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Effect of the Fruit-ripening Mutant Genes *rin* and *nor* on the Flavor of Tomato Fruit[1, 2]

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Abstract. **Organoleptic tests of the non-ripening tomato mutants *rin* and *nor* and their F_1 hybrids with the normal-fruit-bearing cultivar 'Rutgers' indicated that fruits of the *rin* heterozygous plants (*rin*/+) were slightly inferior and that those of inferior in flavor to fruits of the normal genotype (+/+), all sampled 3–5 days after ethylene and CO_2 evolution rates attained maximum levels. The flavor of fruits of the double heterozygote *nor* heterozygotes (*nor*/+) were distinctly *rin*/+,*nor*/+ was poorer than either of the 2 single-gene heterozygotes, while fruits of both homozygous plants, *nor*/*nor* and *rin*/*rin*, were unpalatable. Analyses of pH, titratable acidity, total soluble solids, and reducing sugars did not indicate that any of these parameters is responsible for the inferior flavor of the genotypes containing the non-ripening genes. Comparisons of reciprocal crosses provided no evidence of cytoplasmic inheritance of fruit flavor.**

The ripening mutant genes *nor* (non-ripening) and *rin* (ripening inhibitor) in tomato inhibit, or greatly slow down, a wide range of processes related to ripening of the tomato fruit (3, 8, 13, 22), leading to a markedly extended shelf life (8) and inferior flavor (7). These mutant genes have been described as being recessive (13, 22); however, in plants heterozygous for *rin* and *nor*, several ripening characteristics (e.g., CO_2 and C_2H_4 evolution rates, pectinases activity, softening of the fruit, and carotenogenesis) exhibit levels intermediate between those of the normal and the mutant parents, as reviewed by Tigchelaar et al. (20). Hence, incomplete dominance seems to be a better description of the relationship of these genes with their normal alleles.

Mizrahi et al. (11) have suggested that *rin* and *nor* regulate the whole process of ripening. Since flavor change accompanies ripening, it might also be affected by the mutant genes in the heterozygous condition. The present work was aimed at extending our knowledge regarding the effect of these genes in heterozygous combinations with the normal alleles on tomato fruit flavor.

Materials and Methods

Plant material. The plant material consisted of 'Rutgers', a normal (+/+) cultivar of tomato; the 2 ripening mutants, i.e., *rin* (5th backcross to Rutgers)[6] and *nor* (3rd backcross to Rutgers);[6] and the 6 reciprocal F_1 hybrids among them. Another breeding line of *nor*, *nor*-608, and the normal 'Ailsa Craig' were also used in this study. The plants were grown in soil in heated glasshouses. They were trained to a single stem, and only 2 fruits per cluster were allowed to develop (10). Fruits were tagged at the breaker stage and picked for flavor evaluation on the 5th–6th, 7th–8th, 10th–11th, 14th–15th, and 15th–20th day after the breaker stage for 'Rutgers' and 'Ailsa Craig', the F_1 combinations 'Rutgers' × *rin*, 'Rutgers' × *nor*, *rin* × *nor*, and the 2 homozygous mutants, respectively. These times were chosen so as to test the fruits in each plant type at their "optimal" stage (see discussion).

Flavor. Organoleptic tests were carried out in a darkened room lit with red light. In this way, the bias resulting from fruit color was eliminated. Judges rinsed their mouths with cold water between samplings to clear the palate. Panels of 17–25 judges classified the overall flavor of the samples, using the "score test" (7) according to 3 grades: 1 = bad; 2 = medium, and 3 = good. Sweetness and sourness were also evaluated by organoleptic tests and classified according to 3 grades: 1 = low, 2 = medium, and 3 = high. In each case, the panel tasted thin fruit slices consisting of pericarp and jelly. Each test consisted of only 3–4 fruit types. Comparisons are possible within tests, but not between them. We also conducted a triangle test (14, 19) in which the tasters were requested to differentiate between 2 identical fruit slices and one from the reciprocal cross. Statistical analysis was carried out by the chi-square test performed on the distribution of judges (9).

pH, titratable acidity, total soluble solids and reducing sugars. These parameters were determined in fruits on the 7th and the 14th day after the breaker stage. At each developmental stage, 5 fruits of each genotype were combined and analyzed individually as follows: 10 g tissue consisting of pericarp and jelly were homogenized with 5 ml of double-distilled water. The homogenate was filtered through 4 layers of Miracloth. Five ml of the supernatant were titrated with 0.1 M NaOH to pH 7.0. The concentration of total soluble solids (TSS) was determined with a Karl Zeiss refractometer. Reducing sugars were determined by Sumner's technique (7).

