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Induction of Ammonium Metabolizing Enzymes is Related with Fruit Shelf Life in Tomato Crosses among Cultivated, Mutant and Exotic Germplasms

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ABSTRACT

In a previous report, glutamine synthetase (GS) and glutamate dehydrogenase (GDH) were found to be differentially induced in fruits at two ripening stages among tomato lines with different shelf life (SL). The objective of the present research was to analyze SL and the induction of GS and GDH in the pericarp tissue at the mature-green and the red ripe stages in different tomato genotypes, including a set of hybrids and their parents. The *L. esculentum* parents were the standard ripening cultivar 'Caimanta', which produces round fresh-marketable fruits, and two inbred lines, N and R, which are recessive for the *nor* and *rin* mutations, respectively. The exotic parent 'Ceras' belonged to the wild *L. esculentum* var. *cerasiforme*. Four crosses were assayed: F_1 ('Caimanta' x N), F_1 ('Caimanta' x 'Ceras'), F_1 (N x 'Ceras'), and F_1 (N x R). The concomitant presence of GS and GDH was found in the pericarp of mature-green and red ripe tomato fruits from N, R, 'Ceras', F_1 ('Caimanta' x 'Ceras'), F_1 (N x 'Ceras'), and F_1 (N x R). All these genotypes had a long SL. Particularly, in red ripe fruits of N and R, the genotypes with the longest SL, two isoforms of GDH were detected. The F_1 ('Caimanta' x N) had a quite shorter SL and presented a differential induction pattern: while GS was found just in mature-green fruits, GDH was present at both ripening stages. In 'Caimanta', the genotype with shortest SL, GS was present only at the mature-green stage and GDH at the red ripe stage.

Keywords: *Lycopersicon esculentum*, glutamate dehydrogenase, glutamine synthetase, ripening
Abbreviations: d/a, degree of dominance; **GDH**, glutamate dehydrogenase; **GS**, glutamine synthetase; **SL**, shelf life

INTRODUCTION

The objective of this work was to analyze the induction of glutamine synthetase (GS) and glutamate dehydrogenase (GDH) in the pericarp tissue of fruits at the mature-green and the red ripe stages in a set of tomato genotypes with different shelf life (SL). Our interest was focused in assessing a putative association between both ripening-associated traits (the induction pattern of GS and GDH and the tomato fruit SL) in genetic materials involving cultivated, exotic and mutant germplasms.

In fact, in a climacteric fruit such as the tomato (*Lycopersicon esculentum* Mill.), GS and GDH were inversely induced in the pericarp during ripening of mature fruits (Boggio *et al*. 2000). These changes in the enzymatic pattern were associated with an increase in the relative content of glutamate. On the other hand, in three tomato varieties homozygous for the ripening mutations, *green flesh*, *rin* and *nor*, the GS and GDH polypeptides coexisted under the whole ripening process (Bortolotti *et al*. 2003; Pratta *et al*. 2004). Both *rin* (located on chromosome V) and *nor* (located on chromosome X) homozygotes delay ripening and have pleiotropic effects on ethylene production, color, texture, acidity and SL. These mutations were introgressed into commercial varieties to prolong SL, but a reduction in fruit quality occurred even in the heterozygotes (Mutschler *et al*. 1992).

Long SL tomato genotypes were obtained by Zorzoli *et al*. (1998) and Pratta *et al*. (2000), by means of crossing commercial varieties of *L. esculentum* to the wild relatives *L. esculentum* var. *cerasiforme* and *L. pimpinellifolium*. These fruits had an appropriate marketable quality, with SL values similar to those of the *nor* and *rin* heterozygotes. Recently, it was reported that GS and GDH were detected in the fruit pericarp at the mature-green as well as red ripe stages in two exotic germplasms of *L. esculentum* var. *cerasiforme* accessions (Pratta *et al*. 2004). These induction patterns of GS and GDH in the exotic germplasms resembled those in the mutant varieties.

MATERIALS AND METHODS

Four tomato crosses involving cultivated, exotic and mutant tomato germplasms were assayed: F_1 ('Caimanta' x N), F_1 (N x R), F_1 ('Caimanta' x 'Ceras') and F_1 (N x 'Ceras'). The *L. esculentum* parents were the standard ripening 'Caimanta', which produces round fresh-marketable fruits, and two inbred lines, N and R, which are recessive for the *nor* and *rin* mutations respectively. The exotic parent 'Ceras' belonged to the wild *L. esculentum* var. *cerasiforme*, from which two accessions (LA1385 and LA1673) were randomly sampled. Plants were grown at an experimental field station located at latitude 33°S and longitude 61°W from October to March under greenhouse conditions. Previous to the transplantation, the soil (a typical argiudol) was fertilized with poultry grit. The plants (15 per genotype) were watered twice a week. This level of irrigation was sufficient to avoid water stress during the growing period.

