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Research Article

Grafting in *Capsicum* peppers as a strategy to mitigate the effects of climate change on yield and quality factors

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Abstract

Climate change in the Mediterranean areas is increasing problems on droughts, water availability and salinization of irrigation water. These are probably some of the most limiting factors on farming, especially in vegetables production. *Capsicum* peppers, one the most valuable vegetables in Spain, are quite sensitive to water deficit and particularly to salinity. The use of rootstocks tolerant to these abiotic stresses could be explored as a short/mid-term solution. In this work, we evaluated the ability as rootstocks of several accessions, together commercial F1 'Robusto' and 'Oscos', with the cultivar 'Herminio' as scion, under control, drought (30% decrease irrigation) and salinity (5.8 dS m^{-1}) in Campo de Cartagena area (Murcia, Spain). Yield and fruit weight, and water content, ascorbic acid content (AAC) and total phenolics (TP) at the unripe and fully ripe commercial stages were evaluated. Under control conditions, our rootstocks did not provide extra vigour and yield as none showed higher performance than the non-grafted 'Herminio' in yield (10 kg m^{-2}) and fruit weight (215 g). However, drought and salinity conditions revealed that some accessions might be useful as rootstocks, providing higher yields and/or fruit size than 'Herminio', particularly yield under drought, i.e. 5-7 kg m^{-2} while 'Herminio' only achieved 4 kg m^{-2}, and fruit weight in both stress conditions (i.e. 190-223 g vs 173 g under drought, 187-209 g vs 158 g under salinity). On average, drought increased the levels of antioxidants at both ripening stages, while salinity decreased them, specially AAC. Also, remarkable rootstock × treatment interactions, particularly in phenolics, enabled identifying several rootstocks providing high levels of antioxidants at both ripening stages and under both abiotic stresses, improving those from non-grafted 'Herminio'.

Keywords: ascorbic acid; drought; fruit weight; genotypes; phenolics; rootstock; salinity

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Introduction

Nowadays, climate change is one of the most challenging threats on our planet. Temperature rising, increasing UV levels, unexpected and erratic weather events, among other phenomena, are more frequent in the last decades and they are affecting many activities, particularly farming and food security (FAO, 2015; World Economic Forum, 2023). In this regard, for instance, temperature rising is provoking uncontrolled problems in i) fruit setting, development, ripening and composition, ii) yields, iii) occurrence of new pathogens (FAO, 2015; Singh *et al.*, 2023). Also, changes in rain patterns are provoking floodings or droughts (Trenberth, 2011; Winsemius *et al.*, 2018). These events are particularly dramatic in the Mediterranean area, where due to the climate change, drought is worsening the availability of water, a limiting factor for crop production (Chenoweth *et al.*, 2011; FAO, 2015). In addition, usually, the lower the availability of water the lower the quality as, globally, this kind of water is more concentrated in soluble salts (Ullah *et al.*, 2021; Jeppesen *et al.*, 2023) and, therefore, farmers have no other choice than using salinized water in their crops (Fita *et al.*, 2015; Ullah *et al.*, 2021).

In this frame, the Mediterranean area of Spain is one of the most productive regions of the world, particularly in vegetables. However, their farmers are facing considerable loss of productivity due the lack of water for irrigation and/or its salinity levels (Fita *et al.*, 2015). Some important crops like tomatoes or eggplants are still relatively tolerant to water deficit and salinity and, therefore, farmers can still manage this challenge (Suarez *et al.*, 2021; Ortega-Albero *et al.*, 2023). By contrast, other crops like peppers, the second vegetable in terms of production in Spain, i.e. 1.53 mill t per year from an acreage of 22,000 ha, and an economic value of 1,600 mill €, are more sensitive to these abiotic stresses (Toppino *et al.*, 2021; MAPA, 2023).

Breeding can be helpful to mitigate these problems. However, the introgression of genes of tolerance identified in new sources of variation into commercial varieties may took several years (Fita *et al.*, 2015). As an alternative, the direct use of rootstocks may offer a short-term solution, which, apart from increasing the vigour and productivity of the variety, may also provide a satisfactory adaptation to abiotic stresses (Kumar *et al.*, 2017; Razi and Muneer, 2023). To this regard, materials domesticated in regions affected by hot and dry weathers or saline soils are good candidates to be tried. Finally, apart from productivity parameters, it must be considered that abiotic stress, as well as grafting, may also affect quality factors (Rouphael *et al.*, 2010; Perin *et al.*, 2015; Semiz and Suarez, 2019; Egea *et al.*, 2023). Peppers are in fact recognized for their high antioxidant value, the highest among common fruits and vegetables mainly due to their high levels in ascorbic acid and phenolics (Pellegrini *et al.*, 2003; Morales-Soto *et al.*, 2014; Ribes-Moya *et al.*, 2019 and 2020).

In the present work, we have studied in a comprehensive way the response of a set of accessions of peppers used as rootstocks, against water deficit and salinity conditions, using a reference variety in the Mediterranean area of Spain. The effect of these abiotic stresses and the different grafting combinations on yield, fruit size and the accumulation of the main antioxidants in the fruits at different ripening stages was assessed.

Materials and Methods

Plant materials and grafting combinations

The cv. 'Herminio' (Syngenta Seeds, abbreviated as Herm in tables), a commercial variety of *C. annuum*, corresponding to the Lamuyo type (large bell) and grown profusely in the Campo de Cartagena area (Murcia, Spain) for decades, was utilized as reference in the present experiment. Also, eight *Capsicum* accessions were evaluated as rootstocks: two commercial rootstocks 'Robusto' (Syngenta, Spain) and 'Oscos' (Ramiro Arnedo seeds, Spain); three *C. annuum* landraces from the southern dry area of USA: 'Chimayo' type (abbreviation 'Chimayo'), 'Jalapeno E10397' (JE10397) and 'Numex Heritage' (NMh), originally provided by The Chile Pepper Institute (Las Cruces, New Mexico, USA); and other three *Capsicum* sp. accessions from the Andean region: U19235 and U26484 (*C. chinense*) and U27610 (*C. baccatum*).

Working on these materials, ten different plant materials or combinations were evaluated: non-grafted 'Herminio', self-grafted 'Herminio' ('Herm'/ 'Herm') and the eight grafting's of 'Herminio' (as aerial part) on the different accessions used as rootstocks: 'Herm'/ 'Robusto', 'Herm'/ 'Oscos', 'Herm'/'NMh', 'Herm'/ 'Chimayo', 'Herm'/JE10397, 'Herm'/U19235, 'Herm'/U26484 and 'Herm'/U27610.