Results

The flavor of fruits of 'Rutgers', the 2 ripening mutants *nor* and *rin*, and the 6 reciprocal F_1 hybrids among them was evaluated by a panel of 17–25 people (Table 1). The flavor of the

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[6]Seeds of the 5th backcross of *rin* and seeds of the 2nd backcross of *nor* were kindly supplied by E. C. Tigchelaar, Purdue University, Agricultural Experimental Station, Lafayette, Ind., USA. The 3rd backcross of *nor* was performed in our laboratory.

Table 1. Effect of *rin* and *nor* in homozygous and heterozygous combinations on tomato-fruit flavor as evaluated by a panel of tasters.

Test number	Plant type or cross (♀ × ♂)	Genotype	Days after breaker	No. of judges grading fruits as: good = 3	medium = 2	bad = 1	Average flavor score	Statistical significance[z]
I	Rutgers	+/+	5–6	7	10	0	2.41	a
	nor	*nor/nor*	15–20	0	5	12	1.29	c
	nor x Rutgers	*nor*/+	10–11	5	5	7	1.88	b
II	Rutgers	+/+	5–6	10	7	0	2.59	a
	rin	*rin/rin*	15–20	0	4	13	1.24	b
	rin x Rutgers	*rin*/+	7–8	6	8	3	2.18	a
III	Rutgers	+/+	5–6	16	4	2	2.64	a
	Rutgers x *rin*	*rin*/+	7–8	6	10	6	2.00	b
	Rutgers x *nor*	*nor*/+	10–11	4	10	8	1.82	b
IV	Rutgers	+/+	5–6	16	6	0	2.73	a
	rin x Rutgers	*rin*/+	7–8	13	8	1	2.55	ab
	nor x Rutgers	*nor*/+	10–11	10	11	1	2.41	bc
	nor x *rin*	*nor*/+,*rin*/+	14–15	4	14	4	2.00	c
V	Rutgers	+/+	5–6	6	13	0	2.32	a
	nor	*nor/nor*	15–20	0	6	13	1.32	b
	nor x Rutgers	*nor*/+	10–11	5	7	7	1.89	b
	nor x *rin*	*nor*/+,*rin*/+	14–15	1	13	5	1.79	c
VI	*rin* x Rutgers	*rin*/+	5–6	10	10	2	2.36	a
	nor x Rutgers	*nor*/+	10–11	4	15	3	2.05	a
	rin x *nor*	*rin*/+,*nor*/+	14–15	5	5	12	1.68	b

[z]Chi-square test was performed on the distribution of judges. Flavor of plant types marked by similar letters is not significantly different at P = 5%.

2 mutants, as evaluated by organoleptic tests, was markedly inferior to that of 'Rutgers'. F_1 hybrids between each mutant and 'Rutgers' were of better flavor than the homozygotes but still inferior to 'Rutgers', i.e., in all tests, *rin* in one dose decreased fruit flavor slightly (statistically significant in one test), while one dose of *nor* decreased the flavor markedly (statistically significant in all tests). In all relevant tests, the panel preferred fruits heterozygous for *rin* to those heterozygous for *nor*, and the double heterozygote received the lowest score (Table 1).

The judges who participated in the triangle tests were not able to differentiate between the flavor of fruits of the F_1 hybrids of the reciprocal crosses, at a significance level of 5% (Table 2). Thus, no cytoplasmic inheritance was found to be involved in the control of fruit flavor.

Chemical analysis was carried out on the 7th and the 14th day after the breaker stage (Table 3). The experiment was repeated 4 times with 5 fruits each.

All 4 experiments yielded similar results. Since the reciprocal crosses yielded similar results, only crosses of one direction were presented in the tables. The results indicate that at both stages of development the levels of all parameters tested (pH, TSS titratable acids, and reducing sugars) were within the range of normal cultivars (*see* Discussion).