Tomato SL was evaluated as the number of days elapsed from harvest to the beginning of the fruit softening, which was handled assessed (Zorzoli *et al*. 2000). Fruits (10 per plant) were harvested when the first symptoms of color turn were observed, and stored in a random design in an acclimatized room at 25 ± 3ºC. The mean

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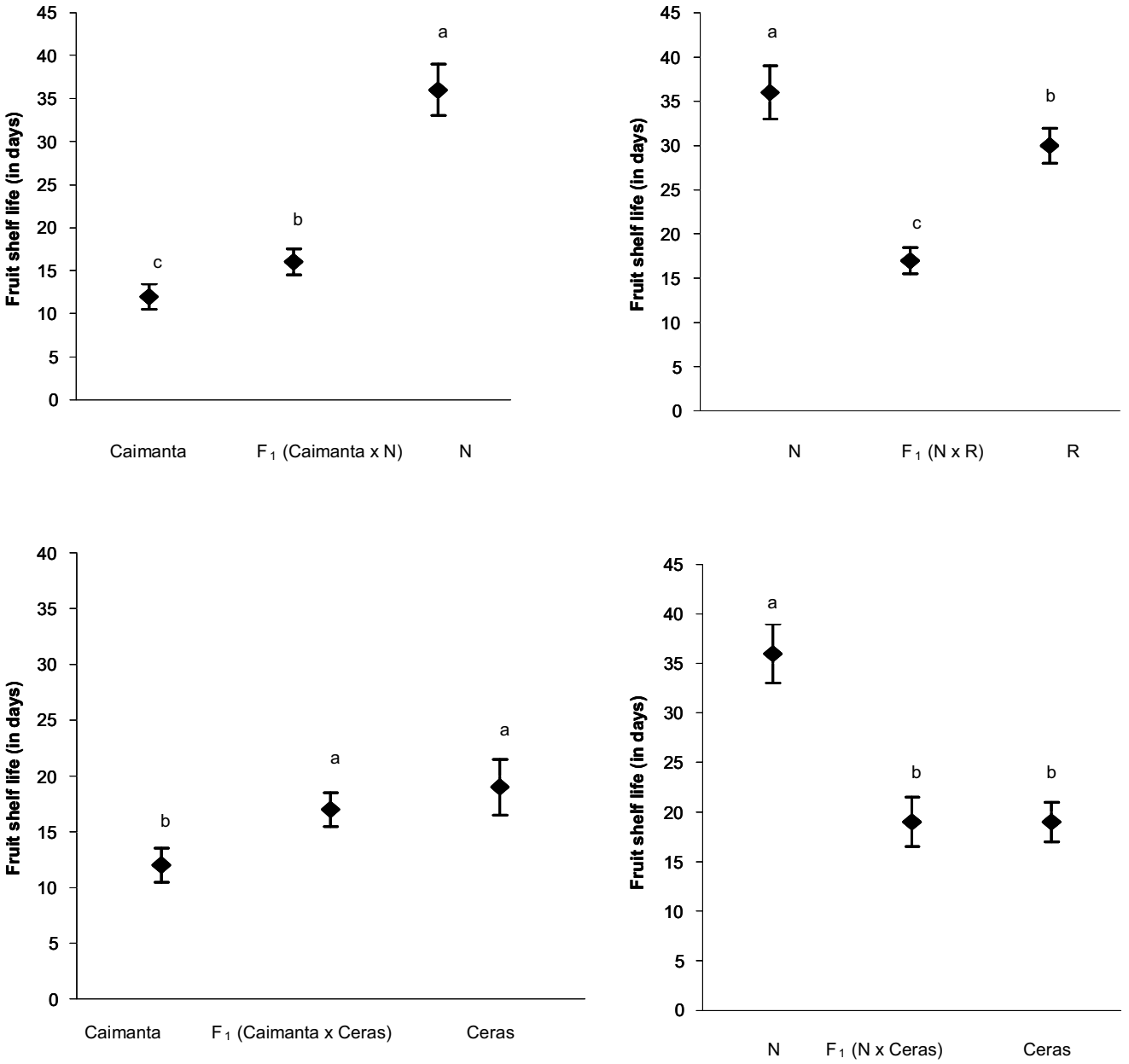

