GENETIC AND PHYSIOLOGICAL STUDIES OF
THE 'ALCOBACA' TOMATO RIPENING MUTANT

BY

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To the memory of my father, Manuel J.

and

to my mother, Ofelia; my wife, Yolanda;

and my children, Jorge Mario, Tatiana,

and Juan Manuel.

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TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	iii
ABSTRACT	vi
INTRODUCTION	1
LITERATURE REVIEW	3
Tomato Fruit Ripening	3
Ripening and Respiration	4
Ethylene and Ripening	7
Cell Wall Metabolism and Ripening	10
Softening and Hydrolytic Enzymes	12
Color and Ripening	15
Tomato Ripening Mutants	16
'Alcobaca' Tomato	23
MATERIALS AND METHODS	26
Pedigree of Studied Materials	26
Storage Life Studies	26
Color Determination	29
Firmness of the Fruits	30
Respiration Studies	31
Ethylene Evolution	32
Polygalacturonase Activity	32
Model To Relate Storage Life with Different Physiological Parameters	33
Genetic Studies	34
Inheritance Study	34
Functional and Recombinational Genetic Tests	35
Inheritance of Storage Life	35
RESULTS	37
Storage Life Studies	37
Preliminary Observation	37
Storage Life of Commercial Varieties and 'Alcobaca'	39
Fruit Storage Life of Hybrids with Ripening Mutants	44
Fruit Storage Life of Hybrids Between 'Alcobaca' and the Ripening Mutants <u>nor</u> , <u>rin</u> , and <u>rin-1</u>	49

	Page
Respiration Study	55
Ethylene Evolution	55
PG Activity Study	63
Model To Relate Storage Life with Different Physiological Parameters	70
Genetic Studies	70
Inheritance of the Abnormal Ripening 'Alcobaca'	70
Allelism Test Between 'Alcobaca' and <u>rin</u>	72
Allelism Test Between 'Alcobaca' and <u>nor</u>	72
Inheritance of Fruit Storage Life	79
DISCUSSION	83
Storage Life Indexes	83
Storage Life Studies	83
Respiration, Ethylene Evolution, and PG Activity	87
Genetic Studies	92
SUMMARY AND CONCLUSIONS	97
LITERATURE CITED	99
BIOGRAPHICAL SKETCH	108

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Genetic and physiological studies were conducted with the ripening tomato mutant 'Alcobaca'. A single recessive gene was found to be responsible for the abnormal ripening. This gene causes a ripening syndrome characterized by an attenuated climacteric pattern, delayed softening of the fruit, low polygalacturonase (PG) activity, and extended shelf life. When the mutant is heterozygous with the wild-type allele, these parameters of ripening are also affected although not to the extent observed in the homozygous condition. Thus the gene might be employed in the heterozygous condition to improve the shelf life of tomato.

Allelism tests and examination of F_2 progenies involving the ripening mutants rin and nor showed that the mutant gene of 'Alcobaca' is allelic to nor. Here it is proposed that the name nor^A be used to refer to the mutant allele of 'Alcobaca'.

The mutant allele nor^A is dominant to the standard recessive allele nor in that nor^A is climacteric. Also, nor^A conditions higher levels of respiration, ethylene evolution, and PG activity. These parameters are intermediate in nor^A/nor heterozygotes. Thus there exist now three alleles at the nor locus. The wild-type allele conditions a normal climacteric and normal ripening. The nor^A allele has a climacteric but does not ripen normally. The recessive nor lacks a climacteric and normal ripening. These alleles should be useful in the study of regulatory mechanisms of ripening. Ethylene peak evolution and PG activity were reduced to a greater extent in the double heterozygous genotype nor⁺/nor^A, rin⁺/rin than in single heterozygous genotypes nor⁺/nor^A, indicating a gene dosage effect for these two mutants.

Correlation studies of storage life and the different ripening parameters of normal and mutant ripening genotypes indicated that PG activity was the parameter that exhibited the most highly significant relationship to storage life.

A quantitative genetic study of the fruit shelf life, employing as parents 'Alcobaca' and the normal ripening tomato 'Florida 1C', indicated that storage life fitted an additive-dominance model, and that the parents differed only at one locus. This, then, agrees with the more qualitative study above in that 'Alcobaca' and 'Florida 1C' carry different alleles of nor.

INTRODUCTION

The improvement of the tomato fruit storage life via different storage conditions has received considerable work. Low temperature (Tomkins 1963), controlled atmospheres (Eaves and Lockhart (1966) Parsons et al. 1970, Salunkhe and Wu 1973), employment of ripening retardants or inhibitors (Babbitt et al. 1973, Kader et al. 1973, Dostal and Leopold 1967, Yang 1980), and use of plastic film wraps (Wu et al. 1972, Gilbert and Hening 1971) have been proposed to extend the shelf life of tomato fruit. Another possibility is genetic manipulation of the ripening process.

The discovery of the tomato ripening mutants rin and nor (Robinson and Tomes 1968, Tigchelaar et al. 1973) has been considered the cornerstone for possible genetic manipulation of the fruit storage life in tomato.

'Alcobaca', another ripening mutant was described previously (Almeida 1961, Leal and Shimoya 1973). This material exhibits a very extended fruit shelf life (Almeida 1961, Leal and Mizubuti 1975, Leal and Shimoya 1973, Leal and Tabim 1974). However, there is a lack of information concerning the genetics and physiology of the abnormal ripening behavior of this material. These investigations were undertaken to study the storage life and some

physiological processes of fruit ripening in 'Alcobaca', in hybrids of this material with normal ripening tomatoes, and in hybrids between 'Alcobaca' and other ripening mutants. Also studies were done to elucidate the inheritance of the 'Alcobaca' abnormal fruit ripening and possible allelic relationships with another ripening mutants.

LITERATURE REVIEW

Tomato Fruit Ripening

Tomato fruit ripening involves a number of chemical and physical changes (Babbitt et al. 1973, Hobson and Davies 1971). The changes are highly synchronized as evidenced by the fact that respiration, ethylene evolution, carotene development, and flavor and textural changes occur in rapid succession during the relatively short period in which fruit ripens (Babbitt et al. 1973, Khudairi 1972). There has been inconsistency in the literature in the use of the terms maturation and ripening. Kader and Morris (1976) stated that maturation occurs during the stage of development in which the fruit attains full growth. Most of the maturation processes occur on the plant, but they can continue after harvest for partially mature fruits. Nagel, cited by Babbitt et al. (1973), defined a mature tomato as one that has reached nearly full external and internal development and has the capacity to ripen within a few days after harvest. Kader and Morris (1976) stated that ripening is a form of senescence that can occur on or off the plant, beginning when maturation is completed. Ripening, then, would include the many processes that make the fruit edible.

Hansen (1966) considered ripening to be a sequential phase in the life of the fruit. It requires induction in order to become active and, once ripening is induced, it proceeds according to a predetermined pattern. Several investigators believe that the period of active ripening begins with the onset of the climateric rise (Biale 1960, Hansen 1966).

Hansen (1966), in an extensive review of postharvest physiology of fruits, stated that energy is required for ripening in contrast to an older view that the ripening processes were catabolic in nature.

Tigchelaar et al. (1978a) reported that four clearly defined changes occur during normal ripening of tomato. These include a) chlorophyll degradation and carotenoid biosynthesis, b) increased respiration and associated ethylene production, c) softening and associated increases in pectolytic enzyme activities, and d) seed maturation. In addition, less well-defined changes in flavor, texture, and aroma are integral parts of the ripening process.

Ripening and Respiration

The modern study of the ripening processes stems from the investigation of the physiology of the apple fruit by Kidd and West in the early 1920s (Rhodes 1970). They found that after picking, the CO₂ production of the fruit fell to a minimum value and then rose rapidly. Subsequently, the

respiration declined. They used the term climacteric to refer to this pattern of respiratory changes. This pattern of respiration is associated with the ripening of many other fruits. The respiratory rise was shown by Gustafson in 1920, cited by Rhodes (1970), for the tomato. Climacteric pattern of respiration on tomato has been described by a number of authors (Clendenning 1942, Biale 1950, Workman et al. 1957, Biale 1960).

Biale (1960) classified the fruits into two categories: climacteric and non-climacteric. In non-climacteric fruits, changes in color and composition are not accompanied by a rise in ethylene or CO_2 production. In climacteric fruits a large increase in respiration and ethylene production accompanies ripening. Ethylene stimulates respiration and ripening of mature unripe fruits, and once stimulated by exogenous or endogenous ethylene, climacteric fruits produce ethylene autocatalytically (Burg and Burg 1965).

Rhodes (1970) pointed out that it is difficult to determine whether there is a distinct difference in the mechanism of ripening in the two types of fruits or whether events which occur slowly over a long period in the so-called non-climacteric types are merely telescoped in the climacteric fruits into a short dramatic period. Tigchelaar et al. (1978a) reported that the mechanisms by which ripening changes are initiated and synchronized remains largely unknown. The initiation of the respiratory rise and

associated ethylene are used to define the onset of ripening for climacteric fruit such as tomato; and these dramatic changes are lacking and ripening is frequently protracted for non-climacteric fruit.

Sacher (1973) postulated that considerable evidence indicates that some aspects of ripening are not dependent on the increase in respiration that occurs in climacteric fruits. Dostal and Leopold (1967) showed that ethylene stimulation of color development in tomatoes is prevented by gibberelic acid, but the stimulation of the respiratory climacteric was not affected. Quazi and Freebairn, cited by Sacher (1973), found that when tomato ripening was initiated with ethylene and the fruit given 2.5% O₂, normal ripening took place in the absence of respiratory climacteric. Reid and Pratt, cited by Sacher (1973), suggested that the respiratory climacteric may be caused directly by the large amounts of ethylene produced during ripening rather than by the energy demands of other phenomena. This view was based on their demonstration that a respiratory rise comparable in magnitude and duration to the climacteric occurred in the non-climacteric orange and potato tuber when each was treated with ethylene. They concluded that the critical difference between climacteric and non-climacteric fruits is in the amount of endogenous ethylene produced during ripening.

Ethylene and Ripening

The role of ethylene in fruit ripening has been discussed for a long time. There are two schools of thought concerning the role of ethylene in fruit maturation (Burg and Burg 1962). The classic view of Kid and West (cited by Burg and Burg 1962) is that ethylene is a ripening hormone. Biale et al., cited by Burg and Burg (1962), postulated that ethylene is a by-product of the ripening process.

Burg and Burg (1965) reported data for several fruits, including tomato, which showed that ample ethylene accumulates before the onset of the climacteric. The quantity accumulated is larger than the threshold value for ripening. The rise in ethylene occurs at the onset of the climacteric rise, suggesting that ethylene might trigger ripening. When ripening is prevented by such conditions as low temperature, ethylene production is also inhibited (Galston and Davies 1970), suggesting that ethylene is the natural fruit-ripening hormone. Abeles (1973) stated that there is no exception to the fact that ethylene can promote ripening if the tissue is in a receptive state, although there is no reason to believe that ripening is controlled by ethylene alone.

The onset of ripening is associated not only with a rise in the ability to biosynthesize ethylene, but also with a marked increase in ethylene responsiveness (Burg and Burg 1966). Work (1929) first demonstrated the relationship

between maturity of fruit and rate of response to ethylene. Immature fruits show a slower response to ethylene than do mature fruit (Lyons and Pratt 1964).

Ethylene concentrations ranging from 100 to 2000 ppm are effective in stimulating fruit ripening (Harvey 1928, Saccher 1973). Lyons and Pratt (1964) stated that ethylene treatment induced a climacteric rise in respiration in tomato fruits of all ages and hastened or induced changes usually associated with ripening. Rosa (1925) first showed that gains of 5 to 8 days could be induced by ethylene in the ripening of mature green tomatoes. Similar data were published by Harvey and Prokosheva, and Babichev, cited by Pratt and Workman (1962). Pratt and Workmann (1962) found that ethylene treatment at 20°C caused mature-green tomato fruits to ripen early. The climacteric rise in respiration was induced immediately after initiation of ethylene treatments. The rise in respiration rate was more rapid and the maximum respiration rate was obtained sooner. In addition, the maximum rate of respiration was higher in ethylene-treated fruits than in control fruits. Similar results were reported by Hobson and Davies (1971), McGlasson (1970), Pratt and Workmann (1962), and Rhodes (1970).

The role of ethylene in the biochemical processes occurring during fruit ripening has not been clearly established. Several hypotheses have been proposed to explain the action of ethylene. These include effects on enzymatic activities,

interaction with nucleic acids, effect on lipo protein membranes, and the formation of complexes with metallo-enzymes (McGlasson 1970).

