compounds (mg 100 g^{−1} FW) in the peel. For each factor, values within a column followed by the different letter are statistically different at the level of $p \leq 0.01$ (capital) and $p \leq 0.05$ (lowercase) according to Duncan's test. * $p \leq 0.05$, ** $p \leq 0.01$, ns, not significant.

Factor	Vit. C	Carotenoids	Polyphenols	Anthocyanins
System				
LI	13.36	24.84	109.96 ^A	0.91 ^a
LI ^{MI}	11.02	23.25	79.76 ^B	0.42 ^b
Genotype				
392	10.49 ^B	25.48	102.26 ^B	0.26 ^b
446	15.05 ^A	22.40	107.83 ^A	0.85 ^a
RG	11.03 ^B	24.27	74.50 ^C	0.88 ^a
Sys × Gen				
392 LI	9.87 ^B	25.70	118.54 ^B	0.51 ^{bc}
446 LI	20.23 ^A	24.65	127.37 ^A	1.48 ^a
RG LI	9.97 ^B	24.18	83.98 ^C	0.74 ^{ac}
392 LI ^{MI}	11.10 ^B	25.25	85.97 ^C	0.02 ^c
446 LI ^{MI}	9.87 ^B	20.15	88.29 ^C	0.21 ^c
RG LI ^{MI}	12.08 ^B	24.35	65.01 ^D	1.02 ^{ab}
Significance				
System	ns	ns	**	*
Genotype	**	ns	**	*
Sys × Gen	**	ns	**	*

The presence of the *hp-2* gene in the line 392 should have increased the level of pigments in the plant with particular reference to the lycopene in the fruit [28]. However, in the peels of line 392, the carotenoids were weakly higher (25.48 mg 100 g^{−1} FW) than in the other genotypes, and no evident effect appeared between the farming systems. The 446 line showed the increase (22.3%) in the carotenoids content in the fruit peel produced in the LI compared to LI^{MI} system, but with any statistical significance.

The data of the pulp confirmed the tendency to produce a higher amount of antioxidant compounds as vitamin C (+44.0%) and polyphenols (+11.0%) when the plants were grown in the LI conditions (Table 7).

Table 7. Content of the main functional compounds (mg 100 g^{−1} FW) in the pulp. For each factor, values within a column followed by the different letter are statistically different at the level of $p \leq 0.01$ (capital) and $p \leq 0.05$ (lowercase) according to Duncan's test. * $p \leq 0.05$, ** $p \leq 0.01$, ns, not significant.

Factor	Vit. C	Carotenoids	Polyphenols	Anthocyanins
System				
LI	19.92 ^A	24.69	33.31 ^a	0.002
LI ^{MI}	13.83 ^B	25.07	30.00 ^b	0.002
Genotype				
392	13.91 ^B	25.10	30.44 ^b	0.002
446	27.43 ^A	25.28	34.43 ^a	0.003
RG	9.29 ^C	24.27	30.18 ^b	0.002
Sys × Gen				
392-LI	13.90 ^C	25.51	34.02 ^a	0.001
446-LI	34.27 ^A	24.36	33.25 ^a	0.004
RG-LI	11.60 ^{CD}	24.21	32.67 ^a	0.002
392 LI ^{MI}	13.92 ^C	24.68	26.86 ^b	0.002
446 LI ^{MI}	20.60 ^B	26.20	35.60 ^a	0.001
RG-LI ^{MI}	6.97 ^D	24.34	27.69 ^b	0.002
Significance				
System	**	ns	*	ns
Genotype	**	ns	*	ns
Sys × Gen	**	ns	*	ns

The vitamin C content in the pulp was higher than the peel while the polyphenols content was lower. At the genotypic level, the line 446 confirmed the highest vitamin C accumulation, especially for the fruits produced under LI conditions. As already observed for the peels, the pulp of 446 fruits showed the highest value (34.43 mg 100 g^{−1} FW) of polyphenols on average. Interestingly, the polyphenols content decreased significantly in the pulp of the 392 and RG genotypes when the fruits were collected from the LI^{MI} plants. As reported for the peels, no factor affected the carotenoids content of the pulp, and the differences did not reach a clear statistical significance.

The distribution of the bioactive components among the fractions and the cultivation system was shown by the PCA plot in Figure 5. The first two axes explained 73.4% of the variability and the areas representing the peel and pulp were clearly separated in the opposite halves of the main component. It is interesting to underline that while for the pulp, the areas belonging to the two systems were overlapping, for the peels, there was a high misalignment between the areas of the LI and LI^{MI} systems. The graph confirmed that the metabolic pathway of polyphenols is active in the peel and is absent in the pulp. On the other side, the direction and loadings of the vitamin C and, to a lesser extent, the carotenoids vectors indicated that their preferential production occurred in the pulp.

