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Research Paper

Genome-wide expression analysis at three fruit ripening stages for tomato genotypes differing in fruit shelf life

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ABSTRACT

The ripening stage at harvest time determines the tomato fruit quality. After the fruit achieves its maximum size several metabolic changes of typically climacteric fruits are produced. Two cultivated genotypes of *Solanum lycopersicum* (Caimanta and 804627), with normal and altered fruit ripening, respectively and two accession, LA1385 of *S. lycopersicum* var. *cerasiforme* and LA722 of *S. pimpinellifolium*, with genes that prolong fruit shelf life, were tested to: 1) characterize and make a comparatively analysis for the transcriptome at different fruit ripening stages in genotypes that differ in fruit shelf life by cDNA-AFLP; and 2) provide further insight into the relationship between the extreme phenotypic differences for ripening among the genotypes through changes at transcriptomic level. Fruits at the breaker stage (B) were evaluated for fruit weight, firmness and fruit shelf life. The elapsed days between mature green (MG) and breaker stages Days (MG-B) as well as the elapsed days between B and red ripe (RR) stages Days (B-RR) were recorded. Comparison among ripening stages showed a great polymorphism related to the changes in gene expression. For all genotypes the transition from B to RR stages had higher polymorphism than the transition from MG to B. It was observed a great genetic variability for the phenotypic traits in agreement with the changes of gene expression. Moreover, it was observed that the transcriptome expression profiles in the initial and intermediate stages during ripening (MG and B) are more important to characterize genotypes. The wild species which have long shelf life do not show as drastic changes in gene expression as the cultivar with altered ripening that carrythe *nor* gene. These results suggest that the expressed or silenced genes could be involved, in some way, in the determination of the phenotypic traits evaluated in this study.

1. Introduction

The cultivated tomato (*Solanum lycopersicum* L.) is an autogamous species in which the fruit quality plays an important role for both producers and consumers. The ripening stage at harvest time determines the final product quality and the maintenance in good conditions during a period of time (Javanmardi and Kubota, 2006). Fruit ripening is the final step in the fruit development. After the fruit achieves its maximum size several metabolic changes of typically climacteric fruits are produced. Fruit quality traits such as color, aroma, flavor, texture and consistence are defined in this final step of ripening. The accumulation of carotenoid pigments and fruit softening allows to distinguish various ripening stages: mature green, breaker, turning, orange, red firm and red ripe (Rick, 1978; Nuez, 1991; Giovannoni, 2004). Mutants affecting the normal ripening process were detected in *S. lycopersicum* such as *rin* (*ripening inhibitor*), *nor* (*non ripening*), *Nr* (*never ripe*), *alc* (*alcobaca*) (Chalukova and Manuehyan, 1991). These genes block or prolong the fruit ripening so they contribute to extend fruit shelf life. However, these mutants also have undesirable effects on fruit quality due to pleiotropic actions of the genes. Fruits from *S. lycopersicum* var. *cerasiforme* (Dunal) (Spooner et al., 1993) and *S. pimpinellifolium* L. wild species have wide variability for attributes such as flavor, aroma, coloration and texture and they also carrying genes for fruit shelf life (Pratta et al., 1996; Zorzoli et al., 1998). The advantage of the fruit shelf life wild genes is that they have no negative effects on organoleptic fruit quality. A wide range of tomato genotypes such as the standard for ripening Caimanta, variety homozygote for the *nor* allele and some relative wild species have been characterized by our research team at phenotypic, proteomic and genomic levels (Rodríguez et al., 2008; Pereira da Costa et al., 2017). In fact, several polypeptides and genomic regions were associated with fruit traits in segregating populations derived from interespecific crosses (Rodríguez et al., 2010; Pereira da Costa et al., 2013), however a whole gene expression analysis to compare the ripening process among genotypes carrying fruit

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Table 1
Restriction enzymes, adapters, pre-amplification primers, selective amplification primers and primer combinations to obtain AFLP-based transcript profiling (cDNA-AFLP) for expression analysis.

Restriction enzymes		*Apo I*	*Mse I*
Adapters	Top Strand	CTCGTAGACTGCGTACC	GACGATGAGTCCTGAG
	Bottom Strand	AATTGGTACGCAGTCTAC	TACTCAGGACTCAT
Pre-amplification primers (0)		CTCGTAGACTGCGTACCAATT	GACGATGAGTCCTGAGTAA
Selective amplification primers (+1)		GACTGCGTACCAATTG (Apo11)	GATGAGTCCTGAGTAAG (Mse37)
		GACTGCGTACCAATTA (Apo12)	GATGAGTCCTGAGTAAT (Mse38)
Combination A	Apo11-Mse37	**Combination C**	Apo11-Mse38
Combination B	Apo12-Mse37	**Combination D**	Apo12-Mse38

Table 2
Mean values and standard error for each genotype and Degree of Genetic Determination (DGD) for each trait. **W**: fruit weight, **Fir**: fruit firmness, **SL**: fruit shelf life, **Days (MG-B)**: days from mature green to breaker stage, **Days (B-RR)**: days from breaker to red ripe stage. **CAI**: Caimanta cultivar of *S. lycopersicum*, **NOR**: accession 804627 of *S. lycopersicum* homozygous for *nor* gene, **LA1385**: accession LA1385 of *S. lycopersicum* var. *cerasiforme* and **LA722**: accession LA722 of *S. pimpinellifolium*. Different letters indicate significant differences. $^{**}p < 0.01$ y $^{***}p < 0.001$.