Grafting, experimental design and growing conditions

Seeds from cv. 'Herminio' were provided by nursery La Sala (San Javier, Campo de Cartagena shire, Spain). Seeds from the different accessions used as rootstocks were provided by the group of *Capsicum* Breeding and the Seedbank of the COMAV Institute (Universitat Politècnica de València). Seeds were sown in October 2020 and managed at the La Sala nursery facilities and all the graftings were performed by La Sala technicians at the plantlet state. Plants were transplanted in December 2020 in a greenhouse at El Mirador experimental (El Mirador, Campo de Cartagena, Spain) and five blocks (n=5) of three plants each were transplanted randomly per plant combination and treatment.

Plant materials were grown by El Mirador staff during the usual autumn-spring season of peppers at the Campo de Cartagena area in 2020-21. Plants were managed following the usual practices of the area. The materials were submitted to three different treatments: i) control fertigation solution (1g L^{-1} of a commercial 15N-2.2P-24.9K water soluble fertilizer, EC 1.1 dS m^{-1}); ii) drought simulation: 30% reduction of the control fertigation; and iii) salinity simulation: control fertigation solution artificially salinized by applying 40 mM NaCl to achieve EC 5.8±0.2 dS/m.

Fruit sampling and studied traits

Yield and fruit weight were recorded on each plant along the harvesting season (starting at the end of march until June 2021). A mean value per block was estimated based on the corresponding three plants. Then a final mean was obtained with the data from the five blocks for each plant combination and treatment (n = 5). Fruits from the three plants of each block were sampled at the unripe and the fully ripe commercial stages to prepare a repetition (n = 5 per plant combination and treatment). Fruit samples were immediately sent to the UPV labs, washed, cut into 3 mm pieces and divided into two subsamples: i) frozen in N and preserved in Falcon tubes at -86 °C for ascorbic acid determination and ii) lyophilized and preserved into a refrigerator (5 °C dry and dark) for total phenolics.

Ascorbic acid content (AAC) and total phenolics (TP) were estimated following different spectrophotometrical methods with high accuracy and adapted to peppers, following the methodology described by Ribes-Moya *et al.* (2018). ACC was estimated reflectometrically, on the basis of yellow molybdophosphoric acid reduction to phosphomolybdene blue, using a reflectometer RQflex plus and a Reflectoquant test (Merck, Darmstadt, Germany). Fresh fruit samples of 15 g were homogenized by means of an industrial blender, mixing with distilled water to facilitate blending. Once homogenized the mixture was filtered with a 0.5 mm mesh in a test tube and filled with distilled water until 50 mL. The results were expressed in mg per 100 g of fresh weight, according to the formula:

$$\text{AAC (mg/100 g)} = 0.1 \times \frac{\text{Volume (mL)} \times \text{Measured value (mg L)}}{\text{fruit weight (g)}}$$

TP determination was done according to Folin-Ciocalteu method, colorimetric reaction quantified by measuring absorbance at 750 nm, referring results to a standard curve of chlorogenic acid, as reported by Ribes-Moya *et al.* (2018). Before the analysis, each sample was submitted to extraction: 125 mg of lyophilized sample plus 5 mL extraction solution (700 mL acetone + 5 mL glacial acetic acid + 295 mL Milli-Q water) placed in 15 mL Falcon centrifuge tubes, incubated and stirred (1 d, darkness, room temperature). Then, samples were centrifuged (3 min, 3500 rpm) and 1.5 mL supernatant was collected in a microcentrifuge tube and stored (-80 °C). For the analyses, samples were centrifuged again (5 min, 10000 rpm) and 70 µL of the sample were mixed with 0.5 mL Folin-Ciocalteu reagent (in 10% water dilution, v/v) and incubated 5 min at room temperature. After that, 0.5 mL of a saturated Na_2CO_3 solution (60 g/L) were added and the mixture incubated 90 min (darkness, room temperature). Then, an aliquot (200 µL) of each sample or standards were placed in 96-well microplate, measuring absorbance at 750 nm, using a microplate reader (Bio-Rad iMarkTM). On the basis of the dry matter content of each sample, results were expressed as mg chlorogenic acid per 100 g fresh weight.

Statistical analysis

An analysis of variance ANOVA of main factors i) plant material (reference cv. 'Herminio' and grafting combinations with 'Herminio' as aerial part), ii) treatment (control, drought and salinity), iii) fruit ripening stage (unripe and fully ripe), as well as iv) their interactions, was performed, in both original and transformed data (to adapt to normal distribution), using Statgraphics Centurion XVI software (StatPoint Technologies, Inc; Warrenton, Virginia, USA). A linear model was used for general ANOVA: $X_{ijkl} = \mu + A_i + B_j + C_k + (\alpha \times \beta)_{ij} + (\alpha \times \gamma)_{ik} + (\beta \times \gamma)_{jk} + E_{ijk(l)}$. Xijkl is the value for fruit sample l from plant material i, treatment j and ripening stage k, being µ the general mean; Ai: effect of genotype i; Bj:effect of growing system j; Ck: effect of ripening stage k; (α × β)ij: interaction between plant material i and treatment j; (α × γ)ik: interaction between plant material i and ripening stage k; (β × γ)jk: interaction between treatment j and ripening stage k and Eijk(l): error term or effect of fruit sample l from the combination of plant material i, treatment j and ripening stage k.

Furthermore, additional ANOVAs were also carried out for analytical traits, AAC and TP, considering separately unripe and fully ripe stages. The linear model used in these cases was $X_{ijk} = \mu + A_i + B_j + (\alpha \times \beta)_{ij} + E_{ij(k)}$, where Xijk is the value in one specific ripening stage for fruit sample k of plant material i and treatment j; being µ the general mean; Ai: effect of plant material i; Bj: effect of treatment j; (α × β)ij: interaction between plant material i and treatment j and Eij(k): effect of fruit sample k from the combination of plant material i and treatment j. No significant differences were found in the ANOVAs comparing original and transformed data and, therefore, the results were displayed considering the original non-transformed data.

Results and Discussion

Analysis of variation

For yield and fruit weight the general ANOVA revealed that all the factors, i.e. plant combination (P), treatment (T) and their interaction (P × T) contributed significantly to the variation found in both traits. According to the magnitude of the corresponding mean squares, the treatment provided the higher contribution, followed by plant material and the interaction (Table 1). No more effects were studied in these traits as all the fruits, regardless their ripening stage, were weighted to estimate fruit weight and the total yield per plant. Therefore, the ripening stage (R) and their interaction with plant combination or treatment were not included in this study.

