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Performance of New Muchamiel Tomato Lines with Virus Resistance Genes Grafted onto Two Commercial Rootstocks

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Abstract: Tomato landraces are regaining interest in Spain because their great fruit quality and value in popular gastronomy. Muchamiel is a traditional tomato variety grown in SE Spain that has been recently improved by the CIAGRO-UMH Tomato Breeding Group, resulting in several lines and hybrids with genetic resistances to virus and most of the original Muchamiel genome. In the current study, two hybrids and one pure line from CIAGRO-UMH and a commercial Muchamiel were grown under conventional conditions to evaluate three different grafting treatments: non-grafting and grafting onto the commercial Beaufort and Maxifort rootstocks. The yield parameters and fruit quality were assessed, and a sensory analysis was performed to evaluate the behavior of every scion/rootstock combination. Overall, significantly worse yield and fruit number in Maxifort-grafted plants were reported; as well as a slight reductions in SSC, fructose, and sucrose; and significant effects on few sensory traits. Instead, Beaufort-grafted plants showed no reduced yield, whereas no differences were reported between grafting treatments in fruit weight, TA, and acid profile, as well as in most of flavor and texture sensory parameters. These results suggest that Muchamiel/Beaufort combination could be suitable under unfavorable conditions, while Maxifort do not seem to provide agronomic nor quality benefits.

Keywords: Muchamiel hybrids; landraces; grafting; yield; quality; sensory analysis

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1. Introduction

In recent years, traditional tomato landraces have been regaining attention in Spain markets due to their great organoleptic quality and as a differentiated product linked to popular culture and gastronomy [1]. In a market saturated of commercial hybrids with generally low quality, some farmers have found a business opportunity in recovering the cultivation of these landraces [2]. Valenciano tomatoes represent a clear example of recovered tomato landrace commercialized under distinguished quality brand, leading to increased cultivated area and production in Comunidad Valenciana (Spain) [3]. Muchamiel landrace (Alicante) is also a traditional tomato type that is very popular in local markets of southeastern (SE) Spain, where its price can be three to six times higher than typical commercial hybrid varieties [4]. In fact, The Association of Producers and Marketers of Muchamiel Tomato was stablished in 2018 with the aim of developing a common production and marketing framework, as well as achieving an official quality brand for this tomato variety. However, growing this landrace continues to entail a high risk for farmers, partly due to their susceptibility to different biotic and abiotic factors. One of them is the incidence of viral diseases, such as those caused by ToMV, TYLCV, and TSWV [5]. The high susceptibility of the landrace implies strong reductions in tomato production, thus ruining the benefits obtained by farmers [2,6,7]. In order to cope with this problem, the plant breeding group at CIAGRO-UMH introgressed three dominant genes: *Tm-2a*, *Ty-1*, and *Sw-5*, which confer resistance to ToMV, TYLCV, and TSWV, respectively into Muchamiel cultivars [8]. Since 2011, several Muchamiel breeding lines and hybrids with genetic resistance to virus have been obtained and officially registered in the Spanish Office of Plant Varieties [9].

Apart from the incidence of viral diseases, other biotic and abiotic factors can hinder the recovery of tomato landraces, since they lack the genetic resistances that can be found in commercial bred varieties [10]. For example, these landraces are usually susceptible to incidence of soil-borne diseases such as bacterial wilt (*Ralstonia solanacearum*), fusarium wilt (*Fusarium oxysporum* f. sp. *Lycopersici*), and nematodes [11–14]. Grafting onto resistant rootstocks has emerged as an effective alternative to face with these problems and to promote a more sustainable cultivation at the same time, as soil fumigation would not represent a requisite for growing them and their use could contribute to reduce fertilizer and water consumption [15]. In this context, grafting is already being used to promote the cultivation of tomato landraces, such as Cuore di Bue in Italy [13], the Catalonian variety Mando [16], Spanish Moruno cultivars [17], or Brandywine [12] and Cherokee Purple [14] American heirlooms. Apart from the benefits obtained in unfavorable environments, grafting is widely used by farmers to improve yield also in conventional conditions (with low or no incidence of biotic or abiotic stresses), with relative success in many cases. However, its use also entails some downsides, as negative effects on fruit quality and sensory traits have been reported in grafted tomato plants across the world, thus impairing the commercial value of the tomatoes [15]. These negative effects have a great dependence on the specific scion/rootstock combinations being considered, as well as the growing system and the specific environmental conditions [18]. It is not clear how quality traits are affected by grafting [19], although some authors have pointed to a dilution effect of compounds caused by a higher water content in fruits from grafted tomato plants [20,21]. Small negative effects in quality could be tolerated under adverse growing conditions as a minor trade off, but they would be unacceptable under conventional conditions for farmers targeting high quality markets. With the aim of offering economically sustainable choices to the farmers, the effects of grafting in yield and fruit quality must be studied using different scion/rootstock combinations, picking out those capable of improving yield without compromising fruit quality and sensory traits.