Methionine was suggested as the precursor of ethylene since this amino acid is the biological precursor of ethylene in all higher plant tissues (Lieberman et al. 1966, Yang 1980). Since ethylene production as well as the conversion of methionine to ethylene ceases in plant tissues placed in an anaerobic atmosphere, and the surge of ethylene production occurs upon return of the tissue to air, it was concluded that an intermediate accumulates during anaerobic incubation and is subsequently converted to ethylene upon exposure to oxygen (Burg 1962). This intermediate was later identified as 1-amino-cyclopropane-1-carboxylic acid (ACC) (Yang 1980). ACC content in preclimacteric fruits of avocado, banana, and tomato is quite low, but a massive increase occurred at the onset of rapid ethylene evolution, suggesting that the inability of preclimacteric fruit tissue to produce ethylene is due to the lack of ability to form ACC (Hoffman and Yang, cited by Yang 1980).

Zobel (1973) indicated a divergence of mechanisms controlling ethylene synthesis in different tissues. The diageotropic mutant of tomato requires exogenous ethylene for normal vegetative growth and development, but fruit maturation and ripening and production of ethylene by flowers is normal. McGlasson et al. (1975b) found that aging leaf

segments of a non-climacteric mutant of tomato produced a normal pattern of ethylene evolution in contrast to the lack of increase in ethylene production during aging in whole fruits and only a very small rise in ethylene production in very old fruits of the mutant. Herner and Sink (1973) working with the non-climacteric mutant tomato rin, found that ethylene production was induced by wounding the fruits. The response to wounding suggests that a) this stress, ethylene, was not produced through the same pathway of the climacteric or normal fruits, or b) cutting or wounding stimulated the synthesis of ethylene through the normal pathway, but exogenous ethylene is unable to do so in the mutant.

Cell Wall Metabolism and Ripening

Cell wall metabolism has recently been implicated in the regulation of the ripening process in tomato (Ng and Tigchelaar 1977, Tigchelaar and McGlasson 1977). An increase in soluble polyuronide has been well documented for a number of fruits and is generally attributed to hydrolysis of insoluble pectic polysaccharides by polygalacturonase (Gross and Wallner 1979).

Mohr and Stein, cited by Pressey (1974), described the changes occurring in the tomato fruit parenchymatous outer pericarp. Enlargement of the cells during fruit development is accompanied by intercellular space enlargement and cell wall separation. The intercellular spaces are small

or nonexistent in very young fruits, but separation of cell walls begins within a week or two after fertilization. This separation starts at the intercellular spaces. The middle lamella surface next to the spaces is the last to separate. Increase in cell size and cell wall separation continues until the fruit approaches maturity. The tomato fruits at the edible stage are composed of senescing tissues. Further breakdown of the pectinaceous middle lamella occurs in mature pericarp tissue to the point where cells may be completely separated.

Studying the degradation of cell wall polysaccharides, Gross and Wallner (1979) found that the only components to decline during tomato fruit ripening were galactose, arabinose and galacturonic acid. Isolated cell walls of ripening fruit contained a water-soluble polyuronide, possibly a product of polygalacturonase action. The ripening-related decline in galactose and arabinose content appeared to be separated from polyuronide solubilization. This idea was supported by the fact that in the ripening mutant rin the loss of these neutral sugars occurred in the absence of polygalacturonase and polyuronide solubilization. Furthermore, Wallner and Bloom (1977) and Wallner and Walker (1975) observed that polygalacturonase was not capable of hydrolyzing the neutral sugars, which have been reported to lend rigidity and intracellular cohesion to cell walls (Knee 1974). Softening of the fruit during ripening was attributed to the solubilization of the pectic materials by

pectinases (Babbit et al. 1973). Wallner and Walker (1975) reported that beta-1,3-glucanase, an enzyme capable of hydrolyzing the neutral sugars, was present in significant quantities in tomato fruits when polygalacturonase was largely absent. However, the major wall-softening modifications occurred.

Mattoo and Vickery (1977) postulated that changes in the degree to which enzymes are bound to membranes may comprise one of the mechanisms by which the activities of the enzymes are controlled in tomato pericarp.

Softening and Hydrolytic Enzymes

Softening reflects chemical and physical changes in the cell walls (Kader and Morris 1976). Early workers established that fruit ripening is accompanied by conversion of protopectin to soluble forms of pectin (Pressey 1974). Softening of the fruit during ripening is attributed to the solubilization of the pectin materials by pectinases (Babbit et al. 1973).

Polygalacturonase and pectinmethylesterase are the major hydrolases involved in fruit softening with cellulase as a secondary contributor (Kader and Morris 1976). Gonzalez and Bretch (1978) suggested that two possible, related, mechanisms of softening are present in tomatoes. The first one can be activated via exogenous ethylene and the second, which seems to operate without the effect of exogenous ethylene

occurs after normal tomatoes reach the turning stage of ripening.

Softening is genetically controlled and affected by cultural practices, environmental conditions, picking stage, plant age and condition, cluster position in the plant, and incidence of diseases (Kader and Morris 1976). Softening starts at the stylar end and proceeds toward the stem scar (Kader and Morris 1976). El-Sayed et al. (1966) postulated that softening of fruit is controlled by a single major gene with complete dominance, but certain modifier genes might have an effect on whole fruit firmness. Hall (1964) found that firmness in tomato was affected by variety, harvest date, and the interaction variety-harvest date.

Pectinesterase catalyzes the removal of the methyl ester groups of pectin (Pressey 1974). This enzyme is present in green tomatoes and increases about 4-fold during ripening (Kertesz 1938). A relationship between firmness and pectinesterase activity was suggested by Hamson (1952a). However, Hall and Denison (1960) and Hobson (1963) showed that there was no correlation between pectinesterase and softening. Pressey (1974) stated that attempts to identify the pectinesterase role in fruit softening by measuring the enzymatic activity are of limited value because the enzyme is present at all stages of development. Evidence for the presence of isozymes of pectinesterase was presented (Pressey and

Avants, cited by Pressey (1974) stated that the presence of multiple forms of pectinesterase in tomatoes and variation in their levels during ripening might suggest specialized roles for the enzymes.

Polygalacturonase (PG) acts to depolymerize or shorten the chain length of the esterified pectin (Ng 1976). Hobson (1964) found no PG activity in green tomato fruits. The activity rose exponentially to the time of the orange stage and increased further to the red stage. Enzyme activity continued to rise as fruit became overripe. Babbit et al. (1973) did not find PG activity in developing tomato fruits until after the fruit had initiated ripening. PG activity was detected at the onset of the climacteric and then increased rapidly. Increases of PG activity by a factor of 10 during tomato fruit ripening were found by McClendon et al. (1959). Hobson (1964) reported an increase in PG activity of up to 200 fold as the tomato fruits pass from the green to the red stage. McColloch et al. cited by Pressey (1974), were able to show a close relationship between the level of red color and the level of PG activity in the tomato fruits. Very similar results were presented by Hobson (1964). Thus PG activity closely followed the normal sequence of coloration, suggesting that the enzyme is intimately connected with the ripening mechanism.

PG activity in tomato is highest in the outer locule wall of the pericarp tissue, followed by the inner locule

walls and the placental tissue (Hobson 1964). No PG activity has been detected in the locular contents (Pressey 1974). Reduced PG activity was found in areas of the tomato fruit affected with "blotchy ripening" (Hobson 1963). A relationship between PG activity and firmness of fruits was demonstrated in tomatoes by Hobson (1965).

Color and Ripening

The color of the mature tomato fruit can be largely attributed to its carotenoid pigments. These compounds increase markedly during ripening while chlorophyll is lost (Spurr 1976). The common red tomato contains three predominant carotenoids, i.e., lycopene, beta-carotene, and gamma-carotene (Tomes 1969, Harris and Spurr 1969). Lycopene accounts for about 90% of the carotenoid fraction (Harris and Spurr 1969). McCollum (1955) showed that color depends upon the content of total carotenoids and the ratio lycopene to carotene. Kattan (1957) found that the stage of harvest modifies the final expression of color in the tomato fruits. Thus, fruit harvested at the mature-green stage did not attain as high color as fruits of other ripening stages.

Khudairi (1972) affirmed that the process of color change in tomatoes during ripening is triggered by red light, followed by a chain of biochemical reactions within the color apparatus (chloroplast-chromoplast), which include

ethylene production, chlorophyll degradation, oxygen uptake, abscisic acid synthesis, and the enzymatic transformation of carotenes to red color.

Kirk and Tilvery-Basset, cited by Spurr (1976), note that in the mature green fruit the plastids are chloroplasts, and as the fruit ripens, they are transformed into chromoplasts.

Tomato Ripening Mutants

The term ripening mutants has been used for single gene mutations with multiple effects on tomato ripening (Tigchelaar 1978). Before the discovery of the ripening mutants, a number of mutants that simply altered the ability to synthesize specific carotenoids were described (Tigchelaar et al. 1978a), but the other ripening processes occurred normally (Mizrahi et al. 1976).

Three ripening mutants have been described: Never ripe (Nr), ripening inhibitor (rin), and non-ripening (nor).

The Nr mutant was found by Harris, cited by Tigchelaar (1978) in a chimera fruit. Part of the tissue was heterozygous for Nr. The Nr mutant is dominant and affects the intensity of fruit pigmentation (Rick 1956). Polygalacturonase activity is reduced and, consequently, the softening rate (Hobson 1967). This gene does not totally inhibit ripening but rather retards the onset of ripening and attenuates the magnitude of specific ripening changes

(Tigchelaar et al. 1978a). Hobson (1980) reported that Nr produced fruits with high acid and low sugar contents. The fruits exhibited a slow ripening and were very firm. Also there were very low activities of PG, phosphofructokinase, and NADP-malic-enzyme in fully developed fruits. The low enzymatic activities offer an explanation for the weak climacteric respiration rise and slow ripening of Nr fruits (Hobson 1980).

The mutant rin, described by Robinson and Romes (1968), was found as a spontaneous mutation in a F_4 line developed by H. M. Munger from a cross between 'Fireball' and his breeding line 54-149. Robinson and Tomes (1968) found that the gene affected color expression. The fruits were green when normal fruits turned red. The mutant fruit became yellow latter in development. Also, Robinson and Tomes (1968) found that the fruits were firmer when compared with normal fruits and that the storage life was extraordinarily increased.

Herner and Sink (1973) and McGlasson et al. (1975a) reported that rin is a non-climacteric mutant. Herner and Sink (1973) classified the mutant as non-climacteric since a) it lacks a respiratory climacteric and a rise in ethylene production for up to 120 days from harvest, b) its response to exogenous ethylene resulted in enhanced respiratory activity only while ethylene was present, c) it showed repeated stimulations of CO_2 evolution by repeated ethylene

treatments, and d) it responded to the ethylene-analogue propylene where CO_2 production was stimulated but ethylene production was not. Herner and Sink (1973) concluded that the rin tomato mutant lacks the capacity for autocatalytic production of ethylene.

Other characteristics exhibited by rin in relation to ripening include extended shelf life (Tigchelaar et al. 1978a), normal pectinesterase activity (Buescher 1977), reduced carotene content (Tigchelaar et al. 1978a), reduced beta-carotene content (Tigchelaar et al. 1978a), and trace amounts of lycopene (Tigchelaar et al. 1978a).

Poovaiah and Nukaya (1979), who followed cell wall degrading enzymes during development of normal and isogenic rin fruits, found no change in PG activity up to 100 days postanthesis in the rin mutant and that short-term ethylene treatment in rin increased cellulase activity but had no effect on PG activity. They suggested that the failure of rin fruits to ripen was related to their low PG activity during maturity.

The fruit ripening mutant nor was described by Tigchelaar et al. (1973). It was found in an accession designated 'Italian Winter' obtained from W. A. Kerr, Vineland Station, Ontario, Canada. The mutant was classified as a recessive gene non-allelic to rin. It exhibited the following characteristics: a) absence of softening during fruit maturation, b) high level of crack resistance, and c) abnormal carotene biosynthesis with orange

pericarp and reddish orange locular jelly and placental tissue.

Ng and Tigchelaar (1977) stated that in many respects the nor mutant resembles rin. Both fail to undergo fruit softening or normal chlorophyll degradation, and both exhibit a prolonged storage life.

Ng (1976) concluded that homozygous fruits of nor exhibit a ripening pattern typical of non-climacteric fruits. They lack a respiratory climacteric and concomitant rise in ethylene at a time when normal fruits undergo ripening. This condition persists up to 110 days after anthesis, twice the time required for normal fruits to mature and ripen. Color development occurs slowly over this period of time, and the fruit eventually attain a yellow appearance with a blush of red color at the blossom end. PG activity is present only in trace amounts in the fruit, and little softening occurs during what would constitute the ripening period of a normal fruit. Ng and Tigchelaar (1977) noted that phytoene, beta-carotene, and neurosporene were the major carotenoids in fruits of this mutant, but in very mature fruits there was some lycopene development, although the content was less than 10% of normal fruits.