	W	Fir	SL	Days (MG-B)	Days (B-RR)
CAI	151.62 ± 12.90 ^{c}	50.67 ± 2.02 ^{a}	6.92 ± 0.65 ^{a}	13.81 ± 0.66 ^{b}	5.26 ± 0.42 ^{a}
NOR	80.37 ± 10.38 ^{b}	63.67 ± 7.88 ^{b}	20.67 ± 4.33 ^{c}	16.22 ± 0.73 ^{c}	10.07 ± 0.49 ^{b}
LA1385	3.16 ± 0.26 ^{a}	57.20 ± 2.15 ^{ab}	17.00 ± 1.26 ^{bc}	10.95 ± 0.76 ^{a}	5.36 ± 0.57 ^{a}
LA722	0.83 ± 0.09 ^{a}	61.56 ± 1.83 ^{b}	13.33 ± 1.47 ^{b}	12.54 ± 0.63 ^{ab}	4.93 ± 0.37 ^{a}
F	53.66 ***	5.32 **	15.85 ***	7.25 ***	29.05 ***
DGD	0.88	0.38	0.68	0.13	0.50

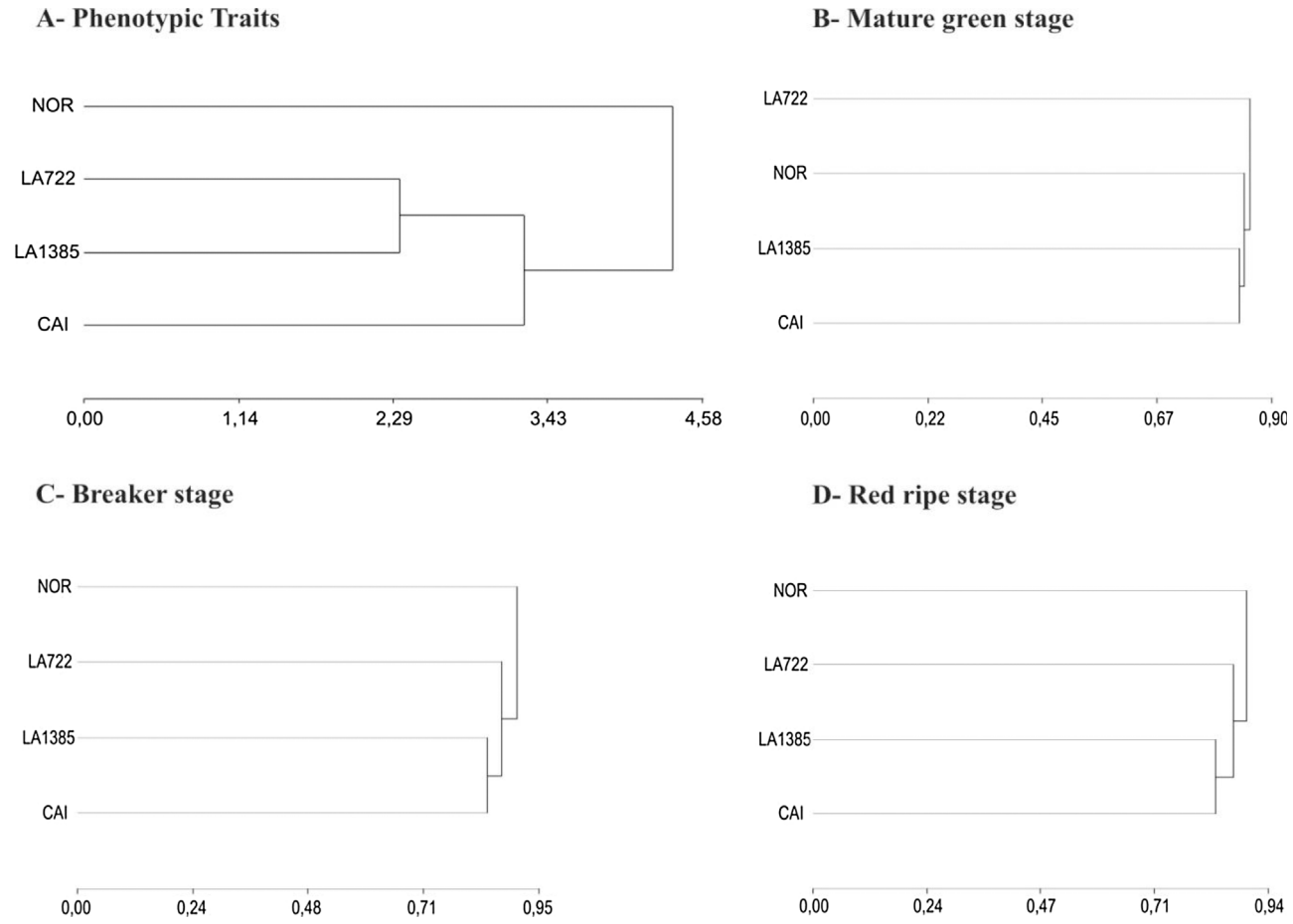

Fig. 1. Analysis of cluster among genotypes from phenotypic traits (*A*) and gene expression profiles at three ripening stages: mature green (*B*), breaker (*C*) and red ripe (*D*). **CAI**: Caimanta cultivar of *S. lycopersicum*, **LA722**: accession LA722 of *S. pimpinellifolium,* **LA1385**: accession LA1385 of *S. lycopersicum* var. *cerasiforme* and **NOR**: accession of *S. lycopersicum* homozygous for *nor* gene.

Table 3
Percentage of polymorphism, number of total transcript derived fragments (TDF) and exclusive of stage for each genotype with four specific primer combinations. **CAI**: Caimanta cultivar of *S. lycopersicum*, **LA722**: accession LA722 of *S. pimpinellifolium,* **LA1385**: accession LA1385 of *S. lycopersicum* var. *cerasiforme* and **NOR**: accession of *S. lycopersicum* homozygous for *nor* gene. **Primer combination A**: Apo11-Mse37, **Primer combination B**: Apo11-Mse38, **Primer combination C**: Apo12-Mse37, **Primer combination D**: Apo12-Mse38, **% pol**: percentage of polymorphism, **TT:** Total number transcript derived fragments (TDFs), **PAA**: exclusive TDFs at mature green stage, **APA**: exclusive TDFs at breaker stage, **AAP**: exclusive TDFs at red ripe stage.

Genotypes	Primer Combination A					Genotypes	Primer Combination B				
	% pol	TT	PAA	APA	AAP		% pol	TT	PAA	APA	AAP
CAI	56.6	53	6	1	3	**CAI**	54.3	35	3	2	6
LA722	63.0	54	9	6	9	**LA722**	86.5	52	13	9	8
LA1385	56.1	66	6	6	4	**LA1385**	55.1	49	9	4	2
NOR	68.5	54	23	0	2	**NOR**	64.7	17	7	0	0
Total	61.1	227	44	13	18	**Total**	65.2	153	32	15	16
Genotypes	Primer Combination C					Genotypes	Primer Combination D				
	% pol	TT	PAA	APA	AAP		% pol	TT	PAA	APA	AAP
