Breeding Long Shelf-Life (LSL) Tomato Landraces to Non-Trellised Culture and Water Deficit Irrigation: The Effect on Yield and Postharvest Storage

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1. Introduction

Many countries, such as those along the Mediterranean basin, are increasingly facing the risk of water scarcity, which imposes serious constraints on agricultural production. This situation is further pushed in the context of predicted population increase, urbanization expansion and economic growth, which will increase the competition for natural resources. Simultaneously, water availability is impeded under future scenarios directed by climate change [1]. Farmers in some Mediterranean areas will suffer an estimated gross increase of 40–250% of irrigation requirements, because of a decrease in the amount of available water and an increase in crop evapotranspiration [2,3]. In this context, it will be necessary to reduce water losses and promote maximal crop growth and productivity per unit of water...
applied (i.e., water-use efficiency, WUE) [4], for instance, by diverting the soil evaporation fraction into the plant transpiration component [5]. To face this challenge, farmers should adopt and combine different technologies related to sustainable water management [3], including the use of drought-tolerant genotypes and efficient irrigation systems (e.g., drip irrigation), the adjustment of irrigation to crop requirements, or the use of water deficit strategies [6]. Soil structural enhancements through conservation of organic matter can play also an important role in increasing soil water and nutrient holding capacity [7]. By combining all these strategies we can achieve water savings higher than 50% in vegetable production systems [8].

Tomato (Solanum lycopersicum L.) is a major horticultural crop worldwide being second in terms of cultivated area (15% of total vegetable production, excluding potatoes [9]). The water footprint of tomato cultivation is slightly low in comparison with the other main agricultural crops [10], although the final result strongly depends on the variety and cultivation system [11]. Under commercial conditions, the WUE usually ranges between 15 and 40 kg m$^{-3}$ (fresh weight, fw) [12]. Fresh market tomatoes cultivated in modern greenhouses exhibit a considerably higher WUE [13], exceeding 65 kg m$^{-3}$ in some systems [12]. Genetic and agronomic improvements made in the last 50 years have drastically increased the WUE in this crop, as described in processing tomato, where for similar irrigation amounts, the yield has increased by more than 50% [14]. Moreover, water-deficit irrigation strategies are gaining interest among farmers as they enable the optimization of WUE and the production of high-quality fruits, in some cases with a minimal penalty on yield [15–17].

The Mediterranean basin is rich in tomato genetic diversity and in this regard, it is considered a secondary center of diversification for this crop [18,19]. Landraces emerged under a wide range of growing conditions and selection criteria as a result of adaptation to local conditions and consumption habits [20]. Among all the Mediterranean landraces, the long shelf-life (LSL) group represents a specific varietal type, which differs from the main tomato horticultural groups (fresh market, processing) regarding the production system and culinary usages [21]. In the Spanish landraces (Penjar, Ramellet), the LSL phenotype has been related to the alcobaça (alc) mutation [22]. These varieties were historically grown in low-input agrosystems (non-trellised and non-irrigated conditions), with fruits harvested at the red-ripe stage and stored under ambient temperature for up to 6 months [22,23]. In Spain, fruits are usually bunched and hung-up over winter, and traditionally consumed to prepare “pa amb tomàquet” (the pulp of the fruit is spread on dry bread) or sauces [24–26]. Because of the non-irrigated farming systems where they evolved, some of these varieties show specific adaptations to drought [27,28].

In Catalonia (NE Spain), Penjar tomato is a popular LSL variety that is characterized by a low fruit weight and a long postharvest storage. Despite these common traits, among the different pure lines cultivated by farmers there exists an important phenotypic diversity regarding agronomic, morphological and fruit quality traits [22,24,29,30]. Penjar landraces are traditionally produced in the open-field, either using unpruned plants in a non-trellised culture or plants pruned to single stem that were trellised by using canes. The recent success of this variety has led to its transfer into modern-cropping systems (protected cultivation, use of rootstocks, fertigation), sometimes losing the quality traits that historically distinguished this variety in the market [31,32].

Non-trellised culture of Penjar tomatoes is gaining interest among farmers, who consider this cropping system an alternative to reduce cultivation costs. Traditional Penjar genotypes normally show an indeterminate growth habit. Under traditional and low-input non-trellised cropping systems, indeterminate Penjar-type plants display a controlled vegetative growth, but under current high-input farming practices instead they show vigorous growth that hampers their management. Thus, farmers use low plant densities (0.5 plants m$^{-2}$) and perform two to three harvests per crop cycle to maximize yield. To adapt this landrace to the modern non-trellised culture there is a need to breed determinate varieties suitable for Penjar production. Among the natural variation encompassed within the Penjar landrace, the phenotypes associated with the determinate growth habit [33]
and compound inflorescence architecture [34] have been previously identified in some accessions [22]. Phenotypical characteristics of these accessions suggest that they could correspond to the self-pruning (sp) [33] and compound inflorescence (s) [34] mutations, although the molecular basis of these traits remain to be confirmed. The sp mutation has been the basis for the development of processing tomato suitable for open-field cultivation and mechanical harvesting [35], and thus can be useful for breeding Penjar tomato adapted to the non-trellised culture. Moreover, the introgression of the “compound inflorescence” trait into a determinate genetic background can offer novel phenotypic variation to improve the yield in non-trellised culture.

In this study, we aim to improve the agronomic behavior and reduce the water footprint of the Penjar tomato when cultivated under a non-trellised system. We identify traditional determinate Penjar genotypes and develop Penjar genotypes that display the double determinate/compound inflorescence phenotype. We characterize (a) the allelic sequence of sp and s genes of the breeding lines; (b) the adaptation to non-trellised culture and single harvest of the different Penjar genotypes; (c) the effect of water deficit on fruit quality, yield, WUE and biomass partitioning; and (d) the combined effect of water deficit and storage conditions on postharvest shelf life.