In spite of the fact that the non-climacteric ripening mutants rin and nor have been reported to be recessive (Robinson and Tomes 1968, Tigchelaar et al. 1973), it has been demonstrated that both mutants exhibit alteration of

several of the ripening processes in heterozygous condition (Buescher et al. 1976, Tigchelaar et al. 1978a). Compared to the normal ripening parents, the hybrids have delayed softening, lower pectolytic enzyme activities, and decreased solubilization of pectic substances (Tigchelaar et al. 1978a). Ng (1976) reported that ripening was delayed and shelf life was improved to a greater extent in nor heterozygotes than in similar rin heterozygotes. Similar results were found by Buescher et al. (1976) in that fruits produced from the nor heterozygotes displayed more than twice the storage life of the normal parent.

Tigchelaar et al. (1978b) showed that the mutants rin and nor each contributes additively as heterozygotes to several aspects of ripening: time from anthesis to respiratory peak, ethylene peak, carotene production, and polygalacturonase activity. The authors suggested that the mode of action of the nor and rin mutants is not directly through endogenous ethylene but probably via a slow change which precedes the ethylene rise and other concomitant ripening changes. The researchers proposed that PG synthesis or activation may represent the primary genetic event which is inhibited in the mutants and attenuated in mutant heterozygotes.

Ng and Tigchelaar (1977) reported that the ripening of nor and heterozygous nor resembles the ripening of normal tomatoes stored under low oxygen atmospheres, suggesting

that ripening is inhibited in the nor mutant by the limited availability of oxygen at sites of active oxidation within the tissue.

The absence of PG activity in nor and rin, its attenuation in the mutant Nr, and its lowered activity in normal fruits with the physiological disorder "blotchy ripening," suggested to Ng and Tigchelaar (1977) that this enzyme plays a vital role in the initiation of the ripening process. Strand et al. (1976) suggest that the absence of other ripening changes in the mutant fruits may be secondary effects which may occur as a result of release of cell-wall-bound enzymes by PG.

Continuous application of the ethylene analogue oleofin propylene to rin mutant tomatoes stimulated respiration in immature fruits. Although respiration reached rates similar to those during the climacteric of normal ripening fruits, there was no change in endogenous ethylene production, which remained at a low level. This suggests that the onset of ripening in normal tomato fruit is not controlled by endogenous ethylene, although increased ethylene is probably an integral part of the ripening process (McGlasson et al. 1975a).

By using reciprocal transplanting of tissues between normal fruits and the mutants rin and nor (Mizrahi et al. 1975d) and reciprocal grafting between normal genotypes and the ripening mutants rin and nor (Mizrahi et al. 1975c), it was concluded that the fruits of the mutants do not

contain translocatable ripening inhibitors or lack translocatable ripening factors. However, the possibility of non-translocatable inhibitors in the mutants was not ruled out. More likely, however, is that the fruits lack the capacity to produce a key cellular component essential for ripening.

Mizrahi et al. (1975a) found that applications of ethephon or ethylene gas to attached rin fruits induced ripening as measured by lycopene development, fruit softening, increased total soluble solids, and tomato flavor. However, rin fruits did not reach the fully ripe stage of normal tomato. Lycopene was 29% of fully ripe normals. Buescher (1977) found that the intensity of ethephon-induced color in rin fruits was dependent on the stage of maturity, elapsed time between harvest and treatment, and ethephon concentration.

Mizrahi et al. (1975b) concluded that endogenous levels of ABA and cytokinins did not account for the lack of ripening in the rin fruit. Suwan and Poovaiah (1978) reported that the rin mutant contains higher levels of bound Ca during advanced stages of fruit development compared to normal fruits, which suggests more preserved membranes, a fact that may contribute to the delay in senescence observed in the rin mutant fruit. Poovaiah et al. (1975) recorded that rin did not show changes in water permeability through cellular membranes, but after the ripening process had been initiated in normal strains, there was an increase in the hydraulic

permeability of cell membranes which occurred after other parameters of ripening had already been altered. The above results were interpreted to mean that the increases in permeability are not causes of the onset of fruit ripening but rather may be involved in the progressive changes involved in ripening. Gonzalez et al. (1976) found similar free methionine levels in rin and normal isogenic tomato fruits, suggesting that the lack of ripening of rin fruits was not due to low methionine levels.

'Alcobaca' Tomato

'Alcobaca' tomato was first described by Almeida (1961) as a material with long fruit storage life, found in the Alcobaca region in Portugal. Leal and Shimoya (1973a) reported that 'Alcobaca' plants have excellent vegetative growth which show more intense leaf color than other cultivars. It has a potato-type leaf. The fruits are yellow and multilocular. The fruits remain on the plant in perfect conditions longer than fruits of other cultivars. After a long period of storage they gradually begin to lose turgidity without becoming rotten and, in the final stage, they become mummified. Long tomato shelf life for this material has been reported by a number of researchers (Almeida 1961, Leal and Liberal 1971, Leal and Shimoya 1973b, Leal and Tabin 1974, Leal and Mizubuti 1975). Leal and Mizubuti (1975) described a variant from the yellow standard 'Alcobaca' which exhibit reddish fruits.

Leal and Mizubuti (1975) produced a series of reciprocal crosses between yellow 'Alcobaca' and normal materials, and conducted studies of fruit storage life. The researchers concluded that 'Alcobaca' is a genotype that exhibits long shelf life. Differences in storage life between direct and reciprocal hybrids were not found. The authors suggested that the inheritance of the long shelf life displayed by the fruits of 'Alcobaca' was quantitative in nature.

Leal and Shimoya (1973b, 1973c) conducted anatomical studies on the pericarp tissue of 'Alcobaca' through storage of the fruits at room temperature conditions. The authors reported that many structural changes take place in the pericarp region of the fruit as time of storage increased, which may account for the prolonged fruit shelf life of this material.

Tigchelaar et al. (1976) noted that 'Alcobaca' is an aberrant ripening cultivar phenotypically distinct from both rin and nor, and suggested, based on unpublished data of allelism tests, that 'Alcobaca' may carry a third allele at the nor locus. Kopeliovitch et al. (1980) did not agree with the above allelism relationship but did not present data to support their argument.

Kopeliovitch et al. (1980) studied several physiological parameters of 'Alcobaca' in comparison to a normal material. They found that the 'Alcobaca' fruits ripened on the vine were similar to those of the variety 'Rutgers' in flavor, pH, concentration of total soluble solids, titrable

acidity, and reducing sugars. The pattern of respiration and ethylene evolution was similar in the two genotypes, but the peak levels were reduced and delayed in time in the mutant 'Alcobaca'. The mutant fruits differed from normal in their prolonged shelf life, their relatively low PG and polymethylgalacturonase activities, and their low level of endogenous ethylene. Also, the authors reported that fruits of the mutant harvested before the onset of ripening failed to reach normal pigmentation and remained yellow; fruits harvested at the onset of the ripening reached an orange color, whereas fruits ripened while attached to the plant reached almost normal pigmentation.

MATERIALS AND METHODS

Pedigree of Studied Materials

Seed of 'Alcobaca' and 'Chonto' were obtained from the Colombian tomato breeding program of the Instituto Colombiano Agropecuario (ICA). 'Alcobaca' was introduced to Colombia from the University of Vicosa (Brazil). 'Chonto' is a Colombian material registered in the tomato breeding program of ICA as MBT-1. The genotypes 'FTE #5', 'FTE #12', 'Tempo', 'Duke', and 'Castlex 1035' are commercial hybrids. Pedigrees and identification of the materials from the University of Florida tomato breeding program are included in Table 1.

Storage Life Studies

Four different experiments were conducted: a) preliminary observation, b) fruit storage life of normal ripening materials in comparison to 'Alcobaca', c) fruit storage life of hybrids between normal ripening tomatoes and the mutants rin, nor, and 'Alcobaca', and d) fruit storage life of hybrids between 'Alcobaca' and the ripening mutants rin and nor.

For the preliminary observation, staked tomato plants were grown on raised beds of sandy soil and covered with plastic mulch at the Agricultural Research and Extension Center at Bradenton during the fall of 1978. Fruits were picked at the "turning" stage for the normal ripening

Table 1. Identification and pedigree of experimental materials included in the study.

Name	Pedigree
'Florida 1C'	U.F. 645
'Hayslip'	U.F. 648
'Burgis'	U.F. 71057
'Florida 1B'	U.F. 631
'Tropic'	(479-6-1 X 18-D2-D4-8-1) _{F₁₁}
'Walter'	1544-3-4-1-Bk-Bk-Bk
'Walter PF'	U.F. 740985-3
'Florida MH-1'	2431-1-1-SpBk-SpBk
'Flora-Dade'	U.F. 908
<u>rin</u>	'East Ithaca'
<u>rin-1</u>	(<u>rin</u> X 'Florida MH-1') _{F₈}
<u>nor</u>	'Italian Winter'
<u>rin</u> F ₁	U.F. 648 X 'East Ithaca'
<u>rin-1</u> F ₁	U.F. 648 X <u>rin-1</u>
<u>nor</u> F ₁	U.F. 648 X 'Italian Winter'
'Hayslip' X <u>rin</u>	U.F. 648 X 'East Ithaca'
'Burgis' X <u>rin</u>	U.F. 71057 X 'East Ithaca'
'Hayslip' X <u>nor</u>	U.F. 648 X 'Italian Winter'
'Florida 1C' X <u>nor</u>	(U.F. 645 X A816)
A816	('Walter' X 'Italian Winter') _{F₃}

materials as well as for the 'Alcobaca' tomato mutant, and at the inception of the yellow color for the ripening mutants rin and nor. The fruits were transported to Gainesville and, 24 hours after harvest, were rinsed with running water, air dried, reclassified for ripening stage, individually weighed, and stored on plastic trays in a ripening room at 20°C. Loss of fruit weight was monitored twice weekly, and those fruits exhibiting decay or excessive softening were discarded.

For the experiments b, c, and d, above, unstaked tomato plants were grown in plastic mulch beds at the University of Florida Horticultural Unit during the spring of 1980 in a randomized block design with four replicates. Each experimental unit was composed of 3 plants in a row. Fruits were harvested at the breaker stage for those genotypes that exhibited a normal color-change pattern, and at the inception of the yellow color for the mutants rin, rin-l, nor, and the hybrid nor X 'Alcobaca'. Within 24 hours, fruits were rinsed with running water, air dried, and selected for uniformity of ripening. Nine to 10 fruits of each genotype and each replication, were selected with the exception of the hybrids between 'Alcobaca' and other ripening mutants (study d), in which case each of the samples was composed by 5 to 6 fruits (in each replication). The selected fruits were completely randomized on plastic trays and were stored in a ripening room at 20°C. The fruits having decay or excessive softening were discarded every 5 days. Fruit weight loss was evaluated every 10 days for study b, every 5 days for study c, and

every 10 days up to 30 days and then every 15 to 20 days for experiment d.

Two measures of fruit shelf life were obtained: accumulative storage index (ASI) and mean storage life (MSL), where

$$ASI = \Sigma \frac{\text{Percentage of remaining fruits} \times \text{days in storage}}{100}$$

and MSL equals the number of days required for 50% of the fruits to be discarded. ASI was calculated from the percentage of fruits remaining in storage every 10 days for studies b and c, and at 10, 20, 30, 45, 60, and 80 days for the experiment d.

For analysis of variance, the percentage of discarded fruits and the percentage of fruit weight loss were transformed by using the angular or inverse sine transformation which is applicable to data expressed as percentages. This is particularly recommended when the percentages cover a wide range of values (Steel and Torrie 1960). Duncan's multiple range test with 95% of confidence was applied to compare the mean values.

Color Determination

The color of the fruits was evaluated with a Hunter Color and Color Difference Meter. The results reported are the ratio a/b, where the higher the value, the more red the fruit (Hall 1964). A white plate with values L = 94.9, a = -1.2, and b = 2.2 was employed as a standard for calibration.

Unstaked tomato plants were grown in plastic mulch beds at the University of Florida Horticultural Unit during the

spring of 1980. Fruits of all the genotypes were harvested the same date at the mature-green stage and stored at 20°C. After two days in storage, fruits at the inception of the yellow color for the ripening mutants rin and nor, and at the breaker stage for the remaining genotypes, were selected. Fruits were allowed to attain yellow color for the mutants rin and nor, reddish color for nor X 'Alcobaca' hybrid, and red color for the remaining genotypes. The above colors are considered to correspond to a fully ripened stage.

Five samples of each genotype of 3 to 5 randomly selected fruits were analyzed. The fruits were sectioned and the stem scar was eliminated. They were then blended in a Waring blender and filtered through a double layer of cheesecloth. An anti-foam agent was added to the juice, and the samples were deaerated by partial vacuum. The deaerated juice was used to measure color.

Firmness of the Fruits

Firmness was determined with the Cornell Pressure Tester (Hamson 1952b), using a 1.9 cm pressure plate and a load of 2000 g for 5 seconds. Pressure was applied to the equatorial section of the fruit, with only one determination for each fruit. Care was taken to align the fruit so that the plunger was applied over an inner wall as judged by external appearance (Hall 1964). The range of the Cornell Pressure

Tester is 0 to 7, where the higher the value, the softer the fruit (Hall 1964).