In this context, the main objective of the current work is to evaluate the performance of four Muchamiel tomato cultivars grafted onto the popular rootstocks Beaufort and Maxifort under conventional growing conditions, with no incidence of biotic nor abiotic stresses. For this purpose, agronomic parameters such as yield and fruit metabolic parameters (sugar and acid profiles of the fruit) were evaluated, and the effect on sensory traits (external appearance, flavor, and texture) was analyzed with a trained panel to assess the possible impact on traits related to consumer preferences. This is the first grafting performed with Muchamiel improved lines and hybrids, and it will offer valuable information to those agents interested recovering the cultivation of tomato landraces.

2. Materials and Methods

2.1. Plant Material

Four different Muchamiel-type cultivars were chosen for the experiments. The first of them, UMH1200, is a breeding line obtained in CIAGRO-UMH Breeding Tomato Program carrying *Tm-2a*, *Ty-1*, and *Sw-5* genes in homozygous state, which confer resistance-tolerance to ToMV, TYLCV, and TSWV, respectively [22]. The Muchamiel hybrids UMH1200×4 and UMH1200×18 were also included, which are crosses between UMH1200 and the traditional accessions Muchamiel 4 and Muchamiel 18 respectively, selected previously in the same breeding program. Both hybrids have the three resistance genes in heterozygous state. Finally, a commercial Muchamiel cultivar (Semillas Batlle, Barcelona, Spain) with no genetic resistances to virus was also selected as control.

Every cultivar was grown in three conditions: non-grafted and grafted onto the interspecific (*S. lycopersicum* L. × *S. habrochaites* S. Knapp & D.M. Spooner) commercial rootstocks Beaufort and Maxifort (De Ruiter Seeds/Monsanto, Bergenschoenk, The Netherlands). Just like seed company claims, both rootstocks provide extra vigor to the crop (especially Maxifort) and resistances to fusarium wilt, verticillium wilt, corky root rot, and root-knot nematodes. These two rootstocks are very popular in tomato farms in the southeast of Spain. Plants were grafted using the Japanese top grafting method by a commercial producer in Albatera (Alicante, Spain).

2.2. Field Experiments

Every *cultivar*grafting* combination was grown in two different locations simulating two repetitions: the CIAGRO-UMH experimental farm located in Orihuela, Alicante province, SE Spain (38°07′ N, 0°98′ W and 25 m a.s.l.) and the Agricultural Experiment Station of Elche (EEA-Elx), also located in Alicante (38°25′ N, 0°69′ W and 86 m a.s.l.). The climate in these areas is arid to semiarid Mediterranean (average annual rainfall of 250 mm). The seeds were germinated in greenhouse and transplanted to the field at the appropriate time. The plants were grown in mesh greenhouse during the spring crop cycle (from April to August) of 2019. Both trials were organized in two replicates of 6–7 plants for each *cultivar*grafting* combination. Plants were grown vertically with one and two stems (with a density of 2.5 plants m^{-2} and 1.25 plants m^{-2}, respectively), according with common cultural practices in this area for each case. To normalize the data from yield parameters, results were provided per area unit (m^{-2}). Growing techniques and conditions were similar in both locations, with the standard fertilization through drip irrigation commonly applied in this area for tomato crops. The soil was fumigated in CIAGRO-UMH field before the experiment using metam-sodium 50% (2000 L/ha) in drip irrigation, to avoid presence of soil-borne pathogens during culture. The soil of EEA-Elx field was not fumigated because we considered it free of soil-borne diseases, since there were no crops being grown during the prior year. Finally, bumblebees (*Bombus terrestris*) were used in EEA-Elx experiment to improve the pollination and, thus, tomato production.

2.3. Agronomic Traits

Yield (weight in kg of all the fruits harvested per plant), the total number of fruits and the fruit weight (average fruit weight considering all the harvested fruits and expressed in g) were calculated on a per plant basis. Then, the results were calculated per unit area (m^{-2}).

2.4. Analysis of Fruit Quality Parameters

To assess solid soluble content (SSC) and titratable acidity (TA), fruits were harvested once a week during the last 4–5 weeks of productive cycle (end of June and whole of July). Four replications per plot of every *cultivar*grafting* combination were selected with 3–4 fruits each one. Fruits were selected considering approximately 50–60% of the surface red in color, which is considered the regular commercial ripening state for this landrace. Fruits were grounded obtaining a biological mean and SSC of the sample was estimated with a PR-100 (Atago, Tokyo, Japan) digital refractometer, in duplicate, and the results were expressed in °Brix. TA was also measured twice with the same samples, using a CRISON pHmatic 23 with 0.01 mol/L NaOH titration to pH 8.1 and expressed as percentage of citric acid.

For deeper metabolic evaluation, samples from CIAGRO-UMH trial with approximately 50–60% of the surface red in color were sent by express transport to Joint Research Unit UJI-UPV Improvement of Agri-Food Quality, where they were blended until obtain a completely homogeneous sample which was divided into two aliquots. The first one was used to calculate fruit dry weight (%) after oven-drying at 65 °C until constant weight.