CAI	34.9	43	2	1	5	**CAI**	54.5	22	5	0	3
LA722	65.1	63	11	5	8	**LA722**	69.8	43	7	0	11
LA1385	60.0	50	4	4	5	**LA1385**	82.8	29	1	0	12
NOR	66.0	50	16	2	1	**NOR**	77.8	27	16	0	0
Total	56.5	206	33	12	19	**Total**	71.2	121	29	0	26

shelf life genes, mutants or wild was not enough studied, specifically at the transcriptome level.

Differential expression studies are fundamental to understand the growth and development process in plants. Transcriptomic analysis provides an approach by which genetic changes can be linked to phenotype. In spite of *S. lycopersicum* and *S. pimpinellifolium* have extremely phenotypic differences, only minor variations were found between them at the genomic level (near to 0.6%). According to this, Michael and Alba (2012) postulated that phenotypic differences are due to protein functions and consequently by the regulation of transcriptome.

The cDNA-AFLP method developed by Fischer et al. (1995) combines differential display and AFLP (Vos et al., 1995). The cDNA-AFLP has been widely used to identify differentially expressed genes in plants and others organisms (Vriezen et al., 2008; Frank et al., 2009; Gamalath et al., 2009). Molesini et al. (2009) examined expression profiles obtained by cDNA-AFLP during early development of parthenocarpic and normal tomato fruits and they detected 212 differentially expressed transcripts. Recently, Zhao et al. (2015) used cDNA-AFLP to identify resistant genes for *Cladosporium fulvium* in tomato. cDNA-AFLP was generally used to study differential expression in a single genotype under changing or contrasting condition of biotic or abiotic stress. However, when more than two genotypes and three ripening stages need to be evaluated, cDNA-ALFP is turned an alternative method cheaper than RNAseq. Therefore, in those situations can definitely be a first approach to the study of the transcriptome.

The aims of this work were: 1) to characterize and make a comparatively analysis for the transcriptome at different fruit ripening stages in genotypes that differ in fruit shelf life by cDNA-AFLP; 2) to provide further insight into the relationship between the extreme phenotypic differences for ripening among the genotypes through changes at transcriptomic level.