Unstaked tomato plants were grown in plastic mulch beds at the University of Florida Horticultural Unit during the spring of 1980. Fruits of all genotypes were harvested at the mature-green stage on the same date. They were stored at 20°C and re-sorted when they reached the breaker stage. The selected fruits were allowed to attain red color for normal genotypes, reddish for 'Alcobaca' and the nor X 'Alcobaca' hybrids, and yellow color for rin and nor. At these stages the first determination of firmness was done on 5 randomly chosen fruits for each genotype. Subsequent determinations were done 1 and 2 weeks later.

Respiration Studies

Five fruits of each genotype were harvested at the mature green stage from plants growing in the greenhouse. The fruits were rinsed with running water, air dried, individually weighed, and stored on plastic trays in a ripening room at 20°C.

To determine the respiration, the fruits were enclosed daily in wide-mouth 500 ml glass jars supplied with a constant flow of atmospheric air at a rate of 50 ml per min. The evolved CO₂ was monitored after 20 min. by passing the effluent air stream through an infrared analyzer Beckman Model 215. The results were recorded by a 24-point

Westronic recorder. A sample of the atmospheric air was monitored daily to correct the obtained readings for CO₂ content in the air circulating through the system.

Respiration was expressed as milliliters of CO₂ produced per kilogram of original fresh weight per hour.

Ethylene Evolution

Ethylene was determined by using a Hewlett Packard 4710A gas chromatograph with a flame ionization detector. A standard of 1 ppm ethylene in nitrogen was used daily to calibrate the results.

Five fruits of each genotype were harvested at the mature-green stage from plants grown in a greenhouse, rinsed with running water, air dried, individually weighed, and stored in a ripening room at 20°C. Ethylene evolution was determined every 24 hours by placing the fruits inside 500-ml glass jars with a rubber stopper in the lid. After 20 min, 0.5 ml of volume was withdrawn from the jar and injected in the gas chromatograph. Ethylene was expressed as microliters of ethylene per kilogram of original fresh weight per hour.

Polygalacturonase Activity

Five fruits of each genotype from plants grown in the greenhouse were harvested at the mature-green stage, stored at 20°C, and sorted for ripening at the breaker stage. After

5 days, equatorial sections of the outer pericarp were obtained and stored at -30°C .

The stored samples were weighed and then macerated with pestle and mortar in an aqueous solution of 5% NaCl and 1% polyvinylpyrrolidone. Three milliliters were used per gm of tissue. The solution was adjusted to pH 9.0, filtered through a double layer of cheesecloth, and after 30 min was centrifugated in a Sorvall Superspeed RC2-B centrifuge with an SS-34 rotor at 10000 rpm for 10 min. Polygalacturonase (PG) activity was determined by a modified method of the one described by Babbitt *et al.* (1973), as described below.

One milliliter of the enzyme extract was added to 5 ml of a 1.2% solution of polygalacturonic acid grade III (Sigma), buffered to pH 5.0 with 0.1 M sodium acetate in a Cannon #200 viscometer held at 31°C in a water bath. Initial readings of viscosity were obtained within 1 min of mixing. The change in viscosity was measured after 30 min. Activity was expressed as percentage loss in viscosity. For statistical analysis the percentage loss in viscosity was transformed by using the angular or inverse sine transformation which is applicable to data expressed as percentages (Steel and Torrie 1960).

Model To Relate Storage Life with Different Physiological Parameters

A multiple regression model to determine the relationship of storage life, measured as ASI, and different

ripening parameters was carried out with the aid of the Northeast Regional Data Center in Gainesville. Two different procedures were used: R-square and Stepwise (SAS Institute 1979).

Genetic Studies

Inheritance Study

In the study of inheritance of the abnormal ripening of 'Alcobaca', the normal ripening material 'Florida IC' was used as a parent. The populations used were 'Florida IC' (P_1), 'Alcobaca' (P_2), 'Florida IC' X 'Alcobaca' (F_1), 'Alcobaca' X 'Florida IC' (RF_1), F_1 X 'Florida IC' (BC_1), F_1 X 'Alcobaca' (BC_2), and 'Florida IC' X 'Alcobaca' F_2 (F_2).

These materials were grown unstaked at the Horticultural Unit during the spring of 1980. A randomized block design with 5 replications was used. The experimental units for the non-segregating populations P_1 , P_2 , F_1 , and RF_1 , were composed of 10 plants in a row, giving a total of 50 plants per genotype. The plots of the backcrosses (BC_1 and BC_2) had 20 plants each, giving a total of 100 plants per backcross. The F_2 population plots were composed of 50 plants each, giving a total of 250 plants.

Fruits were harvested individually from each plant, and, based on the ripe fruit color, the plants were classified as normal or mutant. Also, individual fruits were harvested at the breaker stage, rinsed with running water, air dried,

and stored at 20°C to study the inheritance of the storage life as described below. A goodness of fit test (χ^2) was applied to test the genetic ratios.

Functional and Recombinational Genetic Tests

Allelism tests were conducted between 'Alcobaca' and the ripening mutants rin and nor.

For recombinational analysis, plant materials consisted of 20 plants for non-segregating materials (parents and F_1), and 80 plants for the F_2 generation. Fruits were harvested from each plant and allowed to ripen at 20°C. Plants were then classified as normal or mutant for fruit ripening.

In the allelism test of nor by 'Alcobaca', fruits of the F_1 and F_2 were allowed to obtain maximum color expression in the field since it was evident that the full expression of color was affected by the ripening stage at harvest. Tests of goodness of fit (χ^2) were applied to test the genetic ratios.

Inheritance of Storage Life

Two to 3 fruits from the inheritance study of the abnormal fruit ripening of 'Alcobaca' were harvested by plant, as described previously, and stored at 20°C. Every 5 days the fruits exhibiting decay or excessive softening were discarded and the number of days in storage for each plant for the populations P_1 , P_2 , F_1 , RF_1 , BC_1 , BC_2 , and F_2 was recorded.

The resultant data were fitted to an additive-dominance model including three parameters: m (population mean), h (dominance effect), and d (additive effect), by applying the scaling test, as proposed by Mather (Mather and Jinks 1971), as well as a joint scaling test (Cavalli, cited by Mather and Jinks 1971).

The minimum number of gene pairs differentiating the parents was estimated by both the Castle and Wright (1921) and the Sewall Wright methods (Burton 1951). These methods assume that there is no linkage, one parent contributes only plus factors and the other only minus factors, all genes are equally important, degree of dominance is the same for all plus factors, and no epistasis (Ibarbia and Lambeth 1969). In addition, it was assumed in the present study that all the genotypes were equally affected by decay.

RESULTS

Storage Life Studies

Preliminary Observation

The objective of this experiment was to compare the storage life of the abnormal ripening tomato 'Alcobaca', the ripening mutants rin and nor, hybrids of these mutants with normal ripening tomatoes, and the normal ripening varieties 'Chonto', 'Walter', and 'Flora-Dade'.

The storage life of the genotypes used in the preliminary study are presented in Table 2. Among the normal varieties, and at 30 days in storage, 'Chonto' and 'Walter' exhibited more remaining fruits than did 'Flora-Dade'. At 50 days 'Flora-Dade' displayed the best storage life of the normal genotypes examined. At this time 40% of this material still remained in good condition in comparison to 12 and 24% of 'Walter' and 'Chonto' respectively. At 90 days there were no remaining fruits of the normal ripening varieties. Differences in percentage of remaining fruits between normal ripening genotypes and the ripening mutants rin, nor, and 'Alcobaca' were evident after 50 days in storage. The percentage of remaining fruits of the genotypes rin, rin-1, nor, and 'Alcobaca' was quite similar after 30 and 50 days in storage. At 90 days rin-1 had more remaining fruits in

Table 2. Storage life of normal ripening varieties, ripening mutants, and their hybrids at 20°C. (Fruits picked at turning stage.)

Genotype	Initial sample size	Percentage of remaining fruits			
		Days after storage			
		30	50	57	166
'Walter'	17	82	12	0	0
'Chonto'	25	80	24	0	0
'Flora-Dade'	10	50	40	0	0
<u>nor</u>	21	90	85	57	5
<u>nor F₁</u>	17	100	59	24	0
<u>rin</u>	25	96	92	64	4
<u>rin F₁</u>	16	81	38	0	0
<u>rin-l</u>	23	96	87	78	4
<u>rin-l F₁</u>	11	82	37	9	0
'Alcobaca'	30	90	83	60	5

²Pedigrees included in Table 1.

storage than nor, rin and 'Alcobaca'. At 166 days no differences were found in percentage of remaining fruits between rin, rin-1, nor, and 'Alcobaca' with 4, 4, 5, and 3% of the fruits of these genotypes remaining in storage.

Fruit shelf life was improved to a greater extent in the nor heterozygotes than in the rin and rin-1 heterozygotes. After 30 days, 100% of the nor hybrid fruits remained in good condition in comparison to 81 and 82% of the rin and rin-1 hybrids. At 50 days 59% of the nor F_1 fruits were in good condition in comparison to 38% rin F_1 and 37% rin-1 F_1 remaining fruits. After 90 days 24% of the fruits of the nor heterozygote remained in comparison to 0 and 9% from rin and rin-1 heterozygotes.

Fruit weight loss during storage is included in Table 3. Significant correlations between storage life as percentage of remaining fruits and percentage of weight loss were not detected after 30 and 50 days in storage ($r=0.17$ and $r=-0.25$) respectively, but was detected after 70 days ($r=-0.98$).

Storage Life of Commercial Varieties and 'Alcobaca'

The storage life of the commercial varieties, experimental breeding lines, and 'Alcobaca' mutant used in the study is presented in Table 4. Significant differences in percentage of remaining fruits were found after 20 days in storage. At this time 'Florida 1B', 'Burgis', 'FTE #12', and 'Duke' exhibited a significantly reduced number of

Table 3. Fruit weight loss during storage of different tomato genotypes at 20°C.

Genotype	Initial sample size	Percentage of fruit weight loss			
		Days after storage			
		20	30	50	70
'Walter'	17	3	5	8	*
'Chonto'	25	5	6	10	*
'Flora-Dade'	10	5	7	10	*
<u>nor</u>	21	5	7	8	14
<u>nor F₁</u>	17	7	11	15	23
<u>rin</u>	25	4	6	8	12
<u>rin F₁</u>	16	4	6	10	*
<u>rin-1</u>	23	3	4	6	9
<u>rin-1 F₁</u>	11	5	7	10	13
'Alcobaca'	30	5	7	10	16

*No fruit.

Table 4. Storage life of commercial varieties, breeding lines, and 'Alcobaca' held at 20°C. (Fruits picked at the breaker stage.)

Genotypes	Percentage of remaining fruits ^x						
	Days after storage						
	10	20	30	40	50	60	MSL ^z
'Tropic'	100.0a	95.0ab	45.0ef	5.0cd	5.0cd	2.5c	44.5cd 23.5d
'Walter PF'	100.0a	90.0abc	40.0ef	2.5d	2.5d	0.0d	41.0d 25.7cd
'Florida MH-1'	100.0a	95.0ab	65.0bcd	2.5d	0.0e	0.0d	50.5cd 30.7bc
'Flora-Dade'	100.0a	92.5abc	72.5b	42.5b	40.0b	17.5b	97.7b 35.5b
'Florida 1c'	100.0a	95.0ab	60.0cd	0.0e	0.0e	0.0d	47.0cd 26.0cd
'Florida 1b'	100.0a	60.0d	25.0g	0.0e	0.0e	0.0d	29.5e 23.2d
F1 2432 (ug MH-1)	100.0a	95.0ab	35.0fg	15.0c	10.0cd	0.0d	50.5cd 23.2d
'Burgis'	100.0a	85.0bcd	60.0cd	15.0c	15.0c	5.0c	61.5c 28.5cd
'Hayslip'	100.0a	87.5abc	60.0cd	10.0cd	10.0cd	0.0d	53.0cd 28.2cd
'FTE #5'	100.0a	87.5abc	52.5cd	5.0d	5.0d	2.5c	49.2cd 28.0cd
'FTE #12'	100.0a	82.5cd	45.0ef	12.5c	12.5c	2.5c	52.7cd 25.7cd
'Duke'	100.0a	82.5cd	40.0ef	12.5c	12.5c	5.0d	52.7cd 23.2d
'Tempo'	100.0a	100.0a	70.0bc	0.0e	0.0e	0.0d	51.0cd 31.0bc
'Castlex 1035'	100.0a	92.5abc	75.0b	30.0b	30.0b	17.5b	88.5b 31.0bc
'Alcobaca'	100.0a	100.0a	95.0a	67.5a	67.5a	52.5a	150.7a >60.0a

^xMean separation within columns by Duncan's multiple range test at the 5% level.

^yAccumulative storage index.