2. Materials and Methods

2.1. Plant Materials and Breeding Program

LC215 (Penjar) and LC547 (Ramellet) are two LSL landraces exhibiting a determinate growth habit (collected in Catalonia and in the Balearic Islands, respectively). LC257 is a Penjar landrace showing compound inflorescences (Catalonia). Palamós is a Penjar commercial hybrid (Semillas Fitó). Pera Delta is an open-pollinated processing variety (Mas Pastoret) widely used by organic farmers in the area of study (Table 1).

Table 1. Description of plant materials.

| Genotype    | Varietal Type     | Origin                      | Ripening 1 | Plant Architecture | Inflorescence Type | Fruit Shape 2 | Earliness (DAT) 3 |
|-------------|-------------------|-----------------------------|------------|--------------------|-------------------|---------------|------------------|
| LC215       | Penjar Breeding line | LSL Determinate Compound Heart | 133.3 ± 0.5 |
| LC215       | Penjar Breeding line | LSL Determinate Compound Round | 133.3 ± 0.5 |
| LC257       | Penjar Landrace (Origin: Catalonia) | LSL Determinate Regular Round | 112.0 ± 9.9 |
| LC547       | Ramellet Landrace (Origin: Balearic Islands) | LSL Determinate Regular Flat | 127.3 ± 1.9 |
| Palamós     | Penjar Commercial hybrid (Semillas Fitó) | LSL Indeterminate Regular Round | 104.3 ± 4.9 |
| Pera Delta  | Processing Commercial inbred (Mas Pastoret) | Normal ripening Determinate Regular Long rectangular | 104.3 ± 4.9 |

1 LSL: long shelf life. 2 According to the fruit shape classification proposed by Visa et al. [36]. 3 Number of days from transplant (DAT) to harvest (mean ± SEM, calculated from the harvesting date of the 3 localities).

LC215 (sp-like mutant; formerly CDP00023) and LC257 (s-like mutant; formerly CDP05468), both with confirmed alcobaça (alc) mutation [22], were used as parents to breed a triple alc/sp-like/s-like mutant. With this aim, a genealogic method was used, starting from a single LC215 × LC257 cross, with no selection in the S1 generation (200 plants) and selecting in the S2 the plants conjointly exhibiting a determinate growth habit and compound inflorescences.
(putative sp-like/s-like double homozygous plants). In subsequent generations (S3 to S6), we followed a minimum of 12 families per year and selected on the S6 the genotypes 2.9 and 2.14.

2.2. Plant Genotyping

Total genomic DNA was extracted from LC215, LC257, LC547, 2.9 and 2.14 plants as indicated in Doyle [37]. The SP (Solyc06g074350) and S (Solyc02g077390) genes were amplified by PCR in three technical replicates, divided in three overlapping fragments each, using specific primers (Table S1). PCR products where sequenced and full gene sequences were reconstructed and aligned to the wild type for comparison and mutations’ identification.

2.3. Growth Conditions and Irrigation Scheduling

The deficit irrigation trials were conducted at three proximate locations in the north-east of Spain (distance between farms < 15 km); two organic, Eco-1 (Sant Cugat, 41°27′07.1″ N, 2°02′01.8″ E) and Eco-2 (Molins de Rei, 41°23′51.3″ N, 2°01′26.2″ E), and a third one using conventional practices, Conv-1 (Rubí, 41°30′05.7″ N, 2°01′03.8″ E). Fertilization dose and phytosanitary treatments were those typically employed at each site. One-month-old seedlings were transplanted on 18 April 2019 at each of the three locations; the cultivation period lasted until 30 August 2019 (the last genotype harvested). The reference evapotranspiration (ET₀), hourly and daily average temperature, average relative humidity, accumulated daily precipitation and global solar radiation were recorded at the nearest meteorological measuring station at Castellbisbal (maximum distance to the fields: 10 km). Average minimum and maximum and average temperatures throughout the growing period were 19.3, 32.6 and 25.2 °C, respectively, average relative air humidity was 64.1 ± 10.3%. The accumulated global solar radiation was 3548 MJ m⁻² and the total reference evapotranspiration was 691.8 l m⁻² (Figure S1). The plantlets were grown under open-field conditions in planting beds covered with black polyethylene plastic mulch (120 cm width, 120 g m⁻²) to reduce soil water evaporation and weed growth. No pruning was performed. Soils at all three locations were of a loamy texture, with different levels of fertility (Table S2).

In each locality we used a split plot design, with three replications, where the whole-plot factor was the irrigation regime, and the genotypes were randomized in subplots. Each subplot was composed by seven plants. Two irrigation regimes were applied: normal watered (NW), covering 100% of daily crop evapotranspiration (ETₐ), and water deficit (WD), covering 50% of ETₐ. The plants were established in single rows per planting bed (1.33 plants m⁻²) with separate beds for each irrigation regime side by side divided by walking paths. Field irrigation was facilitated by a drip tape with 5 emitters m⁻¹ and a discharge of 1.18 l emitter⁻¹ h⁻¹. A flow meter was installed to quantify the total water applied to the plants.

Irrigation was applied following the crop evapotranspiration (ETₐ) method based upon soil-water balance (ETₐ = ET₀ × Kc) [38]. The crop factor estimation (Kc) was established to 0.6–0.8 from 0 to 30 days after transplant (DAT); 0.8–1.3 from 31 to 60 DAT; 1.3–1.1 from 61 to 90 DAT and 1.1 as of 90 DAT, based on the approaches of Peet et al. [39] and Allen et al. [38]. Considering the plastic mulch, ETₐ was reduced by 20%. For the first month after transplanting, all the plants were watered at 100% ET₀ in order to allow them to fully develop at the initial stages; subsequently, the two irrigation regimes were applied (Figure S2). Accumulated precipitation (l m⁻²) was 75.5 in May, 22.3 in June, 35.4 in July and 29.0 in August. Total water applied by irrigation was 323 l m⁻² (i.e., 243 l plant⁻¹) in NW and 111 l m⁻² (i.e., 84 l plant⁻¹) in WD treatment. Adding the precipitation, at the end of the cultivation period NW treatment received an average of 490 l m⁻² (i.e., 370 l plant⁻¹) and WD 280 l m⁻² (i.e., 210 l plant⁻¹).