Fig. 1 Mean fruit shelf life of different tomato genotypes grouped by a given hybrid and its parents (the pistillate parent is placed at the left). Different letters indicate significant differences ($p < 0.05$) among genotypes within each group according to the Duncan test. Parental genotypes: Caimanta: standard ripening cultivar of *L. esculentum*, N: homozygote for the mutant *nor* (*L. esculentum*), R: homozygote for the mutant *rin* (*L. esculentum*), Ceras: sampled accession of *L. esculentum* var. *cerasiforme*.

SL values were compared by the Duncan test (Kearsey and Pooni 1996) within each group formed by a given tomato hybrid and its parents. The degree of dominance (d/a) was calculated for each group according to Mather and Jinks (1977).

Also, samples of tomato fruits (four per genotype) were harvested at the mature-green stage (when fruit stops growing but is still green) and red ripe stage (fruit is completely red but still firm). Pericarp tissue of harvested fruits was obtained by removing the locule juice and seeds, and then stored at -80°C until analysis (Valle *et al*. 1998). Proteins from pericarp of frozen fruits (4-10 g fresh weight) were extracted and precipitated with thrichloroacetic acid (final concentration 20%) according to Boggio *et al*. (2000). Protein extracts (20 µg) were fractionated by 10% (w/v) SDS-PAGE and electroblotted to nitrocellulose membranes overnight at 4°C. Immunodetection was carried out with antisera raised in rabbits against *Zea mays* GS2 and *Vitis vinifera* NADH-GDH as previously described (Boggio *et al*. 2000).

Given that the F_1 (N x R) carries two well known monogenic mutants at the heterozygous state, it was considered as the reference genotype. In this context, the remaining genotypes were analyzed as a 'modified-diallel cross', in the sense that the effect of the putative allelic substitution was assessed from their performance for the fruit SL and the induction patterns of both enzymes.

RESULTS AND DISCUSSION

Tomato SL in each group formed by the hybrids and their parents are shown in **Fig. 1**. Considering all the genotypes, the *nor* and *rin* homozygotes had the greatest values (36 ± 6 and 30 ± 4 days, respectively), followed by 'Ceras', F_1 (N x 'Ceras'), F_1 ('Caimanta' x 'Ceras'), F_1 (N x R), and F_1 ('Caimanta' x N) (19 ± 5, 19 ± 4, 17 ± 3, 17 ± 2, and 16 ± 3, respectively). The latter genotype had the lowest value among hybrids, though, as expected, 'Caimanta' had the minimum value (12 ± 3 days) among the set of genotypes under study. The hybrid among both mutant parents had the same SL that the two crosses involving the exotic germplasm, which were not statistically different to their wild parent. When analyzing the groups of each hybrid and its parents, dominant effects of the wild genome ('Ceras') over the cultivated ones ('Caimanta' and N) were detected (d/a = 1 in both cases), and partial dominance (d/a = -0.67) was found for the F_1 ('Caimanta' x N) (**Fig. 1**). Thus, an allelic substitution effect could be noted just in the cross between 'Caimanta' and N, while in the crosses of these genotypes to 'Ceras', the effect of the exotic alleles was prevalent. The reference genotype, F_1 (N x R), showed overdominance (d/a