^zMean storage life.

remaining fruits. At 30 days in storage and thereafter, 'Alcobaca' exhibited a higher percentage of remaining fruits than did the normal ripening materials. After 30 days in storage, the normal ripening materials with the longest storage life were 'Flora-Dade', 'Tempo', and 'Castlex 1035'. At 40 days no fruits remained of 'Tempo'. From 40 to 60 days, the normal genotypes 'Flora-Dade' and 'Castlex 1035' exhibited a significantly higher percentage of remaining fruits than did the other normal ripening materials.

Significant differences in accumulative storage index (ASI) and mean storage life (MSI) were detected between 'Alcobaca' and the remaining genotypes. The normal materials 'Flora-Dade' and 'Castlex 1035' displayed the greatest ASI among the normal genotypes with values for ASI of 97.7 and 88.5. The normal genotypes with higher MSL were 'Flora-Dade', 'Castlex 1035', 'Tempo', and 'Florida MH-1'.

The greatest weight loss after 10 days of storage was exhibited by the genotype 'Florida 1B' (Table 5). After 25 days 'Florida 1C', 'Florida 1B', 'Fl 2432', and 'Burgis' displayed the greatest weight loss. At 40 days the genotypes 'Fl 2432', 'Duke', and 'Castlex 1035' exhibited the greatest weight loss. No significant correlations were found between percentage of remaining fruits and fruit weight loss.

Table 5. Fruit weight loss of commercial varieties, breeding lines, and 'Alcobaca' held at 20°C.

Genotype	Percentage of weight loss ^z		
	Days after storage		
	10	25	40
'Tropic'	1.98ab	3.55bc	**
'Walter PF'	2.15abc	3.86cd	**
'Florida MH-1'	1.32a	2.25a	**
'Flora-Dade'	1.67ab	2.52ab	5.15a
'Florida 1C'	2.09abc	4.88d	*
'Florida 1B'	3.22c	5.01d	*
FL 2432 (ug MH-1)	1.92ab	5.35d	8.32d
'Burgis'	2.53bc	3.96cd	6.64b
'Hayslip'	2.20abc	3.14abc	6.60b
'FTE #5'	2.46bc	4.09cd	**
'FTE #12'	2.06ab	3.40bc	6.51bc
'Duke'	2.10abc	2.77ab	7.37cd
'Tempo'	2.65bc	3.32abc	*
'Castlex 1035'	2.22abc	4.35cd	7.11cd
'Alcobaca'	2.31abc	3.24abc	5.83ab

^zMean separation within columns by Duncan's multiple range test.

*All fruits discarded.

**Less than 10% of fruits remaining.

Fruit Storage Life of Hybrids
with Ripening Mutants

The experiment was carried out to evaluate the potential use of hybrids involving ripening mutants and normal materials in order to improve the fruit shelf life. Also, the phenotypic appearance of the hybrid genotypes was monitored.

Significant differences in storage life appeared 20 days after storage as shown in Table 6. At this time the materials with the highest percentage of remaining fruits were the 'Florida 1C' X nor and 'Hayslip' X rin hybrids. At 30 and 40 days the 'Florida 1C' X nor hybrid had a significantly higher percentage of fruits remaining in storage than the other studied genotypes. After 50 days no differences in percentage of remaining fruits were detected between the 'Florida 1C' X nor and 'Hayslip' X nor hybrids. At this time the above hybrids exhibited the greatest percentage of remaining fruits. From 60 days on the 'Hayslip' X nor hybrid had the greatest percentage of fruits remaining in storage. Differences in percentage of remaining fruits between the studied hybrids and their normal parents were evident after 20 days in storage.

The ASI was improved 3 to 4 fold and the MSL 2 to 3 fold in the hybrids with nor in comparison to the normal parents. The hybrids with 'Alcobaca' had an ASI and MSL with 2 to 3 and 2 fold, respectively, over the normal parents

Table 6. Storage life of hybrids between normal ripening materials and ripening mutants held at 20°C. (Fruits picked at the breaker stage of maturity.)

Genotype	Percentage of remaining fruits ^z							ASI ^{xz}	MSL ^{yz}
	Days after storage								
	10	20	30	40	50	60	70		
'Burgis' <u>rin</u>	100.0a	89.1bc	56.6bc	34.7cd	14.7cd	9.0de	3.7de	74.0bc	32.7b
'Hayslip' X <u>rin</u>	100.0a	94.1ab	76.4b	49.6bc	22.9c	11.8d	6.2cd	96.7b	33.7b
'Florida lc' X <u>nor</u>	100.0a	100.0a	100.0a	87.5a	50.0a	37.5b	37.5b	164.0a	48.0a
'Hayslip' X X <u>nor</u>	100.0a	75.0cd	75.0b	60.0b	55.0a	50.0a	50.0a	164.2a	52.0a
'Hayslip' X 'Alcobaca'	100.0a	78.7cd	73.7bc	38.7cd	25.0b	17.5cd	11.9c	96.7b	35.0b
'Florida lc' X 'Alcobaca'	100.0a	66.2d	51.2c	45.0bc	33.7b	27.5bc	11.2c	97.9b	36.0b
'Florida lc'	100.0a	62.5d	28.1d	28.1d	12.5d	0.0e	0.0e	42.2d	21.7c
'Hayslip'	100.0a	40.0e	30.0d	25.0e	15.0cd	5.0de	0.0e	47.5cd	18.7c

^xAccumulative storage index.

^yMean storage life.

^zMean separation within columns by Duncan's multiple range test at 5% level.

and the hybrids with rin exhibited a 2- and 1.5-fold improvement of ASI and MSL respectively (Table 6).

As can be seen in Table 7, there were slight differences in fruit weight loss between the hybrid genotypes during the 60 days of storage. Some differences were detected only at 10 days in storage, but these differences disappeared at 15 days. Differences between the hybrids and the normal parents were evident after 30 days of shelf life. No significant correlations were found between percentage of remaining fruits and percentage of fruit weight loss during the current experiment.

Significant differences in fruit color as ratio a/b were found between genotypes (Table 8). The normal ripening material, 'Hayslip', exhibited the highest a/b ratio followed by 'Florida 1C' X 'Alcobaca', 'Florida 1C', and 'Hayslip' X 'Alcobaca'. These genotypes had a/b values above 2.0. The mutant ripening genotypes rin and nor exhibited the lowest a/b ratio value (0.2) followed by 'Alcobaca'. The hybrids between normal ripening parents and the ripening mutants rin and nor had a less intense red color. These genotypes displayed a/b ratio values below 2.0.

Significant variation in fruit firmness was found between genotypes at the red stage and 7 and 15 days after the red stage (Table 8). The fruits of the rin and nor mutants were significantly firmer than the remaining genotypes in all of the dates in which firmness was evaluated. 'Alcobaca'

Table 7. Fruit weight loss during storage of hybrids between normal ripening materials and ripening mutants held at 20°C. (Fruits picked at the breaker stage of maturity.)

Genotype	Percentage weight loss ²					
	10	15	25	30	45	60
'Burgis' X <u>rin</u>	1.6b	2.4	3.3a	3.5a	4.6a	7.3a
'Hayslip' X <u>rin</u>	2.0cd	3.0ab	3.8a	4.3b	5.2a	7.6a
'Florida 1C' X <u>nor</u>	1.6b	2.5a	3.3a	3.5a	4.8a	7.0a
'Hayslip' X <u>nor</u>	2.1d	3.0ab	4.0ab	4.1ab	5.3a	7.2a
'Hayslip' X 'Alcobaca'	2.1d	3.0ab	3.9ab	4.3b	5.4a	7.3a
'Florida 1C' X 'Alcobaca'	1.1a	2.7ab	3.5a	3.9a	4.7a	6.7a
'Florida 1C'	1.8bc	3.3b	4.7b	5.4c	6.9b	*
'Hayslip'	1.9bc	4.8c	6.9c	8.0d	10.2c	*

²Mean separation within columns by Duncan's multiple range test at 5% level.

*No fruits remaining.

Table 8. Fruit color and firmness of normal ripening varieties, ripening mutants, and their parents.

Genotype	Firmness ^{Yz}			
	Color ^{xz} a/b	Red stage	7 days after	15 days after
'Florida 1C'	2.1c	-	-	-
'Hayslip'	2.4a	2.8c	3.3b	2.2e
'Alcobaca'	1.1h	3.0c	3.2b	3.8bc
<u>rin</u>	0.2i	2.4ab	2.6a	2.6a
<u>nor</u>	0.2i	2.2a	2.3a	2.4a
'Burgis' X <u>rin</u>	-	3.4cde	3.9bcd	4.1cd
'Hayslip' X <u>rin</u>	1.8e	3.3cde	3.4b	3.6bc
'Hayslip' X <u>nor</u>	1.8e	3.2cd	3.3b	3.6bc
'Florida 1C' X <u>nor</u>	1.7f	3.2cd	3.6bc	4.1cd
'Hayslip' X 'Alcobaca'	2.0d	3.5cde	3.8bcd	4.4d
'Florida 1C' X 'Alcobaca'	2.2b	3.9e	4.4d	4.4d

^xHigher value indicates a more intense red color.

^yLower value indicates a firmer fruit.

^zMean separation within columns by Duncan's multiple range test at 5% level.

fruits were firmer than those of the hybrid 'Florida 1C' X 'Alcobaca' at the red stage and 7 and 15 days after the red stage. 'Alcobaca' fruits were firmer than those of the 'Hayslip' X 'Alcobaca' hybrid at the red stage and 7 days after the red stage. 'Alcobaca' fruits were firmer than 'Hayslip' X 'Alcobaca' fruits 15 days after the red stage. 'Hayslip' did not differ from the 'Hayslip' X rin, 'Hayslip' X nor, and 'Hayslip' X 'Alcobaca' hybrids in fruit firmness at the red stage. Seven days after the red stage, 'Hayslip' fruits were as soft as those of the hybrid 'Hayslip' X nor. Fifteen days after the red stage 'Hayslip' exhibited the softest fruits of all of the studied genotypes. No significant correlation was found between firmness at the red stage and 7 days after the red stage with storage life, but a significant correlation was obtained between firmness 15 days after the red stage and storage life.

Fruit Storage Life of Hybrids Between 'Alcobaca'
and the Ripening Mutants nor, rin, and rin-1

The experiment was carried out to evaluate the possibility of using hybrids between 'Alcobaca' and other ripening mutants in order to improve the fruit storage life.

Data of percentage of remaining fruits and ASI for the genotypes included in the study are presented in Table 9.

Differences between genotypes for percentage of remaining fruits were detected at 30 days of storage. At this

Table 9. Fruit shelf life of normal ripening varieties, ripening mutants, and hybrids of these mutants with 'Alcobaca' held at 20°C. (Fruits picked at the beaker stage.)

Genotype	Percentage of remaining fruits ^z					
	Days after storage					
	10	20	30	45	60	80
'Florida MH-1' ^x	100.0	95.0	65.0	2.5	0.0	0.0
'Flora-Dade' ^x	100.0	92.5	72.5	42.5	17.5	0.0
<u>nor</u>	100.0a	100.0a	100.0a	100.0a	95.8a	95.8a
<u>rin</u>	100.0a	87.5a	70.8c	70.0cd	48.3c	25.0cd
<u>rin-1</u>	100.0a	90.0a	75.0bc	75.8bcd	41.7c	40.0c
'Alcobaca'	100.0a	91.7a	87.5b	83.3bc	70.8b	66.7b
<u>nor</u> X 'Alcobaca'	100.0a	91.6a	91.6ab	87.5b	83.3b	66.7b
<u>rin</u> X 'Alcobaca'	100.0a	91.6a	79.2b	83.3bc	25.0d	12.5d
<u>rin-1</u> X 'Alcobaca'	100.0a	85.0a	75.0b	65.0d	35.0cd	25.0cd
						97.2c

^xNormal ripening varieties stored at the same time but from a different experiment.

^yAccumulative storage index.

^zMean separation within columns by Duncan's multiple range test at 5% level.

time the ripening mutant nor and the nor X 'Alcobaca' hybrid exhibited the highest percentage of remaining fruits. At 45 days in storage and thereafter nor displayed a significantly higher percentage of remaining fruits than any of the other studied genotypes. 'Alcobaca' had a significantly higher percentage of remaining fruits than did rin and rin-1 at 60 and 80 days in storage. Differences in percentage of remaining fruits between 'Alcobaca' and the nor X 'Alcobaca' hybrid were not found throughout the time of storage. Significant differences in percentage of remaining fruits were detected between 'Alcobaca' and the hybrid rin X 'Alcobaca' after 60 days in storage. Also, significant differences in this respect were found between 'Alcobaca' and the hybrid rin-1 X 'Alcobaca' after 45 days in storage. The normal ripening varieties, 'Florida MH-1' and 'Flora-Dade', had a reduced percentage of remaining fruits after 30 and 45 days, respectively, and thereafter in comparison to the genotypes included in this study. 'Florida MH-1' and 'Flora-Dade' did not belong to the current experiment but they were stored at the same time in the ripening room. It should be noted that 'Flora-Dade' displayed the longest fruit shelf life of 14 normal ripening materials in a previous experiment (Table 4).