In the case of fruit composition traits, the general ANOVA detected significant contribution of all the main factors, i.e. plant material, treatment and ripening stage, as well as their paired interactions, i.e. P × T, P × R and T × R, to the variation observed, with the only exception of T × R in total phenolics. Furthermore, the magnitude of the mean squares revealed a very high contribution of the ripening effect, considerably much higher than plant combination and treatment. Also, the interactions reached levels of contribution similar to the plant material, or even higher like T × R, suggesting that plant materials might behave differently when changing among treatments, or that the changes of composition with the ripening process may be different among plant materials. For such reason, and considering the great contribution of the ripening stage, ripening stage-specific ANOVA were also carried out to simplify the interpretation of the components of variation.

The ANOVA for unripe fruits found that all the factors, plant combination, treatment and their interaction contributed significantly to the variation of water content, ascorbic acid and phenolics, particularly the treatment and, to a very lesser extent, plant material and the P × T interaction. This trend was particularly remarkable in AAC, where the mean square was 15-fold and 40-fold those of the plant material and the P × T interaction (Table 1). Finally, a similar trend was found for fully ripe fruits, where all the factors contributed significantly to the variation of analytic traits, and particularly the treatment in AAC (Table 1).

Table 1. ANOVA table corresponding to the mean squares of each main factor and interactions between them general (all factors considered together. upper) and specific for each fruit ripening stage (unripe and fully ripe. lower) for yield. fruit weight. water content (WC). ascorbic acid content (AAC) and total phenolics (TP)

Factor	**d.f.**	**Yield**		**FW**		**d.f.**	**WC**		**AAC**		**TP**	
	General											
Plant material (P)	9	21.82	***	235	**	9	8.64	***	1221	***	6207	***
Treatment (T)	2	76.47	***	456	***	2	18.44	***	64259	***	55542	***
Ripening (R)		---		---		1	302.91	***	116336	***	398146	***
Interactions												
P×T	18	15.64	***	164	**	18	4.85	***	609	*	8273	***
P×R		---		---		9	4.07	***	801	**	3272	***
T×R		---		---		2	8.97	***	8178	***	203	NS
Error	129	3.67		65		258	1.02		312		721	
Unripe fruits	**Ripening stage-specific**											
Plant material (P)						9	2.72	***	827	***	5771	***
Treatment (T)						2	19.20	***	13319	***	26468	***
Interactions												
P×T						18	2.03	***	358	*	5606	***
Error						120	0.39		166		417	
Fully ripe fruits												
Plant material (P)						9	9.99	***	1195	**	4213	***
Treatment (T)						2	8.21	***	59118	***	31036	***
Interactions												
P×T						18	11.98	***	1005	**	4761	***
Error						120	0.42		391		827	

NS and *. ** and *** indicate not significant for a probability p >0.05 and significant for p< 0.05, 0.01 and 0.001, respectively, according to the statistical F ratio.

Productivity traits

Yield

A range of yield values was found under control conditions for the different plant materials evaluated, suggesting different levels of compatibility, highly depending on the genotype used as rootstock. The cultivar 'Herminio' used as reference, reached satisfactory yield levels (10 kg/m^2) and many rootstocks provided similar or even slightly higher yields, like the commercial 'Oscos' and the experimental 'Numex Heritage', 'Chimayo', U26484 and U27610 (Table 2). Finally, the other commercial rootstock 'Robusto' provided slightly lower yields, while jalapeno JE10397 and particularly U19235, as well as self-grafted 'Herminio', showed the poorest yield performance (Table 2).

Table 2. Mean values of yield (kg m^{-2}) and fruit weight (g) under control, water deficit. and saline conditions

Plant material	**Control**		**Drought**		**Saline**	
Yield (kg m^{-2})						
Herm (non-grafted)	10.05	d	4.14	b	2.90	ab
Herm/Herm	5.38	a	4.13	ab	2.60	a
Herm/Robusto	8.12	b	4.38	b	2.98	ab
Herm/Oscos	10.76	d	6.51	d	4.27	c
Herm/NMh	9.94	d	5.60	c	3.57	b
Herm/Chimayo	10.29	d	3.06	a	3.39	b
Herm/JE10397	6.32	ab	2.41	a	3.38	b
Herm/U19235	5.38	a	3.14	a	2.81	ab
Herm/U26484	10.14	d	4.87	bc	2.25	a
Herm/U27610	9.40	c	5.15	bc	4.01	bc
Mean	8.58	C	4.34	B	3.22	A
Fruit weight (g)						
Herm (non-grafted)	215	b	173	b	158	b
Herm/Herm	220	b	175	b	135	a
Herm/Robusto	242	c	192	c	149	b
Herm/Oscos	183	a	155	ab	187	cd
Herm/NMh	176	a	195	c	209	e
Herm/Chimayo	162	a	196	c	169	c
Herm/JE10397	229	bc	223	d	197	d
Herm/U19235	224	bc	165	a	164	bc
Herm/U26484	235	c	176	bc	132	a
Herm/U27610	196	ab	165	b	187	d
Mean	208	C	182	B	169	A

Different letters within the same growing conditions indicates means significantly different at $p < 0.05$, according to the Newman-Keuls statistic test. Upper case letters indicate significant differences among treatment means at $p < 0.05$, according to the Newman-Keuls statistic test.

In many cases, rootstocks have been demonstrated (and commercially used) to improve vigour and yield, particularly in tomato and eggplants (Djidonou *et al*., 2020; Grieneisen *et al*., 2018; Cerruti *et al*., 2021). However, our results confirm that variety 'Herminio' is a variety well-adapted to the greenhouse conditions of the Campo de Cartagena of Spain, being a reference of the 'Lamuyo' varietal type in this production area for years (López-Marín *et al*., 2013; Syngenta Vegetables Seeds Global, 2024). In fact, none of the grafting combinations improved 'Herminio' yield (10-11 kg m^{-2}) (Table 2). Another explanation could be the fact that, usually, commercial rootstocks are F1s and, therefore, many of them might provide their hybrid heterosis to the aerial part, while our experimental materials are still merely landraces. Probably, crossings with other sources would improve their vigour as rootstocks.

Moreover, the dramatic decrease on yield observed in self-grafted 'Herminio' provides an estimation of the stress that grafting may cause in the scion. By contrast, as mentioned before, other genotypes including accessions from other species like *C. chinense* U26484 and *C. baccatum U27610*, showed a similar performance as rootstocks than 'Herminio' itself. Although other authors have not reported significant decreases in the yield of self-grafted varieties (Hollick and Kubota, 2022), our results suggest that, in the case of some varieties of peppers, self-grafting may provoke injuries in the rootstock-scion connection, while paradoxically other materials used as rootstocks may show better compatibility by providing a higher efficiency in restoring the phloem and xylem and then promoting a better mobilization of water and nutrients (Ropokis *et al*, 2019).

