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THE EFFECTS OF ALLELES AT THE "NEVER RIPE" LOCUS ON THE RIPENING OF TOMATO FRUIT

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Abstract—The tomato variety "Potentate" has been modified by the introduction of the dominant allele (*Nr*) at the "Never ripe" locus. Fruit from the resulting plants ripen and soften much more slowly than control fruit. This behaviour is associated with a much reduced solubilization of the pectic substances and a considerable attenuation in the activity of the pectic enzyme polygalacturonase. There is evidence of incomplete dominance at the "Never ripe" locus. The analyses provide additional evidence for an association between the action of the pectic enzymes and the ripening processes of tomato fruit.

INTRODUCTION

AMONG tomato stocks grown in California in 1950, Rick and Butler[1] noted one line in which the fruit coloured and softened much more slowly than usual. It was found that this retardation in the ripening processes was due to a dominant allele which was christened "Never ripe" (*Nr*).

As part of a project being undertaken by the Plant Breeding Department of this Institute for the study of genes controlling fruit pigmentation in isogenic backgrounds, the dominant allele at the "Never ripe" locus has been introduced into the tomato variety "Potentate" by means of a backcross breeding programme. If completely successful, "Never ripe" plants should only differ from "Potentate" plants in one gene or gene complex and this would facilitate an accurate assessment of the effect of different alleles at the "Never ripe" locus on the fruit-ripening processes. Although comparisons of gene action may be carried out on material of diverse genetic origin, the use of isogenic lines enables specific effects of gene action to be studied without the complication of widely differing genetic backgrounds.

This contribution describes the influence of these alleles on the activities of the pectic enzymes pectinesterase and polygalacturonase. Additional data on the extent of solubilization of the pectic substances and on the firmness of tomato fruit samples drawn from the various genotypes has also been obtained.

RESULTS

Analyses of fruit taken from heterozygous (*Nr*/*nr*) plants in family 24, from red-fruited segregants (*nr*/*nr*) in the same family, and from red-fruited "Potentate" plants are presented in Table 1. The latter variety had served as a recurrent parent in the backcross programme. The activity of the enzyme pectinesterase was not significantly affected by the introduction of one *Nr* allele, but the protein content of the enzyme extract from "Potentate" fruit was significantly higher than either of the other two solutions. Hence the specific activity of pectinesterase in the fruit of variety "Potentate" was significantly lower than that of the *Nr*/*nr* family.

[1] C. M. RICK and L. BUTLER, *Advan. Genet.* **8**, 267 (1956).

TABLE 1. EFFECT OF THE INTRODUCTION OF A DOMINANT ALLELE AT THE "NEVER RIPE" LOCUS ON TOMATO FRUIT OF THE VARIETY "POTENTATE"

	Genetic constitution of the fruit*				
	Nr/nr from family 24	*nr/nr* from family 24	*nr/nr* variety "Potentate"	Significance of the *F*-test	Least significant difference ($P=0{\cdot}05$)
Pectinesterase (units/100 g fresh tissue)	4·29	3·82	3·92	—	0·86
Protein (mg/100 g fresh tissue)	58·5	73·9	110·4	$P<0{\cdot}001$	23·0
Specific activity of pectinesterase (units/mg of protein)	0·076	0·053	0·039	$P<0{\cdot}01$	0·026
Polygalacturonase† (units/100 g fresh tissue)	102 (2·09)	2880 (3·46)	3090 (3·49)	$P<0{\cdot}001$	0·57
Dry matter (%)	6·20	6·67	6·32	$P<0{\cdot}001$	0·25
Total pectic substances‡	0·45	0·39	0·40	—	0·12
Insoluble pectic substances‡	0·39	0·23	0·22	$P<0{\cdot}01$	0·10
Insoluble as % of total pectic substances	86·7	59·0	52·5		

* Each figure is based on the mean of six determinations.

† These values were obtained by averaging the logarithms of the individual results, in which form statistical analyses were carried out so that the error variances were kept about the same. The means in logarithmic form are given in parentheses.

‡ Expressed as % (w/w) anhydrouronic acid in fresh tissue.

The activity of polygalacturonase in normal red "Potentate" fruit quoted in Table 1 was somewhat lower than has been found in previous studies,[2] probably because the plants were grown outside the normal season. Nevertheless, the activity in fruit from plants containing one *Nr* allele was very much lower than in either fruit from the *nr/nr* segregant or in red "Potentate" fruit and was, in fact, close to the level usually associated with tomatoes near the beginning of the ripening process.

There were small but significant differences in the dry matter content between the various sources of fruit material considered, but this property is a particularly difficult and variable factor to determine.[3]

Following this preliminary examination of the effect of the introduction of one *Nr* allele into variety "Potentate", a more detailed survey covering the complete range of heterozygous and homozygous material was carried out. The results are set out in Table 2.