The second one was centrifuged, and supernatants were then diluted 1:20 with ultrapure water and filtered using a 0.22 µm cellulose acetate centrifuge tube filter (Costar Spin-X, Corning, NY, USA). The quantification of fructose; glucose; sucrose; and malic, citric, and glutamic acids was performed using a 7100 CE system equipped with diode array detector and thermostatted sample compartment (Agilent Technologies, Waldbronn, Germany) and following the methodology described by Cebolla-Cornejo [23]. Uncoated fused silica capillaries with 67 cm total length, 60 cm effective length, 375 µm outer diameter, and 50 µm internal diameter (Polymicro Technologies, Phoenix, AZ, USA) were used for the separation. Prior to the first use, capillaries were flushed with NaOH 1 M during 300 s at 50 °C, NaOH 0.1 M during 300 s, and water during 600 s. Each run started with a rinse of the capillary during 120 s with sodium dodecyl sulphate (SDS) 58 mM followed by 300 s with running buffer (20 mM 2,6-pyridinedicarboxilic acid solution with 0.1% (*w*/*v*) hexadimethrine bromide (HDM) adjusted to pH 12.1). A hydrodynamic injection at 3400 Pa during 10 s was used. The voltage applied for separation was −25 kV at 20 °C with indirect detection at 214 nm. Sucrose equivalents were calculated as described in a previous work [24], as the weighted sum of sugar concentration using the relative sweetening power of each sugar: 1.73 for fructose and 0.74 for glucose. Sucrose was only found at traces under the limits of quantitation.

2.5. Sensory Analysis

The sensory analysis was performed by a trained panel from the research group Food Quality and Safety (Universidad Miguel Hernández de Elche, Orihuela, Alicante, Spain), in two repeated sessions during two consecutive weeks. In the first session, 13 panelists (aged 25–45; 8 men and 5 women) formed the trained panel, whereas 10 panelists (aged 25–45; 5 men and 5 women) set the second session. The panelists were all familiar with taste panel procedures and the terminology used, as well as the majority of them had participated previously in other sensory analysis in tomato.

The evaluation was done only in the experiment from CIAGRO-UMH field. In every session, a set of 10–15 tomatoes in commercial ripening state (approximately 50–60% of the skin red in color) were collected from the second to fifth trusses of every *cultivar*grafting* combination, meaning 12 different samples in total. The selected fruits were washed with fresh tap water and dried with absorbent paper before the analysis.

Panelists evaluated a set of five visual, seven flavor, and nine texture traits closely related with tomato-consumer preferences, which appear in Table 1, including a precise definition of every one and the scale used by the panel to score the samples, based in Hongsoongnern and Chambers IV [25]. The visual parameters were evaluated with the whole tomatoes (except for the amount of columella), while flavor and texture traits were assessed from sliced portions of those same tomatoes. Most of parameters were scored by a numerical 0–10 scale with increments of 0.5 units, where 0 represents no intensity and 10 extremely strong. The rest of them were evaluated through specific smaller scales agreed by the panelists before the analysis. The references for the extremes and intermediate values of every trait were stablished by consensus in each session take into account the global set of samples collected.

Table 1. Description and score scale for all the evaluated traits.

Trait	Description	Scale
Visual		
Color	Visual evaluation of the optimal skin color of the tomatoes	0–10
Homogeneity	General homogeneity of the sample	0–10
Ribbing	Intensity of the ribs at calyx end	1–4
Green shoulder	Intensity of the green trips at calyx end	1–4
Columella	Amount of columella evaluated at longitudinal cut of the sample	1–3

Table 1. *Cont.*

Trait	Description	Scale
Flavor		
Sweet	Taste stimulated by sugars	0–10
Acid	Taste stimulated by acids	0–10
Salty	Taste stimulated by presence of salty substances	0–10
Tomato ID	Aromatics reminiscent tomato characteristic flavor	0–10
Fruity	Aromatics reminiscent fruity flavor	0–10
Vegetal	Aromatics reminiscent vegetal flavor	0–10
Aftertaste	Time the flavor of tomato remains in mouth after swallowing	0–10
Texture		
Hardness	The force required to bite completely through the sample with molar teeth	0–10
Crunchiness	The intensity of audible noise at first bite with molars	0–10
Juiciness	The sensation of moisture released by the tomatoes during the first bites	0–10
Juice density	Thickness of the tomato juice during the first bites	1–3
Flesh	Amount of pulp detected in mouth after the first bites	0–10
Peel	Thickness of the pericarp evaluated during the first bite	0–10
Seeds	Quantity of seeds of the sample	0–10
Adhesiveness	The degree to which product sticks on the surface of teeth.	0–10
Residual peel	Amount of skin remaining on teeth after swallowing the sample	0–10

2.6. Statistical Analysis

Firstly, Shapiro–Wilk and Levene statistics were performed to test the normality and homogeneity of the parameters evaluated, respectively. Results were evaluated using a two-way analysis of variance (ANOVA), considering the cultivar (C) and grafting (G) as fixed factors and including the interaction between both of them (C*G). Differences were considered statistically significant at $p < 0.05$. Later, a post-hoc Newman–Keuls's multiple-range test was performed to compare the means in the case of yield parameters and fruit quality traits, whereas a Tukey's multiple-range test was considered for sensory traits evaluated in CIAGRO-UMH experiment. ANOVAs and multiple-range tests were performed using Statgraphics Centurion XVII v. 17.2.00.