The results in Table 3 suggest an apparent discrepancy between the levels of chemical constituents in the mutants, which are in the normal range, and the flavor inferiority (Table 1). Therefore, we compared *nor* fruits with fruits at 2 ripening stages of a normal cultivar with respect to organoleptic evaluations, distinguishing between sweetness, sourness, and overall flavor, in parallel to analyses of chemical constituents (Table 4). In this way, we may find out what is the relation among the scores of sweetness, sourness, and overall flavor, and how these scores relate to the chemical parameters. The sweetness score of *nor* fruit was intermediate between the scores of the pink and red fruits of the normal cultivar, whereas its sourness score was higher than those of both pink and red normal fruits. The flavor of *nor* fruits was much inferior to that of the normal fruits, at both ages, without an apparent relation to the scores of either sweetness or sourness. It was clear that in normal fruits higher values of TSS and reducing sugars were accompanied with higher sweetness score, while high concentration of acids and low pH were accompanied with sourness. In *nor*, the high values of TSS and reducing sugars as related to those of the red normal fruit do not agree with the difference in the sweetness score between the 2 plant types. Like in the normal fruits, the sourness score was related to high titratable acidity.

Discussion

The F_1 hybrids of *rin* and *nor* x 'Rutgers' ripen at different rates (12, 21). Therefore, the chronological age of fruits (or the number of days after incipience of ripening) cannot be used as a basis for comparison of fruits of different genotypes. In preliminary tests (data not shown), flavor of fruits of various genotypes was evaluated at various time intervals after the onset of ripening. In each genotype, fruits reached their best flavor at a specific time, corresponding to 3–5 days after ethylene and CO_2 evolution rates attained their maximal levels (unpublished data). On the basis of these preliminary tests, we were able to evaluate fruit flavor of each genotype at or near its highest value.

Table 2. Comparison of reciprocal crosses by organoleptic triangle tests of *rin* and *nor* tomato F_1 hybrids.[z]

Genotypes	Plant types compared	No. of correct answers/ No. of judges[y] Test I	Test II
rin/+	Rutgers x *rin* ≠ *rin* x Rutgers	7/25	6/20
nor/+	Rutgers x *nor* ≠ Rutgers x *nor*	4/19	7/20
rin/+,*nor*/+	*rin* x *nor* ≠ *nor* x *rin*	9/19	4/20

[z]The tests were run separately for fruits of each genotype.
[y]All data of the triangle tests were not significant at P = 5%.

Table 3. Effect of *rin* and *nor* in homozygous and heterozygous combinations on pH, total soluble solids (TSS), titratable acidity, and reducing sugars in tomato fruits.[z]

Plant type or cross (♀ x ♂)	Genotype	Harvested 7 days after breaker stage				Harvested 14 days after breaker stage			
		pH	TSS (%)	Titratable acids (meq/g fresh wt)	Reducing sugars (mg/g fresh wt)[y]	pH	TSS (%)	Titratable acids (meq/g fresh wt)	Reducing sugars (mg/g fresh wt)
Rutgers	+/+	4.29 ± 0.04	4.03 ± 0.06	0.050 ± 0.004	31.69 ± 2.65	4.40 ± 0.00	4.15 ± 0.12	0.038 ± 0.002	32.78 ± 2.24
rin	*rin/rin*	4.30 ± 0.06	4.10 ± 0.46	0.057 ± 0.004	37.68 ± 5.23	4.43 ± 0.03	4.80 ± 0.72	0.053 ± 0.003	41.27 ± 5.55
nor	*nor/nor*	4.13 ± 0.03	3.52 ± 0.12	0.070 ± 0.010	31.96 ± 3.93	4.08 ± 0.04	4.03 ± 0.40	0.050 ± 0.002	32.51 ± 1.46
rin x Rutgers	*rin*/+	4.16 ± 0.07	4.40 ± 0.27	0.061 ± 0.005	36.75 ± 1.24	4.25 ± 0.08	4.30 ± 0.27	0.065 ± 0.003	40.01 ± 2.43
nor x Rutgers	*nor*/+	4.03 ± 0.03	3.87 ± 0.47	0.076 ± 0.010	27.93 ± 5.19	4.18 ± 0.02	4.87 ± 0.58	0.068 ± 0.010	32.17 ± 2.07
rin x *nor*	*rin*/+ ,*nor*/+	4.31 ± 0.06	4.10 ± 0.53	0.062 ± 0.050	21.48 ± 2.15	4.35 ± 0.03	4.05 ± 0.43	0.059 ± 0.002	23.50 ± 2.17

[z]The table represents 1 experiment out of 4 similar experiments. The numbers are means ± SE of 5 replicates.
[y]Calculated from a glucose standard.