2.4. Plant Phenotyping

2.4.1. Agronomic traits and Water Use Efficiency

A single harvest per plant was performed when 80–100% of fruits were at the red-ripe stage, considering common commercial practices. Four middle plants of each plot were
sampled for agronomic characterization. Fruits and above-ground vegetative tissue were separated and weighed, obtaining the following variables: total yield (kg m\(^{-2}\) fw), total fruit number, commercial yield (weighing solely disease-free and ripen fruits without symptoms of sunscald, cracking or blossom end rot (BER); kg m\(^{-2}\) fw). Non-commercial fruits were separated into classes and weighed according to the incidence of physiological disorders (cracking, sunscald, BER; in %). Unripe fruits were estimated by subtraction (in %). Average fruit weight (g) was calculated by dividing the total yield by the fruit number per plant (i.e., considering solely commercial fruits). Epigeous plant tissue (stem, leaves, and inflorescences) was weighed (g plant\(^{-1}\) fw) and subsequently moved into a thermo-ventilated oven (70 °C, 72 h) to obtain the dry weight (g plant\(^{-1}\) dw). Biomass partitioning between fruits and vegetative biomass was calculated as the ratio of total yield and vegetative biomass and expressed in fw and dw (%). WUE (kg m\(^{-3}\)) was calculated as the ratio of total yield (fw and dw) by total water applied, following procedures described in previous works [40].

2.4.2. Quality Traits

In each locality, six commercial fruits from each subplot were randomly selected for fruit quality analyses. Fruits were individually analyzed for color, firmness and total soluble solids (TSS). Color was evaluated in the equatorial section of each fruit with a colorimeter (Konica Minolta CR-410; Minolta, Osaka, Japan) and given as Chroma and Hue coordinates from the CIELAB color space. Firmness was measured at two opposite points in the equatorial part of the fruit with a durometer (Agrosta Durofel, Compainville, France) and the average value per fruit was expressed as a percentage. TSS were analyzed with a portable refractometer (Erma, Tokyo, Japan) at 20 °C from juice drops of cut fruits (expressed in °Brix). For the analysis of pH, titratable acidity (TA) and dry matter, the six fruits per replication were bulked and blended into a homogenate sample; pH was determined with a glass electrode pH-meter (CRISON microPH 2001); for TA the sample was titrated using 0.1 M NaOH up to pH = 8.1, and expressed as g citric acid 100 g\(^{-1}\) fw; fruit dry matter was measured by drying samples in a thermo-ventilated oven air to constant weight (65 °C, 72 h) and expressed in %.

2.5. Postharvest Conservation and Analysis of Shelf Life

The effects of locality, genotype and watering treatment on postharvest shelf life were assessed under three storage conditions: farmer storage facilities (ambient conditions), laboratory-controlled conditions (24 ± 2 °C; 62 ± 11% relative humidity) and cold room (8 ± 2 °C, 95 ± 5% relative humidity). Three replicates of 30 commercial fruits per genotype, treatment and locality were collected and distributed in perforated, stackable plastic boxes at each storage condition. Fruits were checked weekly for signs of decay and removed when applicable during a period of 90 days. Shelf life was expressed as the percentage of sound fruits at 30, 60 and 90 days.

2.6. Statistical Analysis

All analyses were performed using R software (v. 3.6.1; R Core Team, Vienna, Austria). To assess the effects of the irrigation regime and the genotype on agronomic, fruit quality and postharvest traits we used the Analysis of Variance (ANOVA) by implementing the following linear mixed model (LMM):

\[ Y_{ijk} = \mu + \alpha_i + \eta_{k(i)} + \beta_j + (\alpha\beta)_{ij} + \gamma_l + \epsilon_{ijkl} \]  

where \( \mu \) is the grand mean; \( \alpha_i \) is the fixed effect of the irrigation regime; \( (\alpha\beta)_{ij} \) is the corresponding interaction term; \( \eta_{k(i)} \) is the whole-plot error; \( \beta_j \) is the fixed effect of the genotype; \( \gamma_l \) is the random effect of locality; and \( \epsilon_{ijkl} \) is the random error distributed. The ANOVA was performed with the lmer function of the “lme4” package [41]. For the postharvest traits, the LMM was implemented for the data from the laboratory storage conditions at each postharvest time. Subsequently, we performed a one-way ANOVA for each locality–genotype interaction in each postharvest time, in order to study the impact
of the irrigation regime on the shelf life. The impact of the storage conditions on shelf life was assessed with a one-way ANOVA within each genotype (i.e., merging the results of the different localities). Differences between means were evaluated for significance using the Tukey multiple range test. Graphs were elaborated with the “ggplot” package [42].

To select the best genotypes based on the commercial yield during the postharvest we calculated the “yield after storage” (i.e., commercial yield*shelf life) at 30, 60 and 90 days. A GGEbiplot analysis was performed at each storage period, using the mean versus stability procedure described in Olivoto and Lucio [43]. To estimate the broad-sense heritability (H²) for this variable, we used a mixed model with genotype and genotype–environment interaction as random effects and estimated the H² as the contribution of the genetic variance to the total phenotypic variance. Both analyses were performed with the “metan” package [43].

3. Results
3.1. Plants Obtained in the Breeding Program Contain a Known Allele of the SP Gene and a New Allele of the S Gene

Previous studies on the Penjar tomato revealed the existence of accessions with determinate growth habit or compound inflorescence [22]. Phenotypes strongly resembled published descriptions of self-pruning (sp) [33] and compound inflorescence (s) [34] mutants. LC215 (alc/sp-like) and LC257 (alc/s-like) were used to breed a triple alc/sp-like/s-like mutant. In subsequent generations, lines 2.9 and 2.14 were selected based on their sp-like/s-like phenotype (Table 1).