3. Results

3.1. Agronomic and Basic Quality Traits

The results from ANOVA and Newman–Keuls's multiple range test are shown in Table 2. On average, the Muchamiel hybrids and commercial Muchamiel showed a significant better performance in all the agronomic traits and TA than UMH1200 at both CIAGRO-UMH and EEA-Elx locations, being UMH1200×4 the most productive cultivar. Considerably worse yields were observed in UMH1200 compared to both Muchamiel hybrids (−20% to −40%), an effect related to a decreased fruit weight. Instead, UMH1200 showed statistically significant higher SSC than the rest, but only in the CIAGRO-UMH trial.

As grafting is considered, a significantly worse behavior was observed in plants grafted onto Maxifort in Total yield, with reductions of 12% in CIAGRO-UMH trial and 21% in EEA-Elx, as well as in number of fruits, with reductions of 13% and 18%, respectively. No differences were observed between non-grafted tomatoes and plants grafted onto Beaufort EEA-Elx trial, while a small significant reduction (3%) was found in the CIAGRO-UMH trial. In addition, non-grafted plants showed significant higher SSC than grafted ones in both trials (+8% and +11%, on average), but no differences were observed in fruit weight and TA between grafting combinations. Finally, the C*G interaction was not significant in any case.

Table 2. Effect of cultivar and grafting (p-values from ANOVAs and Newman–Keuls's multiple range test) in agronomic and basic quality traits (SSC and TA) evaluated at CIAGRO-UMH and EEA-Elx. ComMuch: Commercial Muchamiel. Bold format indicates statistical significance.

	Yield		Fruit Quality		
Factor	**Total Yield (kg m^{-2})**	**Number of Fruits m^{-2}**	**Fruit Weight (g)**	**SSC (°Brix)**	**TA (%)**
CIAGRO-UMH					
ANOVA *p*-values [x]					
Cultivar (C)	***	***	***	*	***
Grafting (G)	0.055	*	0.703	***	0.102
C×G	0.069	0.054	0.125	0.682	0.249
Newman–Keuls's Multiple Range Test [y]					
Cultivar					
UMH1200	**6.59 a**	**61.0 a**	**109.0 a**	**5.21 b**	**0.34 a**
UMH1200×4	**10.91 c**	**73.2 b**	**151.8 c**	**4.99 a**	**0.37 b**
UMH1200×18	**8.41 b**	**56.4 a**	**154.4 c**	**4.96 a**	**0.37 b**
ComMuch	**7.9 b**	**58.8 a**	**138.3 b**	**5.17 ab**	**0.42 c**
Grafting					
Non-grafted	**8.88 b**	**65.7 b**	136.8	**5.30 b**	0.37
Beaufort	**8.63 ab**	**64.2 b**	137.8	**5.02 a**	0.38
Maxifort	**7.84 a**	**57.3 a**	140.6	**4.92 a**	0.38
EEA-Elx					
ANOVA *p*-values [x]					
Cultivar (C)	***	***	**	0.492	***
Grafting (G)	***	**	0.091	***	0.093
C×G	0.057	0.170	0.649	0.439	0.768
Newman–Keuls's Multiple Range Test [y]					
Cultivar					
UMH1200	**10.52 a**	**60.5 a**	**173.9 a**	4.27	**0.33 a**
UMH1200×4	**15.59 b**	**78.1 b**	**198.4 b**	4.37	**0.39 b**
UMH1200×18	**14.93 b**	**78.5 b**	**189.7 b**	4.20	**0.35 a**
ComMuch	**14.83 b**	**74.8 b**	**198.3 b**	4.30	**0.42 b**
Grafting					
Non-grafted	**14.91 b**	**78.3 b**	190.1	**4.48 b**	0.39
Beaufort	**15.16 b**	**76.5 b**	197.2	**4.23 a**	0.37
Maxifort	**11.83 a**	**64.6 a**	182.8	**4.15 a**	0.37

[x] Significant p-values: *, **, and ***, at $p < 0.05$, 0.01, and 0.001, respectively. [y] Means followed by the same letter, within the same column and factor, were not significantly different ($p > 0.05$).

3.2. Organic Acids and Sugars

A further metabolic analysis related to fruit quality was performed in the CIAGRO-UMH experiment, and the individual content of main tomato organic acids and sugars was quantified (Table 3). The largest differences between cultivars were found in malic and citric acids, both of them being significantly higher in the commercial Muchamiel than in the rest (+20% and +18% on average, respectively). Small significant differences were observed in SSC, fructose and glucose content between cultivars, but with no clear pattern. On the other hand, no differences were observed for glutamic acid content, sucrose equivalents, and dry matter between cultivars.

Regarding the effect of grafting, significant differences were found only in fructose and sucrose equivalents between grafting combinations, with grafted plants accumulating lower contents of these compounds in the fruit than non-grafted plants. The reduction in fructose content in fruits of the grafted plants was on average lower than 7% with the use of the Beaufort rootstock and lower than 10% in the case of Maxifort compared to the non-grafted controls. This reduction led to lower sucrose equivalents, which were 6% and 10%, lower than the non-grafted controls respectively. This reduction was not significant for glucose content. Thus, the effect in SSC was limited. Although fructose reductions were coherent the observed slightly lower SSC values of grafted plants, these differences were not significant, though p-value was close to the threshold ($p = 0.096$). The C*G interaction was not significant, indicating a similar response to grafting in all the cultivars.

