Pectinesterase, Polygalacturonase, Cx-Cellulase Activities and Softening of the rin Tomato Mutant

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Abstract. Pectinesterase (PE), polygalacturonase (PG), and cellulase (Cx form) activities and softening of 4 physiological maturities were compared in normal and rin tomatoes (Lycopersicon esculentum Mill cv. Rutgers). PG, PE, and Cx-cellulase activities increased during ripening of normal fruits. In rin fruits, PG activity was not detected, PE activity remained relatively constant, and Cx-cellulase activity increased during ripening. The lack of softening in rin fruits appears to be associated with the lack of PG activity.

The fruit ripening inhibitor (rin) mutant in tomato lacks the normal climacteric (5), does not develop characteristic carotenoids and fails to soften (12). Other characteristics of fruit ripening affected by this mutation have not been investigated. The long storage life associated with this recessive gene is of particular interest. In this report we compare changes in PE, PG and Cx-cellulase, and softening during ripening of normal and rin tomato fruits.

Isogenic normal and rin stocks were developed by 5 successive backcrosses with 'Rutgers' as the recurrent parent. Flowers from 'Rutgers' and isogenic rin plants were tagged at anthesis and one fruit was allowed to develop per cluster. Field-grown fruits were harvested 41, 46, 49, and 54 days from anthesis, intervals which corresponded to distinct maturity stages (mature-green to ripe) of 'Rutgers'. Harvested fruit were washed in water containing 0.1% sodium hypochlorite, air-dried and separated into 3 uniform lots of 10 fruits each. An Asco Firmness Meter set at 1 kg prestress and 1.5 kg linear stress for 30 sec was used for determining firmness of each fruit (1). Enzymes were extracted by homogenizing tomato sections in aqueous solution containing 1% polyvinylpyrrolidone and 1 M NaCl and by filtering through 8 layers of cheese-cloth. Cx-cellulase was desorbed from the cell walls prior to filtering as described by Dickinson and McCollum (3). The action of PE on citrus pectin, PG on pectic acid, and Cx-cellulase on carboxymethyl-cellulose (hercules 7HP) was determined by the methods described by Rouse and Atkins (13), Kertesz (10), and Dickinson and McCollum (3), respectively. Cannon-Fenske No. 200 viscometers were used for PG and Cx-cellulase assays. Enzyme units are presented as microequivalents/g fresh wt/min for PE, % change in viscosity of 15 ml 1% pectic acid/2.5 g fresh wt/15 min for PG and % change in viscosity of 15 ml 1% carboxymethyl-cellulose/2.5 g fresh wt/3 hr for Cx-cellulase.

A close association has been established between normal tomato softening and activities of PE and PG (2, 6, 7, 8). We show this relationship for 'Rutgers' and rin (Fig. 1). Softening increased concomitantly with increased PE and PG activity in 'Rutgers'. In contrast, rin fruits remained firm, no PG activity was detected, and PE activity remained relatively constant. Incubation of predetermined levels of active PE and PG with homogenates of rin fruits did not affect the enzyme activities (unreported data) which indicates that lower PG and PE activities in rin fruits are not a result of the presence of inhibitors.

Lowered PG activity has also been reported in tomato fruit tissue affected by the fruit ripening inhibitor (rin) mutant in tomato (5). The fruit lacks the normal climacteric (5), does not develop characteristic carotenoids and fails to soften (12). Other characteristics of fruit ripening affected by this mutation have not been investigated. The long storage life associated with this recessive gene is of particular interest. In this report we compare changes in PE, PG and Cx-cellulase, and softening during ripening of normal and rin tomato fruits.

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Fig. 1. Changes in firmness (Asco units), PG, PE, and Cx-cellulase activity of 'Rutgers' and rin tomatoes as related to physiological maturity.
Correlation Between Methods to Determine the Protein Content of Potato Tubers

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Abstract. Tubers of potato (Solanum tuberosum L.) ranging from 6.9 to 18.6% total amino acids, on a dry weight basis, were analyzed by 6 methods which determine protein content: 1) total amino acids minus free amino acids determined by column chromatography, 2) Kjeldahl N x 5.7, 3) Kjeldahl N x 5.7 minus ninhydrin determined free amino acids, 5) ninhydrin total minus ninhydrin free amino acids, and 6) Folin-Lowry analyses. The results of the latter 5 methods were correlated to the data of methos 1 (column chromatography). All of the methods, except 4, had correlation coefficients above the 99% level of significance. When compared to the data obtained from column chromatography, methods 6 and 4 gave low, while method 3 gave approximately equal protein values. Method 2 is excellent for determining total tuber amino acids (r = 0.96**).and is the method of choice for screening large numbers of tubers for protein, (r = 0.92**). Kjeldahl N x 5.7 correlates high with tuber protein, because there is a high correlation between tuber free protein and bound amino acids, (r = 0.74**). However, Kjeldahl N x 5.7 gives high tuber protein values. Based upon their correlation coefficients, methods 2, 5 and 6 are applicable for screening, but their individual shortcomings must be considered when interpreting results from tubers.

Breeders and physiologists attempting to measure potato tuber protein often require a rapid, reliable screening technique to determine the protein content of numerous samples. Kjeldahl analyses are often used, but potatoes may contain 37-64% of their nitrogen as non-protein nitrogen (6) with as much as 32-46% in the form of amide-nitrogen (9). Consequently, Kjeldahl data may lead to high estimations of tuber protein. Folin-Lowry analyses can be used (2), but the method is laborious, and protein can be lost in the washing procedures (personal communication, Sharon Desborough, Univ. of Minnesota). Tuber proteins and amino acids are best analyzed with an amino acid analyzer, to determine the precision of each protein screening technique.

To produce samples with a wide range of tuber protein, numerous genotypes which differ in their protein content were grown in moderate (25/18°C, day/night) and cool (11/7°C, day/night) regimes. Entire tubers, including the peel, were sliced, quick-frozen with liquid nitrogen, and freeze-dried. Samples were ground to pass through a 177 μ mesh screen and the resulting powder was stored at −20°C until analysis.

Column chromatography analyses of total and free amino acids. Total amino acids were obtained by hydrolyzing 10 mg of tuber powder in an evacuated ampule containing 1 ml of 6 N HCl at 110°C for 22 hr. After hydrolysis, the HCl was removed by flash-evaporation and the resulting residue was redissolved in phosphate buffer (pH 2.2) filtered, and analyzed. Free amino acids were extracted from 100 mg of tuber powder with 10 ml of 10% TCA (trichloroacetic acid) by stirring for 1 hr. Cellular debris was removed by centrifugation and the TCA was removed by three, 10 ml ether extractions followed by flash-evaporation to dryness. The resulting residue was redissolved in phosphate buffer (pH 2.2) and analyzed. All amino acid analyses were conducted by the automated procedure of Moore et al. (5) with a Beckman, Model 120, Automatic Amino Acid Analyzer.

Kjeldahl analyses. Kjeldahl nitrogen was analyzed by the technique of Brenner (1) using 125-150 mg of tuber powder.

Ninhydrin analyses of total and free amino acids. Total amino acids were obtained by hydrolyzing 20 ± 4 mg of tuber powder as described above. After hydrolysis, the solution was brought up to pH 5.00 with 1N NaOH, diluted to 20-25 ml with distilled water, and analyzed by the technique of Rosen (8) using leucine as a standard. Since salts change the ninhydrin color yield, the standard was dissolved in a NaCl solution of a normality equal to that of the amino acid extract. Free amino acids were extracted as described above and analyzed by the technique of Rosen (8).