In comparison, stress conditions provoked considerable decreases in yield, particularly salt stress. Thus, on average, water deficit and salt stress decreased yield 50% and 62% respectively compared to control conditions and also all the plant materials showed remarkable decreases, particularly under salt stress (Table 2). Considering water deficit, yield values ranged from 2.41 to 6.51 kg m^{-2} of JE10397 and 'Oscos', respectively, while cv. 'Herminio' and its self-grafting reached about 4 kg m^{-2}. In comparison, several rootstocks like the commercial 'Oscos', followed by 'Numex Heritage', *C. chinense* U26484 and *C. baccatum U27610* reached ≥5 kg m^{-2}) (Table 2). Finally, commercial rootstock 'Robusto' gave intermediate values (4 kg m^{-2}), similar to that of 'Herminio', and the performance of the rest of rootstocks was very poor (2-3 kg m^{-2}) (Table 2). These results suggest that *Capsicum* genotypes can differ in water use efficiency under drought conditions and that such ability can be also conferred through grafting, as reported not only in peppers, but also in other crop species (Djidonou *et al*., 2013; Ropokis *et al*., 2019; Padilla *et al*., 2021; Taha *et al*., 2022)

Salt stress conditions decreased yield dramatically, although significant differences were still found among accessions. The performance among genotypes was quite similar than that observed in water deficit. In this regard, yield values were comprised between 2.25 and 4.27 kg m^{-2} of U26484 and 'Oscos', respectively, while cv. 'Herminio' and its self-grafting achieved 2.5-3.0 kg m^{-2} (Table 2). In comparison 'Oscos' and *U27610*, followed by and 'Numex Heritage' and to a lesser extent *Chimayo* and JE10397, showed yields around 3.5-4.0 kg m^{-2}) (Table 2). Finally, the worst performance under salt conditions corresponded to the *C. chinense* rootstocks and the own variety 'Herminio' (< 3 kg m^{-2}) (Table 2). In comparison to drought, salinity on average and in most combinations provoked a higher impact, indicating that peppers are more sensitive to the latter, but also that genotypes used as rootstocks might differ in the way they metabolise salt ions as reported by Singh *et al.* in tomatoes (2020).

Among the combinations evaluated, we identified some accessions with a satisfactory performance as rootstocks in terms of yield. Thus, the commercial 'Oscos', 'Numex Heritage' and *C. baccatum U27610* exhibited a remarkable performance in yield, similar or slightly higher than the reference 'Herminio' in control conditions, and 25-50% higher under water deficit and saline conditions, while the rest of accessions showed a poor performance (Table 2). All these results indicate a variable performance against water deficit and even salinity conditions, depending on the genotype, and therefore some accessions can be efficient in mitigating the effect of these abiotic stresses in pepper cultivation through the grafting strategy.

Fruit weight

Fruit weight was also variable under control conditions among the different combinations evaluated, ranging from 162 to 242 g in rootstocks 'Chimayo' and 'Robusto', respectively (Table 2). In contrast to yield, cv. 'Herminio' fruit weight (215 g) was similar to the mean value and, in comparison, some rootstocks like 'Robusto' and *C. chinense* U26484, and at a lesser extent JE10397 and U19235, provided and increasing effect to this trait (Table 2). On the contrary, 'Oscos', 'Numex Heritage' and 'Chimayo', provided a decreasing effect on the fruit weight. These findings suggest that the increase in yield provided as rootstocks by some genotypes was to the detriment of fruit weight, while the contrary was found for the genotypes which enabled low yields, i.e. the increasing effect in the yield (biomass) could have provoked smaller fruits, which must be considered with caution by producers, particularly when aimed at certain export markets which prefer large sizes. Actually, this effect is unclear as some authors have reported that grafting may increase both yield and fruit size and number, while other authors found different results (Kyriacou *et al*., 2017), suggesting that this effect could be highly dependent on species and/or the scion-rootstock combination.

Regarding the performance under the stress conditions, both drought and salinity caused a significant mean decrease in fruit weight in comparison to control conditions, particularly the latter (Table 2). This effect was also found among most combinations. Thus, in the case of water deficit, fruit weight decreased in most cases, with the exception of 'Numex Heritage', 'Chimayo' and JE10397. Nevertheless, some accessions, including the mentioned ones and the commercial 'Robusto', could mitigate as rootstocks the impact of drought in this trait, as they achieved higher values than 'Herminio' (≥190 g) in these conditions, particularly JE10397 (Table 2). Saline conditions also decreased the size of the fruits in all the combinations, with the only exception of 'Numex Heritage'. In fact, the weight of the reference 'Herminio' decreased to 158 g, which is about the minimum for marketing. Nevertheless, several rootstocks provided a beneficial effect under these highly stressful conditions, enabling a fruit size considerably higher than 'Herminio' (≥190 g), namely the commercial 'Oscos', 'Numex Heritage', JE10397 and *C. baccatum* U27610 (Table 2).

These findings suggest that some accessions can be very useful as rootstocks, providing similar or even higher yield and fruit sizes under control conditions, but particularly when the variety (scion) is grown under more limiting conditions, when the real potential of these genotypes can be observed (Kyriacou *et al*., 2017). In this regard, it must be noted that genotypes like 'Numex Heritage', 'Chimayo' or JE10397 belong to varietal types profusely grown in the southern USA and northern Mexico, i.e. arid areas of Sonora and Chihuahua deserts, and even the *C. chinense* and *C. baccatum* accessions are also original from the arid areas of Ecuador or the Andean Altiplano (Pereira-Dias *et al*., 2019). Thus, as a result of adaptation to these conditions, these materials could have accumulated an advantageous genetic background to face drought and/or salinity when grown under commercial conditions.

Fruit composition. Unripe fruits

Water content

Under control conditions, water content in the unripe fruits was quite similar among the different combinations, although slight significant differences were found. Thus, the unripe fruits from non-grafted cv 'Herminio' showed 92% of water content, while fruits from most of the grafting combinations reached 93-94%, including self-grafted 'Herminio' (Table 3). This fact suggests that, paradoxically, the injuries caused by grafting might result in a beneficial response at tissue level which increases the mobilization of water towards aerial organs like fruits, as reported by Ropokis *et al* (2019) and Gisbert-Mullor *et al* (2020).

Considering the stress conditions, on average, both drought and salinity caused a significant, but low, decrease of water content in unripe fruits (0.77-1.23%) (Table 3), which, can be also considered as an increase on dry matter, i.e. 6.63% under control conditions vs. 7.86% and 7.40% under drought and salinity, respectively. These results are in agreement with several reports (e.g. Van de Wal *et al*. 2017; Coban *et al*., 2020; Li *et al*., 2023), which described an increase on dry matter in the aerial structures due to drought and/or salinity conditions. Under these conditions, unripe fruits from non-grafted 'Herminio' showed similar levels than those found under control conditions (around 92%), although the grafting combinations caused different patterns of variation in this trait. This was more evident in self-grafted 'Herminio', commercial 'Robusto' or JE10397, which provided higher water content in the fruits (92.8-93.5%), while others like 'Chimayo' and the *C. chinense* and *C. baccatum* rootstocks provided lower water content (91-92%) (Table 3).