2. Materials and methods

2.1. Plant materials

The genotypes tested were Caimanta (hereafter referred as CAI) and the accession 804627 (homozygous for *nor* mutant gene, hereafter referred as NOR) of *S. lycopersicum* with normal and altered fruit ripening, respectively. The wild genotypes, LA1385 of *S. lycopersicum* var. *cerasiforme* and LA722 of *S. pimpinellifolium,* with genes that prolong fruit shelf life (Rodríguez et al., 2010; Pratta et al., 2011a) were the other genotypes tested. Seeds from these four genotypes were germinated in seedling trays and transplanted to greenhouse after 45 days in a completely randomized design. Ten plants by genotypes were distributed with a distance between plants of 40 cm and row spacing of 1 m. Field assays were conducted at the Experimental Station 'José F. Villarino' (33°S and 61°W, Universidad Nacional de Rosario, Argentina).

2.2. Phenotypic traits

Five fruits per plant (total number of fruits = 133) at the breaker stage (B) defined by Giovannoni (2004) were harvested and evaluated for fruit weight (W, g), firmness (Fir) and shelf life (SL) according to Pereira da Costa et al. (2014). Other six fruits per plant (total number of fruits = 162) were labeled at mature green stage (MG) and the elapsed days between mature green and breaker stages (Days MG-B) and elapsed days between breaker and red ripe (RR) stages (Days B-RR) were recorded.

2.3. Tissue collection and total RNA extraction

A single fruit from three different plants (biological replications) per genotype were harvested at MG, B, and RR stages. Total number of fruits per genotype was nine. Pericarp was separated and immediately immersed in liquid nitrogen. Total RNA was extracted with TriPure Isolation Reagent following the instruction suggested by the manufacturer (Roche, Basel, Switzerland). A mortar and pestle were used to powdering 500 mg of pericarp with 1 ml of TriPure Isolation Reagent. Then, equal volume of chloroform was added to separate the aqueous phase, containing RNA. Finally, RNA was precipitated with 0.5 ml of isopropanol.

2.4. cDNA synthesis and obtaining of cDNA-AFLP profiles

First strand cDNA synthesis was carried out from 1 μg of total RNA according with ImPromII™ Reverse Transcriptase protocol (Promega, Madison, WI, USA). The reaction conditions were: 1X of ImProm-II 5X reaction buffer, 3 mM of $MgCl_2$, 0.5 mM of dNTP mix (10 mM each one) and 35 ηg/μl of biotinylated Oligo dT_{25} in 20 μl final volume. The mix reaction for second strand synthesis was: 50 U of DNA polymerase I (New England BioLabs® Inc.), 1.6 U of Ribonuclease H (Ambion®), 1X DNA polymerase I buffer, 0.20 mM of dNTPs mix and 0.004 mM of DTT in 140 μl final volume. mRNA was extracted from total RNA by mean of