Table 3. Effect of grafting in the metabolic profile (organic acids, sugars, and dry matter content) of the fruits grown at CIAGRO-UMH (ANOVA and Tukey's multiple-range test). ComMuch: Commercial Muchamiel. Bold format indicates statistical significance.

Factor	Malic Acid ($mg\ g^{-1}$)	Citric Acid ($mg\ g^{-1}$)	Glutamic Acid ($mg\ g^{-1}$)	Fructose ($mg\ g^{-1}$)	Glucose ($mg\ g^{-1}$)	Sucrose Equivalents	SSC (°Brix)	Dry Matter (%)
ANOVA *p*-values [x]								
Cultivar (C)	**	***	0.800	*	*	0.063	*	0.159
Grafting (G)	0.071	0.396	0.483	*	0.110	*	0.096	0.069
C×G	0.071	0.139	0.601	0.082	0.879	0.250	0.943	0.536
Newman–Keuls's Multiple Range Test [y]								
Cultivar								
UMH1200	**0.69 a**	**3.24 a**	2.64	16.77	18.18	**42.85 ab**	**5.16 b**	4.92
UMH1200×4	**0.66 a**	**3.41 a**	2.83	18.95	18.87	**46.74 b**	**5.05 ab**	4.97
UMH1200×18	**0.68 a**	**3.13 a**	2.81	17.76	16.83	**43.17 ab**	**4.78 a**	4.68
ComMuch	**0.81 b**	**3.84 b**	2.76	16.99	16.41	**41.16 a**	**5.12 b**	4.94
Grafting								
Non-grafted	0.66	3.35	2.83	**18.63 b**	18.48	**45.91 b**	5.17	5.04
Beaufort	0.74	3.5	2.81	**17.48 ab**	17.5	**43.19 ab**	4.99	4.82
Maxifort	0.72	3.37	2.64	**16.74 a**	16.73	**41.34 a**	4.91	4.78

[x] Significant p-values: *, **, and ***, at $p < 0.05$, 0.01, and 0.001, respectively. [y] Means followed by the same letter, within the same column and factor, were not significantly different ($p > 0.05$).

3.3. Sensory Analysis

The sensory analysis was performed in the EPSO-UMH trial during two repeated sessions. Overall, panelists visually distinguished the different cultivars and grafting treatments with no doubt. On the other hand, results for liking traits (flavor and texture) were much less clear, showing statistically significant differences only in few cases, as well as in one of both sessions.

Accordingly, significant differences between the cultivars and grafting treatments were found in all the visual traits (Table 4). The commercial Muchamiel scored below the rest plants in homogeneity and green shoulder, whereas non-grafted tomatoes showed lower ribbing and higher green shoulder than grafted ones. There were not robust results in the rest of the visual traits, since there was not correspondence between both sessions. The noticeable differences in the homogeneity of the samples found between both sessions (6 and 7 points on average in session 1 and session 2, respectively) could be explaining the signification of the C*G interaction in the visual parameters, which will be discussed later.

Significant differences between cultivars were found for 8 out of 16 flavor and texture liking-traits. Differences in the descriptors tomato acidity and hardness were detected in both sessions, but only in one session for the descriptors sweet, tomato ID, vegetal, aftertaste, crunchiness, and adhesiveness. In the case of acid taste, no clear pattern was found, whereas a large difference between sessions was observed in UMH1200×18 (4.44 and 1.98 in session 1 and 2, respectively). In tomato hardness, UMH1200 scored the worst on average. Finally, lower significant differences were observed between grafting treatments in liking parameters. Specifically, the acidity, tomato ID, fruity, juiciness, adhesiveness, and residual skin (6 out of 16) were affected by the grafting. However, these differences did not seem to be robust enough, as they were detected only in one of the sensory evaluation sessions.

Table 4. Effect of grafting on sensory traits evaluated in CIAGRO-UMH trial (ANOVA and Tukey's multiple-range test). Se: Session; C: Cultivar; G: Grafting; CMuch: Commercial Muchamiel. Bold format indicates statistical significance.

