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GENETIC DETERMINATION OF MATURATION PROCESSES IN CLIMACTERIC FRUITS
A review

T. KÁLLAY*

Research Institute for Fruitgrowing and Ornamentals, H-1223 Budapest, Park u. 2. Hungary

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There are unexpected cases in apple or pear storage practice, i.e. external effects such as high or low temperatures, CO_2 stress and intensive oxygen pull down may disturb normal ripening of fruits. Physiological consequences of these disturbances are expressed in certain cases only in incomplete ripening, while in irrevocable cases they are manifested as serious tissue damages such as non-parasitic storage disorders known as core flush, soft scald, and internal or external CO_2 injuries.

It has been widely demonstrated in pome fruits that in a transient phase of maturity, during the pre-climacteric, ripening of pome fruits is preceded by changes in gene expression, detachment of new mRNA-s and the synthesis of compounds that are certainly essential for ripening processes. It is likely that this transient phase is closely related to changes on molecular level, and therefore, it may be considered as critical when external effects influence fruit life.

Keywords: apple, carbon dioxide injury, controlled atmosphere storage, ripening

Many signs indicate that ripening of climacteric fruits is determined by genetic factors. The basis of the determination’s hypothesis is the ripening sequence of certain fruit varieties, and this can be considered as constant and independent of the growing region and season. In case of apples ripening sequence of the actual assortment grown in Italy lasts 54 days; Golden Delicious is the basis of comparison in the middle of the period (SANSAVINI et al., 1994). The fruit’s suitability for storage depends basically on harvest time; climacteric fruits seem to be able to resist the temperature and gas combination that is close to the lowest limit of their tolerance after the pre-climacteric phase only, in a certain state of maturity (SMOCK & NEUBERT, 1950).

The hypothesis of genetic determination of fruit ripening is not new at all. TUKEY (1942) and HALLER (1944) determined the development of state of maturity suitable for storage with the method “Days after Full Bloom”. The scientific world was in a hurry to confute their report (SMOCK & NEUBERT, 1950, FIDLER et al., 1973). During the evaluation of confutation it turned out that there were uncertainties about the designation of the “day of full bloom” and the “day of optimal maturity”, so their value are as uncertain as the report itself.

* Tel/Fax: +36(1)3621568; E-mail: kallica@mail.matav.hu

Technology of fruit storage underwent a spectacular and rapid development. Long-term storage of apple and pear is feasible only in controlled atmosphere. Fashionable trend of present times is the oxygen-poor atmosphere (ULO), where the fruit is exposed to triple stress: by cooling, by elevation of CO_2 level and by the rapid reduction of oxygen.

In this report we try to summarize the latest results contributing to a better understanding of the genetic determination of fruit ripening, and by means of that information, we attempted to find explanation for storage anomalies that had been unanswerable by science at the time when they arose.

The genetic alarm clock

It seems that initiation of maturation processes at a certain time are induced by changes on the molecular level transcribed from the genetic code. CHRISTOFFERSEN and co-workers (1982) suppose the existence of "maturity genes" that regulate the process of maturation. We can add to this hypothesis that the genetic determination of ripening means also the timing of the functioning of these "maturation genes", similarly to setting an alarm clock in the previous night, for example to six o'clock. The clock is ticking all night unexpectedly and awakes us at the preset time with loud jingling.

The same genetic timing can be observed at the end of pregnancy, it results in the *de novo* synthesis of oxytocin, which after all conduces to the initiation of birth. Many of similarly timed development processes could be cited. In case of fruits, the "setting of the alarm clock" happens most possibly at the time of fertilization.

Beginning of the genetic program

The possible day of fertilization referring to the whole yield of an apple tree was expressed as the day of the "full bloom" by the literature. The respective phenological state of "full bloom" means that all flowers in the same cluster are fully blooming and the first flowers are in the phase of petal-fall. This definition can be used for the determination of the date of fruit setting, if the period of blooming is very short (few days only). In case of protracted blooming the difference between the dates of fertilization of flowers may spread over a period as long as several weeks, in this case inflorescences are in "full bloom" at different times. The only result obtained from the initial experiments concerning this object shows that fruits from earlier fruit sets were larger and looked more mature than others fertilized later (SULLIVAN, 1965; MAGGS, 1975).

Results obtained both from New Mexico and Australia are influenced by the fact that apples with different length of growing season were examined at the same harvest time. OLSEN and MARTIN (1980) produced a 50 day long flowering course with artificially advanced and delayed blooming. Similar results had been achieved concerning the growth of fruits of manual pollination and taking the same length of growing season.

Surprisingly, apples originated from earlier blooming were firmer and usually had higher acid content than fruits from later fruit sets. Apples from earlier fertilizations retained their quality advantage also during storage. Data of the experiment verify that maturation processes are programmed, because apples set earlier during the same length of growing period did not ripen earlier or “faster” than others from later fruit sets, though their maturity time was in the second part of August, contrary to the maturity time of the delayed yield in October. On the basis of their data, synthesis of new materials involved in scald appearance, even in susceptible varieties, seems to be independent of the maturity program but depends on meteorological stress (heat days).

End of the genetic program: the initialisation of ripening

On the basis of experiments on maturation biology ethylene climacteric was suggested – among others – by KNEE and his co-workers (1975), and DILLEY (1980) for signalling maturation processes and optimal maturity for harvesting. Ethylene climacteric may be good for signalling genetic timing of ripening, if the autocatalytic ethylene synthesis could be correlated with the time of fertilization of individual fruits.