Significant differences in storage life as ASI were detected between genotypes. The mutant nor exhibited the highest ASI value. No differences in ASI were detected

between rin and rin X 'Alcobaca' and between rin-1 and rin-1 X 'Alcobaca'. 'Alcobaca' did not differ in ASI from the hybrid nor X 'Alcobaca', but the ASI value of 'Alcobaca' was significantly higher than that of rin X 'Alcobaca' or rin-1 X 'Alcobaca'. The normal ripening varieties 'Florida MH-1' and 'Flora-Dade' displayed a very low ASI in comparison to nor, rin, rin-1, 'Alcobaca', and the hybrids nor X 'Alcobaca', rin X 'Alcobaca', and rin-1 X 'Alcobaca'.

Variation in fruit weight loss was detected between genotypes (Table 10). The mutant rin and the hybrid rin X 'Alcobaca' had the greatest weight loss. Differences in fruit weight loss were found between rin and rin-1 after 20 days in storage. The genotypes that exhibited the lowest fruit weight loss during storage until 80 days were nor, rin-1 X 'Alcobaca', and nor X 'Alcobaca'.

The hybrids rin X 'Alcobaca' and rin-1 X 'Alcobaca' had fruits of normal color with an a/b ratio above 2.0 (Table 11). The hybrid nor X 'Alcobaca' had an a/b ratio 0.7, which was closer to the ratio of 'Alcobaca' (1.1) than that of nor (0.2).

The hybrid fruits nor X 'Alcobaca' were firmer than the ones of 'Alcobaca' 7 and 15 days after the red stage and as firm as those of nor at the red stage and 7 and 15 days after the red stage (Table 11). The rin X 'Alcobaca' fruits were softer than the fruits of its parents (Table 11). The fruits of the hybrid rin-1 X 'Alcobaca' were as firm as the ones of 'Alcobaca' and softer than rin-1 fruits.

Table 10. Fruit weight loss during storage of ripening mutants and hybrids of these mutants with 'Alcobaca'.

Genotype	Percentage of weight loss ^z				
	Days after storage				
	10	20	45	60	80
<u>nor</u> X 'Alcobaca'	2.6c	3.4bc	5.7a	9.1b	13.1ab
<u>rin</u> X 'Alcobaca'	2.3bc	3.3bc	9.5bc	13.6d	17.5c
<u>rin-1</u> X 'Alcobaca'	1.3a	2.4a	6.3a	9.1b	11.2a
<u>nor</u>	1.4a	2.2a	5.4a	6.6a	10.2a
<u>rin</u>	2.4bc	4.3c	9.1c	14.5d	21.1d
<u>rin-1</u>	1.8ab	2.8ab	6.7ab	9.2b	15.2bc
'Alcobaca'	1.5a	3.8c	7.7bc	10.9c	16.1bc

^zMean separation within columns by Duncan's multiple range test at 5% level.

Table 11. Fruit color and firmness of ripening mutants and hybrids of these mutants with 'Alcobaca'.

Genotype	Color ^{xz} a/b	Firmness ^{yz}		
		Red stage	7 days after	15 days after
<u>nor</u> X 'Alcobaca'	0.7e	2.5bc	2.6a	2.8a
<u>rin</u> X 'Alcobaca'	2.0c	3.8d	4.2c	4.5c
<u>rin-1</u> X 'Alcobaca'	2.1b	3.5cd	3.7bc	4.0bc
<u>nor</u>	0.2f	2.2ab	2.3a	2.4a
<u>rin</u>	0.2f	2.4ab	2.6a	2.6a
<u>rin-1</u>	0.2f	1.8a	2.0a	2.4a
'Alcobaca'	1.1d	3.0c	3.2b	3.8b
'Hayslip'	2.4a	2.8c	3.4b	5.2d

^xHigher values indicate a more intense red color.

^yLower values indicate firmer fruit.

^zMean separation within columns by Duncan's multiple range test.

Respiration Study

The genotypes 'Florida 1C', 'Hayslip', and 'Chonto' had a typical climacteric pattern of respiration (Figures 1, 2, 3). The 'Alcobaca' mutant also exhibited a climacteric pattern. This material did not display the sudden rise and fall in respiration of 'Florida 1C', 'Hayslip', and 'Chonto' but rather a smooth rise and gradual reduction (Figures 1, 2, 3, 4, 5, 6). The hybrids between 'Alcobaca' and the normal ripening genotypes 'Florida 1C', 'Hayslip', and 'Chonto' showed a typical climacteric pattern but with a reduced CO₂ peak intermediate between 'Alcobaca' and the normal ripening parent (Figures 1, 2, 3; Table 12).

The rin X 'Alcobaca' and rin-1 X 'Alcobaca' hybrid fruits had a respiration pattern similar to that of the normal ripening genotypes (Figures 4, 5). The respiratory peak of the hybrids was greater than those of 'Alcobaca', rin, and rin-1 (Table 12). The nor X 'Alcobaca' hybrid had an attenuated climacteric respiratory pattern (Figure 6). The respiratory peak of this hybrid was similar to that of 'Alcobaca' (Table 12). The ripening mutants rin, rin-1, and nor displayed a typical non-climacteric pattern or respiration (Figures 4, 5, 6).

Ethylene Evolution

'Florida 1C', 'Hayslip', and 'Chonto' had a typical climacteric pattern for ethylene evolution (Figures 7, 8, 9).

Figure 1. Respiration pattern of 'Alcobaca', 'Florida IC', and 'Alcobaca' X 'Florida IC' fruits.

Figure 2. Respiration pattern of 'Alcobaca', 'Hayslip', and 'Alcobaca' X 'Hayslip' fruits.

Figure 3. Respiration pattern of 'Alcobaca', 'Chonto', and 'Alcobaca' X 'Chonto' fruits.

Figure 4. Respiration pattern of 'Alcobaca', 'rin', and 'Alcobaca' x 'rin' fruits.

Figure 5. Respiration pattern of 'Alcobaca', rin-1, and 'Alcobaca' X rin-1 fruits.

Figure 6. Respiration pattern of 'Alcobaca', nor, and 'Alcobaca' X nor fruits.

Table 12. Tomato fruit maximum respiration, maximum ethylene evolution, and polygalacturonase (PG) activity for different genotypes.

Genotype	Maximum respiration ml X kg ⁻¹ X hour ⁻¹	Maximum ethylene μl X kg ⁻¹ X hour ⁻¹	PG activity ^z % decrease viscosity
'Florida LC'	22.3±1.0	22.2±1.8	19.5b
'Hayslip'	18.6±1.4	18.9±1.7	14.3d
'Chonto'	31.1±1.8	32.2±2.5	24.2a
'Florida LC' X 'Alcobaca'	18.3±1.1	16.1±1.0	10.3e
'Hayslip' X 'Alcobaca'	17.8±1.5	18.8±1.9	12.0de
'Chonto' X 'Alcobaca'	21.3±1.7	22.4±1.8	16.6c
<u>rin</u> X 'Alcobaca'	18.3±1.3	9.0±1.0	8.0f
<u>rin-l</u> X 'Alcobaca'	22.9±1.5	8.6±1.2	7.3f
<u>nor</u> X 'Alcobaca'	14.1±1.3	4.1±0.3	0.8g
'Alcobaca'	14.2±1.0	11.2±0.7	1.5g
<u>rin</u>	9.1±1.5	*	**
<u>rin-l</u>	15.2±2.2	*	**
<u>nor</u>	8.9±1.0	*	**

^zMean separation by Duncan's multiple range test at 5% level.

*No ethylene detected.

**No PG activity detected.

'Alcobaca' also exhibited a climacteric pattern for ethylene production but, as in the case of respiration, 'Alcobaca' did not have the sudden rise and reduction of ethylene evolution but rather a gradual rise and reduction (Figures 7, 8, 9, 10, 11, 12). The "Florida 1C" X 'Alcobaca', 'Hayslip' X 'Alcobaca', and 'Chonto X 'Alcobaca' hybrids exhibited a climacteric pattern for ethylene evolution (Figures 7, 8, 9) but with a reduced ethylene peak that was intermediate between 'Alcobaca' and the normal ripening parent (Table 12).

Ethylene evolution was attenuated in the hybrids rin X 'Alcobaca' and rin-1 X 'Alcobaca' (Figure 10, 11). In these hybrids the ethylene peak was less than the one of 'Alcobaca' (Table 12). No ethylene was detected in the rin and rin-1 fruits. The nor X 'Alcobaca' hybrid fruits had a highly reduced ethylene evolution (Figure 12). The ethylene peak of this hybrid was lower than that of 'Alcobaca'. No ethylene was detected in the nor fruits.

PG Activity Study

No PG activity was detected in the mutants rin, rin-1, and nor. 'Alcobaca' had very low PG activity (Table 12). The 'Florida 1C' X 'Alcobaca', 'Hayslip' X 'Alcobaca', and 'Chonto' X 'Alcobaca' hybrids had reduced PG activity. These hybrids exhibited values intermediate between both parents for PG activity (Table 12). The PG activity of the rin X 'Alcobaca' and rin-1 X 'Alcobaca' hybrids was greater

Figure 7. Ethylene production of 'Alcobaca', 'Florida 1C', and 'Alcobaca' X 'Florida 1C' fruits.

Figure 8. Ethylene production of 'Alcobaca', 'Hayslip', and 'Alcobaca' X 'Hayslip' fruits.

Figure 9. Ethylene production of 'Alcobaca', 'Chonto', and 'Alcobaca' X 'Chonto' fruits.

Figure 10. Ethylene production of 'Alcobaca', 'Alcobaca' X rin, and rin fruits.

Figure 11. Ethylene production of 'Alcobaca', 'Alcobaca' x rin-1, and rin-1 fruits.

Figure 12. Ethylene production of 'Alcobaca', 'Alcobaca' X nor, and nor fruits.

than that of 'Alcobaca' but much lower than that of hybrids between normal ripening materials and 'Alcobaca' (Table 12). The PG activity of the nor X 'Alcobaca' hybrid was intermediate between nor and 'Alcobaca'.

Model To Relate Storage Life with Different Physiological Parameters

A multiple regression model to relate fruit storage life measured as ASI against a/b ratio, firmness at the red stage, firmness 7 days after the red stage, firmness 15 days after the red stage, maximum respiration, maximum ethylene, and PG activity indicated that a high determination coefficient (R^2) for one independent variable was obtained for PG activity. Once PG activity was included in the model, none of the other independent variables were significant enough to be included in the model. The obtained value for R^2 in the PG-ASI model was 0.69.

Genetic Studies

Inheritance of the Abnormal Ripening 'Alcobaca'

The abnormal fruit ripening 'Alcobaca' was found to be due to a single recessive gene. The segregating generations, BC_2 and F_2 fit the model with a probability of 0.7-0.5 (Table 13).

Although this mutant fit a single recessive gene model, it was found by studying the 'Florida 1C' X 'Alcobaca' hybrid

Table 13. Inheritance of the abnormal tomato fruit ripening 'Alcobaca'.

Population	Number of plants	Classification		Expected ratio	χ^2
		Number of Normal	plants Mutant		
'Florida LC' (P_1)	40	40	0	1:0	-
'Alcobaca' (P_2)	40	0	40	0:1	-
'Florida LC' ($P_1 \times P_2$)	40	40	0	1:0	-
Rec F_1 ($P_2 \times P_1$)	40	40	0	1:0	-
BC_1	80	80	0	1:0	-
BC_2	82	39	43	1:1	0.7-0.5
F_2	211	154	57	3:1	0.7-0.5

that in the heterozygous condition several of the ripening processes were affected. The respiratory and ethylene peaks were reduced 18.0 and 30.5%, respectively, PG activity was lowered 47.1%, and storage life (ASI) was increased more than 2 fold. The hybrid fruits had a normal appearance and good red color (Table 14).

Allelism Test Between 'Alcobaca' and rin

Allelism tests between 'Alcobaca' and two different sources of rin (rin and rin-1) indicated that the mutant gene carried by 'Alcobaca' was not allelic to rin. The fruits of the F_1 plants were normal and the F_2 segregation was 9 normal : 7 mutant, indicating 2 non-allelic recessive genes for abnormal ripening (Tables 15, 17).

The F_1 plants of the rin X 'Alcobaca' and rin-1 X 'Alcobaca' hybrids had normal red fruits (a/b/ ratio above 2.0), greater respiration peak, and greater PG activity than either parent. The ethylene peak was reduced and was similar to the one of 'Alcobaca' (Tables 16, 18).

Allelism Test Between 'Alcobaca' and nor

The nor X 'Alcobaca' F_1 fruits had a mutant phenotype similar to that of 'Alcobaca'. The F_2 segregated 3 'Alcobaca'-type fruits : 1 nor ($p=0.3-0.5$), indicating that 'Alcobaca' carries another allele of nor. This allele is dominant to the standard nor recessive allele in phenotypic appearance (Table 19).