The plants used and obtained in the breeding program were genotyped in order to identify the mutations that they contained. LC257 was considered the wild-type line for the SP gene. Lines LC215, 2.9, 2.14 and LC547 presented a mutation in SP gene previously described [33], which consisted of a C to T nucleotide change that results in a proline into leucine amino acid change in position 76 (Figure 1a). Regarding the S gene, LC215 line was considered the wild type, whereas lines LC257, 2.9, 2.14 were found to present the s-classic mutation previously described by Lippman et al. [34], in which a G to A nucleotide change resulted in a glycine into aspartic acid amino acid change in position 69 (Figure 1b). Moreover, these lines presented another C to A nucleotide change that resulted in a threonine into lysine amino acid change in position 291 of the S protein sequence (Figure 1b). Therefore, our s-like plants presented two different mutations in the sequence of the S gene.

Figure 1. Schematic representation of the SP and S genes and the missense mutations found in them. (a) SP gene where the mutation caused a proline (P) into a leucine (L) amino acid change. (b) S gene showing the s-classic mutation where a glycine (G) changed into an aspartic acid (D) and a second mutation consisting in a threonine (T) into lysine (K) amino acid change. The numbers indicate the aminoacidic positions inside the proteins affected by the mutations. White boxes, untranslated regions (UTRs); grey boxes, exons; red font, nucleotide changes found in the mutant alleles. WT, wild type; MUT, mutant.
3.2. Performance of Penjar Tomato under Non-Trellised Culture Depends on Irrigation and Genotypic Factors

We evaluated the effects of the irrigation regime (NW and WD), locality (Eco-1, Eco-2 and Conv-1) and genotype on different agronomic traits of five genotypes of the Spanish LSL varietal group: two breeding lines (2.9; 2.14), two determinate growth landraces (LC215; LC547), and one commercial hybrid (Palamós). We also included an open-pollinated processing variety (Pera Delta) (Table 1; Figure S3).

We found that irrigation, genotype and locality significantly affected the tomato crop (Table 2). Scant genotype-by-irrigation interactions caused significant effects (solely for incidence of cracking). Incidences of the physiological disorders BER (<2%) and cracking (3–8%) were very low in the overall experiment.

Table 2. Effect of irrigation regime (I), genotype (G) and locality (L) on agronomic performance. The level of significance is reported following *p < 0.05; **p < 0.01; ***p < 0.001; ns, not significant (p > 0.05). Within columns and for each fixed factor (I, G) different letters indicate significant differences according to Tukey multiple-range test (p < 0.05). NW, normal watering; WD, water deficit. Blossom end rot (BER) was absent (<2%) and is not shown in this table. Genotypes: 2.9 and 2.14, Penjar breeding lines; LC215 (Penjar) and LC547 (Ramellet), LSL traditional lines; Palamós, Penjar modern hybrid; Pera Delta, processing variety.

| Irrigation regime (I) | Number of Fruits Per Plant | Yield (kg m⁻²) | Commercial Yield (%) | Cracking (%) | Sunscald (%) | Unripen Fruits (%) | Fruit Weight (g) |
|-----------------------|---------------------------|----------------|----------------------|--------------|--------------|-------------------|-----------------|
| NW                    | 66.4                      | 5.1 a          | 57.6 a               | 4.7          | 13.1 b       | 21.0              | 58.7 a          |
| WD                    | 68.9                      | 4.3 b          | 55.2 b               | 4.2          | 19.0 a       | 18.9              | 47.9 b          |
| *p value*             | ns                        | ***            | ns                   | ns           | ***          | ns                |                 |
| Locality (L)          |                           |                |                      |              |              |                   |                 |
| Eco-1                 | 62.1                      | 4.0            | 57.9                 | 5.9          | 9.9          | 18.6              | 48.3            |
| Conv-1                | 75.1                      | 4.9            | 56.5                 | 3.2          | 19.8         | 21.6              | 49.9            |
| Eco-2                 | 66.5                      | 5.5            | 54.4                 | 4.1          | 19.2         | 19.7              | 63.2            |
| *p value*             | **                        | ***            | **                   | **           | ***          | ns                | ns              |
| Genotype (G)          |                           |                |                      |              |              |                   |                 |
| ‘2.9’                 | 83.5 a                    | 4.7 ab         | 52.4 bc              | 4.2 ab       | 24.0 a       | 20.0 ab           | 41.9 b          |
| ‘2.14’                | 70.1 ab                   | 4.2 b          | 55.6 bc              | 3.8 ab       | 17.9 b       | 17.9 ab           | 45.2 b          |
| ‘LC215’               | 60.3 b                    | 4.4 b          | 54.8 bc              | 5.7 ab       | 18.5 ab      | 16.4 ab           | 56.0 a          |
| ‘LC547’               | 55.6 b                    | 4.5 b          | 34.0 c               | 7.2 a        | 27.1 a       | 16.1 b            | 62.1 a          |
| ‘Pera Delta’          | 67.4 ab                   | 6.1 a          | 66.0 b               | 3.5 ab       | 7.1 bc       | 20.1 ab           | 67.0 a          |
| ‘Palamós’             | 67.7 ab                   | 4.3 b          | 71.2 a               | 3.2 b        | 5.1 c        | 27.1 a            | 46.7 b          |
| *p value*             | ***                       | ***            | ***                  | ***          | ***          | ns                | ns              |
| GxI                   |                           | ns             | ns                   | ***          | ns           | ns                | ns              |

The irrigation regime significantly affected fruit weight, yield, commercial yield and sunscald incidence (four out of seven measured traits). Total yield decreased by 15.7% under WD, mainly caused by a reduction in fruit weight (18.4% reduction under WD), rather than an effect on fruit number per plant, which was not affected by the irrigation regime. Among the physiological disorders analyzed, only sunscald was significantly affected: WD treatment increased its occurrence by 45%. Locality affected all the agronomic traits. Sunscald was the major disorder in all the localities with incidences ranging from 9.9% (Eco-1) to 19.8% (Conv-1) of fruits affected. On the other hand, the genotype affected all the parameters analyzed. Major differences were found between the processing tomato (Pera Delta) and the LSL varieties. Pera Delta showed better performance in terms of total yield, and a lower incidence of cracking and sunscald. With respect to this last parameter, a clear difference in sunscald was observed between modern controls (Palamós, Pera Delta, incidence <8%) and LSL traditional and breeding lines (LC547, LC215, 2.9, 2.14, incidence 18–27%). Differences among the LSL varieties (control, traditional, and breeding genotypes) were small regarding the total yield (range 4.2–4.7 kg m⁻²), although important in the
yield-related components' fruit weight (41.9–62.1 g), commercial yield (34.0–71.2%) or the number of fruits per plant (55.6–83.5). The double mutants sp/s (2.9, 2.14) showed the highest number of fruits per plant (83.5 and 70.1, respectively), but this was not translated into a higher yield, because of their reduced fruit weight. Thus, in this case, the combined trait phenotype of the breeding lines seems not to improve the agronomic behavior and adaptability of LSL Penjar tomato to processing management practices.

