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BRIEF COMMUNICATION

Protein profiling in F_1 and F_2 generations of two tomato genotypes differing in ripening time

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Abstract

Pericarp polypeptide profiles were analyzed at three ripening stages in the F_1 hybrid and the F_2 population from the cross between the accessions: LA1385 (*Lycopersicon esculentum* var. *cerasiforme*) and 804627 (*L. esculentum,* a homozygous genotype for the *nor* mutant). Six polymorphic polypeptides were observed in LA1385, while no polymorphic polypeptides among ripening stages was observed in 804627. On the other hand, some polypeptides in the F_1 hybrid were not observed in the parents whereas others were present in both parental genotypes and were unnoticeable in the hybrid genotype. From a cluster analysis on the protein profiles of the F_2 population, the differential expression of proteins allowed to distinguish mature green (MG) stage from the others two stages, while for breaker stage (BR) and red ripe stage, the genetic background was more important in forming groups. The differential expression of proteins could be associated with fruit morphology traits such as a 72 kDa polypeptide present in MG stage with fruit diameter, height and mass and a 47 kDa polypeptide found in BR with fruit shelf life.

Additional key words: fruit shelf life, *Lycopersicon esculentum* var. *cerasiforme*, *nor* mutant, SDS-PAGE, tomato fruit ripening.

Tomato fruit ripening is the result of highly synchronized biochemical and physiological changes that occur during a relatively short period. These changes allow to distinguish three different ripening stages: MG (fully expanded unripe fruit with mature seeds), BR (first visible carotenoid accumulation), and RR (near complete the carotenoid accumulation) (Giovannoni 2004). Some genes affecting the ripening process have been found and characterized in tomato [*Lycopersicon esculentum* (L.) Mill.] mutants such as *rin* (*ripening inhibitor*), *Nr* (*never ripe*), and *nor* (*non ripening*) (Tigchelaar *et al.* 1978). These tomato mutants, which might prolong fruit shelf life, have pleiotropic effects on carotenoid synthesis and other ripening processes reducing the overall fruit quality (Mutschler *et al.* 1992). In contrast, some lines of *L. esculentum* var. *cerasiforme* (Dun.) Gray and *L. pimpinellifolium* (Jusl.) Mill., as well as their hybrids to normal ripening genotypes of *L. esculentum*, presented a longer shelf life than some commercial cultivars without diminishing other quality traits (Pratta *et al.* 2003). According to Grierson and Tucker (1983), protein synthesis appears to be an essential component of the ripening process. Therefore, SDS-PAGE of proteins from individuals at different stages of ripening might be able to detect differences closely related to this biological process. Although many authors have characterized inbred lines of tomato at different ripening stages by SDS-PAGE technique (Pratta *et al*. 2001, Bortolotti *et al*. 2003), this methodology has not still been extended to segregating populations such as F_2 individuals.

The objective of this research was to analyze changes in the polypeptides profiles at MG, BR and RR stages of the pericarp tomato tissue in two genotypes that differ for their ripening time, and then in the F_1 and F_2 generation

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Abbreviations: BR - breaker stage; MG - mature green stage; Mr - molecular mass range; RR - red ripe stage; SDS-PAGE - sodium dodecyl sulfate polyacrylamide gel electrophoresis.
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obtained between them. In addition, the purpose was to determinate how some differentially expressed polypeptides during ripening process are associated with trait related to fruit development and quality.

The accession LA1385 of *Lycopersicon esculentum* var. *cerasiforme* (L.) Mill. was crossed as the pistillate parent with the accession 804627 of *L. esculentum*, a *nor* homozygous mutant (Germplasm Bank of EEA INTA La Consulta, Argentina). Both parents, the F_1 hybrid and the F_2 population were grown at the field station "José F. Villarino" (Facultad de Ciencias Agrarias UNR, Zavalla, Argentina, 33º SL and 61º WL) in a completely randomized design. During anthesis, flowers from the parent, the F_1 hybrid and 67 F_2 plants were tagged. Fruits from the tagged flowers were collected at three different developmental stages (MG, BR and RR). Fruit tissue was conditioned for protein extraction by removing the locular tissue and the seeds were discarded while the pericarp was cut into small pieces, quick-frozen in liquid nitrogen and stored at -80 °C.

Protein extractions were carried out following Hurkman and Tanaka (1986). Electrophoresis was run in a *Mini-Protean II* (*Bio-Rad*, Hercules, CA, USA) apparatus at a 35 mA during 1.5 h. Equal amount of protein (30 μg) was loaded onto each well. Proteins ranging from 25 to 105 kDa were electrophoresed in a stacking gel (4 % polyacrylamide) followed by a separating gel (10 % polyacrylamide). Running calibration proteins (ranging from 30 to 97 kDa) were used as molecular mass marker. Proteins were then stained with 0.1 % Coomasie Brilliant Blue R-250 solution.