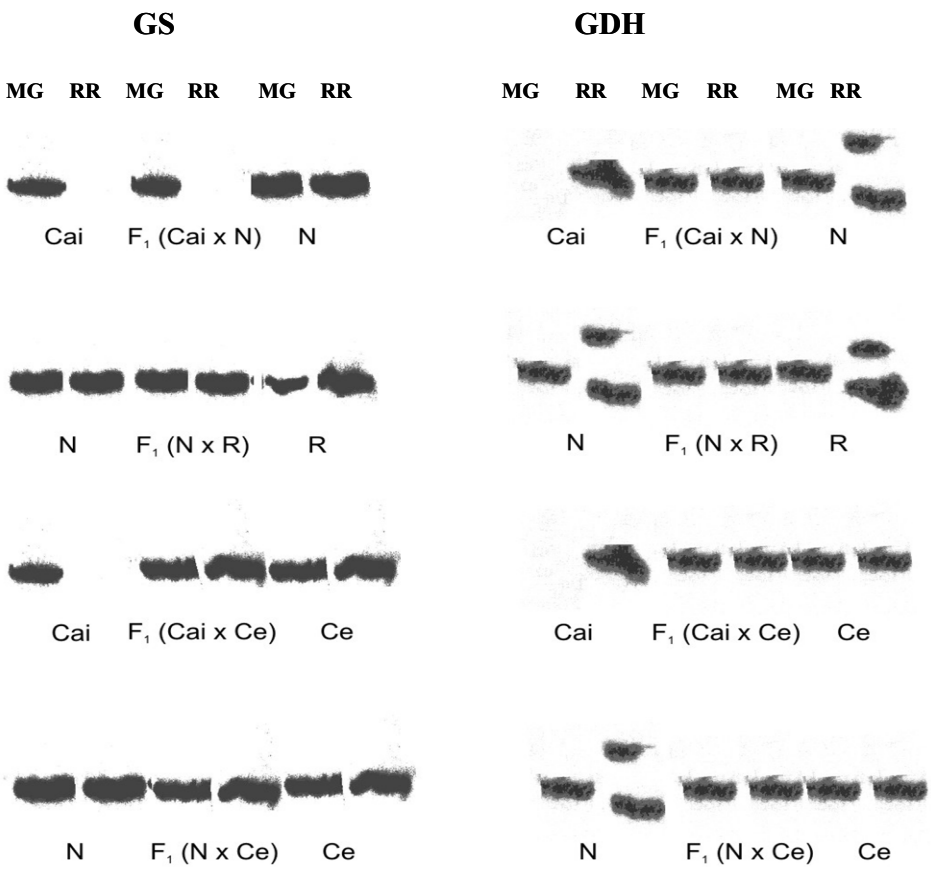

Fig. 2 Changes in polypeptide pattern of glutamine synthetase (GS) and glutamate dehydrogenase (GDH) in fruits at the mature-green (MG) and red ripe (RR) stages in different groups of tomato genotypes formed by a given hybrid and its parents (the pistillate parent is placed at the left). Parental genotypes: Caimanta: standard ripening cultivar of *L. esculentum*, N: homozygote for the mutant *nor* (*L. esculentum*), R: homozygote for the mutant *rin* (*L. esculentum*), Ceras: sampled accession of *L. esculentum* var. *cerasiforme*.

= -5.33), which was expected considering that both mutations are recessive. The allelic substitution effect of both mutants determines a phenotype more similar to the standard genotype. These results indicated that, though the final phenotypes of the four hybrids yield a longer SL than the standard ripening genotype, allelic effects in each cross are different, and provide enough evidence about the different gene action of the 'long SL' genes carried by the exotic *L. esculentum* genotypes in comparison to the mutants.

Results from Western analysis are shown in **Fig. 2**. Parents' performance was the same as described by Pratta *et al*. (2004). In 'Caimanta', GS was found at the mature-green stage while GDH was observed at the red ripe stage. In the mutants N and R both enzymes were detected in pericarp extracts of fruits at either the mature-green or the red ripe stages, while accession of the exotic germplasm *L. esculentum* var. *cerasiforme* also presented GS and GDH in the pericarp of fruits at both mature stages. An opposite induction of GS and GDH was reported by Boggio *et al*. (2000) and Scarpeci *et al*. (2007) in the pericarp of var. Platense and 'Micro-tom', other standard ripening tomato cultivars, respectively. In addition, 'Micro-tom' fruits modified the GS/GDH induction pattern when plants were irrigated by high nutrition (Scarpeci *et al*. 2007). Altogether these results suggest that standard cultivated varieties such as Caimanta, Platense and 'Micro-tom' exhibit similar induction pattern of GS (in green) and GDH (in red) in mature fruits. The different GS and GDH induction patterns showed by fruits of exotic as well as mutant plants, which all grew in the same nutritional conditions, suggest that genetic factors are involved in the expression of both enzymes in these fruits. While GS acts as an ammonium-fixing enzyme using glutamate as substrate, GDH can participate in the amination or deamination of glutamate. From both enzymes, GDH could particularly contribute to regulate the internal glutamate concentration in fruits through a GDH-shunt, satisfying the cell needs for carbon or nitrogen compounds (Miflin and Habash 2002). Glutamate is known to increase in red ripe fruits and it is also involved in signaling pathways (Valle 2004).