A major factor in reducing production costs in LSL Penjar tomato is to harvest the fruits in a single operation. In our experiment, a single harvest was performed when each genotype reached full maturity (80–100% of red-ripe fruits), and the remaining immature fruits were counted (%). The higher value for immature fruits at harvest was recorded for the indeterminate variety (Palamós, 27.1%), while the lower values were recorded in the sp mutant landraces (LC215, 16.4%; LC547, 16.1%). Thus, our results indicate that within the LSL Penjar tomato exists an important genetic variability for adaptation to processing tomato production practices.

3.3. Harvest Index Is Neither Affected by Environmental Conditions nor by the Introgression of the Double sp/s Mutation

We next analyzed the effect of the irrigation regime, locality and genotype on the total biomass and the harvest index traits (HI, the ratio of fruits to total plant biomass). Total plant biomass was affected by all the factors but not by the interaction between irrigation regime and genotype (Table 3). WD reduced the total biomass by 16.1% (fw) and 7.8% (dw) with respect to NW, showing a similar pattern of reduction than yield (15.7% (fw), 8.0% (dw), Table 2). HI was significantly different among genotypes, which ranged 61.4–77.5% fw and 48.8–60.9% dw (Table 3). This trait was neither affected by locality nor by irrigation regime when studied on a dw basis, but showed significant variations across localities when expressed as fw. Processing variety (Pera Delta) and LC215 reached the higher HI values (60.9% and 59.1% dw, respectively), while the indeterminate variety (Palamós) and the determinate landrace LC547 presented the lowest values (48.8% and 49.7% dw, respectively). In all, 2.9 and 2.14 genotypes, carrying the double sp/s mutation, with intermediate values of HI (55.4% and 51.3% dw, respectively), did not show a different pattern for this trait with respect to the rest of determinate genotypes. Altogether, our results evidence that biomass partitioning between fruits and vegetative organs is not dependent on plant vigor. Moreover, the introgression of the compound inflorescence into a determinate genetic background does not alter the HI, suggesting that this conjoint alteration in the inflorescence and plant architecture does not change resource allocation between reproductive and vegetative organs either.

3.4. Deficit Irrigation Has the Potential to Combine High WUE and Yield in Penjar Tomato

Water use efficiency (WUE), here expressed as the ratio of total yield per unit of water applied, is a key variable for evaluating deficit water strategies [44]. In our study, WD received on average 42.9% less water with respect to NW (difference between irrigation plus precipitation received, 490 NW versus 280 WD l m⁻², Figure S2). Under these conditions, we found that the irrigation regime and genotype had the strongest effects on WUE (Figure 2a; Table S3). WD treatment significantly increased the WUE in comparison to NW (43.0% and 55.0% on a fw or dw basis, respectively), implying that plants used water in a much more efficient way under restricted watering regimes. This means an increase of 4.9 kg m⁻³ fw of production efficiency under WD with respect to NW. The WUE between genotypes ranged from 19.3 (Pera Delta) to 11.1 kg m⁻³ fw (2.14), and between 1.32 (Pera Delta) to 0.87 kg m⁻³ dw (2.14), with scant differences among them. These results fall into the intermediate zone of the range of variation described in the literature for fresh market [45], processing [16] and LSL tomatoes [31]. Although all the genotypes responded positively to WD, the capacity of improvement of WUE under WD was different. For instance, LC547 (9.7 (NW), 15.8 kg m⁻³ fw (WD)) and Palamós (10.5 (NW), 16.4 kg m⁻³ fw (WD)) showed
an increase of WUE higher than 50% due to water shortage, while 2.9 increased WUE solely by 20% (11.3 (NW), 13.6 kg m\(^{-3}\) fw (WD)).

Table 3. Effect of irrigation regime (I), genotype (G) and locality (L) on biomass partitioning (expressed in fresh (fw) and dry (dw) weight). The level of significance is reported following * \(p < 0.05\); ** \(p < 0.01\); *** \(p < 0.001\); ns, not significant (\(p > 0.05\)). Within columns and for each fixed factor (I, G) different letters indicate significant differences according to Tukey multiple-range test (\(p < 0.05\)). NW, normal watering; WD, water deficit. Genotypes: 2.9 and 2.14, Penjar breeding lines; LC215 (Penjar) and LC547 (Ramellet), LSL traditional lines; Palamós, Penjar modern hybrid; Pera Delta, processing variety.

|                        | Total Biomass (kg Plant\(^{-1}\), fw) | Harvest Index (%, fw) | Total Biomass (kg Plant\(^{-1}\), dw) | Harvest Index (%, dw) |
|------------------------|--------------------------------------|------------------------|----------------------------------------|------------------------|
| Irrigation regime (I)  | NW                                   | 5.6 a                  | 69.3                                   | 0.51 a                 | 54.4                   |
|                        | WD                                   | 4.7 b                  | 69.6                                   | 0.47 b                 | 53.8                   |
| \(p\) value           | ***                                  | ns                     | *                                      | ns                     |
| Locality (L)           | Eco-1                                | 4.0                    | 71.2                                   | 0.39                   | 54.9                   |
|                        | Conv-1                               | 5.3                    | 69.7                                   | 0.52                   | 54.0                   |
|                        | Eco-2                                | 5.9                    | 67.7                                   | 0.54                   | 53.6                   |
| \(p\) value           | ***                                  | *                      | ***                                    | ns                     |
| Genotype (G)           | ‘2.9’                                | 5.0 b                  | 71.5 ab                                | 0.52 ab                | 55.4 abc               |
|                        | ‘2.14’                               | 4.8 b                  | 65.9 bc                                | 0.48 ab                | 51.3 bc                |
|                        | ‘LC215’                              | 4.4 b                  | 73.9 ab                                | 0.40 b                 | 59.1 ab                |
|                        | ‘LC547’                              | 5.0 ab                 | 67.1 bc                                | 0.47 ab                | 49.7 c                 |
|                        | ‘Pera Delta’                         | 5.9 a                  | 77.5 a                                 | 0.49 ab                | 60.9 a                 |
|                        | ‘Palamós’                            | 5.4 ab                 | 61.4 c                                 | 0.54 a                 | 48.8 c                 |
| \(p\) value           | ***                                  | ***                    | ***                                    | ns                     |