Time from transplantation to flowering (F), from flowering to MG stage (EM1), from MG to BR stage (EM2) and from BR to RR (EM3) traits were evaluated in all genotypes. Another set of traits were evaluated in 10 fruits per plant: diameter (D), height (H), fresh mass (FM) and shelf life (S). To evaluate shelf life, fruits were harvested, stored at 25 °C and examined three times each week, and those commercially unacceptable (decay or excessive softening) were discarded (Schuelter *et al*. 2002).

Polyacrylamide gels were analyzed by quantifying the total number of polypeptides and by calculating the percentage of polymorphic polypeptides at each stage for the parental genotypes, F_1 and F_2 plants. Polypeptide profiles from F_2 population could be obtained in 35 plants from MG stage, 37 plants from BR stage and 33 plants from RR stage. Cluster analysis using the Ward's method with Jaccard distances was performed using polymorphic polypeptides from fruit tissue of 11 F_2 plants at three ripening stage.

Then, χ^2 test was used to verify the Mendelian segregation 3:1 of the polypeptides in the F_2 plants at each ripening stage (Snedecor 1964). For each trait, mean values and standard deviations (SD) were determined and the normality was assessed by Shapiro-Wilk test (1965). Associations between each trait and polymorphic polypeptides were estimated by one-way *ANOVA*, in which the independent variable was the presence or absence of the polypeptides while the dependent variable was the given trait (Collard *et al.* 2005). The mean values between the individuals with absence of polypeptides and individuals with presence of polypeptides were compared by Duncan test (Snedecor 1964).

Twenty-four polypeptides were detected in all the genotypes. In the LA1385 genotype, six polymorphic polypeptides were found and they represented 25 % of the total number of polypeptides. Their M_r were: 30, 43, 59, 69, 94 and 97 kDa respectively, while in the 804627 genotype no polymorphism among the different ripening stages was observed. Fruits carrying the *nor* gene retained the appearance of mature green fruit and this characteristic agrees with the absence of polymorphisms among ripening stages. In contrast, the polypeptide polymorphisms found in the exotic genotype agree to the noticeable changes that take place among the different ripening stages. Similar results were found by Piechulla *et al*. (1987) evaluating the cherry line of *Lycopersicon esculentum* (VFNT LA1221). Fruits of the F_1 generation were also polymorphic among the different ripening stages. Differences were not only observed among ripening stages in each individual genotypes as well as they were observed among genotypes in a particular ripening stage. When 804627 and LA1385 genotypes and the F_1 hybrid were analyzed, 12 polymorphic polypeptides were observed among the three ripening stages that was 50 % of the whole detected polypeptides. Previously, Pratta *et al.* (2001) reported differentially expressed polypeptides among exotic (LA1385 and LA722 of *L. pimpinellifolium*), mutant (804627), cultivated ('Caimanta') genotypes and their hybrids. On the other hand, some polypeptides detected in the parents were not observed in the F_1. This fact could be due to interactions resulting of the genic contributions of both parental. According to this, Fender and O´Connell (1990) demonstrated that the F_1 between *L. esculentum* and *L. pennellii* did not display both sets of parental profiles of heat shock proteins (HSPs). The F_1 displayed all of the HSPs unique to the *L. pennellii* parent, and many but not all of the HSPs unique to *L. esculentum* parent.

In the F_2 plants, the stage specific comparisons allowed the identification of polypeptides that were differentially expressed in all individuals. Polypeptides of 52 and 56 kDa were monomorphic at the three analyzed ripening stages. A polypeptide of 58 kDa was only present at MG stage, while a polypeptide of 67 kDa was only present at the BR stage. The BR and RR stages could be characterized by the presence of polypeptides of 45, 63 and 105 kDa, while MG and RR stages could be characterized by a polypeptide of 80 kDa. The cluster analysis of 11 F_2 individuals at the three ripening stages had a cophenetic correlation of 0.42, with distances varying from 0.08 to 0.78 (Fig. 1). In contrast, Castro *et al.* (2006) found a great homogeneity (distances varying from 0.91 to 1) in the protein composition of two *Cucurbita* species by SDS-PAGE. In this analysis, one cluster was mainly conformed by all individuals at