Table 4. Organoleptic tests of taste components and chemical constituents in *nor* and the normal cultivar 'Ailsa Craig' at 2 stages of ripening.[z]

Plant type	Genotypes	Days after breaker	Organoleptic score: Mean sweetness	Mean sourness	Mean overall flavor	Chemical constituents: T.S.S. (%)	Reducing sugars, (mg/g fresh wt)[y]	Titratable acidity (meq/g fresh wt)	pH
Ailsa Craig	+/+	3–4 (pink)	1.60	1.72	2.30	4.55	31.52	0.069	3.95
Ailsa Craig	+/+	6–7 (red)	2.35	1.46	2.40	4.75	34.04	0.065	4.10
nor (608)	*nor/nor*	15–20	1.90	1.96	1.61	7.60	64.21	0.075	4.01

[z]In each organoleptic taste 25 tasters participated. The values of the chemical constituents are means of 5 fruits analyzed individually.
[y]Calculated from a glucose standard.

Our results indicate that a single dose of *rin* slightly reduced fruit flavor, while the effect of a single dose of *nor* was more deleterious. It could be argued that the degree of isogenicity may affect the results. However, similar results (i.e., a slight reduction of flavor by a single dose of *rin*) were obtained with *rin* at a more advanced backcross (6th backcross, which is over 95% isogenic). Moreover, fruit flavor in *nor* hybrids with 5 normal cultivars other than Rutgers was inferior to the flavor of the corresponding normal cultivars as well as to that of the corresponding *rin* heterozygotes (data not shown). The effect of the 2 genes seems additive, as the combination of 1 dose of each of these genes in the double heterozygote *(rin*/+, *nor*/+) reduced the flavor even more (Table 1). The flavor of this combination was, however, much better than that of *nor* homozygotes, which was definitely the worst (Table 1). When the ripening process was evaluated by the determinations of CO_2 and ethylene evolution rates, polygalacturonase activity, lycopene concentration, and shelf life duration, it was found that a single dose of *nor* was much more inhibitory than a single dose of *rin*, in parallel to their effects on flavor. It thus supports the previous suggestion (6, 11) that *rin* and *nor* act as ripening regulatory genes, flavor being one of the ripening parameters affected by them.

In the last few years, it has been suggested that *rin* and *nor* genes may be used in breeding programs for the production of commercial cultivars having extended shelf life (2, 8, 20, 21). Our flavor evaluations indicate difficulties in utilizing *rin* and *nor* for breeding purposes. This drawback may be overcome by crossing *rin* and *nor* with normal parents of superior flavor, which might yield more acceptable F_1 hybrids. In order to prepare parents with more desirable characters, the mutant genes also could be transferred into cultivars of good quality by a regular backcross program.

A number of studies (1, 5, 17, 18) have suggested a good correlation between high sugar and acid levels in tomato fruits and good taste. In our material, however, we were not able to relate the flavor scores (Table 1) to the values of the chemical constituents (Table 3). The values of sugar and acid concentration were similar in all fruit types tested: in the tasty fruits of normal cultivars as well as in fruits of the homozygous *rin* and *nor* which are unpalatable and the dihybrid heterozygote which has significantly poor taste. Moreover, in the homozygous *nor*, high scores of sweetness and sourness were found to be accompanied by high levels of TSS, sugars, titratable acidity, and low pH even though the score of overall flavor was low (Table 4).

Usually, the sugar content of tomato fruit increases with ripening. However, its increase starts at early developmental stages, before the initiation of the ripening process (as defined by the rise of the ethylene evolution rate and the climacteric). The acid content also starts increasing before the initiation of the ripening process, reaching a peak at the orange stage. Thus, in our opinion, it is questionable whether these 2 parameters really belong to the ripening process.

While all genotypes with *rin* and *nor* genes were inferior in flavor to fruits of the normal cultivar, their reducing sugars and acidity levels were within the range of normal cultivars (15, 18, 23). It is, therefore, possible to conclude that *rin* and *nor* do not affect fruit flavor via the modification of these parameters. This conclusion is further supported by the fact that both sugar content and total acidity start increasing already before the onset

of ripening (4) and, hence, may not be an integral part of the ripening process. In view of the role of volatile compounds in the determination of tomato flavor (16), it is tempting to correlate the flavor inferiority of *rin* and *nor* genotypes with the lack of some volatile compounds. One could also postulate the existence of increased levels of undesirable volatile compounds responsible for the unpalatability of the homozygous mutants.