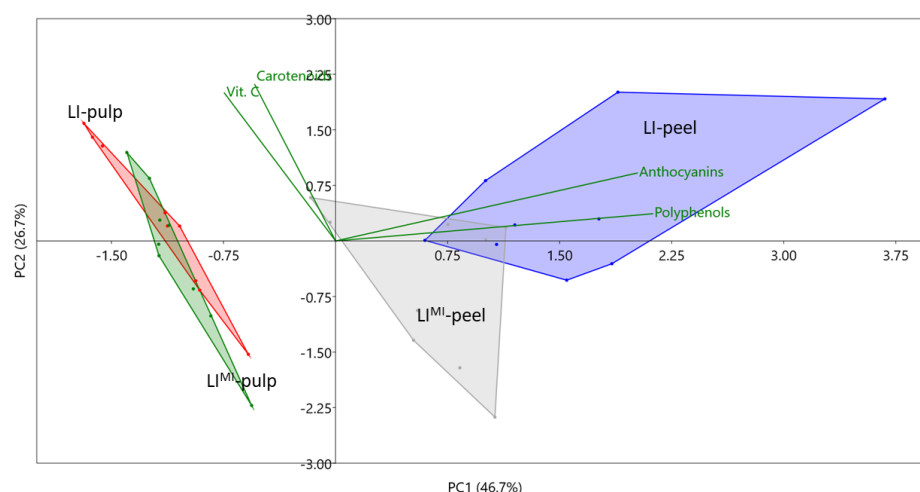


Figure 5. PCA of the amount of bioactive compounds in LI and LI^{MI} farming and for their distribution in peel and pulp.

4. Discussion

Tomato lines accumulating bioactive compounds (carotenoids and polyphenols) in the fruits have been assessed for their suitability to low-input (LI) farming and low-input farming integrated with mulching and intercropping (LI^{MI}).

The farming systems caused a differentiation in the physiological cycle: LI^{MI} plants grew more slowly and then recovered gradually through a longer vegetative cycle. The integration of mitigation elements can require a longer period of adaptation to reach a threshold of green biomass, triggering a sustainable sink-source balance. A certain degree of timing mismatch in the later stages of growth has been also observed and discussed for organic farming by [29]. Tomato grown under organic system showed a delay in the activation of sink-source mechanisms leading to fruit ripening because they needed to accumulate the photosynthates (source) released successively at the filling of fruit (sink). In fact, the highest leaf greenness index of the LI^{MI} system was achieved just before harvesting when the LI plants had already begun to disassemble from the photosynthetic machinery.

The marketable yield in processing tomato increased progressively with the release of the latest varieties compared to RG [30]. Depending on factors such as the genotype, climate conditions, and type of cultivation, the yield can range from 50 to over 150 t ha⁻¹. In our study with an investment of 3.5 plants m⁻², the potential average yield ranged from 76.8 t ha⁻¹ on the LI conditions to 95.1 t ha⁻¹ on the LI^{MI} conditions. However, the distribution of the yield was different between LI and LI^{MI} because the plants grown with the LI^{MI} system concentrated the production in the last harvest (September). The yield level in LI^{MI} agreed with those reported by [31,32] in Italian studies, where mitigation elements such as mulching or an organic source of fertilization were tested. The effect of the year [31,32] as well as the genotype [31] has been observed but, in both cases, the positive effect of mulching was clear. Soil protection can improve the biological cycle of the microbial fauna, the preservation of organic matter (and, hence, the carbon storage), the availability of nutrients, the control of weeds, and the soil-plant interaction. Our results open a further space for discussion regarding the use of wood chips as a mulcher. In a comparison of eight different mulch materials [33], it was observed that the bark or wood chips and wheat straw increased the organic carbon content, aggregate stability, and pH, showing the largest and long-time impact on the soil's properties. In addition to the cited effect, the wood chips of different species improved the weed control through the release of allelopathic chemicals [34].

In this sense, the proposed systems can provide some clues for a more respectful soil management according to the principle of sustainable agriculture [2] and natural horticulture [21]. However, it also presents a further element of interest linked to the use of genotypes improved for the content of bioactive compounds in the fruits. As

discussed below, indeed, the environmental milieu where these lines are grown can affect the accumulation rate of the compounds. Therefore, a rational integration between farming systems and genotypes can allow for obtaining high-quality healthier food, improving the agroecological status. As a corollary, we must reiterate that low-input farming systems suffer a lack of purposefully selected lines since most varieties are developed from breeding programs for high-input conditions [35,36]. The study showed that even without selecting lines for traits aimed at a low-input system with low-input breeding programs or low-input on-farm breeding, it is possible to identify lines with valuable traits. RG (a commercial processing variety) and the breeding line 392 were the most productive and both were high-yielding in low-input conditions.