MG stage while the other one it was conformed only by individuals at BR and RR stages. Regarding to this, Carbone *et al*. (2005) found differential expression of genes encoding enzymes of the carotenoid biosynthesis pathway, primary metabolism, photosynthesis and cell wall metabolism at different ripening stages. Recently, Alba *et al*. (2005) have confirmed through transcriptome and selected metabolite analyses that fruit development

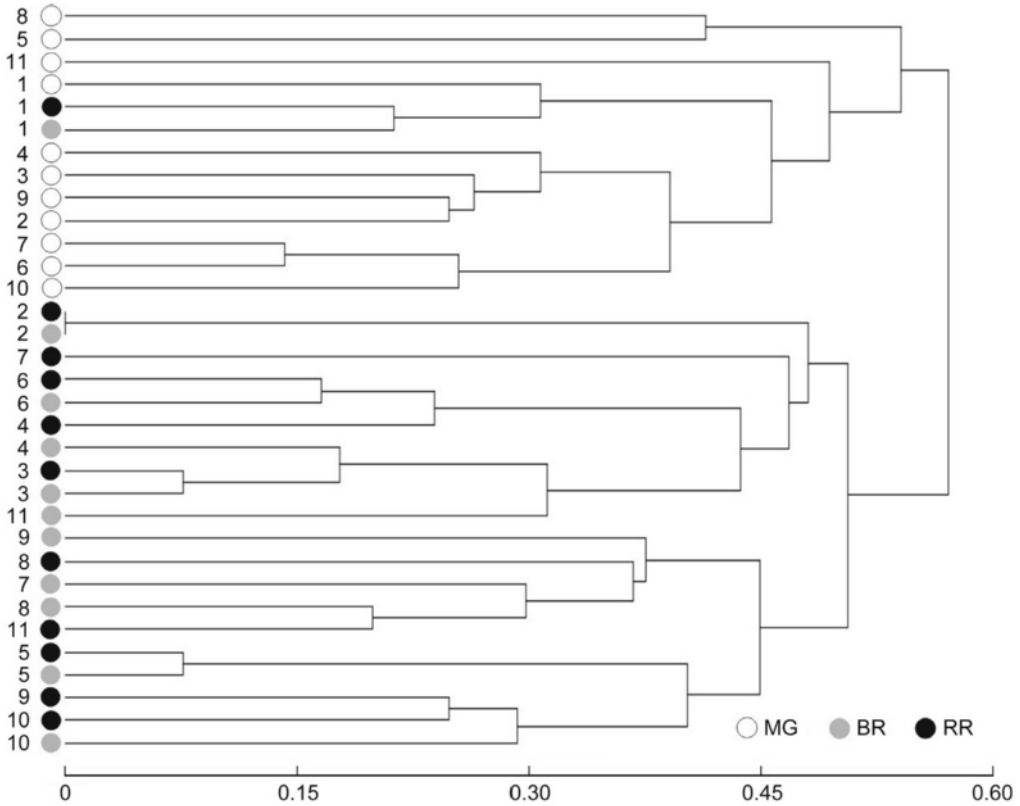

Fig. 1. Cluster analysis of 11 F_2 individuals at MG, BR and RR ripening stage.

Table 1. Quantitative traits associated to polymorphic polypeptide in the F_2 generation at three ripening stages and proportion of explained variance (R^2). F - time from transplantation to flowering; EM1 - from flowering to mature green stage; EM2 - from mature green to breaker stage; EM3 - from breaker to mature red; D - fruit diameter; H - fruit height; FM - fruit fresh mass and S - shelf life. Number of individuals and mean values of each trait for the group of F_2 individuals defined by the presence or absence of each polypeptide. Mean values followed by different letters indicate significant differences at 5 % according to Duncan´ test.

Trait	F_2 mean value	Protein Mr [kDa]	Ripening stage	R^2	P	Absent		Present	
						n	mean	*n*	mean
F	73.95 ± 21.70	65	BR	0.21	0.0026	10	64.36b	27	83.75a
		80	RR	0.12	0.0491	9	65.30b	24	80.40a
EM1	31.90 ± 4.49	45	BR	0.13	0.0215	14	28.33b	23	31.63a
		65	BR	0.11	0.0316	10	31.66a	27	28.68b
EM2	14.70 ± 5.27	39	MG	0.26	0.0039	5	17.50a	30	10.96b
		47	MG	0.14	0.0450	5	7.75b	30	12.46a
		69	MG	0.34	0.0007	11	8.25b	24	13.63a
EM3	5.70 ± 4.68	34	MG	0.20	0.0270	8	4.60b	27	7.60a
D	2.40 ± 0.35	47	MG	0.13	0.0470	5	1.84b	30	2.20a
		72	MG	0.13	0.0360	11	1.98b	24	2.24a
H	1.90 ± 0.35	39	BR	0.21	0.0034	11	2.11a	26	1.85b
		72	MG	0.24	0.0036	11	1.79b	24	2.10a
FM	5.45 ± 2.83	39	BR	0.11	0.0416	11	6.57a	26	5.17b
		72	MG	0.19	0.0110	11	4.53b	24	7.17a
S	36.20 ± 14.70	47	BR	0.10	0.0461	23	31.09b	14	38.95a

involves many loci encoding translational machinery, transcription factors, and signal transduction components. Genes associated with primary metabolism, photosynthesis, cell wall metabolism, and hormone responses are differentially expressed.