GxI \(p\) value ns ** ns ns

Figure 2. Effect of irrigation regime on water use efficiency in LSL tomato, in relation to yield. (a) Histogram showing the comparison between normal watering (NW) and water deficit (WD) treatments; (b) relationship between yield (kg m\(^{-2}\)) and water use efficiency (WUE, kg m\(^{-3}\)). Results are expressed on a fresh weight (fw) basis.
To further explore the differential behavior of the genotypes under NW and WD, we constructed a yield–WUE scatterplot (Figure 2b). Besides the negative effect on yield (Table 2), the graph shows that lowering the irrigation amount clearly improves the WUE, and that some plants achieve similar yields under WD and NW. Differences in WUE between NW and WD increase sharply for high yields, indicating that under a water scarcity scenario WD strategies can promote the sustainable production of Penjar tomato, as reported previously for the same variety using a single-stem trellised culture [31].

3.5. Induced Water Stress Increases Fruit Quality While Genotype-Specific Characteristics Are Retained

At harvest, we analyzed the effect of the irrigation regime, locality and genotype on different fruit quality traits (Table S4). It has been described that several quality parameters in tomatoes are positively affected by WD [9,46]. We found that external color measured as Chroma, TSS, TA and fruit dry matter content were significantly higher under WD with respect to NW; by contrast, no effect was observed for Hue, firmness and pH. Differences between localities were significant for color coordinates (Chroma and Hue), firmness and TA. Among genotypes, differences were recorded for all studied quality parameters. The highest values for fruit dry matter and TSS were obtained for Palamós and the breeding lines 2.9 and 2.14, which scored significantly higher than the other genotypes. TA and Chroma clearly differentiated the LSL genotypes with the processing control (Pera Delta), being LSL genotypes characterized by high contents of acids and low values of Chroma. The high content of TA in LSL tomatoes has been described previously [23], as well as the pleiotropic effect of the alc mutation on carotenogenesis of the fruit, which diminishes the intensity of the external color (i.e., Chroma) [47].

3.6. Specific Genotype*Preharvest Conditions Drive High Shelf Life in the Short Term, While Genotype Is Critical for Long-Storage

Fruit shelf life is an important trait for the marketability of the Penjar tomato as their storage can last up to several months. In our study, we analyzed the shelf life every 30 days during three months under controlled conditions. We found that the genotype significantly affected postharvest shelf life during all the storage, while locality was significant at the early stage (30 days), and irrigation regime at later stages (60, 90 days) (Table S5). The processing variety, Pera Delta, which does not carry any ripening mutation, showed a very short shelf life (22% of sound fruits after 30 days) in all the experimental conditions (Figure 3). Differences between LSL genotypes became evident after 60 days, when the shelf life of the Penjar commercial hybrid (Palamós) and the Ramellet landrace LC547 decreased sharply. Storage performance of Penjar breeding lines (2.9, 2.14) and the Penjar landrace (LC215), with 80.0, 70.9, and 70.7% of sound fruits after 90 days, respectively, was very good and showed similar behavior in all the localities (Figure 3; Table S5).

Some authors reported a beneficial impact of environmental stressors such as WD on fruit shelf life [48], as it has been reported in the case of LSL Ramellet tomato [26]. In a previous study of our group [31], we showed that the positive effect of WD was genotype-dependent and that shelf life is a trait largely affected by Genotype × Environment × Management (G×E×M) interactions. In the present work, WD had a positive and significant effect on shelf life for some genotype-by-locality combinations, being more pronounced in the later stages of postharvest (Figure 3; Table S5). For instance, the shelf life of 2.14, 2.9 and Palamós was not affected by WD, whereas in the case of LC215 and LC547 it was positively affected in some localities.

Postharvest losses have a profound impact on the profitability of Penjar tomato. Farmers are interested in selling the fruits during the first four months of storage, which has been described as the period during which this variety maintains its singular sensory profile [25]. With the aim of selecting the most appropriate genotypes for selling Penjar tomatoes after a storage period, we calculated the variable “yield after storage” (i.e., what a farmer can sell after storage period). The broad-sense heritability (H²) of this trait was 0.250, 0.492 and 0.596 at 30, 60 and 90 days, respectively, showing that the contribution
of the genotype to the phenotypic variation of this variable increased during the storage. Thus, in our study, environmental factors affect the storability of Penjar fruits more during the first postharvest period, while the long storage is more dependent on the genotype. This is not contradictory to the results showing that the effect of WD increases throughout postharvest, given that environmental effects include irrigation regime as well as multiple other uncontrollable factors (e.g., temperature, relative humidity, irradiance, etc.).

![Figure 3](image-url)

**Figure 3.** Effect of irrigation (NW, normal watering; WD, water deficit) on 90-day postharvest shelf life (percentage of sound fruits) of LSL genotypes grown in 3 localities (Conv-1, Eco-1, Eco-2). Storage in laboratory conditions (24 ± 2 °C; 62 ± 11% relative humidity). Error bars represent the SEM calculated on the basis of the 3 biological replicates. Significant differences between watering regimes are presented by an * ($p < 0.05$). Genotypes: 2.9 and 2.14, Penjar breeding lines; LC215 (Penjar) and LC547 (Ramellet), LSL traditional lines; Palamós, Penjar modern hybrid; Pera Delta, processing variety. NA: not available.