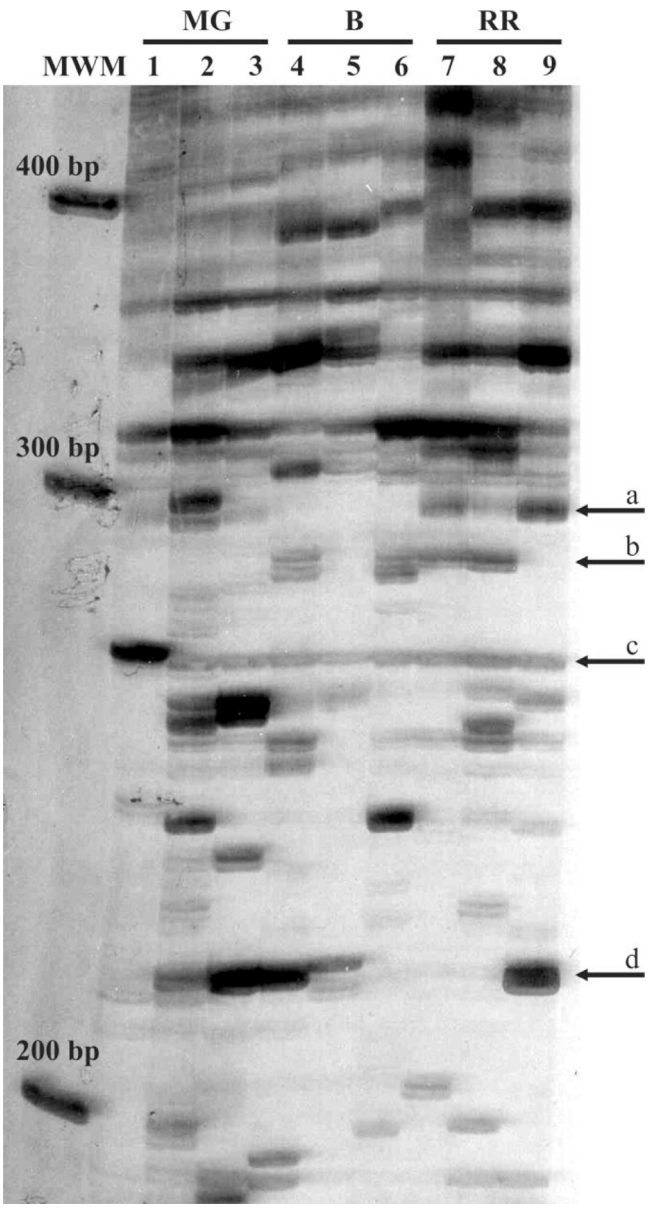

Fig. 2. Transcript profiles obtained from primer combination A for the accession LA1385 of *Solanum lycopersicum* var. *cerasiforme* in a denaturing polyacrylamide gel at 5% w/v. **MWM**: molecular weight marker, **bp**: base pairs, **MG**: mature green stage, **B**: breaker stages, **RR**: red ripe stage, **1, 2, 3**: three biological repetitions at MG stage, **4, 5, 6**: three biological repetitions at B stage, **7, 8, 9**: three biological repetitions at RR stage, **a**: example of a TDF present in MG, absent in B and present in RR stage, **b**: example of a TDF absent at MG and present at B and RR stages, **c**: example of a monomorphic TDF, **d**: example of a TDF present at MG and B stages and absent at RR stage. The presence or absence was considered when two or more biological replicates had or not had the TDF, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

magnetic capture using a commercial kit PolyATract® mRNA Isolation Systems (Promega, Madison, WI, USA). Synthesis of first and second strand, adapter sequences, ligation and amplification conditions were carried out according to the protocol proposed by Vuylsteke et al. (2007). Primers +0 for pre-amplification and primers +1 for selective amplification were used. Primer sequences are shown in Table 1. The primer combinations for selective amplifications were named as A (Apo11-Mse37), B (Apo11-Mse38), C (Apo12-Mse37) and D (Apo12-Mse38). Restriction enzymes were selected based on Stölting et al. (2009) study. The amplified fragments were separated by denaturing polyacrylamide gels (5% w/v) at room temperature and detected with a commercial silver staining kit (Silver Sequence™ Staining Reagents, Promega, Madison, WI, USA). Electrophoresis was carried out at 50 W constant for 3 h. For all genotypes and ripening stages the presence and absence of bands (TDF, Transcript Derived Fragments) were determined. The presence or absence was considered when two or more biological replicates had or not had the TDF, respectively. Different ripening stages in each genotype were compared. On another hand, the expression profiles among genotypes in each ripening stages was analyzed. Total number of TDFs and polymorphic TDFs per ripening stage and genotypes were calculated.

2.5. Statistical analysis

The normal distribution of phenotypic traits was evaluated by the Shapiro-Wilk test (Shapiro and Wilk, 1965). To test the mean value differences among genotypes for all phenotypic traits, an analysis of variance (ANOVA) was performed and then Duncan's multiple range test was used to compare sets of mean values. Degree of Genetic Determination (DGD) was calculated to estimate the proportion of phenotypic variation explained by genotypic variation for each trait in this set of tomato genotypes (Kearsey and Pooni, 1996). The comparison between DGD and polymorphism percentage during fruit ripening was use to probe the consensus between phenotypic variability and transcriptomic profiles (genetic variability). A chi-square test was used to test if total number of TDFs detected at each ripening stage was independent from each genotype. To test if the exclusive TDFs were homogenously represented in each genotype another chi-square test was used. A multivariate cluster analysis with average linkage as grouping method was carried out using Euclidean distances for phenotypic traits and Jaccard's distances for molecular data.

3. Results and discussion

3.1. Phenotypic traits

All phenotypic traits were normally distributed. The genotypes showed highly significant differences ($p < 0.01$) for all traits. Table 2 shows the mean values per genotype for each phenotypic trait. The cultivated genotypes showed heavier fruits than wild ones. CAI genotype had fruit firmness values lower than the NOR and LA722 genotypes but both later genotypes had not significant differences from LA1385. However, LA1385 and NOR had the longest fruit shelf life suggesting that firmness is not directly related with fruit shelf life in this genetic background. These results have a great coincidence with previous studies (Pereira da Costa et al., 2009; Rodríguez et al., 2010; Pratta et al., 2011a), but some differences in the mean values for each trait can be explained by a genotype by environmental interaction (G × E) affecting fruit traits in tomato (Pratta et al., 2011b; Panthee et al., 2012). Regarding to the period MG to B, NOR showed the highest mean value for it but there is not significant differences between the wild genotypes in this period. In the period B to RR, NOR showed a mean value twice greater than the rest of genotypes.

All DGD values were significant ($p < 0.01$, Table 2). The highest value (0.88) was estimated for fruit weight, intermediate values (0.68–0.50) were found for fruit shelf life and Days (B-RR) respectively. The lowest values were found for fruit firmness (0.38) and for Days (MG-B) (0.13). These DGD values have indicated the presence of genetic differences among these genotypes for those traits that are involved in the fruit ripening process.

Fig. 1*A* shows the cluster analysis obtained from phenotypic data. The NOR genotype was different from the rest, being SL and Days (B-RR) the classificatory traits. This genotype, carrying *nor* gene in homozygosis, had the highest SL value and the longest period of times between the evaluated ripening stages. The value of SL was higher in wild species than CAI but they were not different of this cultivar for Days (MG-B) and Days (B-RR).