Fruit from the heterozygous plants *nr/Nr* contained significantly higher pectinesterase activity than that from the reciprocal cross *Nr/nr* and from the homozygous recessive *nr/nr*. Figures for the protein content of the enzyme extracts were lowest in fruit of the homozygous dominant and highest in the homozygous recessive plants; all the differences were significant except between the *Nr/Nr* and the *Nr/nr* groups of plants. It follows that the specific activity values were highest in the *Nr/Nr* fruit and lowest in the *nr/nr* samples. Both of these figures were significantly different from the intermediate values of the heterozygous fruit. The figures appeared to follow closely the number of dominant alleles in the genetic make-up of the plants.

[2] G. E. HOBSON, *Biochem. J.* **92**, 324 (1964).

[3] D. M. MASSEY and G. W. WINSOR, *Rep. Glasshouse Crops Res. Inst.*, 1956, p. 52 (1957).

TABLE 2. EFFECT OF DOMINANT AND RECESSIVE ALLELES AT THE "NEVER RIPE" LOCUS ON TOMATO FRUIT OF THE VARIETY "POTENTATE"

	Genetic constitution of the fruit*				Significance of the *F*-test	Least significant difference ($P=0.05$)
	Nr/Nr	*Nr/nr*	*nr/Nr*	*nr/nr*		
Pectinesterase (units/100 g fresh tissue)	9·23	8·39	9·59	8·59	$P<0.05$	0·87
Protein (mg/100 g fresh tissue)	65·7	73·0	91·9	118·1	$P<0.001$	12·5
Specific activity of pectinesterase (units/mg of protein)	0·142	0·115	0·106	0·073	$P<0.001$	0·017
Polygalacturonase† (units/100 g fresh tissue)	32 (1·51)	420 (2·62)	306 (2·49)	8220 (3·92)	$P<0.001$	0·17
Dry matter (%)	6·45	6·15	8·05	7·10	$P<0.001$	0·77
Compression under a standard load (mm)	2·04	2·48	2·66	3·20	$P<0.001$	0·19
Compression under a standard load (%)	6·74	8·53	9·40	11·46	$P<0.001$	0·58

* Each figure is based on the mean of six determinations.
† These values were arrived at by similar methods to those detailed in Table 1.

The activity of polygalacturonase in "Never ripe" (*Nr/Nr*) fruit was at a very low level whereas the fruit from heterozygous segregants (*Nr/nr* or *nr/Nr*) displayed about ten times more activity. The enzyme in the red fruit from homozygous recessive plants (*nr/nr*) was even more highly active at about twenty times the average for heterozygous samples or two hundred and sixty times that of "Never ripe" fruit. As in Table 1 the dry matter percentages were rather variable.

A quantitative study of fruit firmness was carried out using an instrument for assessing the compressibility of tomatoes under a standard load. The values quoted in Table 2 show that the fruit firmness increased progressively and significantly with the number of dominant alleles no matter which of the two methods for expressing the compression measurements was used.[4] There was also a significant difference in the firmness between the two heterozygous groups of plants when the percentage compression but not the actual compression was considered. Occasional inconsistencies between the two methods for expressing the results are inevitable and the merits of each have been discussed.[4] Nevertheless, it was possible to establish highly significant ($P=0.001$) positive relations between the logarithm of the polygalacturonase activities in the various genotypes and either the actual compressions ($r=0.849$) or the percentage compressions ($r=0.913$).

DISCUSSION

The fruit used to obtain the results given in Table 1 were from plants grown in the autumn of 1962, but the more detailed survey reported in Table 2 was carried out with fruit grown in the spring of the following year. The activities of the pectic enzymes in the *nr/nr* fruit of this second trial were twice as high as in the first one, and this suggests a strong interaction between the activities of these enzymes and the environmental conditions under which the plants are grown. This in no way, however, invalidates comparisons between values within each of the tables.

[4] S. A. SHAFSHAK and G. W. WINSOR, *J. Hort. Sci.* **39**, 284 (1964).

The "Potentate" fruit were included in the first experiment (Table 1) in order to see how effective the backcross breeding material had been in retaining the general characteristics of the recurrent parent in the *nr/nr* derivatives. There were no significant differences in six of the eight characteristics considered, but differences just obtained significance for the protein content of the enzyme extract and the percentage of dry matter. It has already been pointed out that this latter property is a rather variable characteristic. However, the evidence does suggest that the backcross method as used in this study has not been entirely effective and possibly further backcrosses should have been made. Alternatively, there may be pleiotropic effects of the gene being investigated.

