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Original Paper

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Abstract   Fleshy fruits are important worldwide crops that are rich sources of useful and functional compounds in the human diet. Although fruit ripening has been extensively studied, early fruit development has not been paid much attention despite its contribution to the sensorial and nutritional quality of the fruit. This study aimed at identifying candidate genes involved in early fleshy fruit development that can contribute to the control of final fruit size and composition by comparative analysis of tomato and grape genes. By mining public sequences and microarray database, we identified 23 transcription factors belonging to 14 classes (AP2-EREBP, ARF, bHLH, bZIP, C2C2-GATA, FHA, GeBP, GRAS, HB, LIM, MYB, PBF-2-like, SBP and WRKY) as candidate regulatory genes for early fruit development. The function of these candidate genes will be confirmed by several reverse genetic approaches using the miniature tomato cv. Micro-Tom.

Key words: Tomato, grape, early fruit development, transcription factor.

Fleshy fruits are important worldwide crops that are rich sources of useful and functional compounds in the human diet. These compounds (e.g. sugars, flavonoids, and volatile compounds) are mainly produced and/or accumulated at the ripening stage of fruit development (Giovannoni 2004). Extensive studies on fruit ripening have been done especially using tomato (*Solanum lycopersicum*) and have identified several key regulatory genes for the fruit ripening processes (Bemer et al. 2012; Karlova et al. 2011; Manning et al. 2006; Vrebalov et al. 2002, 2009). In contrast, early stages of fruit development have not been paid much attention despite their large contribution to the visual, sensorial and nutritional quality of the fruit. After fruit set, successive cell division and cell expansion events mostly determine final fruit size (Gillaspy et al. 1993). At the same time, sugars, amino acid and organic acids accumulate during the cell expansion phase (Baxter et al. 2005; Carrari et al. 2006; Mounet et al. 2009). Although a few regulatory genes for fruit size and shape have been characterized (Cong et al. 2002; Frary et al. 2000; Xiao et al. 2008), molecular mechanisms underlying the regulation of fruit growth and metabolism during early fruit development are not fully addressed.

To identify candidate genes regulating early fruit development, we focused on grape (*Vitis vinifera*) which bears berry type fleshy fruits like tomato. In both tomato

and grape, young fruits which have a green color present a rapid size increase by cell division and expansion. The transition phase to ripening is called "veraison" in grape, and breaker stage in tomato. After that, ripening stage consists in color changes, sugars accumulation and fruit softening, in both tomato and grape in spite of a main difference considering that tomato is climacteric and grape is non-climacteric. While grape has some unique characteristics such as the accumulation of tartaric acid instead of ascorbate (DeBolt et al. 2006) and the absence of endoreduplication-associated cell growth (Chevalier et al. 2011), tomato and grape have similar fruit developmental processes. In addition, comparative analysis of full-length cDNA sequences of Micro-Tom tomato cultivar with other plants indicated that tomato had higher similarity to grape than to other fleshy fruits like apple and orange (Aoki et al. 2010). Accordingly, a digital expression profiling revealed that some regulatory genes are up-regulated both in tomato and grape during fruit ripening (Fei et al. 2004).

In this study, we searched for candidate genes involved in early stages of fruit development that can contribute to final size and taste of fleshy fruit. After identification of tomato and grape common genes, transcriptome data mining highlighted several transcription factors having known and unknown biological role were found as the candidate genes.

Materials and methods

Identification of common genes between tomato and grape

To identify common genes between tomato and grape, unigene data sets of the Tomato SGN Unigene (SGN build Tomato200607 #1) and the NCBI Vitis vinifera UniGene (Build #9) were retrieved from SGN and NCBI ftp site, respectively. Using the TBLASTX program (Altschul et al. 1990) with a cutoff e-value at 1e-10, the unigene sequences of tomato were compared with those of grape and vice versa. Reciprocal best hit pairs were determined as common gene pairs.

Transcriptome data mining

Publicly available microarray data of fruit development series in tomato and grape were obtained from TFGD (http://ted.bti.cornell.edu/cgi-bin/TFGD/miame/experiment.cgi?ID=E025; data set E025; Osorio et al. 2011) and PLEXdb (http://www.plexdb.org/modules/PD_browse/experiment_browser.php?plex_name=GrapePLEX; data set VV5; Deluc et al. 2007), respectively. For TOM1 cDNA spotted array data set, the print-tip LOWESS normalized data were used for further expression analysis. For grape microarray data set VV5, only the data of Cabernet Sauvignon berries from well-watered plants were used. The Affymetrix raw CEL files were processed for background correction, quantile normalization and a median polish with the robust multiarray averaging (RMA) method using RMAexpress program (Bolstad et al. 2003). Differentially expressed genes during fruit development were determined by one-way ANOVA with a false discovery rate (FDR) correction ($p<0.05$; Benjamini and Hochberg, 1995). Pearson's correlation k-means clustering of the differentially expressed probes corresponding to the common genes were performed using TM4: MeV 4.3 software (http://www.tm4.org; Saeed et al. 2003).