Table 3. Mean values of water content (WC, %), ascorbic acid content (AAC, mg 100 g-1 fw) and total phenolics (TP, mg 100 g^{-1} fw) in unripe fruits under control, water deficit and saline conditions

Plant material	**Control**		**Drought**		**Saline**	
WC (%)						
Herm (non-grafted)	91.99	a	92.48	cd	92.26	b
Herm/Herm	93.53	b	93.45	f	92.50	bc
Herm/ Robusto	93.81	b	92.83	de	93.05	ef
Herm/ Oscos	92.85	ab	92.29	c	91.59	a
Herm/NMh	93.83	b	92.14	c	92.84	de
Herm/Chimayo	93.91	b	91.44	ab	92.63	cd
Herm/JE10397	93.44	b	93.22	ef	93.29	f
Herm/U19235	93.69	b	90.98	a	92.80	cde
Herm/U26484	93.16	ab	91.57	b	92.21	b
Herm/U27610	93.44	b	90.97	a	92.81	cde
Mean	93.37	C	92.14	A	92.60	B
AAC (mg 100 g^{-1} fw)						
Herm (non-grafted)	103.20	ab	115.34	de	89.51	d
Herm/Herm	94.65	ab	108.50	cd	79.92	cd
Herm/ Robusto	89.33	a	103.79	bcd	66.01	abc
Herm/ Oscos	95.33	ab	81.08	a	59.60	a
Herm/NMh	108.05	b	88.78	ab	64.85	a
Herm/Chimayo	101.17	ab	97.67	abc	71.80	abc
Herm/JE10397	107.19	b	108.22	cd	70.90	abc
Herm/U19235	105.81	ab	101.62	bcd	79.06	bcd
Herm/U26484	94.00	ab	107.80	cd	72.85	abc
Herm/U27610	95.72	ab	129.60	e	83.92	cd
Mean	99.45	B	104.24	B	73.88	A
TP (mg 100 g^{-1} fw)						
Herm (non-grafted)	146.65	c	116.99	b	118.36	f
Herm/Herm	138.86	bc	96.82	ab	99.69	de
Herm/ Robusto	91.40	a	113.64	ab	80.25	bc
Herm/Oscos	95.35	a	84.40	a	60.26	a
Herm/NMh	107.20	a	88.90	ab	105.32	ef
Herm/Chimayo	94.36	a	152.00	c	75.05	ab
Herm/JE10397	115.47	ab	115.15	b	96.28	cde
Herm/U19235	115.31	ab	241.84	e	82.05	bc
Herm/U26484	104.64	a	210.15	d	84.94	bcd
Herm/U27610	117.60	abc	147.93	c	105.65	ef
Mean	112.68	B	136.78	C	90.79	A

Different letters within the same growing conditions indicates means significantly different at $p < 0.05$, according to the Newman-Keuls statistic test. Upper case letters indicate significant differences among treatment means at $p < 0.05$, according to the Newman-Keuls statistic test.

Ascorbic acid content

Ascorbic acid content (AAC) in unripe fruits under control conditions was comprised between 89 and 108 mg 100 g^{-1} fresh weight (fw), with non-grafted 'Herminio' showing 103 mg 100 g^{-1} fw (Table 3). Some differences due to grafting combinations were found, but none offered significant differences in comparison to the non-grafted 'Herminio'. Only the levels of 'Numex Heritage' and JE10397 (107-108 mg 100 g^{-1}) were higher than those from 'Robusto' (Table 3). On average, drought conditions did not appear affecting, on average, the levels of AAC compared to control conditions. However, a remarkable effect of the grafting combination was found within this stress, suggesting that the occurrence of water deficit may provoke a differential response, depending on the genotype used as rootstocks, with rootstocks like 'Oscos', 'Numex Heritage' or 'Chimayo' provoking a remarkable decreasing effect (80-98 mg 100 g^{-1}), while others like *U27610* favoring the accumulation of ascorbic acid in the unripe fruits (130 mg 100 g^{-1}) (Table 3). By contrast, some authors have described the beneficial effect of endogenous ascorbic acid or exogenous applications on alleviating the effects of drought, although these studies have also revealed a reduction on AAC in the fruits (Seminario *et al*., 2017; Gisbert-Mullor *et al*., 2020). Probably, the diversity used in our study has allowed to identify some materials that, used as rootstocks, may favor AAC.

In comparison, saline stress caused a dramatic deleterious effect on AAC in unripe fruit, not only on average (74 mg 100 g^{-1} fw vs. 100-104 mg 100 g^{-1} fw in control and drought conditions), but also in all the combinations (Table 3). Thus, although there were significant differences among combinations, this stress decreased AAC in all of them, from 15% in the fruits of non-grafted 'Herminio', self-grafted 'Herminio' or U27610 to other rootstocks like 'Oscos' or 'Numex Heritage', with decreases of about 50% (Table 3). These findings indicate that salinity is very negative for AAC in the fruits of peppers, even more aggressive than drought, suggesting that plants use very high amounts of this antioxidant by means of ascorbate oxidase to prevent oxidative stress in the tissues, causing therefore a remarkable decrease in the fruits, as reported by Abdelgawad *et al*. (2019).

Total phenolics content (TP)

The content of phenolics in unripe fruits under control conditions ranged considerably between 91 and 147 mg 100 g^{-1} fw, with the highest values corresponding to the non-grafted cv 'Herminio' and its self-grafted control (around 140 mg 100 g^{-1} fw) (Table 3). Therefore, despite some significant differences were found among grafting combinations, none of them offered levels similar to the nongrafted cv 'Herminio' and even some combinations like 'Robustos', 'Oscos' or 'Chimayo' provided very low levels (< 100 mg 100 g^{-1} fw) to the fruits (Table 3). In comparison, drought increased TP on average, although the exposure to this stress showed very variable behaviors among combinations. In fact, in contrast to AAC and the average TP increase due to drought, it decreased considerably TP in non-grafted and self-grafted cv 'Herminio' (30-40%). Moreover, a great variation was found among the different grafting combinations, from a few cases of very low levels and comparatively lower than control conditions like 'Oscos' and 'Numex Heritage' (< 90 mg 100 g^{-1} fw) to other grafting combinations that remarkably increased TP in unripe fruits under these drought conditions like U19235 and U26484 (>200 mg 100 g^{-1} fw) (Table 3). In general, these findings suggest, in a clearer way than that observed for AAC, that drought can be beneficial for antioxidant accumulation in most cases, with some genotypic variation, as reported by several authors (Gharibi *et al*., 2016; Sarker and Oba, 2018, Laddomada *et al*., 2021).