Table 14. Physiological parameters obtained from fruit of 'Florida 1C', 'Alcobaca', and the hybrid between these genotypes.

Genotype	Maximum respiration	Maximum ethylene	Color ^z a/b	PG activity	ASI ^z
'Florida 1C'	22.31	23.20	2.15b	19.49a	42.2c
'Florida 1C' X 'Alcobaca'	18.31	16.11	2.25a	10.30b	97.9b
'Alcobaca'	14.25	11.19	1.06c	1.48c	185.7a

^zMean separation within columns by Duncan's multiple range test at 5% level.

Table 15. Allelism test between 'Alcobaca' and rin.

Population	Number of plants	Classification		Expected ratio	χ^2
		Normal	Mutant		
<u>rin</u>	20	0	20	0:1	-
'Alcobaca	20	0	20	0:1	-
<u>rin</u> X 'Alcobaca' (F_1)	20	20	0	1:0	-
<u>rin</u> X 'Alcobaca' (F_2)	78	41	37	9:7	0.5-0.3

Table 16. Physiological parameters obtained with rin, 'Alcobaca', and the hybrid between these materials.

Genotype	Maximum respiration	Maximum ethylene	Color ^z a/b	Firmness ^z			PG activity	ASI ^z
				Red stage	7 days after	15 days after		
<u>rin</u>	9.1	*	0.2c	2.4a	2.6a	2.6a	**	107.9b
<u>rin</u> x 'Alcobaca'	18.2	9.0	2.0a	3.8c	4.2c	4.5c	8.0a	90.8c
'Alcobaca'	14.2	11.2	1.1b	3.0b	3.2b	3.8b	1.5b	161.7a

^z Mean separation within columns by Duncan's multiple range test at 5% level.

**No PG activity detected.

Table 17. Allelism test between 'Alcobaca' and rin-1.

Population	Number of plants	Classification		Expected ratio	χ^2
		Number of plants Normal	Mutant		
<u>rin-1</u>	20	0	20	0:1	-
'Alcobaca'	20	0	20	0:1	-
<u>rin-1</u> X 'Alcobaca' (F_1)	20	20	0	1:0	-
<u>rin-1</u> X 'Alcobaca' (F_2)	70	38	32	9:7	0.9-0.8

Table 18. Physiological parameters obtained with rin-1, 'Alcobaca' and the hybrid between these materials.

Genotype	Maximum respiration	Maximum ethylene	Color ^z a/b	Firmness ^z			PG activity	ASI ^z
				Red stage	7 days after			
<u>rin-1</u>	15.2	*	0.2c	1.8a	2.0a		**	117.7b
<u>rin-1</u> X 'Alcobaca'	22.9	8.6	2.1a	3.5b	3.7b		7.3a	97.2c
'Alcobaca'	14.2	11.2	1.1b	3.0b	3.2b		1.5b	161.7a

^zMean separation within columns by Duncan's multiple range test at 5% level.

**No PG activity detected.

Table 19. Allelism test between 'Alcobaca' and nor.

Population	Number of plants	Classification		Expected ratio	χ^2
		'Alcobaca' type	<u>nor</u> type		
<u>nor</u>	20	0	20	0:1	-
'Alcobaca'	20	20	0	1:0	-
<u>nor</u> X 'Alcobaca' (F_1)	20	20	0	1:0	-
<u>nor</u> X 'Alcobaca' (F_2)	66	46	20	3:1	0.5-0.3

The physiological parameters measured for nor and the nor X 'Alcobaca' hybrid are presented in Table 20. Most measured parameters of the hybrid have values which fall between both parents. Maximum ethylene production, color ratio a/b, firmness at the red stage, firmness 15 days after the red stage, and PG activity exhibited intermediate values between 'Alcobaca' and nor. Maximum respiration and ASI values were closer to 'Alcobaca' than to nor.

Inheritance of Fruit Storage Life

The joint scaling test and the scaling test applied to the data obtained for storage life of different generations of the 'Florida 1C' X 'Alcobaca' hybrid indicate that the values obtained fit an additive-dominant model (Tables 21, 22).

The minimum number of gene pairs differentiating both parents was found to be close to 1, calculated by the methods of Castle and Wright (1921) and Sewall Wright (Burton 1951) (Table 22).

Table 20. Physiological parameters obtained with nor, 'Alcobaca', and the hybrid between these materials.

Genotype	Maximum respiration	Maximum ethylene	Color ^z a/b	Firmness ^z			PG activity	ASI ^z
				Red stage	15 days after			
<u>nor</u>	8.9	*	0.2c	2.2a	2.4a	**		201.9a
<u>nor</u> X 'Alcobaca'	14.1	4.1	0.7b	2.5ab	2.8a	0.8a		171.0b
'Alcobaca'	14.2	11.2	1.1a	3.0b	3.8b	1.5a		161.7b

^zMean separation within columns by Duncan's multiple range test at 5% level.

**No PG activity detected.

Table 21. Joint Scaling Test (JST) for storage life inheritance.

Population	Number of plants	Observed mean	Expected mean	Standard error mean	JST (χ^2)	Proba- bility
'Florida LC'	57	28.4	27.0	2.4	0.07	-
'Alcobaca'	36	75.8	69.7	3.9	0.53	-
F_1 (*)	40	59.1	50.1	3.6	1.62	-
BC_1	80	37.9	38.5	2.6	0.04	-
BC_2	74	54.6	53.5	3.7	0.02	-
F_2	193	45.6	49.2	2.0	0.26	-
Combined					2.54	0.5-0.7

(*) = pooled values from direct and reciprocal F_1 .

Table 22. Scaling test, values for m, h, and d and number of pairs of factors for fruit storage life.

Scaling test

A	-11.7 n.s.
B	-12.2 n.s.
C	-9.8 n.s.

Joint scaling
parameters

m	48.37
d	-21.34
h	1.70

Number of factors	1.24 (*)
	1.16 (**)

(*) = Castle method.

(**) = S. Wright method.

n.s. = not significant.

DISCUSSION

Storage Life Indexes

Accumulative storage index (ASI) is considered here to be a more representative index of storage life than mean storage life (MLS). There was no difference in MSL in a number of cases between genotypes but significant differences between the same genotypes were found for ASI. This is illustrated with a comparison of storage life between the varieties 'Flora-Dade' and 'Florida MH-1' (Table 3). There was no significant difference in MSL between these varieties but a significant difference in ASI. After 30 days in storage both genotypes had about the same percentage of fruits remaining, and that was true when 50% of the fruits of both materials still remained in storage. Between 30 and 40 days of shelf life, however, most fruits of 'Florida MH-1' were discarded, but after 40 days 42.5% of the 'Flora-Dade' fruits remained. After 50 days none of the fruits of 'Florida MH-1' remained in storage in contrast to 40% of 'Flora-Dade'. These results indicate that ASI is a more accurate measurement of fruit shelf life than MSL.

Storage Life Studies

'Alcobaca' fruits exhibited a prolonged shelf life in all studies. 'Alcobaca' fruits had a similar pattern in

storage life to the ripening mutants rin (rin and rin-1) and nor, and significantly longer fruit storage life than normal ripening materials.

The long shelf life of 'Alcobaca' fruits was reported by a number of researchers (Almeida 1961, Leal and Liberal 1971, Leal and Shimoya 1973a, Leal and Tabim 1974, Leal and Mitzubuti 1975), and the long storage life of 'Alcobaca' fruit was reportedly due to structural pericarp changes that take place during the storage of this fruit (Leal and Shimoya 1973b). Kopeliovitch et al. (1980) presented data of different ripening parameters of 'Alcobaca'. They suggested that 'Alcobaca' is a slow-ripening mutant.

Shelf life improved in hybrids between normal ripening materials and the ripening mutants rin, rin-1, nor, and 'Alcobaca'. The nor hybrids had a longer shelf life than the other mutant hybrids. Buescher et al. (1976) and Ng (1976) reported that the storage life of the nor hybrids improved 2 to 3 fold over the normal ripening genotypes. The storage life of the hybrids between normal lines and 'Alcobaca' improved by 2 fold in these studies.

The extended shelf life of hybrids with the mutant nor has been explained by the pleiotropic effects of this mutant in heterozygous condition on different ripening events that include the respiration rate, ethylene production, PG activity, softening rate, and red color development (Ng and Tigchelaar 1977, Tigchelaar et al. 1978a).

The nor hybrids have the longest shelf life, but these hybrids have poor red color (a/b ratio below 2.0). The carotene accumulation in hybrids with the ripening mutants rin and nor ultimately approximate that of normal ripe fruit, but the time required from breaker stage to full ripeness as estimated by color development is particularly long for nor mutant hybrids (Ng and Tigchelaar 1977, Tigchelaar et al. 1976). In contrast, the fruits of the hybrids between normal ripening genotypes and 'Alcobaca' had normal red color, and the values for the a/b ratio were above 2.0. This factor, in addition to the extended shelf life, points up the possibility of using 'Alcobaca' in hybrid combination.

Fruit storage life, measured as ASI, was also extended in hybrids between 'Alcobaca' and the ripening mutants rin, rin-1, and nor in comparison to that exhibited by normal genotypes. The rin X 'Alcobaca' and rin-1 X 'Alcobaca' hybrids had normal red color (a/b ratio above 2.0) but not the nor X 'Alcobaca' hybrid. The color pattern obtained with the rin X 'Alcobaca' and rin-1 X 'Alcobaca' hybrids was different from that for the double heterozygous mutant rin X nor reported by Tigchelaar et al. (1978b). In the latter, the fruits did not achieve full ripe color even after prolonged periods on the vine or in storage.

The color expression in the nor X 'Alcobaca' hybrid was attenuated, and the hybrid had less color than 'Alcobaca'.

The use of this hybrid is of no practical value in spite of its long fruit storage life.

'Alcobaca' and the hybrids with this material, except nor X 'Alcobaca', produce soft fruits. The decrease in firmness for these genotypes was not as pronounced as the one shown by normal ripening tomatoes. The softness of the 'Alcobaca' fruits is due to a certain degree of puffiness which causes low readings for firmness.

The slow softening pattern manifested by 'Alcobaca' from the reddish stage up to 15 days after the reddish stage, and the attenuated softening in hybrids with 'Alcobaca', may be caused by the reduced activity of pectolytic enzymes, specifically PG activity. PG activity is absent in the ripening mutants nor and rin and reduced in hybrids with these ripening mutants (Tigchelaar et al. 1978a).

Significant correlations between fruit shelf life and fruit weight loss were evident only after prolonged periods of storage, suggesting that the weight loss was concerned initially with decrease in fresh weight. Leal and Mitzubuti (1975) reported that factors besides weight loss influenced the deterioration of tomato fruits. The correlation coefficient between weight loss and fruit deterioration increased from 0.53 at 33 days of fruit shelf life to 0.64 after 53 days.

Respiration, Ethylene Evolution,
and PG Activity

The results obtained for respiration and ethylene production demonstrated that 'Alcobaca' has a typical climacteric pattern of ripening, but the respiratory and ethylene patterns are more gradual and extended than that of normal ripening genotypes. Similar results with a reddish 'Alcobaca' were found by Kopeliovitch et al. (1980).

'Alcobaca' was very low in PG activity. The fully ripened fruits did not attain the same red intensity as normal ripening genotypes. These characteristics and the modified respiration and ethylene production during the climacteric indicate that 'Alcobaca' is a ripening mutant similar to the gene Never Ripe (Nr), in which there is reduction of ethylene and CO_2 (Tigchelaar et al. 1978a), reduced lycopene content (Tigchelaar et al. 1978a), and slow softening of the fruit (Hobson 1967). 'Alcobaca' differs from rin and nor by the fact that these mutants are non-climacteric (Herner and Sink 1973, McGlasson et al. 1975a, Ng 1976, Ng and Tigchelaar 1977). Similar results with rin and nor were obtained in this study.

The hybrids between 'Alcobaca' and normal ripening materials have normal fruit color appearance, but several aspects of the ripening of these hybrids are modified. The respiratory peak and ethylene peak are reduced, and the amount of reduction depends on the normal parent. The

softening of the fruit takes place at a slower rate in comparison to normal ripening genotypes, and the PG activity is reduced. The storage life of the hybrid fruit is improved.

Quantitative modification of different ripening processes was reported for the rin and nor mutants (Buescher et al. 1976, Herner and Sink 1973, Sink et al. 1974, Ng 1976, Tigchelaar et al. 1976, Ng and Tigchelaar 1977). Tigchelaar et al. (1978a) indicate that the ripening mutants in the heterozygous stage exert their effect largely on the rate at which specific ripening modifications take place.