To allow the selection of the best genotypes regarding the “yield after storage” we produced a GGE biplot for the 30, 60 and 90 days of storage (Figure 4). This analysis shows the ranking of the genotypes and their stability [43]. At 30 days, the Palamós commercial variety had the higher “yield after storage”, but manifested a high instability. LC215 and 2.9 showed slightly lower absolute scores but higher stability, and thus can be more suitable as they offer a more predictable “yield after storage” to farmers. For longer storage (90 days), 2.9 shows the higher “yield after storage”, followed by LC215, and 2.14. These genotypes show a high instability for this trait. At 90 days, the Palamós commercial variety shows a low “yield after storage”, which is highly stable across localities and irrigation regimes. In summary, considering the good agronomic performance of Palamós and 2.9, farmers can combine both genotypes for commercial purposes: Palamós directed to fresh commercialization (0–30 days) and 2.9 to the commercialization of aged, conserved fruits (>30 days).
3.7. Cold Storage Impedes LSL Phenotype

Shelf life is affected by a range of postharvest conditions. In the case of LSL varieties, farmers usually store the fruits in shady spaces under uncontrolled environmental conditions [22,23]. To dig into the postharvest room conditions that affect shelf life, we compared different storage environments: controlled conditions in the laboratory, cold room storage and farmers’ facilities (Figure 5). Low temperatures and high humidity caused a dramatic negative effect on the storage of LSL tomato: all genotypes showed significantly lower shelf life compared to the other means of storage. Interestingly, there were no significant differences between laboratory conditions and farmer facilities.

Figure 4. Mean performance vs. stability GGE biplot of the yield after storage (i.e., commercial yield * % shelf life) during the 90-days postharvest period. The single arrowed horizontal line indicates increasing yield after storage, whereas the vertical projections on the line indicate stability. The greater the projection, the higher is the instability. Red, genotypes; green, localities (E1, Eco-1; E2, Eco-2; C1, Conv-1), and irrigation regimes (NW, normal watering; WD, water deficit). Genotypes: 2.9 and 2.14, Penjar breeding lines; LC215 (Penjar) and LC547 (Ramellet), LSL traditional lines; Palamós, Penjar modern hybrid; Pera Delta, processing variety.

Figure 5. Effect of storage environment on the postharvest shelf life of 5 LSL genotypes after 90 postharvest days at each irrigation regime (NW, normal watering; WD, water deficit). Storage environments: laboratory conditions (24 ± 2 °C; 62 ± 11% relative humidity), farmer storage facilities (ambient conditions), and cold room storage (8 ± 2 °C, 95 ± 5% relative humidity). Columns represent mean values per genotype and conservation environment and error bars represent the standard error of the mean (SEM). Within each genotype, different letters indicate significant differences between storage conditions (p < 0.05, Tukey multiple range tests). Genotypes: 2.9 and 2.14, Penjar breeding lines; LC215 (Penjar) and LC547 (Ramellet), LSL traditional lines; Palamós, Penjar modern hybrid; Pera Delta, processing variety.
4. Discussion

In this work we have conducted a breeding program in which we have obtained a triple \( alc/sp/s \) mutant that carries the \( sp \) and \( s \)-classic mutations already described by other authors [33,34], as well as a new mutation (to our knowledge) at the end of the \( S \) gene which causes another amino acid change in position 291 of the \( S \) protein sequence. Phenotypically, the plants with the \( sp/s \) double mutation displayed the characteristic traits of both mutations, with a reduction in the length of sympodial units and the production of two terminal successive inflorescences caused by the \( sp \) mutation [33], and a highly branched inflorescence and delayed flowering earliness (Table 1) caused by the \( s \) mutation [49]. Although the \( SP \) gene has been reported not to be involved in the regulation of reproductive structures’ development [33,49], the \( sp/s \) mutant plants exhibited a reduced branching of the inflorescence compared to the original mutant \( s \) line, suggesting possible gene interactions or the effect of genetic background in the phenotypic expression of the \( s \) mutation [49,50].

The \( sp/s \) phenotype did not consistently improve the performance of the Penjar tomato when cultivated under a non-trellised system, as the new genotypes (2.9, 2.14) showed a yield and a harvest index similar to the original determinate landrace (LC215) from which they were derived. On the other hand, the recurrent selection for higher postharvest shelf life during the breeding program has resulted in the new varieties, 2.9 and 2.14, that show a high shelf life and “yield after storage”, which seem appropriate for farmers interested in selling the fruits after an aging period.

Mirroring the progress achieved by the processing tomato industry, our results point out that breeding for high-yield and compact plant architecture is needed for a competitive non-trellised culture of Penjar tomato. This culture is gaining interest among farmers, because of the reduction in production costs in comparison with the generalized single-stem-trellised cropping system [31]. The benefits of non-trellised cropping systems are optimized when using a single-harvest strategy, which implies a penalization of 10–20% in marketable yield [35,51], results that are similar to those reported in our work with Penjar tomatoes (16–20% unripen fruits at harvest). Nonetheless, unlike conventional Penjar tomato growing systems where cracking and BER are the major physiological disorders diminishing the profitability of this landrace [31], the main problem of Penjar cultivated under a non-trellised culture is sunscald incidence (>20% in some genotypes). Sunscald can result from prolonged exposure of the fruit surface simultaneously to heat and intense light [52], which is mitigated by the leaf canopy. The shading function of the canopy is related to leaf biomass and leaf area index (LAI), which decrease under water stress conditions [53]. In our study, when we submitted the genotypes to WD, sunscald was increased. Although LAI and leaf biomass measurements were not recorded, we hypothesize that the reduced total biomass obtained under WD diminished the leaf canopy, which may eventually have induced the high sunscald incidence recorded. For this, leaf coverage is an important trait to be considered in the breeding programs.