Result and discussion

Comparison of unigene sequences between tomato and grape

To identify common genes between tomato and grape, we compared 34,829 unigene data sets of tomato to 23,166 of grape. The TBLASTX reciprocal best hit method determined 8,229 unigenes as common gene pairs. This number should however be treated with caution and also take into account the redundancy derived from a same gene and the underestimate for paralogous gene families. Nevertheless, functional classification of common genes into Mapman BIN structure of tomato (Urbanczyk-Wochniak et al. 2006) showed higher proportion of common genes in the vital functions like those coding for co-factor and vitamin metabolism, C1-metabolism, mitochondrial electron transport/ ATP synthesis, biodegradation of xenobiotics and tetrapyrrole biosynthesis (Supplemental Figure 1). In contrast lower proportion of common genes was found in stress, glycolysis, photosynthesis, cell wall and not assigned categories.

Comparative transcription analysis of common genes

To identify common genes expressed in early stage of fruit development in tomato and grape, data-mining of publicly available microarray data of tomato and grape was performed. Among the 8,229 unigenes common to both tomato and grape, 2,503 unigenes (represented by 2,757 spotted probes) were present on tomato TOM1 array and 4,977 unigenes (represented by 6,203 spotted probes) were present on grape Genechip array. ANOVA analysis with a FDR cutoff of 5% identified 1,713 probes in TOM1 and 3,922 probes in grape Genechip corresponding to genes differentially expressed throughout fruit development in tomato and grape, respectively. Among these, 885 probes representing genes included in both the tomato TOM1 and the grape Genechip were used for subsequent analysis.

Figure 1 shows transcription profiles along fruit development in tomato and grape resulting from k-means clustering for the 885 probes selected. The dot-lines on each panel represent the transition phase to ripening, breaker at 42 DPA in tomato and veraison (8 to 9 weeks after flowering) in grape. Based on this transition phase, 188 probes (21% of the total number of probes analysed) represent genes induced during early development in both tomato and grape (clusters C, L, P and S). Conversely, 146 probes represent genes induced during ripening in both fruits (clusters D, E and F). The other probes did not show the same transcription profile in tomato and grape. Indeed the probes grouped in clusters H, M and R represent genes induced during ripening in tomato but repressed during ripening in grape.

Mapman classification of the 182 genes (188 probes) expressed in early stages included e.g. 15 genes in the Photosynthesis category, 7 genes in Cell wall, 6 genes in Amino acid metabolism, 6 genes in Hormone metabolism, 5 genes in Stress, 20 genes in Protein synthesis, 8 genes in Protein degradation (see Supplemental Table S1 for exhaustive list). Comparison of apple and tomato gene expression data by Janssen et al. (2008) highlighted 41 genes expressed during early fruit development in both species. Only five of these genes were also found in the present study. They included 4 genes related to photosynthesis (SGN-U313179, SGN-U317999, SGN-U313194 and SGN-U312690) and one gene related to protein degradation (SGN-U313166). This may reflect actual variations in the control of early fruit development in the various fleshy fruit species, e.g. variations linked to fruit ontogeny (tomato and grape are berries while the fleshy tissues from apple arise from flower receptacle). However, possible
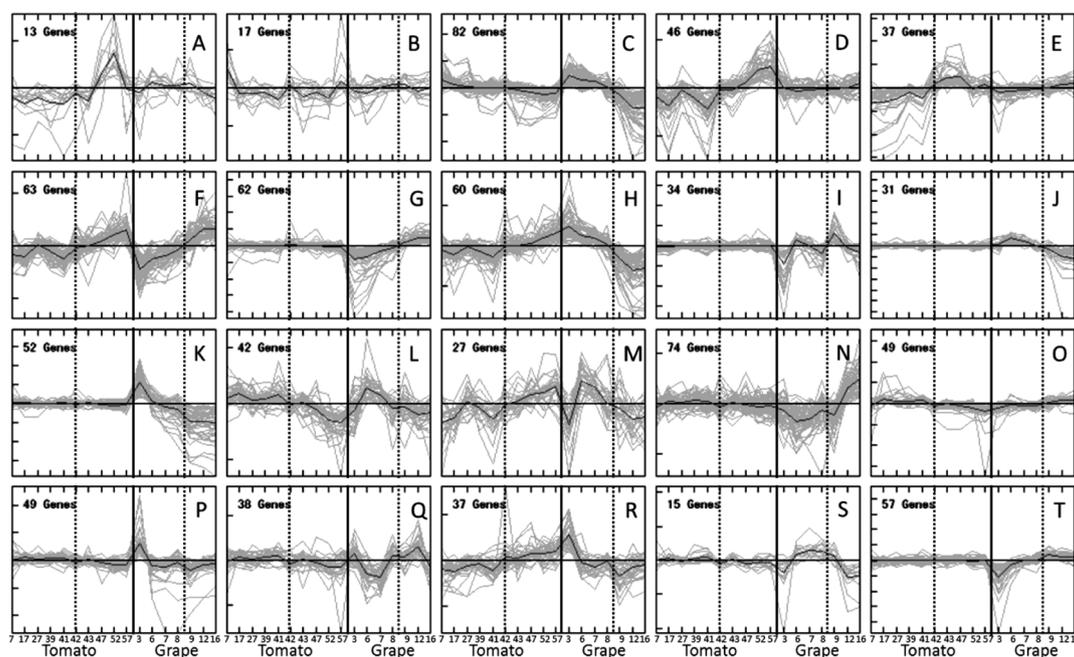
Figure 1. *k*-means clustering of tomato and grape common genes during fruit development. The dot-lines on each panel represent the breaker and veraison in tomato and grape, respectively. The scales on *x*-axis indicate the days post anthesis (DPA) and the weeks after flowering (WAF) in tomato and grape, respectively. The number indicated on the upper left corner indicates the number of probes in each cluster.