The role of breeding in increasing the content of functional compounds was evident. Because of its genetic makeup, the tomato purple line 446 (*Atv*, *aft*) confirmed its capacity to store polyphenols in the peel. Owing to the presence of the *hp-2* gene, the carotenoids level of the line 392 was expected to be higher than the other genotype. Thus, the lack of significance both in the peel and pulp is surprising and can suggest a masking effect on the differences by experimental variability. However, the *hp-2* gene affects positively additional functional compounds with antioxidant activity, including phenolics, flavonoids, and vitamin C [10,28]. The figure was confirmed by the content of polyphenols in the LI system both for the peel and pulp, which is in agreement with the results of other authors [10,37,38].

The data of the present study confirmed the existing difference between the peel and pulp for the presence of bioactive compounds [39,40], thus supporting the interest for the recovery of processing waste [25,41,42]. Peel is recognized as the preferential site hosting the synthesis of polyphenols in tomato fruit because the metabolic pathway in the flesh is lacking [43]. The data agreed with the localization of the polyphenols and anthocyanins in the fruit peel. Just for the polyphenols in the peel, we observed an effect of the cultivation system on their accumulation because the polyphenols were significantly higher in the LI system. This behavior partially disagrees with some observations of several authors working on organic farming. A common outcome of works comparing conventional and organic farming is the absence of a clear role of the cultivation system for the accumulation of bio-compounds. Rather than the growing system, the genotype and the growing year are the main factors driving the production of vitamin C, carotenoids, and polyphenols [44–46]. In a three years trial on conventional, organic, and biodynamic cultivation, [47] pointed out the role of the growing year in determining differences on the phenolic acid content because of the sensitivity of the biosynthesis to the solar radiation.

However, although the cultivation method had generally minor impact on the content of phenolic compounds, the effect on individual polyphenols may be more complex [48]. In a three years experiment on four tomato cultivars, [45] reported a positive influence of organic farming on the content of apigenin acetylhexoside, phloretin dihexoside, and caffeic acid hexoside I, while [44] found differences among conventional, organic and hydroponic for caffeic and ferulic acid content. On the other side, [49] observed no influence of farming on caffeic acid for the cv Perfect-peel. Therefore, the higher content of polyphenols observed in the peel of LI plants suggests the involvement of physiological mechanisms in the LI conditions, involving the role of polyphenols as well as the influence of the LI^{MI} conditions in the polyphenols' synthesis. The genotypes were different for the values of vitamin C, polyphenols, and anthocyanins, but it should be underlined as for each genotype the content of polyphenols in the peel decreased significantly passing from LI to LI^{MI} conditions. Peel is an interface between plants and environment and comprises a cuticle, a layer of epidermal cells, and two-four layers of hypodermal cells [50]. Phenols and anthocyanins produced in peels have, among others, a protective function against oxidant stress generated by biotic or abiotic agents as radiation or insects and pathogens [51,52].

The tendency to produce more polyphenols in the LI system stimulates further investigation. The introduction of mitigation elements (LI^{MI} system) increased the crop biodiversity (polyculture), reduced the competition with weeds improving the plant-soil

relationship (wood chip mulching) and established an equilibrium within the microsystem of the single plot. These complex interactions may have reduced the need for the plant to synthesized protective barriers against the environmental stress. On the other side, the less complex LI system led the plants to produce and use natural antioxidant systems (polyphenols and anthocyanins) to counterfeit biotic and abiotic offenders, thus increasing the content of bioactive compounds.

5. Conclusions

The cultivation of processing tomato lines can provide satisfying results in adopting schemes of sustainable agriculture as low-input farming conditions (LI), with the application of solely cow manure, or with the addition of further mitigation elements such as wood chip mulching and intercropping (LI^{MI}). This responds to the first hypotheses of our study and provides an indication of the feasibility of introducing solutions inspired to sustainable agriculture to improve conventional farming systems even without starting long and delicate conversion processes.

Sustainable agroecological systems may enhance the production of bioactive compounds, but the influence of additional mitigation elements (such as mulching and intercropping) is questionable and requires deeper consideration. The LI farming system induced the plant to produce protective systems that stimulated the production of antioxidant substances, while the ecological micro-equilibrium brought about by mulching and polyculture reduced this need. However, in both cases, the positive aspect concerns the capacity of plants in improving their tolerance of biotic (production of antioxidants) and abiotic stress (improvement of the fitness), thus lowering the need of external sources as chemicals. Finally, farmers attentive about environmental issues should not leave out the association between farming systems and genotypes which can improve the trade-off between the adoption of sustainable farming methods and the production of high-quality healthier food.

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