HI is an important trait as it explains how biomass is partitioned into yield [35,54]. HI has been an important breeding target for processing tomato, whereas it has not been considered for the recent improvement of fresh market tomato [55,56]. With the goal to adapt the Penjar tomato to a non-trellised culture, HI is a character to be taken into consideration. In processing tomatoes, HI falls in a range between 57–67% dw [57], with high-yielding cultivars scoring values around 65% [58]. In our experiments we found that for the determinate Penjar genotypes, HI ranges between 49.7–59.1% dw, therefore, there is margin for improvement of this trait in breeding. Regarding the environmental effects, HI was stable among environments (localities and irrigation regimes) on a dw basis, but showed significant variations across localities when expressed as fw. This variability was probably due to the different water status of the plants in each field when harvested, as fruits accumulate more water than other organs [58].

WUE is a key trait to adapt the tomato crop to the changing climatic conditions. Most authors state that a tomato crop does not benefit from fulfilling total water requirements for the entire growing season and stress the ability to improve plant water use by reducing
irrigation [16,59]. In our study, lowering the irrigation by 42.9%, we achieved an increase of WUE of 43.0% (fw) and 55.0% (dw). It is noteworthy that we observed that the improvement of WUE under WD was genotype-dependent, indicating differential genotype tolerances to water scarcity conditions. In fact, different sensitivities to WD stress have already been described in the different cultivar groups of tomato (e.g., cherry vs. fresh market [60]). In the case of the LSL, for which advantageous leaf physiological adaptations to drought stress have been described in some accessions [61], there is wide diversity in the response to water restrictions ([27,31,62]. Indeed, Fullana-Pericàs et al. [63] described that some LSL genotypes can be suitable for use as a rootstock due to tolerance to water stress.

Regarding the agronomic traits, WD reduced the total plant biomass and yield to similar degrees. This decline is not surprising as tomatoes are water-demanding crops and a negative effect on yield [64–66] and plant biomass [64] of WD strategies have been frequently reported. As described elsewhere, the negative effect of WD on yield is more related to a reduction of fruit weight than to the number of fruits per plant [67], which was also observed in our trials. Other works have shown that the decrease depends on the severity of the treatment and the plant cycle stage when WD is applied [16,64]. For instance, Patanè et al. [16] found no differences in total biomass production in processing tomato with a 50% ETc reduction strategy applied from flowering onwards compared to 100% ETc. Thus, finding appropriate scheduling in water shortages is a major challenge for Penjar tomato growers in order to optimize the amount of water used in the crop.

Extended shelf life is the key trait that distinguishes LSL varieties. Despite the fact that the genetic architecture of the LSL trait in Spanish landraces has been widely studied [21–23,47], the effect of preharvest factors on this trait remains fairly unknown. Considering the myriad of factors that affect plants in the field, and the high intra-batch variability that is found when studying postharvest shelf life, it is very difficult to dissect the main factors that drive a higher shelf life [31,68]. Moreover, scant studies have dealt with the preharvest factors involved in altering the physiological processes governing shelf life [69]. Our study has addressed this topic for the Penjar tomato, by analyzing the effect of genotype, irrigation, locality and postharvest conditions on shelf life. Overall, we have concluded that: (a) shelf life is under strong GxE interactions; (b) locality (i.e., environmental and management factors) affects shelf life during the first storage period (<30 days), irrigation during the long storage (90 days), while the genotype is the main factor that drives shelf life during the whole storage period (>30 days), increasing its effect throughout the postharvest period; (c) WD has the potential to enhance the shelf life of the Penjar tomato, but this positive response is observed in some genotypes grown under specific ExM conditions; (d) cold storage and high humidity impedes the LSL phenotype. The negative effect of low temperatures on Penjar tomato shelf life contrasts with general recommendations for other cultivar groups, where temperatures below 15 °C are recommended to extend shelf life [68], and highlight the singularity of Penjar tomato regarding its postharvest conservation. High shelf-life performance recorded under farmer storage facilities was comparable to laboratory-controlled conditions. This might be due to the past selection of farmer varieties in favor of traditional storage practices, which entails fruit being hung up under ambient temperature for availability during winter. This result confirms farmer storage as a suitable and cost-efficient alternative of storage for Penjar growers.

5. Conclusions

The utilization of natural variation encompassed within the LSL landraces for the adaptation to non-trellised crop management systems has scarcely been explored in modern plant breeding. This study is a first step towards the adaptation of Penjar LSL landrace to modern non-trellised culture. Altogether, our results set the basis for future breeding programs intended to increase competitiveness and improve the WUE of Penjar tomato. The harvest index, a trait that underpins the high performance of processing tomato, might be an important breeding target for the adaptation of Penjar tomato to modern non-trellised and sustainable management systems.
**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12102312/s1, Figure S1: Seasonal overview of climatic data for temperature (daily means, °C), relative humidity (daily means, %) and accumulated global solar irradiation (MJ m−2); Figure S2: Accumulated water requirements (crop evapotranspiration minus precipitation (ETc-P)) and irrigation volumes for normal watered (NW) and water deficit (WD) treatments at the three different growing locations; Figure S3: Representative images of some of the LSL genotypes used in the field trials; Table S1: Primer sequences for genotyping SP and S genes. Sequences for the forward (Fw) and reverse (Rev) primers used in PCR reactions for the amplification of the wild-type and mutant sequences; Table S2: Soil analysis for the three experimental field locations; Table S3: Effect of irrigation regime (I), genotype (G) and locality (L) on water use efficiency; Table S4: Effect of irrigation regime (I), genotype (G) and locality (L) on fruit quality traits; Table S5: Effect of irrigation regime (I), genotype (G) and locality (L) on postharvest shelf life (% sound fruits) during extended storage of the fruits (30, 60, and 90 days) under laboratory conditions (24 ± 2 °C; 62 ± 11% relative humidity).

**Author Contributions:** I.R.-V. and J.C. conceived the study; P.S. and J.B. performed the field and postharvest experiments and organized the database; A.R. coordinated fruit quality analysis; I.R.-V. and S.C. performed the molecular analyses; P.S., J.B. and J.C. performed the statistical analyses; P.S., I.R.-V. and J.C. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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