Literature Cited

1. Bisogni, C. A. and G. Armbruster. 1976. Quality comparisons of room ripened tomato fruits. Food Sci. 41:333–338.
2. Buescher, R. W., 1977. Fruits from *rin* and *nor* tomato mutants. Ark. Farm Res. 26:14.
3. Buescher, R. W. and E. C. Tigchelaar. 1977. Utilization of *nor* tomato hybrids for extending storage-life and improving processed quality. Lebensm-Wiss. U. Technol. 10:111–113.
4. Hobson, G. E. and T. N. Davies. 1971.The tomato. p. 437–482.In: A. C. Hulme (ed.). The biochemistry of fruits and their products. Academic Press, New York.
5. De Bruyn, J. W., F. Garretsen, and E. Kooistra. 1971. Variation in taste and chemical composition of the tomato (*Lycopersicon esculentum* Mill.). Euphytica 20:214–277.
6. Kopeliovitch, E., Y.Mizrahi, N. Kedar, and H. D. Rabinowitch. 1978. A suggested mode of action for *rin* and *nor* ripening mutants of tomato. Plant Physiol. 61:98. (Abstr.).
7. Kopeliovitch, E., Y. Mizrahi, H. D. Rabinowitch, and N. Kedar. 1980. Physiology of the tomato mutant alcobaca. Physiol. Plant. 48:307–311.
8. Kopeliovitch, E., H. D. Rabinowitch, Y. Mizrahi, and N. Kedar. 1979. The potential of ripening mutants for extending shelf life of the tomato fruit. Euphytica 28:99–104.
9. Little, T. M. and F. J. Hills. 1978. Agricultural experimentation design and analysis. Wiley, New York. p.268–278.
10. Lyons, J. M. and H. K. Pratt. 1964. Effect of stage of maturity and ethylene treatment on respiration and ripening of tomato fruits. Proc. Amer. Soc. Hort. Sci. 84:491–500.
11. Mizrahi, Y., H. C. Dostal, and J. H. Cherry. 1976. Descriptive physiology and biochemistry of the abnormally ripening tomato fruit *(Lycopersicon esculentum)* cv. Snowball. Physiol. Plant. 38:309–312.
12. Ng, T. J. and E. C. Tigchelaar. 1977. Action of the nonripening *nor* mutant on fruit ripening of tomato. J. Amer. Soc. Hort. Sci. 102:504–509.
13. Robinson, R. W. and M. L. Tomes. 1968. Ripening inhibitor: a gene with multiple effects on ripening. Tomato Genetics Coop. 18:36–37.
14. Roessler, E. B., J. Warren, and J. F. Guymon. 1948. Significance in the trinagle taste tests. J. Food Sci. 13:503–505.
15. Simandle, P. A., J. L. Brogdon, J. P. Sweeney, E. C. Mobley, and D. W. Davis. 1966. Quality of six tomato varieties as affected by some compositional factors. Proc. Amer. Soc. Hort. Sci. 89:532–538.
16. Stevens, M. A. 1970. Inheritance and flavor contribution of 2-isobutylthiazole, methyl salicylate and eugenol in tomatoes. J. Amer. Soc. Hort. Sci. 95:9–13.
17. Stevens, M. A., and A. A. Kader. 1977. Tomato quality. Annu. Rpt. 1976/77 Calif. Fresh Market Tomato Advisory Board, Bakersfield, Calif. p.16–35.
18. Stevens, M. A., A. A. Kader, and M. Albright. 1979. Potential for increasing tomato flavor via increased sugar and acid content. J. Amer. Soc. Hort. Sci. 104:40–42.
19. Stevens, M. A., A. A. Kader, M. Albright-Holton, and M. Algari. 1977. Genotypic variation for flavor and composition in fresh market tomatoes. J. Amer. Soc. Hort. Sci. 102:680–689.
20. Tigchelaar, E. C., W. B. McGlasson, and R. W. Buescher. 1978. Genetic regulation of tomato fruit ripening. HortScience 13:508–513.
21. Tigchelaar, E. C., W. B. McGlasson, and M. J. Franklin. 1978. Natural and ethephon-stimulated ripening of F_1 hybrids of the ripening inhibitor *(rin)* and non ripening *(nor)* mutants of tomato (*Lycopersicon esculentum* Mill.). Austral. J. Plant Physiol. 5:449–456.
22. Tigchelaar, E. C., M. L. Tomes, E. A.Kerr, and R. J. Barman. 1973. A new fruit ripening mutant, nonripening *(nor)*. Tomato Genetics Coop. 223:33–34.
23. Winsor, G. W. 1964. Some factors affecting the composition, flavor and firmness of tomatoes. Scientific Hort. 18:27–35.