biases cannot be excluded, though the tomato TOM1 microarrays, cultivar and fruit stage of development were identical in the tomato/apple and tomato/grape experiments. Nevertheless, the 182 genes identified by current comparative transcript analysis of tomato and grape represent the major biological processes previously identified in early developing fruit (Gillaspy et al. 1993; Lemaire-Chamley et al. 2005; Mounet et al. 2009). Although several transcription factors (TFs) such as a Myb (SGN-U317694) and a bHLH (SGN-U320741) were included in the 182 early-expressed genes (Supplemental Table 1), no likely candidate regulatory gene controlling these biological processes was identified. The main reason was the lack of enough expression data, which resulted from the low number of probe sets corresponding to common genes that were spotted on the tomato TOM1 array (2,757 probes) compared to the grape Genechip array (6,203 probes).

Candidate transcription factors involved in early fruit development

To obtain further information on TFs expression during fruit development, we analyzed the data from Rohrmann et al. (2011) who accurately quantified the expression of approximately 1,000 tomato TFs using a high throughput quantitative real-time PCR platform and of Sweetman et al. (2012) who reported on the global transcriptome profiling of grape berry development by RNA-seq analysis. By mining the gene expression profiles obtained from the microarray analyses and from these references, we found 23 TFs which belong to 14 TF classes (AP2-

EREBP, ARF, bHLH, bZIP, C2C2-GATA, FHA, GeBP, GRAS, HB, LIM, MYB, PBF-2-like, SBP and WRKY; Perez-Rodriguez et al. 2010), which were expressed during early stages of fruit development in both tomato and grape (Table 1). Some of them have already been functionally characterized in tomato or in *Arabidopsis*, thus validating this tomato/grape comparative data-mining approach for the identification of candidate gene for early fruit development. As an example, the auxin response factor *SlARF4* (SGN-U319581) has been shown to be involved in the control of chlorophyll accumulation in the young fruit, in pericarp cell division, in fruit firmness and cell wall composition (Guillon et al. 2008; Jones et al. 2002). Recently, the involvement of *SlARF4* in the control of sugar metabolism has also been reported (Sagar et al. 2013). Thus it is clear that *SlARF4* is one of the important regulatory genes involved in early fruit development in tomato.

Among the 23 candidate TFs identified here (Table 1), five belong to the bHLH TFs class (SGN-U319163, SGN-U319813, SGN-U319853, SGN-U327501 and SGN-U320741) were found. The homologous genes of SGN-U319163 and SGN-U320741 in *Arabidopsis* are involved in brassinosteroid and auxin signaling (Rampey et al. 2006; Yin et al. 2005). Although the detailed role of brassinosteroid in fruit development is still unclear, BIM1 homolog (SGN-U319163) might be involved in early fruit growth. Indeed, brassinosteroids are produced during early stages of fruit development in both tomato and grape (Montoya et al. 2005; Symons et al. 2006). In addition, recent studies have demonstrated that bHLH

Table 1. List of candidate transcription factors involved in early fruit development.