Among hybrid genotypes, GS was found only at the mature-green stage while GDH was observed at the mature-green and red ripe stages in fruits of the F_1 ('Caimanta' x N). In fruits of the other three hybrids, GS and GDH were detected at both ripening stages (**Fig. 2**). Though in N and R both enzymes were detected in pericarp extracts of fruits at either the mature-green or the red ripe stages, in those red ripe fruits the GDH pattern showed two bands of different electrophoretic mobilities, which were not present in any other genotype (**Fig. 2**). Two GDH isoforms were also observed in maize leaves (Becker *et al*. 2000) and in leaves and fruits of the tomato mutant *gf* (Bortolotti *et al*. 2003). But the three hybrids involving crosses with mutants, F_1 ('Caimanta' x N), F_1 (N x Ceras) and F_1 (N x R), only presented one band in the present experiments (**Fig. 2**). This result indicates that *nor* and *rin* loci at the homozygous state are required for the expression of different GDH isoforms. The *rin* locus was reported as a MADS-Box gene necessary for fruit ripening that acts upstream of the ethylene-mediated ripening control. So, these non-climacteric fruits could present changes in many ripening associated traits, such as the GS and GDH induction patterns, when compared to the wild-type fruits.

For the induction pattern of GS and GDH in each group of genotypes, an allelic substitution effect could be noted again just in the cross among 'Caimanta' and N, as this hybrid had a unique performance, rather different from the others. In the crosses of these genotypes to 'Ceras', the exotic alleles were dominant, the patterns of hybrids being the same as that of the wild parent. The reference genotype, F_1 (N x R), showed an induction pattern different from both parents. Under the hypothesis that *nor* and *rin* have a pleiotropic effect on the induction pattern of GS and GDH, and considering that both mutations are recessive, it was expected that expression of these ammonium metabolizing enzymes is different among the hybrid (in which the dominant alleles would be regulating the expression) and the parents (in which the respective mutant alleles would do it). In this case, the allelic substitution of both mutants determines a phenotype more similar to the exotic genotype.

Genotypic variations for GS and GDH induction patterns in the pericarp tissue could be correlated with the tomato fruit SL. Genotypes with long SL values, N, R, 'Ceras', F_1 (N x 'Ceras'), F_1 ('Caimanta' x 'Ceras') and F_1 (N x R), had a concomitant presence of both ammonium metabolizing enzymes in the mature-green and the red ripe stages. Particularly, in red ripe fruits of N and R, the genotypes with the longest SL, two isoforms of GDH were detected (**Fig. 2**). This unique pattern was not observed in any of the other genotypes. The F_1 ('Caimanta' x N) had a distinctive induction pattern of the enzymes, because GS was present just in green-mature fruits and GDH was detected at both ripening stages. 'Caimanta', the genotype with the standard fruit SL, had also the standard induction patterns of GS and GDH previously reported for tomato (Valle *et al*. 1998; Boggio *et al*. 2000). Moreover, the effects of allelic substitution for fruit SL also correlated with the effects of allelic substitution for the induction pattern of GS and GDH.

Marmiroli *et al*. (1989) found that few hot-stress related proteins had a uniparental heredity, whereas others showed an F_1-specific pattern of expression as a probable consequence of new regulatory interactions which took place in the hybrid between the respective genes of two barley parents. Thus the protein profile of that F_1 was more similar to either of its parents, as also found for the induction of GS and GDH in our experiment. Also, de Vienne *et al*. (1994) observed that the relative abundance of proteins in coleoptiles of maize F_1 hybrids deviated from the high parental values. Padmavathi *et al*. (2001) were able to associate the presence/absence pattern of three polypeptides with resistance to green leafhopper in rice. In this report, the presence/absence pattern of both GS and GDH at the mature-green and red ripe stages could be associated to differences in the fruit SL – a trait that measures the time to ripening off the vine – among the cultivated, exotic and mutant germplasms. So this differential induction pattern could be proposed as a potential biochemical genetic marker of tomato ripening.

The experiment reported here pointed out that for two ripening stages, the molecular polymorphism among genotypes could be related to biological events involved in fruit ripening, such as the fruit SL. Furthermore, this physiological and genetic difference confirms that wild tomato germplasm could be incorporated to a breeding program to modify fruit SL as an alternative to avoid the effect of the spontaneous ripening mutants.

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