Finally, and similarly to observed for AAC, salinity had a remarkable decreasing effect on TP, both on average (91 mg 100 g^{-1} vs 113 mg 100 g^{-1} in control conditions) and in most combinations (Table 3). In this regard, with the only exception of 'Numex Heritage' as rootstock, the decreases due to salinity ranged from 10-15% in non-grafted 'Herminio', 'Robusto' or *U27610* to 30-35% in self-grafted 'Herminio', and 'Oscos' or U19235 (Table 3), and consequently, none of the grafting combinations offered a better performance than the non-grafted variety. These results on phenolics, very similar to those from ascorbic acid, confirm that saline conditions would require a very intense antioxidant-consuming response of the plants, to the point of decreasing remarkably phenolics and ascorbic acid levels in the fruits (Linić *et al*., 2019). Also, at least considering unripe fruits, the use of graftings may be chosen with care as, depending of the rootstock used, the AAC and TP can be decreased in comparison to the non-grafted variety, particularly under saline conditions, or on the contrary may help to increase AAC and specially TP, particularly under water stress.

Fruit composition. Fully ripe fruits

Water content

In general, compared to unripe fruits, water content in fully ripe fruits was considerably lower in all the treatments, i.e. 89-93% among combinations and treatments, 90-91% on average (Table 4), indicating that ripening increases dry matter content. Also, in comparison to the unripe stage, the fully ripe stage exacerbated differences among varieties. Thus, in the case of control conditions, water content in the fruits from non-grafted cv 'Herminio' was 90.4%, similar to the average (90.7%), while fruits from the rest the grafting combinations was comprised between 88.9% of 'Oscos' and 93.1% of self-grafted 'Herminio' (Table 4). Such differences suggest that the real effect of the rootstocks on this trait may appear when the fruits are exposed to the rootstock in a longer period, i.e. more advanced ripening stages, in agreement with the findings of Ribes-Moya *et al.* (2018), when comparing the effects of different growing conditions in the composition of *Capsicum* fruits from several genotypes.

Table 4. Mean values of water content (WC, %), ascorbic acid content (AAC, mg 100 g^{-1} fw) and total phenolics (TP, mg 100 g^{-1} fw) in fully ripe fruits under control, water deficit and saline conditions

Plant material	**Control**		**Drought**		**Saline**	
WC (%)						
Herm (non-grafted)	90.40	b	90.73	cd	93.19	f
Herm/Herm	93.10	d	89.13	b	91.96	e
Herm/ Robusto	89.20	a	92.09	ef	93.36	f
Herm/ Oscos	88.91	a	88.14	a	91.24	de
Herm/NMh	91.81	c	89.91	bc	90.43	bc
Herm/Chimayo	92.60	cd	90.76	cd	91.37	de
Herm/JE10397	92.58	cd	89.36	b	89.14	a
Herm/U19235	90.35	b	92.22	f	90.08	b
Herm/U26484	89.03	a	89.29	b	90.95	cd
Herm/U27610	88.95	a	91.21	de	89.21	a
Mean	90.69	B	90.28	A	91.09	C
AAC (mg 100 g^{-1} fw)						
Herm (non-grafted)	153.12	abc	166.89	cd	100.34	bc
Herm/Herm	139.46	abc	134.64	ab	94.38	abc
Herm/ Robusto	133.52	abc	165.21	cd	106.53	c
Herm/ Oscos	128.47	ab	180.08	d	96.08	bc
Herm/NMh	149.31	abc	161.48	cd	100.39	bc
Herm/Chimayo	125.06	a	147.95	bc	83.72	ab
Herm/JE10397	163.42	c	121.95	a	82.23	ab
Herm/U19235	142.53	abc	163.25	cd	74.20	a
Herm/U26484	160.03	bc	157.18	c	84.16	ab
Herm/U27610	156.24	abc	178.64	d	106.73	c
Mean	145.12	B	157.73	C	92.88	A
TP (mg 100 g^{-1} fw)						
Herm (non-grafted)	241.05	d	197.47	bc	172.24	bc
Herm/Herm	184.51	b	170.87	ab	164.51	abc
Herm/ Robusto	189.78	bc	227.74	cd	159.92	abc
Herm/ Oscos	164.00	ab	230.55	cd	156.91	abc
Herm/NMh	175.69	ab	219.59	cd	190.29	c
Herm/Chimayo	138.57	a	233.20	d	160.33	abc
Herm/JE10397	175.14	ab	150.75	a	135.82	ab
Herm/U19235	180.93	b	270.62	e	129.68	a
Herm/U26484	195.12	bc	240.01	de	147.80	ab
Herm/U27610	224.57	cd	207.54	cd	190.34	c
Mean	186.62	B	213.40	C	160.78	A

Different letters within the same growing conditions indicates means significantly different at $p < 0.05$, according to the Newman-Keuls statistic test. Upper case letters indicate significant differences among treatment means at $p < 0.05$, according to the Newman-Keuls statistic test.

On average, the stress conditions, caused significant but very low and different changes on water content. Thus, drought slightly decreased water content (90.3%), while salinity caused a slight increase of water content (91.1%) (Table 4). Nevertheless, differences were so low among treatments (0.80% between the averages) that a real effect of stress cannot be assumed, while by contrast, the main contribution to differences corresponded to grafting combinations within each treatment. Thus, under drought conditions the fruits from non-grafted 'Herminio' showed similar levels than those from control conditions (around 90.5%), while grafting combinations ranged from 88% of 'Oscos' to 92% of 'Robusto' and U19235 (Table 4). In the case of salinity conditions, non-grafted 'Herminio' showed the highest water content, 93.2%, while values in graftings were comprised between 89% of JE10397 and U27610 and 93.4% of 'Robusto' (Table 4). These results indicate that, with the exception of 'Robusto' (increasing effect under stress conditions) there is not a clear effect in the rest of rootstocks among the treatments, suggesting a genotype × treatment interaction in this trait at the fully ripe stage, as found in the ANOVA (Table 1).