Sensory Trait	Se	ANOVA p-Values [x]			Tukey's Multiple Range Test [y]						
					Cultivar				Grafting		
		C	G	C*G	1200	1200×4	1200×18	CMuch	Non-Graft	Beauf	Maxif
Visual											
Color	1	***	***	***	**3.73 a**	**5.07 b**	**5.24 b**	**5.96 c**	**4.76 a**	**4.86 a**	**5.38 b**
	2	***	***	***	**5.69 c**	**3.94 a**	**5.83 c**	**4.63 b**	**4.96 b**	**5.8 c**	**4.3 a**
Homogeneity	1	***	***	***	**6.72 c**	**5.78 b**	**6.6 c**	**5.36 a**	**6.5 b**	**5.48 a**	**6.36 b**
	2	***	0.577	***	**7.13 b**	**7.67 c**	**7.04 ab**	**6.63 a**	7.17	7.03	7.15
Ribbing	1	***	***	***	**1.36 a**	**2.67 c**	**2.19 b**	**1.55 a**	**1.75 a**	**1.69 a**	**2.4 b**
	2	***	***	***	**2 b**	**1.67 a**	**1.67 a**	**2.59 c**	**1.63 a**	**2.16 b**	**2.16 b**
Green shoulder	1	***	***	***	**2.92 c**	**2.64 b**	**2.92 c**	**2.14 a**	**3 b**	**2.46 a**	**2.5 a**
	2	***	***	***	**2.04 b**	**3 c**	**3.13 c**	**1.52 a**	**2.75 b**	**2.33 a**	**2.18 a**
Columella	1	*	*	**	**2.09 ab**	**1.87 a**	**2.02 ab**	**2.25 b**	**2.22 b**	**1.89 a**	**2.07 ab**
	2	**	*	***	**1.53 a**	**1.76 ab**	**2.07 b**	**1.74 ab**	**1.68 a**	**1.64 a**	**2.02 b**
Flavor											
Sweet	1	0.4	0.169	0.145	4.31	4.35	4.33	3.9	4.12	4.51	4.04
	2	*	0.659	0.923	**3.98 b**	**2.96 a**	**3.76 ab**	**3.57 ab**	3.61	3.66	3.43
Acid	1	***	***	0.527	**3.35 a**	**3.87 ab**	**4.44 bc**	**4.81 c**	**3.6 a**	**4.17 ab**	**4.58 b**
	2	***	0.058	0.875	**3.23 b**	**3.93 b**	**1.98 a**	**3.87 b**	3.1	3.64	3.01
Salty	1	0.632	0.065	0.748	1.69	2.12	2.03	2.19	1.61	1.97	2.44
	2	0.779	0.401	0.952	1.13	0.792	1.06	1.13	0.804	1.23	1.05
Tomato ID	1	0.628	*	0.498	4.56	4.88	5.03	5.01	**4.27 a**	**5.25 ab**	**5.08 b**
	2	**	0.237	0.917	**4.32 ab**	**3.75 a**	**5.39 b**	**4.67 ab**	4.4	4.9	4.29
Fruity	1	0.711	0.457	0.861	4.53	4.42	4.45	4.13	4.51	4.48	4.15
	2	0.027	*	0.982	5.37	4.71	5.52	4.77	**4.9 ab**	**5.57 b**	**4.79 a**
Vegetal	1	0.628	0.211	0.803	3.37	3.62	3.4	3.69	3.74	3.53	3.29
	2	*	0.401	0.061	**3.05 ab**	**2.27 a**	**3.04 a**	**3.28 b**	2.98	3.07	2.69
Aftertaste	1	0.371	0.389	0.841	3.88	4.46	4.17	4.01	3.98	4.37	4.05
	2	***	0.239	0.883	**3.85 bc**	**2.88 a**	**4.85 c**	**3.9 ab**	3.87	4.14	3.59
Texture											
Hardness	1	*	0.419	0.755	**3.08 a**	**3.77 ab**	**3.72 ab**	**3.81 b**	3.43	3.6	3.75
	2	**	0.629	0.912	**3.92 a**	**3.65 a**	**5 b**	**4.4 ab**	4.24	4.38	4.1
Crunchiness	1	0.505	0.038	0.684	2.99	3.48	3.29	3.37	3.06	3.07	3.72
	2	**	0.292	0.848	**2.93 ab**	**2.25 a**	**3.91 b**	**3.23 ab**	3.28	3.18	2.78
Juiciness	1	0.823	**	0.095	6.19	6.28	6.08	6.04	**6.62 b**	**5.89 a**	**5.93 a**
	2	0.794	0.989	0.936	5.68	5.46	5.81	5.45	5.61	5.62	5.57
Juice density	1	0.981	0.991	0.206	1.85	1.91	1.91	1.92	1.9	1.88	1.91
	2	0.093	0.489	0.465	1.53	2.04	1.48	1.8	1.6	1.84	1.7
Flesh	1	0.361	0.224	0.846	5.45	5.63	6	5.92	5.95	5.85	5.45
	2	0.614	0.878	0.923	5.72	5.63	6.04	5.82	5.75	5.88	5.77
Peel	1	0.923	0.645	0.967	3.22	3.28	3.17	3.41	3.3	3.4	3.11
	2	0.25	0.789	0.975	3.15	2.85	2.41	2.87	2.92	2.7	2.84
Seeds	1	0.815	0.199	0.7	2.72	2.62	2.67	2.44	2.58	2.38	2.87
	2	0.345	0.503	0.813	1.87	1.34	1.63	1.7	1.68	1.75	1.47
Adhesiveness	1	0.844	**	0.831	6.05	6.32	6.1	6.21	**6.52 b**	**6.38 b**	**5.61 a**
	2	***	0.903	0.999	**6.43 b**	**7.63 c**	**7.02 bc**	**5.7 a**	6.75	6.7	6.64
Residual skin	1	0.742	*	0.997	4.79	4.68	4.77	5.19	**5.38 b**	**4.98 ab**	**4.21 a**
	2	0.721	0.333	0.989	3.28	3.29	2.91	3.13	3.35	2.88	3.23

[x] Significant p-values: *, **, and ***, at $p < 0.05$, 0.01, and 0.001, respectively. [y] Means followed by the same letter, within the same row and factor, were not significantly different ($p < 0.05$).