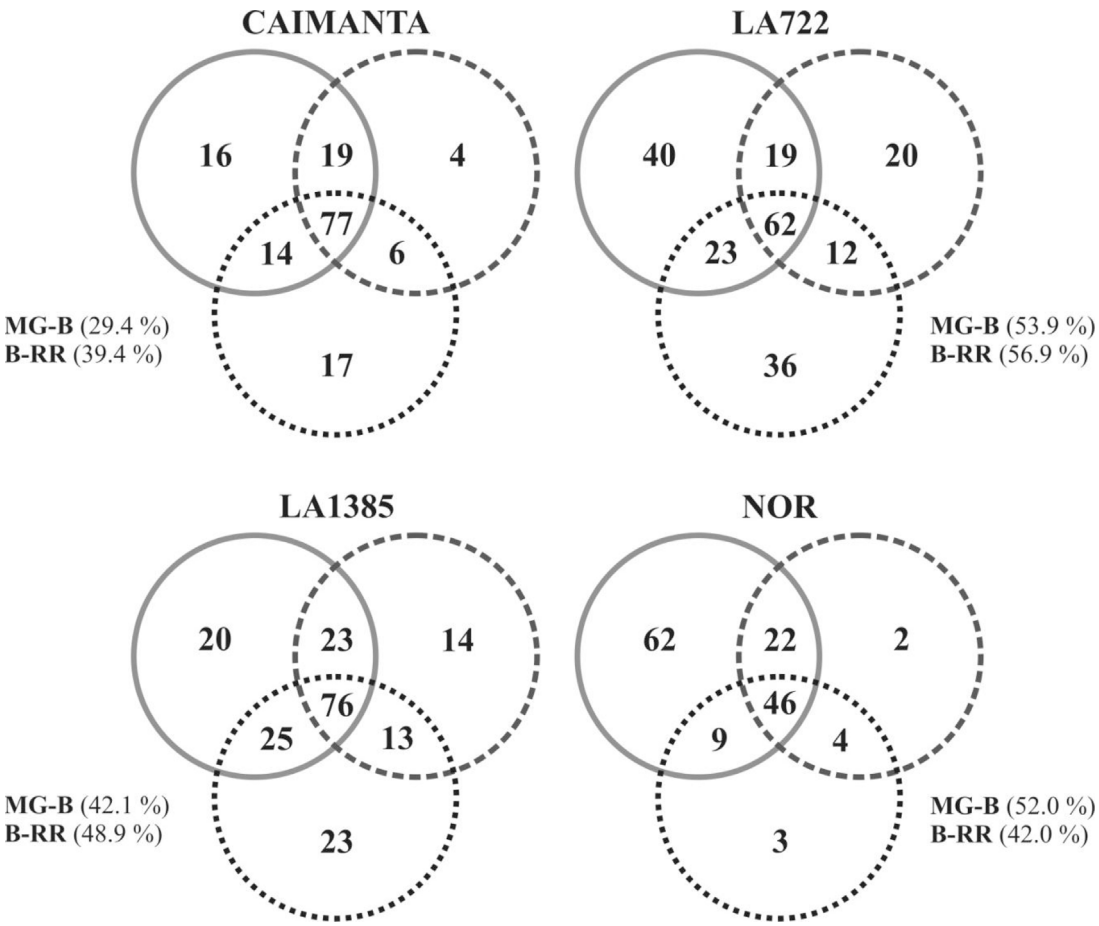

Fig. 3. Venn diagram of number of total and exclusive transcript derived fragments obtained by AFLP-based transcript profiling (cDNA-AFLP) with four specific primer combinations in each genotype. **Caimanta**: Caimanta cultivar of *S. lycopersicum*, **LA722**: accession LA722 of *S. pimpinellifolium,* **LA1385**: accession LA1385 of *S. lycopersicum* var. *cerasiforme* and **NOR**: accession of *S. lycopersicum* homozygous for *nor* gene. Different kind of circle indicates each ripening stage (solid line: MG, dash line: B and dotted line: RR). The numbers into the circle intersection indicate total monomorphic TDFs between ripening stages, while exclusive TDFs of each ripening stages are indicated outside of circle intersections. **MG-B**: indicates the polymorphism percentage for transition from mature green to breaker and **B-RR**: indicates the polymorphism percentage for transition from breaker to red ripe. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Expression profiles by cDNA-AFLP

3.2.1. Comparison among fruit ripening stages for each genotype

A total number of 707 TDFs were detected. An average of 44 TDFs by genotype and primer combination with a 63.5% of polymorphism were found. The primer combination C showed the lowest polymorphism in Caimanta cultivar (Table 3). The Fig. 2 shows the TDF of primer combination A for the accession LA1385 of *S. lycopersicum* var. *cerasiforme* in a denaturing polyacrylamide gel at 5% w/v. These results are in agreement with *in silico* simulation for the estimation of TDFs per primer combination made by Stölting et al. (2009). Then, those results demonstrate that the pair of used restriction enzymes was useful to generate only one fragment per each transcript. Therefore, we also can assume that each TDF corresponds to only one transcript derived from one gene.