Ascorbic acid content

AAC at the fully ripe stage, was considerably higher than at the unripe stage, not only on average, but also in all the combinations apart from very few exceptions (Tables 3 and 4), which agrees with other authors, who have reported remarkable increases in ascorbic acid of different pepper varieties due to ripening (Rodríguez-Burruezo *et al.*, 2009; Ribes-Moya *et al.*, 2018 and 2020). The fruits produced in control conditions showed a range of variation comprised between 125 and 163 mg 100 g^{-1} fw, with non-grafted 'Herminio' reaching 153 mg 100 g^{-1} (Table 3). Grafting combinations provided significant differences, although none was significantly different to the values found on the fully ripe fruits of non-grafted 'Herminio'. Among grafting combinations, AAC in 'Oscos', and 'Chimayo', with values <130 mg 100 g^{-1}, were considerably lower than those recorded in JE10397 and U26484, which increased considerably AAC reaching >160 mg 100 g^{-1} (Table 4).

On average, drought conditions increased significantly AAC, although at 10% in comparison to control conditions. In this regard, higher increases must be found in specific combinations. Thus, under this stress conditions, the fully ripe fruits of non-grafted 'Herminio' increased until 167 mg 100 g^{-1} and some grafting combinations like 'Oscos' or *U27610* provided even higher values (around 180 mg 100 g^{-1}) (Table 4). For the rest of grafting combinations, AAC values were similar or lower than that of the non-grafted 'Herminio'. Nevertheless, most combinations increased AAC in comparison to control conditions, particularly 'Robusto' (25%), 'Oscos' (40%) and *U27610* (15%), and only JE10397 decreased significantly AAC due to drought (-25%) (Table 4). Moreover, the occurrence of this stress provoked more significant differences among combinations than those observed under control conditions. This fact suggests that differences at the genetic level, potentially silenced under non stress conditions, could activate when the materials are exposed to drought, as reported in other cases in peppers (Ahmed *et al.*, 2014; Kopta *et al.*, 2020; Borrás *et al.*, 2021).

In comparison, and as observed at the unripe stage, the occurrence of saline stress also caused a remarkable decrease on AAC in fully ripe fruits, even higher than in unripe fruits, both on average (93 mg 100 g^{-1} fw vs. 145 and 158 mg 100 g^{-1} fw in control and drought conditions), and in all the combinations (Table 4). The effect of salinity on AAC was even more aggressive than that recorded at the unripe stage, so that AAC in the fruits of the non-grafted 'Herminio' decreased 34% in comparison to control conditions (from 153 to 100 mg 100 g^{-1}) and many grafting combinations decreased 33-50% (Table 4). Probably a longer exposure of fully ripe fruits to this stress (harvested >3 months since setting) may explain why the decrease is much higher than that recorded at the unripe stage (2 months) as suggested by Ribes-Moya *et al* (2018) in pepper fruits grown under organic farming. However, some combinations were able to mitigate this effect, providing values similar or slightly higher than those from non-grafted 'Herminio', like 'Robusto', 'Numex Heritage' or *U27610* (Table 4).

Total phenolics content (TP)

In a similar way than AAC, TP were much higher at the fully ripe stage, on average as well as in all the combinations and treatments (Tables 3 and 4), which has been also reported by other authors. Thus, not only ascorbic acid, but also other antioxidants, including phenolics, carotenoids, etc. are usually higher at the fully ripe stage in most varieties of peppers (Rodríguez-Burruezo *et al.*, 2009; Ribes-Moya *et al.*, 2018), and therefore this stage is the most complex and interesting from the nutraceutical perspective. Within control conditions the different combinations evaluated accumulated quite high and variable levels of phenolics. TP values varied from 138 to 241 mg 100 g^{-1} fw. Similarly to AAC in both stages and TP at the unripe stage in control conditions, grafting combinations provided significant differences, although none provided higher values than those found on the fully ripe fruits of non-grafted 'Herminio' (241 mg 100 g^{-1} fw) (Table 4). Thus, some rootstocks like 'Robusto', U26484 and U27610 enabled TP values of 190-225 mg 100 g^{-1} fw, while by contrast others like 'Oscos' and, particularly, 'Chimayo' offered considerably lower values (140-165 mg 100 g^{-1} fw) (Table 4).

The behavior of grafting combinations under stress conditions was similar to that observed in AAC. Thus, compared to control conditions, drought increased TP levels in both on average (213 vs 187 mg 100 g^{-1} fw) and in most materials, whilst salinity have a remarkable deleterious effect (average of 161 mg 100 g^{-1} fw). Nevertheless, regarding conditions of drought, fully ripe fruits of non-grafted 'Herminio' behaved in a different way than that observed in most graftings as well as in AAC, decreasing 18% in TP in comparison to control conditions (197 vs 241 mg/100 g fw). This behavior in non-grafted 'Herminio' (i.e. decreasing effect in phenolics due to drought), as well as self-grafted 'Herminio', was also observed in their unripe fruits (Table 3), and differs from that found in AAC values, which increased with drought at both stages and suggests that the response of non-grafted plants to drought may differ depending on the antioxidant agent as reported in some cases (Kyriacou *et al.*, 2017; Mahmood *et al.*, 2021). In this frame, several grafting combinations could provide an improving effect, increasing considerably TP levels (>220 mg 100 g^{-1} fw), particularly U19235 and U264840 (Table 4). In fact, drought increased TP levels in most combinations compared to control conditions, particularly 'Oscos' (40%), 'Chimayo' (70%) or U19235 (50%).

Finally, despite saline stress also caused a remarkable decrease on TP in fully ripe fruits, both on average (161 mg 100 g^{-1} fw vs. 187 and 213 mg 100 g^{-1} fw in control and drought conditions, respectively), and in most materials, some rootstocks like 'Numex Heritage' or 'Chimayo' were quite resilient (Table 4). Moreover, in comparison to the unripe stage, the effect of salinity on TP at the fully ripe stage was a bit less aggressive. Thus, TP in the fruits of the non-grafted 'Herminio' decreased 25% in comparison to control conditions (from 241 to 172 mg 100 g^{-1}), the decrease of many grafting combinations was around 15-30%, and even grafting combinations like 'Oscos', or the above mentioned 'Numex Heritage' or 'Chimayo' showed similar or even higher TP levels under saline conditions (Table 4).

Interaction of stress conditions with the ripening process on fruit composition

The measurements on how drought and salinity altered the levels of both AAC and TP (i.e. ratios drought/control and salinity/control) in the two ripening stages offered interesting results for both antioxidant parameters. Regarding AAC, the comparison between drought and salinity showed clearly at both stages: i) how the former increased in most cases the levels of ascorbic acid compared to control conditions (i.e. ratio values >1.00) and ii) how the latter decreased dramatically ascorbic acid in all cases (i.e. ratio values < 1.00) (Figure 1). Also, on the whole, the changes on AAC due to drought were quite similar in both ripening stages as some of the combinations appeared below the slope = 1 (i.e. AAC changes higher at the unripe stage), while others appeared over the slope = 1 (i.e. AAC changes due to drought higher at the fully ripe stage), and the total mean appeared close to slope = 1 (i.e. AAC change due to drought equal at both ripening stages) (Figure 1). By contrast, the decrease on AAC due to salinity were in most cases higher at the unripe stage as the ratios of most combinations and the total mean appeared below slope = 1 (Figure 1).