4. Discussion

4.1. Yield Traits

Grafting is usually an excellent strategy to improve yield and fruit quality in crops affected by soil-borne diseases such as those caused by fungi and nematodes, as well as in presence of abiotic stresses (e.g., salinity or high temperatures) [18]. However, this positive effect can remain unnoticed under standard growing conditions not affected by these stresses [26]. In our experiments, soil was disinfected in CIAGRO-UMH trial before the plantation and no crops were growth in EEA-Elx field before current experiment. In addition, there were not important stresses, and the fertilizer supplies were conventional in both locations, so it could be considered that the tomatoes had been grown under standard non-stressing conditions. In fact, the higher total yield obtained in EEA-Elx compared to CIAGRO-UMH was probably due to use of bumblebees during cultivation, which improved the pollination and, thus, production.

A clear reduction in yield in plants grafted onto Maxifort compared with non-grafted plants was detected in the present study, while the use of Beaufort as rootstock led to yields comparable to non-grafted plants. Maxifort and Beaufort are rootstocks capable to improve growth and yield in infected soils with fusarium wilt [11] and nematodes [27], as well as in presence of mineral stressors accumulation [28], salinity [26,29], or water deficiency [30]. However, previous results indicate that this improvement is not clear when plants are grown under non-stressing conditions. Grafting onto Maxifort, which appears in literature as the most commonly used tomato rootstock in grafting research, resulted in extremely variable performance that led to variable yields that depended on the cultivar used as scion, being most of these grafted combinations questionable from an economic point of view [15]. Indeed, in different experiments performed under conventional conditions, Maxifort-grafted plants showed either better yield performance than non-grafting or self-grafting [31], did not show any improvement [14,19,21] and or led to lower yields in a few cases [15]. The same applied to the use of the Beaufort rootstock, as the choice of the scion cultivar is crucial for agronomic performance [16,32]. In the present study, no differential performance of the cultivars as response to grafting or the rootstocks used was detected as no significant C*G was detected. However, it should be considered that all the evaluated cultivars belong to Muchamiel landrace varietal type, with a low genetic diversity [33]. Similarly, the interaction *population x rootstock* was also not significant in a study performed with different populations of the Moruno Spanish landrace grafted onto three commercial rootstocks [17].

Growing season also seems to affect yield performance on grafted plants. For example, in previously studies, grafting onto Maxifort and Beaufort improved yield especially when production was sustained in the late season [11,31]. These rootstocks are known for promote vegetative growth and increase photosynthetic areas, which usually prolongs the harvest period and leads to an improved performance compared to non-grafted plants in long crop cycles [13,31]. In the present study, a short spring–summer cycle was used. It is therefore possible that rootstock/scion combinations used in current work would not be able to promote and increased expected vigor leading to enhanced productivity in longer cycles.

The yield decreases reported in Maxifort-grafted plants in this study were associated to significant reductions in fruit number, and no statistical differences were detected for fruit weight. This result agrees with a previously work using the commercial tomato Belle (Enza Zaden) grafted onto Beaufort in conventional conditions, with no important biotic or abiotic stresses [32]. In other studies using Maxifort in which yield increases are reported, the effects were related with both increased fruit number [16], and fruit weight [14].

Regarding the cultivar effect, the reductions found in yield parameters and TA reported in UMH1200 compared to Muchamiel hybrids totally agree with previously works performed with the same cultivars [34,35]. In fact, these effects would be related with the introgression in homozygosis of the *Ty-1*, which comes from wild tomato relative *Solanum chilense* and confers tolerance to TYLCV. This gene adversely affected some agronomic and quality traits, probably due the negative side effects of linked genes introduced along with the resistant gene that could not be removed during backcrossing due to the effects of linkage drag [36].

4.2. Quality Traits

It is important to stress that high concentrations of both sugar and acid compounds in fruit are extremely desirable in order to offer the best flavor in tomato. Therefore, an important factor to consider in the use of grafting in tomato is the influence on the metabolic profile involving these compounds. Especially in those cases in which the production is targeted to quality markets. Indeed, this would be the case of tomato landraces conserved in high quality local markets and specifically in the case of the Muchamiel landrace used in this study. In presence of biotic or abiotic stress environments, it is known that grafting can have beneficial effects on fruit quality as compared to the non-grafted plants [26,28], but in conventional non-stressing conditions its use may reduce the contents of sugars, acids, and other important compounds related with tomato flavor [14,17,19,21]. Our results seem to agree with this idea of negative side effects in the case of SSC and fructose, while for glucose and sucrose equivalents statistical values were close to the established threshold. However, we did not observe differences between grafting treatments for TA nor the acidity components nor dry matter. Other authors have described a higher impact. It would be the case of Riga [19], who reported a higher SSC reduction (around 16%) with no effects on TA in Jack tomato variety grafted onto Maxifort and Pogonyi et al. [37], who found lower SSC and no significant differences in TA in Beaufort-grafted plants (Lemance F1) in comparison with non-grafted ones. Krumbein et al. [21], described significant reductions around 6–10% in total sugar, glucose and fructose, and slightly increases (6%) in TA in Maxifort-grafted Piccolino and Classy varieties. Negative effects due to grafting have been reported not only in sugars and acids, but also on fruit compounds determining the functional quality of tomato as vitamin C [13] or β-carotene [38], although few studies also described positive effects on content of some carotenoids [17]. Many other experiments using different scion/rootstock combinations (including Beaufort and Maxifort rootstocks and different tomato landraces as scion varieties) reported similar results between grafted and non-grafted plants in several quality traits and fruit compounds [13,15,19,31]. Our findings agree with most of the literature, since it seems clear that the amount of quality traits affected and the way they are affected by grafting is highly dependent on the rootstock/scion combination and growing conditions.