Of the range of characteristics that alter with the ripening of various kinds of fruits,[5] changes in firmness appear to be almost universal. For many years this property has been closely linked with the pectic enzymes,[6] although cellulases[7] may also be involved. In general the present results re-emphasize the importance of the pectic constituents in the ripening of tomatoes. Moreover they provide further evidence for the long-standing association between the degradation of pectins and the firmness of the fruit.[8]

The conclusion from the compression measurements on the three general tomato genotypes is that the introduction of one and then two dominant alleles at the "Never ripe" locus resulted in progressively firmer fruit. The general uniformity in the levels of pectinesterase throughout these trials confirms its non-limiting role in the softening process.[9] The close correlation between the firmness of the four genotypes cited in Table 2 and the logarithms of their polygalacturonase activity suggests that it is this enzyme that is the controlling factor in the loss of firmness, and this view is supported by previous experimental observations.[10]

This gradation in physical properties and polygalacturonase activity with the number of dominant alleles possessed by the genotypes indicates that there is incomplete dominance at the "Never ripe" locus. Whether this locus is exclusively or even primarily concerned in the biosynthesis of polygalacturonase is a matter for speculation. Although there is, perhaps, a *prima facie* case for considering that low polygalacturonase activity is the immediate cause for the slow softening of "Never ripe" tomatoes, there could also be pleiotropic action at the *Nr* locus with an effect upon a completely different part of the metabolic machinery. All that is certain at the moment is that where a dominant allele is present at the "Never ripe" locus, the synthesis of polygalacturonase is severely retarded, and this observation coincides with delays in the appearance of characteristics associated with the normal ripening processes. Further study, especially at the sub-cellular level, may reveal the point in metabolism at which this interesting allele exerts its influence.

EXPERIMENTAL

Pectic Substance Determinations and the Activities of the Pectic Enzymes

Methods for determining the amounts of pectic substances and for assessing the activities of the pectic enzymes have been recorded in detail elsewhere.[2, 9, 11] The procedures used for protein measurements and dry-matter determinations were also as previously published.[9]

[5] R. G. TOMKINS, *J. Sci. Food Agr.* **5**, 161 (1954).
[6] J. B. BIALE, *Handbuch der Pflanzenphysiologie* (Edited by W. RUHLAND), Vol. 12/2, p. 536. Springer Berlin (1960).
[7] D. B. DICKINSON and J. P. MCCOLLUM, *Nature* **203**, 525 (1964).
[8] C. W. WOODMANSEE, J. H. MCCLENDON and G. F. SOMERS, *Food Res.* **24**, 503 (1959).
[9] G. E. HOBSON, *Biochem. J.* **86**, 358 (1963).
[10] G. E. HOBSON, *J. Hort. Sci.* **40**, 66 (1965).
[11] G. E. HOBSON, *Nature* **195**, 804 (1962).

Compression Measurements

The apparatus used for assessing the firmness of fruit has already been described.[4]

The "Never ripe" (Nr) Character in Tomato Plants

The dominant allele *Nr* was introduced into the variety "Potentate" by means of a backcross breeding programme. On self-pollination following the fourth backcross, "Never ripe" and red-fruited plants were recovered in the expected ratio. The "Never ripe" plants were then further self-pollinated to determine whether they were homozygous (*Nr*/*Nr*) or heterozygous (*Nr*/*nr*). One family (number 24) resulting from the self-pollination of a single plant, was used to obtain the data given in Table 1. One-quarter of the plants in the family were red-fruited, indicating that the parent plants were heterozygous for "Never ripe". A further quarter of the plants hardly changed colour from the unripe condition, whilst the remaining half of the total number of plants gradually became orange in colour. It was suspected that these orange-fruited plants were heterozygous for "Never ripe", and they were used to obtain the data given in column 1 of Table 1. Subsequent breeding tests confirmed that they were, in fact, heterozygous. Fruit from the red segregants (which had the genetic constitution *nr*/*nr*) of family 24, and also the normal red fruit of variety "Potentate", were sampled at the same time; the results are given in columns 2 and 3 in Table 1.

The very slow ripening plants from family 24 were used to produce material for the second series of experiments. First of all, they were shown to be true-breeding for the "Never ripe" condition (i.e. they were shown to be *Nr*/*Nr*) and, following a further generation of self-pollination, plants were crossed reciprocally with the variety "Potentate" to obtain F_1 hybrids. Comparisons are made in Table 2 of analyses of fruit from "Never ripe" plants (*Nr*/*Nr*), the reciprocal F_1 hybrids (*Nr*/*nr* and *nr*/*Nr*), and the red-fruited plants derived from family 24 (*nr*/*nr*).

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