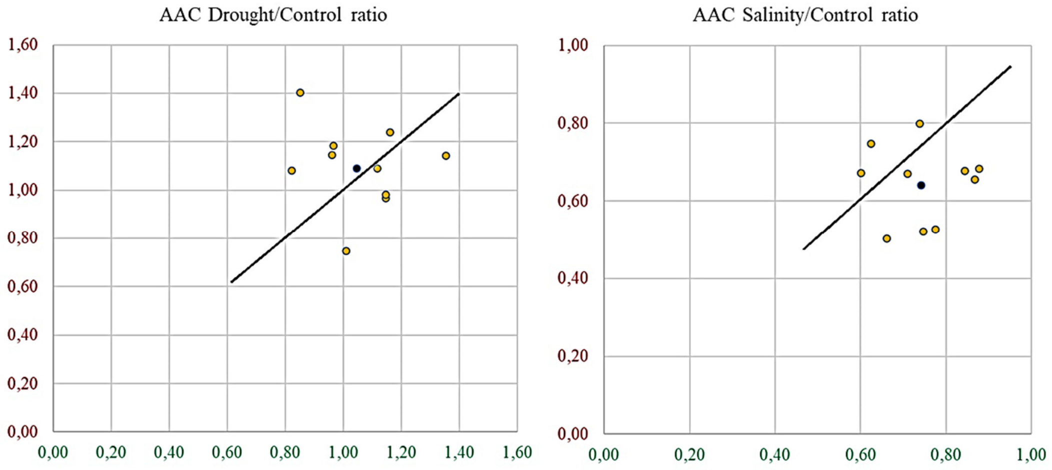

Figure 1. Regression between unripe (green) and fully ripe (red) stages of ascorbic acid content (AAC) Drought/Control (left) and Salinity/Control (right) ratios of the plant materials evaluated. Orange points correspond to the value of each plant material evaluated and black point correspond to the mean value. The black line indicates slope = 1 (i.e. same ratio at unripe and fully ripe stages)

In the case of phenolics, the comparison between drought and salinity showed that drought increased in most cases the levels of phenolics compared to control conditions and, furthermore, higher than AAC as many combinations showed ratios ≥1.20 at both stages, while AAC in most combinations had ratios ≤1.20 (Figures 1 and 2). By contrast salinity decreased phenolics in most cases (Figure 2), although at a lower extent than ascorbic acid, i.e. ratio values for most combinations in TP were ≥ 0.80 while in AAC were ≤ 0.80 Figures 1 and 2). Also, and similarly to AAC, there was not a clear effect of the ripening stage on the TP increase as due to drought as some combinations appeared below the slope = 1, while others appeared over the slope = 1, and the total mean appeared close to slope = 1 (Figure 2). By contrast, the decrease on TP due to salinity were in most cases higher at the unripe stage as the ratios of most combinations and the total mean appeared below slope = 1 (Figure 2).

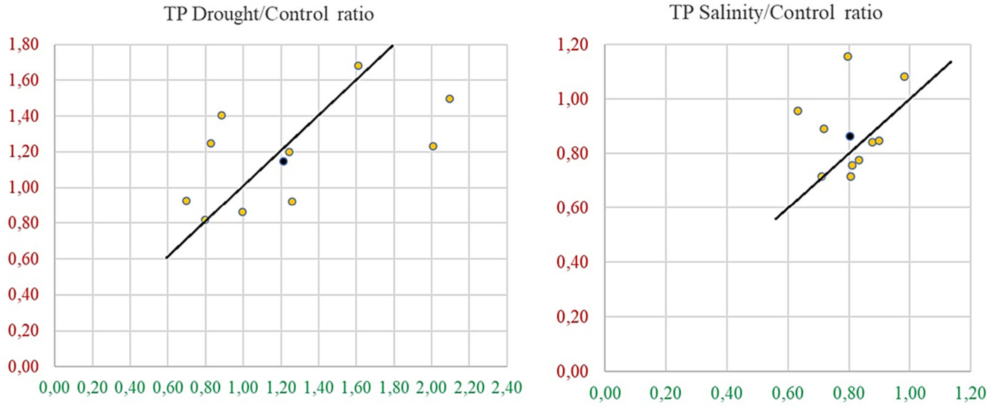

Figure 2. Regression between unripe (green) and fully ripe (red) stages of total phenolics (TP) Drought/Control (left) and Salinity/Control (right) ratios of the plant materials evaluated. Orange points correspond to the value of each plant material evaluated and black point correspond to the mean value. The black line indicates slope = 1 (i.e. same ratio at unripe and fully ripe stages)

Conclusions

Despite the use of rootstocks provides higher vigour and yield to the scion in some vegetables, this was not the case of our materials. At least under control conditions, with the non-grafted variety showing similar or higher performance in yield and fruit weight. However, the occurrence of severe abiotic stress, like drought and/or salinity, revealed that some of the accessions evaluated could allow a considerable mitigation of their impact, providing higher yields and/or fruit size than the variety of reference, particularly yield under drought and fruit weight in both stress conditions. Nevertheless, our results can be considered satisfactory as our experimental rootstocks have been evaluated preliminarily as mere accessions. Thus, it can be expected that their hybrid combination with other sources could improve their vigour in next experimental stages. Finally, it was found that, on average, drought increased considerably the levels of antioxidants at both ripening stages, while salinity provoked dramatic decreases on these compounds, although more remarkable in the case of ascorbic acid. Nevertheless, there was also a considerable rootstock × treatment interaction, particularly in phenolics, which revealed several rootstocks providing very high levels of antioxidants at both ripening stages and under both abiotic stresses, improving those from the non-grafted reference variety.

Authors' Contributions

The authors have contributed to this work as follows: Conceptualization (CMA, AF, MDD, ARB); Data curation (CMA, AdLM, CGR); Formal analysis (CMA, AF, CGR, MDR; VBG); Funding acquisition (AF, ARB); Investigation (CMA, AF, AdLM, CGR, MDR; MDD, ARB); Methodology (AdLM, MDR, MDD, ARB); Project administration (ARB); Resources (AF, ARB); Software (CMA, CGR, VBG); Supervision (AF, MDD, ARB); Validation (AF, AdLM, MDR, VBG); Visualization (CMA, VBG, ARB); Writing - original draft (CMA); Writing - review and editing (CMA, AF, AdLM, CGR, MDR, VBG, MDD, ARB). All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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