4.3. Sensory Analysis

Apart from sugar and acid content in tomato fruit, which have an important effect on consumer preferences, the actual taste of tomato is also influenced by many other parameters, such as the visual aspect, the presence of volatile compounds or the texture in the mouth. Consequently, in order to assess the real effect on consumer perception, 21 sensory traits associated with these factors were evaluated in sensory evaluations, with the aim of further assessing the effect of grafting on the quality of Muchamiel tomatoes.

All the visual traits evaluated showed significant differences between cultivars and grafting treatments. The interaction C*G was also significant for all the parameters, indicating a differential response of each cultivar to the treatments. Other authors have also detected a high dependence of the specific scion/rootstock combination in the effect of grafting on fruit morphology [16]. Nonetheless, it should be considered that the results of the two evaluation sessions for visual parameters differed and thus, a lack of uniformity observed in the tomatoes harvested for each session could also have distorted the real response of the grafted plants. In this sense, it should be considered that Muchamiel tomatoes, as other landraces usually show great variability in tomato ripening and fruit morphology, which obviously affects the global homogeneity of the samples and makes it difficult to collect a high number of fruits in exactly the same ripening stage for the sessions. Nonetheless, the results were more robust for ribbing and green shoulder parameters, confirming an effect of grafting—at least for these parameters. As a results tomatoes grown in plants grafted onto Beaufort and, especially onto Maxifort, may show increased intensity of fruit ribbing and decreased intensity of green shoulder. Both traits are desirable in this landrace, therefore although high ribbing intensity is desirable, the effects on green should result in a visually less attractive tomato.

Regarding flavor and texture perception, three flavor and three texture parameters showed significant differences between grafting treatments, although only in Session 1 (except fruity flavor, significant in Session 2). Again, this result might be related with a lack of complete uniformity in the samples selected for the evaluation. Consequently, the results would indicate a null or very low effect of grafting in fruit flavor and texture for scion/rootstock combinations evaluated in the present work. Only few studies have previously been performed regarding sensory analysis in the assessment of the effects of grafting on flavor and texture qualities of the tomato fruit. In agreement with the results presented here, Di Gioia [13] also found no significant differences in six sensory traits between the Cuore di Bue landrace grafted onto Beaufort and Maxifort and non-grafted plants. On the other hand, cultivars Mando (Spanish landrace), Montgí (Spanish pure improved line), and Egara (commercial hybrid) grafted onto Beaufort showed worse sensory profile than non-grafted plants under conventional non-stressing conditions, being the landrace Mando the most affected [16]. Barrett et al. [39] concluded grafting the Brandywine landrace onto Multifort and Survivor rootstocks had negative effects on sensory profiles but only in the one of the two experiments, and, as in the present study, they related this effect with a low uniformity of the samples used in the trials. It seems clear then that the effects of grafting on sensory traits in conventional non-stressing conditions would be dependent on the scion/rootstock combination.

5. Conclusions

Growing our Muchamiel cultivars grafted onto Beaufort and Maxifort rootstocks under favorable conditions resulted in significantly worse yield and fruit number in plants grafted onto Maxifort, slightly but significant reductions in SSC, fructose, and sucrose equivalents, and significant effects on some sensory traits (especially the visual ones), with no consensus between sessions in most of them. Instead, plants grafted onto Beaufort showed similar yield than non-grafted ones and no differences were reported between grafting treatments in fruit weight, TA, and the acid profile, as well as in most of flavor and texture sensory parameters. Overall, all the scion/rootstock combinations showed a similar behavior in the two trials carried out (CAIGRO-EPSO and EEA-Elx) for yield and main quality traits, so that GxE interaction was low in our specific locations. In addition, *cultivar*grafting* interaction was no significant in any parameter evaluated, except for some of the sensory traits.

According with most of the literature, the accurate choice of scion/rootstock combination seems to be crucial, especially for grow traditional tomato varieties under favorable environments. Demand of these tomatoes in markets is rising and the European agriculture is demanding more sustainable and resilient systems, so that studies of this type are necessary to provide reliable and attractive alternatives to farmers. In that way, we suggest growing Muchamiel/Beaufort combination only under unfavorable environments (presence of soil-borne diseases), using the standard crop conditions for tomato for the spring–summer cycle in SE Spain. Grafting these cultivars onto Maxifort would be an unsuitable option due to the significant decreases reported in yield.

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