The association between polymorphic polypeptides at each ripening stage and quantitative traits is shown in the Table 1. A polypeptide of 72 kDa, at the MG stage, was associated with two fruit size-related traits. This 72 kDa polypeptide explained 19 % ($P < 0.01$) of the phenotypic variation for fruit fresh mass and 13 and 24 % for diameter and height fruit respectively. Fruit size is controlled at the beginning of the fructification process (Frary *et al*. 2000) and this was corroborated with the associations found between proteins and size-related traits in the early ripening stage. These results at protein expression level could be related to other findings at the DNA level. Alpert and Tanksley (1996) found a QTL which have explained from 5 to 30 % of the phenotypic variation for fruit mass in population from crosses between wild species and commercial cultivars. Time elapsed between different ripening stage was also explained by the expression of specific proteins at MG stage. Two polypeptides of 39 and 69 kDa respectively explained 26 and 34 % of the phenotypic variation for days elapsed between MG and BR stages. In addition, a polypeptide of 34 kDa explained 20 % of phenotypic variability for days elapsed between BR and RR stages. The expression of these polypeptides would be related to metabolic pathways involved in ethylene production, fruit softening and carotenoid synthesis since they finally establish the different ripening stages in the tomato fruit. Another important association was found between a polypeptide of 47 kDa at BR stage and shelf life trait. These results suggest that proteins being expressed at harvest time would determine long fruit shelf life. The lower value for R^2 in this association is according to previous studies where we demonstrated that for this type of cross the diameter, height and fruit mass presented high values of heritability, while shelf life showed an intermediate value (Rodríguez *et al*. 2005). According to this, Pratta *et al.* (2006) suggested that the relatively low proportion of phenotypic variance explained by a DNA molecular marker indicates that additive gene effects are not predominant in the expression of the traits.

As a conclusion, tomato pericarp protein profiling allows to distinguish between mature green and breaker or red ripe stage. One of the most important facts found in this experiment is that once the ripening process has begun the individual genetic constitution is more important in the protein differential expression than the ripening stage. This study show that protein differential expression could be associated with some fruit quantitative traits.

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Palaniswamy, U.R.: **Asian Crops and Human Dietetics.** - The Haworth Press, Taylor and Francis Group, New York - London 2008. 206 pp. ISBN: 978-1-56022-312-2.

All humans need sufficient energy and nutrients for living, but peoples, cultures, families, and individuals fulfil this basic need in diverse ways. This book comprehensively reviews plants used in the Asian diet and made significant beneficial contributions to nutrition and health. Migration of populations out of their native lands caused expansion of many useful plants all over the world. Nowadays, most of the species mentioned in this book are grown and used in different parts of the world and not only in Asia.

After general Introduction, the book is divided into seven chapters. The first chapter "Grains" is focused on two most cultivated plants wheat and rice, and further on different millets and buckwheat. The second chapter "Vegetables" deals with cucurbits, brassicas, alliums, green leafy vegetables, legumes and tubers. The main fruits mentioned in chapter three are apple, pear, peach, apricot, cherry, mango, banana, litchi, rambutan, longan, tamarind, jackfruit, mangosteen and citrus. "Fat and oils" (Chapter 4) are mostly extracted from sesame, coconut, flax and rapeseed. "Beans and nuts" (Chapter 5) are important sources of proteins. Red gram, chickpea, fava bean, lentil, adzuki bean, cowpea, mung bean, blackgram, soybean, velvet bean rice bean, almond, pistachio and walnut are mentioned in this chapter. Large collection of plant species is described in chapter 6 "Spices". Finally, a small chapter is devoted to herbal beverages (tea) and subsequents (areca nut and betel).

For each plant species, the author provides Latin and common names, family, origin, places of cultivation, importance in human diet and in medicine, use in different dishes or in food-processing industry.

This readable book contains a lot of information interesting not only for specialist of agronomy, food industry or pharmacology but mainly for nonexperts. It is a great pity that illustrations are missing.

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