T F family	SGN-ID	iT AG-ID	Vv Unigene	Ref=seq ID	E-value[1]	Homologue in Arabidopsis	Gene name in Arabidopsis	Expression in tomato[2]	Expression in grape[3]
AP 2-EREBP	SGN-U313853	Solyc07g064890	Vvi.7125	XM_002275856	2E-30	AT1G50640	ATERF3	A	A
AP 2-EREBP	SGN-U319303	Solyc02g077370	Vvi.5349	XR_077949	4E-37	AT3G23240	ATERF1	A	A
ARF	SGN-U319581	Solyc11g069190	Vvi.20194	XM_002284983	4E-74	AT5G60450	ARF4	S	S
bHLH	SGN-U319163	Solyc03g114720	Vvi.505	XM_002273238	4E-38	AT5G08130	BIM1	S	A
bHLH	SGN-U319813	Solyc07g018010	Vvi.7115	XM_002263963	3E-62	AT5G57150		A, S	S
bHLH	SGN-U319853	Solyc09g083360	Vvi.15310	XM_002284195	2E-44	AT5G43650	BHLH92	S	S
bHLH	SGN-U327501	Solyc12g100140	Vvi.14484	XM_002282963	5E-71	AT1G51140	FBH3	S	S
bHLH	SGN-U320741	Solyc10g006510	Vvi.1904	XM_002282691	2E-79	AT5G54680	BHLH105, ILR3	A	A
bZIP	SGN-U318351	Solyc08g006110	Vvi.6616	XM_002283023	4E-41	AT5G24800	ATBZIP9	S	A
C2C2-GATA	SGN-U321735	Solyc05g056120	Vvi.24337	XM_002273466	1E-51	AT3G54810	BME3, GATA8	S	S
FHA	SGN-U318938	Solyc01g009080	Vvi.1391	XM_002273213	2E-45	AT2G21530		S	A
FHA	SGN-U337751	Solyc06g083350	Vvi.12002	XM_003634962	1E-65	AT3G07220		A	A
GeBP	SGN-U323492	Solyc02g068010	Vvi.2544	XM_002265084	5E-91	AT5G14280		A	A, S
GRAS	SGN-U328504	Solyc02g085340	Vvi.3301	XM_002267104	1E-114	AT5G66770		S	A, S
HB	SGN-U313977	Solyc04g074700	Vvi.14123	XM_002285707	3E-38	AT2G22430	ATHB6	S	S
HB	SGN-U315023	Solyc05g007180	Vvi.199	XM_002276853	2E-80	AT1G69780	ATHB13	S	A, S
HB	SGN-U316935	Solyc02g085630	Vvi.23029	XM_002272971	2E-53	AT4G36740	ATHB40	S	S
LIM	SGN-U320808	Solyc04g077780	Vvi.20672	XM_002285319	1E-107	AT1G10200	WLIM1	S	S
MYB	SGN-U317694	Solyc10g055410	Vvi.3225	XM_002278186	7E-97	AT4G38620	ATMYB4	A, S	A
PBF-2-like	SGN-U320703	Solyc05g007100	Vvi.2264	XM_002277242	1E-101	AT1G14410	ATWHY1	A	A
SBP	SGN-U323360	Solyc03g114850	Vvi.19942	XM_002265167	2E-67	AT1G69170		S	S
SBP	SGN-U342074	Solyc10g078700	Vvi.16055	XM_002278476	5E-62	AT2G42200	ATSP L9	S	S
WRKY	SGN-U315227	Solyc08g008280	Vvi.8889	XM_002280995	2E-39	AT4G23810	ATWRKY53	S	A, S

[1] E-value for the t blast x comparison bet ween tomato and grape unigene sequences. [2] Reference data to identify genes expressed in early fruit development in tomato. A; array data set E025, S: Supplemental data from Rohrmann et al. (2011). [3] Reference data to identify genes expressed in early fruit development in grape. A; array data set VV5, S: Supplemental data from Sweetman et al. (2012).

TFs play important roles in the control of cell elongation in *Arabidopsis* (Bai et al. 2012; Ikeda et al. 2012; Zhang et al. 2009). Furthermore, cross-species TF network analysis revealed that the other bHLH TF SGN-U319813 (Solyc07g018010) which was highly expressed in fruit tissue was correlated with photosynthetic tissue specific TFs (Rohrmann et al. 2012), suggesting that SGN-U319813 would be involved in the regulation of photosynthesis in the fruit. Taken together, these results suggest that the 5 bHLH TFs identified here are likely important candidate genes for the regulation of biological processes taking place in early developing fruit.

This study attempted to identify genes involved in the regulation of early fruit development by focusing on genes displaying similar patterns of expression in tomato and grape. Despite the lack of exhaustive gene expression data on early fruit development available in tomato and grape at the time of that study, we found using this approach one already known ARF gene with important biological role in fruit development and several other candidate genes. The role of these candidate genes in early fruit development will be confirmed by reverse genetic approaches including stable transformation, Tilling or VIGS in the miniature tomato cv. Micro-Tom.

Acknowledgements

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