TDFs with four primer combinations in each genotype are shown in Fig. 3. Different kinds of circles indicate each ripening stage (solid line: MG, dash line: B and dotted line: RR). The numbers into the circle intersection indicate total monomorphic TDFs between ripening stages, while exclusive TDFs of each ripening stages are indicated outside of circle intersections. Genotype taken together, 138, 40 and 79 TDFs exclusives of MG, B and RR respectively were found. The NOR genotype presented the highest number, equal to 62, of exclusive TDFs at MG stage, while LA722 had the highest number of TDFs at B and RR stages with 20 and 36, respectively (Fig. 3).

A high polymorphism percentage, related to the changes in gene expression, was observed in the transition from the stage B to RR. This value was higher than in the transition MG to B for all genotypes except for NOR (see Fig. 3). Carbone et al. (2005) found differential gene expression in genes encoding enzymes involved in biosynthesis of carotenoid pigment, primary metabolism, photosynthesis and metabolism of wall cell, considering the same ripening stages evaluated here, in Money Maker cultivar. It was found a low polymorphism in the transition from MG to B that agrees with the lowest DGD value for Days (MG-B) (DGD = 0.13). Consequently, it is interesting to note that the great polymorphism from B to RR had the great DGD for Days (B-RR) (DGD = 0.50). These changes found in the polymorphisms (in the different stages of ripening) suggest that the phenotypic variability observed in these genotypes, and supported by the genetic variability detected, are in concordance with changes on gene expression during the transition among stages. Mahuad et al. (2013) demonstrated the consensus between phenotypic variability and DNA markers (SRAP, Sequence-Related Amplified Polymorphism) highly related to expressed sequences to characterize five tomato RILs and the hybrids among them. On the other hand, Rodríguez et al. (2010) and Pereira da Costa et al. (2014) detected polypeptides and DNA marker associated with traits related to fruit ripening. Therefore, with the transcriptome characterization of the genotypes evaluated in this work together with proteomic and genomic studies on the same plant material made in previous works (Rodríguez et al., 2008; Pereira da Costa et al., 2017), three different flow levels of genetic information were covered for the tomato ripening process.

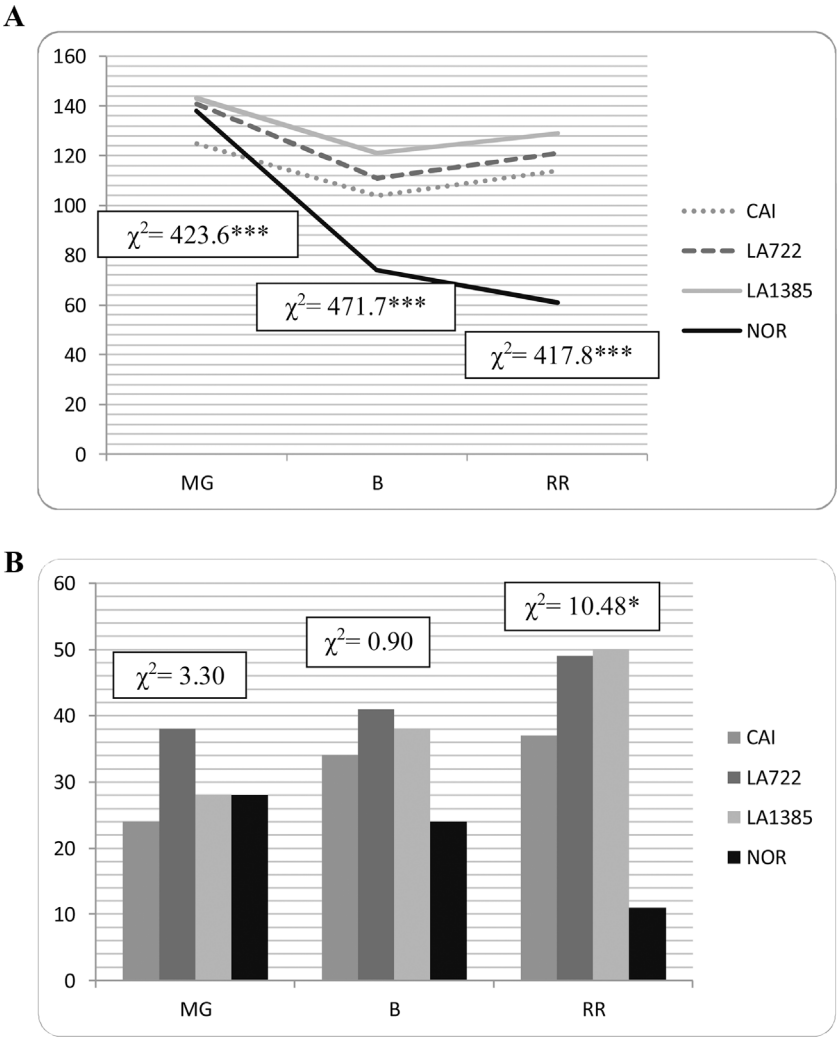

Fig. 4. Total number of Transcript Derived Fragments (TDFs) at each ripening stage per genotype (*A*). Number of TDFs exclusive of genotype for each ripening stages (*B*). **CAI**: Caimanta cultivar of *S. lycopersicum*, **LA722**: accession LA722 of *S. pimpinellifolium*, **LA1385**: accession LA1385 of *S. lycopersicum* var. *cerasiforme* and **NOR**: accession of *S. lycopersicum* homozygous for *nor* gene. **MG**: mature green, **B**: breaker, **RR**: red ripe. * $p < 0.05$, *** $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2.2. Comparison among genotypes at each ripening stage