The ripening processes most affected in hybrids between 'Alcobaca' and the ripening mutant rin (rin and rin-1) were the ethylene pattern and PG activity. The ethylene peak was greatly reduced, in comparison to normal ripening material and lower than those of 'Alcobaca' and hybrids between 'Alcobaca' and normal ripening tomatoes. PG activity was higher than that of 'Alcobaca' but was reduced 66% in comparison to normal ripening materials and was reduced 41% when compared with the PG activity obtained with hybrids between 'Alcobaca' and normal ripening genotypes. Respiration was less affected in hybrids of 'Alcobaca' and the ripening mutants rin and rin-1 with an average of 22% reduction in comparison to normal ripening materials. The respiratory peak was higher than the one of 'Alcobaca', rin and rin-1.

In the nor X 'Alcobaca' hybrid all of the ripening processes were affected. The respiratory peak was similar

to that obtained for 'Alcobaca', and the ethylene peak was reduced 63% in relation to that of 'Alcobaca'. The PG activity was lower than the PG activity obtained for 'Alcobaca'.

A comparison of the hybrids between 'Alcobaca' and normal ripening materials and the hybrids between 'Alcobaca' and the ripening mutants rin and rin-1 suggests gene dosage effects. In the hybrids between 'Alcobaca' and normal ripening tomatoes, the ethylene peak was reduced 22% in comparison to normal ripening materials, and in the hybrids 'Alcobaca' and ripening mutants rin and rin-1, ethylene peak was reduced 66% in comparison to normal genotypes. PG activity was reduced 33% in the hybrids normal X 'Alcobaca' and 60% in the hybrids rin and rin-1 X 'Alcobaca' in comparison to normal ripening tomatoes.

Gene dosage effects for the genes nor and rin in double heterozygotes were reported by Tigchelaar et al. (1978b) for respiration, peak ethylene, carotene production, and PG activity. In the present studies gene dosage effects were not found in the 'Alcobaca' X rin (rin-1) hybrids for respiration and color expression. These hybrids had normal red color (a/b ratio above 2.0).

The dosage effects reported for 'Alcobaca' and the ripening mutants rin and rin-1 may have a certain degree of bias because isogenic lines were not used.

A multiple regression model to correlate storage life of the fruits with the different ripening parameters including color ratio a/b, firmness at the red stage, firmness 7 days after the red stage, firmness 15 days after the red state, maximum respiration, maximum ethylene, and PG activity indicated that the most closely related factor was PG activity. Once this variable was included in the model, none of the remaining parameters was significant. The above results do not indicate that PG activity is the only factor that enhances fruit shelf life because the different ripening processes are not independent but appear to be highly synchronized (Tigchelaar et al. 1978a).

The close relationship between PG activity and storage life could be explained by the action of this enzyme on other ripening processes. Strand and Musell (1975) and Strand et al. (1976) indicated that PG induces release of cell wall-bound enzymes which are responsible for a number of processes such as ethylene production, carotene synthesis and respiratory control. Tigchelaar et al. (1978a) postulated that both rin and nor mutants produce defective PG, and this enzyme (or enzyme complex) is required to condition fruit tissue to undergo subsequent changes associated with the ripening process. Sacher (1962) and Salomos and Lati, cited by Tigchelaar et al. (1978a), indicated that this conditioning process may entail compartmentation changes required for the complex sequence of events

associated with the ripening process. Tigchelaar et al. (1978a) indicated that aberrancies in respiratory behavior, ethylene production, and carotene synthesis in the mutants represent secondary effects caused by failure of PG-induced modifications in cellular compartmentation.

Based on previous studies in which applications of ethylene or the analogue oleoffin propylene did not induce normal ripening of the rin and nor fruits (McGlasson et al. 1975a), Frenkel and Garrison (1976) concluded that ethylene does not control the onset of ripening in tomato fruit. Tigchelaar et al. (1978b) supported this idea and suggested that a slow change must precede the burst in ethylene production and other concomitant ripening changes, and Tigchelaar et al. (1978b) indicated that PG activity could be the key event which occurs prior to the respiratory and ethylene rise.

One criticism of the idea formulated by McGlasson et al. (1975a) and Tigchelaar et al. (1978b) that ethylene does not control the onset of ripening is that the lack of response to ethylene in the ripening mutants does not necessarily mean that ethylene is not the triggering mechanism for the onset of ripening. Some genetic block in the sequential events of ripening may exist prior to or after the onset of ripening that could account for the lack of response to this oleoffin. This causes the fruits to have abnormal ripening. Tigchelaar et al. (1978a) reported that the

genes nor and rin are operating prior to the onset of the respiratory or ethylene rise, which is generally regarded as the signal for the initiation of ripening.

Lin (1978) proposed that fruit ripening is caused by changes in the hormone pool size inside the tissues. A ripening-induced signal is generated when the composition of the constituents attains a proper ratio which can be modulated by different environmental factors. The loss of ripening ability in rin tomato could be due to a lack of a gene or gene regulator to receive the ripening-onset signal from "the balance of hormone pool," followed by no ripening enzyme activation or formation

Genetic Studies

The abnormal fruit ripening 'Alcobaca' was found to be due to a recessive gene, which exerts effect on a number of ripening processes including fruit color, softening rate, respiratory and ethylene peak, PG activity, and storage life of the fruit. Single-gene mutations have been found to act as ripening mutants. The dominant climacteric mutant Nr and the recessive non-climacteric genes rin and nor are included in this category (Rick 1965, Robinson and Tomes 1968, Tigchelaar et al. 1973).

In spite of the recessive nature of the mutant carried by 'Alcobaca', this gene, in heterozygous condition, modifies several of the ripening processes. The respiration

and ethylene peaks are reduced and the softening rate is delayed. The PG activity is reduced and the fruits have a normal red color with a prolonged shelf life in comparison to the normal parent. Modifications of the ripening processes were reported for the heterozygous genotypes involving rin and nor. In rin heterozygotes the respiratory climateric was delayed and the respiratory peak was similar to that of the normal parent (Herner and Sink 1973, Ng 1976). The ethylene peak was 35 to 50% of the normal parent (Herner and Sink 1973, Ng 1976, Ng and Tigchelaar 1977), and PG activity was 25 to 60% of the normal (Buescher et al. 1976, Ng 1976, Tigchelaar et al. 1978b). In nor heterozygotes a reduced respiratory peak (Ng 1976, Tigchelaar et al. 1978b), reduced ethylene peak (Ng 1976, Ng and Tigchelaar 1977, Tigchelaar et al. 1978b), lowered PG activity (Buescher et al. 1976, Ng 1976, Ng and Tigchelaar 1977) in comparison to the normal parents were reported. The primary effect of the ripening mutants has not been clearly established. It has been suggested that they produce defective PG and that this enzyme is required to condition fruit tissue to undergo subsequent changes associated with the ripening process (Tigchelaar et al. 1978a).

The abnormal ripening mutant 'Alcobaca' is not allelic to the mutant gene rin as shown by allelism tests and segregation in the F_2 . In the nor X 'Alcobaca' hybrid the F_1 generation had a mutant phenotype similar to the one of 'Alcobaca', and in the F_2 generation no normal fruits

were found and a segregation ratio of 3 'Alcobaca' type to 1 nor type was observed. These results indicate that they are allelic and that the allele carried by 'Alcobaca' is dominant to nor. Physiological experiments provided further evidence for the allelic relationship between nor and the gene carried by 'Alcobaca'. In the cross nor X 'Alcobaca' the values for the respiratory and ethylene peaks, color (ratio a/b), firmness at different stages, PG activity, and storage life (ASI) were between the parents.

The dominant nature of 'Alcobaca' over nor is supported by the fact that the nor X 'Alcobaca' hybrid had a climacteric pattern for respiration and ethylene evolution, although it was attenuated, and some PG activity was detected in the hybrid fruit. Phenotypically the allele of nor carried by 'Alcobaca' is dominant to nor, but the alleles are co-dominant when physiological parameters are examined.

Tigchelaar et al. (1976) suggested that 'Alcobaca' may carry a third allele at the nor locus, but they provided no data to support the idea. Kopeliovitch et al. (1980) indicated that 'Alcobaca' and nor were not allelic, but no data were presented to support that view. Two other alleles have been reported at the nor locus and were designated nor² and nor³ (Soresi, cited by Tigchelaar 1978). The designation for the abnormal ripening 'Alcobaca' used in this study is nor^A.

Three different kinds of alleles have been identified at the nor locus in this study. They are nor⁺, which is the normal ripening fruit with a typical climacteric pattern; nor^A, which develops some red color in ripening and is climacteric in nature; and nor, which has no red color or only a blush of red color in old fruits and a non-climacteric ripening pattern. These alleles provide a tool for the study of the ripening regulation mechanism. The use of near-isogenic lines will permit the elimination of variation caused by different genetic backgrounds.

Examination of the fruit storage life of the reciprocal F₁ crosses between 'Alcobaca' and 'Florida 1C' indicated no maternal effects. Similar results were obtained by Leal and Mitzubuti (1975) in a series of crosses between 'Alcobaca' and normal ripening cultivars. An additive-dominance model fits the observed data, indicating absence of epistatic or non-allelic interactions. Assuming the methods of calculation for minimum number of gene pairs that a) linkage is absent, b) one parent contributes only plus factors and the other only minus factors, c) all genes are equally important, d) degree of dominance of all plus factors is the same, and e) there is no epistasis (Ibarbia and Lambeth 1969), both parents differed by at least one pair of genes. It was assumed that all of the studied genotypes were equally affected by decay, which could be not exactly true since different population sizes were stored from the different

populations. Similar results were obtained studying the inheritance of storage life (quantitative approach) and the ripening behavior of 'Alcobaca' and 'Florida 1C' (qualitative approach) in that both parents differed at one locus.

SUMMARY AND CONCLUSIONS

'Alcobaca' tomato has a mutant phenotype. This material exhibits reddish color at full ripeness, climacteric pattern for respiration and ethylene evolution, very low PG activity, delayed softening of the fruit, and long fruit storage life. Hybrids of 'Alcobaca' with normal ripening materials have reduced respiratory and ethylene peaks, delayed softening of the fruit, normal red color, and improved fruit storage life. Hybrids between 'Alcobaca' and the ripening mutant rin exhibit normal red color and increased fruit shelf life. The above results indicate possibilities of using 'Alcobaca' hybrids with normal ripening tomatoes or with the ripening mutant rin to improve fruit shelf life.

The most highly correlated parameter with storage life, measured as ASI, was polygalacturonase.

The abnormal ripening of 'Alcobaca' tomato is conditioned by a single recessive gene which affects several aspects of fruit ripening. Tests of allelism indicated that the mutant gene carried by 'Alcobaca' is allelic to nor and phenotypically dominant over the standard recessive allele. The mutant nor is non-climacteric and no PG activity or only traces of this enzyme are found in homozygous fruits or this mutant. The nor X 'Alcobaca' hybrid has a phenotype similar to 'Alcobaca'. The hybrid fruits are climacteric

and exhibit some PG activity. Quantitatively, the dominance of 'Alcobaca' is partial over nor, since the ripening processes are attenuated in the nor X 'Alcobaca' hybrid and the values fall in between both parents. It is proposed that the allele found in 'Alcobaca' be termed nor^A. There are now three different alleles at the nor locus: nor⁺, which conditions a normal climacteric and normal ripening; nor^A, which conditions climacteric but the fruits do not ripen normally; and nor, which lacks a climacteric and produces abnormal ripening. These alleles should be useful in the study of regulatory mechanisms of ripening.

Gene dosage effects for ethylene and PG activity were found for the mutants rin and nor^A. This gene dosage effect was detected by comparing normal genotypes (nor⁺/nor⁺), nor^A heterozygotes (nor⁺/nor^A) and the double heterozygote mutant nor^A X rin (nor⁺/nor^A, rin⁺/rin).

A quantitative genetic study of the storage life indicated that 'Alcobaca' and the normal ripening material 'Florida 1C' differed at one pair of alleles for this characteristic. This agrees with the qualitative study in that 'Alcobaca' and 'Florida 1C' carry different alleles of nor.

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BIOGRAPHICAL SKETCH

The author was born on December 25, 1944, in Circasia, Colombia. He finished his high school at the Instituto Universitario, Manizales, Colombia. In 1962 he entered the Universidad de Caldas, Manizales, Colombia, and in 1966 he completed his studies for the degree of Ingeniero Agrónomo. In 1967 he was employed by the Instituto Colombiano Agropecuario (ICA) as researcher in vegetable crops on the Atlantic coast of Colombia. In 1969 he moved to the "Tulio Ospina" experimental station at Medellín, Colombia, to work in tomato breeding. In 1972 he entered the Graduate School of the Universidad Nacional-ICA at Bogotá, Colombia. He received the degree of Master of Science with a major in plant breeding in December 1973. He continued working at Medellín in tomato breeding. In 1976 he was appointed ICA Regional Director of Research for Region Number 4. In 1977 he was appointed simultaneously as National Director of the Vegetable Crops Research Program of ICA. In March 1978 he entered the Graduate School of the University of Florida with a Rockefeller Foundation scholarship to study for the degree of Doctor of Philosophy in the Vegetable Crops Department. He has been a member of the Tomato Genetics Cooperative and was appointed ad honorem coordinator of

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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