A total number of 791 TDFs were detected of which 290, 248 and 253 corresponded to mature green, breaker and red ripe stage, respectively. Genotypes showed high level of polymorphism. The total number of TDF strongly decreased through ripening process in NOR (Fig. 4*A*), being the total number of TDFs independent from each genotype at the three ripening stage (see chi-square values on Fig. 4*A*). Exclusive TDFs per genotype were present at each ripening stage. The LA722 genotype had the high number of exclusive TDF for MG and B ripening stages. Also the LA722 and LA1385 genotypes had the great amount of exclusive TDFs at red ripe stages, while NOR genotype had the lowest at the same stage. As the ripening process goes on an increasing in the number of exclusive TDFs was observed for all genotypes, but NOR genotype was the exception showing an opposite behavior (Fig. 4*B*). It must be considered the fact that the amount of exclusive TDFs was not homogenously distributed among genotypes at red ripe stage (see chi-square values in Fig. 4*B*) in agreement with the increasing on polymorphism percentage in the transitions period MG-B and B-RR (Fig. 3). Exclusive TDFs of NOR genotype decreased at B and RR stages (Fig. 4*B*) according to the lower polymorphism percentage in the transition B-RR (Fig. 3) and with the higher value for the trait Days (B-RR). It must be considered the fact that NOR mutant blocks the normal ripening process, because *nor* encode a transcription factor which may stop gene expression at early stages during fruit ripening (Liu et al., 2015). Osorio et al. (2011) found that transcripts associated with cell wall degradation are down regulated in other *nor* mutant genotype. Moreover, Liu et al. (2015) suggest a more global effect of *nor* on ethylene-related gene expression in climacteric fruits. On the other hand, the wild species which have long shelf life do not appear to show drastic changes in gene expression, so the wild genes that prolong fruit shelf life would be specific genetic pathways of specific traits and they do not affect the global transcriptome like cultivar carrying *nor* gene done.

3.2.3. Differences among genotypes throughout the fruit ripening process

Fig. 1*B–D* shows the cluster analysis obtained from transcriptomic data. The cluster analysis from expression profiles at mature green stage shows two groups, with LA722 in one of them and CAI, NOR and LA1385 in the other (Fig. 1*B*). The conformed groups at both breaker and red ripe stages (Fig. 1*C* and *D*) were similar, but different from the groups obtained at mature green. At breaker and red ripe stages, NOR genotype was in a group, but CAI, LA722 and LA1385 genotypes in another one. NOR genotype obviously was different from the other genotypes at ripening stages which is the stage when organoleptic fruit quality is defined. On the other hand, LA722 was different of the others at ripening stage where fruit size and weight are defined. Our results indicate that the expression profiles during ripening phases (final step of fruit development), the initial (MG) and intermediate stages (B) are determinant for the genotype because of the different cluster analysis showed while at final ripening stage (RR) there are no any differences.

It is also important to note that the different clustering can be explained by the amount of common TDFs among genotypes. For instance, at MG, LA722 only shares smallest number of TDFs (47) with the rest of genotypes. While, at B and RR stages was NOR genotype in

this condition (22 and 21, respectively). Similarly, CAI and LA1385 genotypes had the greatest number of shared TDFs and they clustered together at the three ripening stages (Fig. 1).

Due to the phenotypic traits are evaluated at B and RR stages, it seems logical that the clusters by gene expression profiles from these ripening stages are in agree with the clustering obtained by phenotypic traits.

Our results are indicating that changes in gene expression are related to phenotypic changes. We assumed that the expressed or silenced genes would be involved on the expression of these phenotypic traits. NOR genotype showed a great difference with the other genotypes of normal fruit ripening in the different stages of the ripening process. This fact was strongly supporting by the results obtained in our experiments. Moreover the clustering of genotypes at MG could be linked to genetic origin of genotypes here evaluated. The wild accession LA722 of *S. pimpinellifolium* was the genotype early differentiated from the others of *S. lycopersicum*.

4. Conclusions

The expression profiles obtained by cDNA-AFLP are useful to analyze genome-wide expression and to characterize different fruit ripening stages when several genotypes are studied. A fraction of phenotypic variability that is highly explained by genetic variability would be related to the changes of gene expression, as it was demonstrated for the transition from B to RR stage. Moreover, the transcriptome expression profiles indicate that the initial and intermediate stages during ripening (MG and B) are important to characterize genotypes. Finally, the clusters analysis obtained from different set of data are indicating that expression changes are related to phenotypic changes for these fruit traits. The wild species which have long shelf life do not show as drastic changes in gene expression as the cultivar with altered ripening that carry the *nor* gene. Some expressed or silenced genes could be involved in the phenotypic traits evaluated here.

Acknowledgments

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