Ethylene can be well measured in the fruit, while it is still on the tree. BLANPIED (1982) attempted to investigate by artificial illumination and darkening whether the number of days or length of irradiation plays a role in the induction of maturation processes. No result in timing of ethylene climacteric or in exogenous ethylene treatment – relating to differences in dates of maturity – occurred in his experiments. On the basis of field experiments in apple orchards (BLANPIED, 1985, 1986) ethylene climacteric means a rise of concentration starting above $0.5\,\mu l\,l^{-1}$. Ethylene concentration of fruits in pre-climacteric state is usually lower than $0.1\,\mu l\,l^{-1}$. Ethylene concentration, following the start of its auto-catalytic synthesis, is not informative about the state of maturation, the only information is that ripening processes have been started. It seems obvious, that instead of the average of ethylene concentrations the rate of apples with a concentration over a threshold value is suitable for the characterization of the maturation state of a basic multitude.

Genetic preliminaries of ripening

In the physiology of fruit ripening synthesis of new compounds are observed. The *de novo* protein synthesis is preceded by changes in gene expression and by the copying of new mRNA-s (SACHER, 1973). CHRISTOFFERSEN and co-workers (1982) detected significant quantitative increase in case of three mRNA-s, LAY-YEE and co-workers (1990) observed six new mRNA-s beside a simultaneous decrease of a single mRNA. DILLEY and co-workers (1994) proved that the rise in the activity of 1-

aminocyclopropane-1-carboxyl acid (ACC) synthase and simultaneously the autocatalytic synthesis of ethylene are preceded by the quantitative increase of ACC oxidase enzyme. On the basis of their results it is possible that the monoclonal-based ELISA test for ACC oxidase suggested by them could be an indicator of fruit ripening in the future, since their method is also adaptable for quantitative definitions.

However, ethylene always plays an active role in ripening processes. Since it is present also before the genetic phase of maturation, it was supposed that ethylene could display its activity by increasing membrane permeability (RHODES, 1981). In reality certain maturation processes, e.g. degradation of chlorophyll or formation of red pigments (LARA & VENDRELL, 2000) and degradation of starch (DILLEY et al., 1994) are enacted well before ethylene climacteric, so enzymes of these processes are active also before ripening. These processes are accelerated significantly by the appearance of ethylene. Quantitative rise of ethylene could be induced by stress caused by removal of fruits from the tree and then holding them either in cold store or at room temperature of 20 °C (DILLEY et al., 1994).

Increasing of respiration and RNA synthesis could be induced by exogenous ethylene in avocado fruit (TUCKER & LATIES, 1984), and the inhibition of ACC synthase could be relieved in pre-climacteric apples (BUFLER, 1984). It is likely that ethylene is able to accelerate quantitative increase or activity of enzymes playing a role in its synthesis. However, the normal ripening of stored fruits may be counteracted by the extraction of ethylene at low (1%) oxygen level, with a minimal concentration (0.5%) of carbon dioxide present (SHAIK, 1994).

Autocatalytic ethylene synthesis or ethylene climacteric is supposed to be a result of convergent processes. For controlling ethylene activity – within a certain range of concentration – increase of carbon dioxide concentration in tissues seemed to be effective, with the simultaneous reduction of the activity of ACC synthase (BUFLER, 1984). Importance of abscissic-acid (ABA) is not quite clear in this aspect (LARA & VENDRELL, 2000).

Polygalacturonase (PG) has an extra importance within enzymes formed evidently via *de novo* synthesis during the genetic phase of maturation. This enzyme plays a role in the destruction of the polysaccharides in the middle lamella of the cell wall (TUCKER & GRIERSON, 1982). Presumably, *de novo* synthesized maturation "catalysts" may also play a role in the synthesis of aromatic materials and flavours, though no respective data are available.

Critical phase of maturation

There are signs that the transient phase from maturity to ripening may be critical for fruits in storage conditions. For the illustration of the critical phase of maturation, we mention three events.

Table 1. Incidence of carbon dioxide injury in Jonathan apples, randomised according to storage chambers and growing sites

Store number: Days of store load: Growing site	Code	1. 7-13 Sept. %	2. 10-15 Sept. %	3. 14-20 Sept. %	4. 21-25 Sept. %	5. 24-29 Sept. %	6. 30 Sept.- 3 Oct. %	Incidence by orchards %	Number of samples Pieces
Bács	1	10.0	46.0	0.8				20.0	235
Bács	2	0.3	0.9	1.4	0	0		0	4880
Bács	3	1.5	1.3	0				0.8	308
Bács	4	1.2	2.4					1.5	875
Bács	5	0.6	5.6					1.5	1194
Bács	6			10.0		6.0		7.1	140
Csongrád	7	0	1.5	4.1	0.1			1.3	304
Pest	8		15.0	4.0	0	0		3.3	297
Pest	9				6.8	0		6.2	160
Somogy	10			2.0		0	9.0	3.4	118
Somogy	11						4.5	4.5	22
Békés	12	0	0					0	111
Békés	13			0	0	0		0	133
Heves	14		24.0	11.4	3.7	10.0		13.5	1002
Heves	15		5.0	0				3.7	132
Pest	16			1.6	4.7	5.0		3.4	410
Pest	17			0	0	0		0	98
Szabolcs	18					0	0	0	88
Szabolcs	19				5.0	0		0	110
Szabolcs	20					0	2.0	1.2	169
Szabolcs	21					6.7		6.7	74
Szabolcs	22					0		0	61
Szabolcs	23						2.5	2.5	40
Borsod	24			0		0	0	0	330
Szabolcs	25			0		3	4.0	2.8	315
Szabolcs	26					0		0	30
Szabolcs	27					0		0	30
Szabolcs	28					0		0	38
Szabolcs	29					10.0		10.0	89
Szabolcs	30						2.6	2.5	40
Szabolcs	31						2.5	2.5	39
Szabolcs	32						10.0	10.0	20
Szabolcs	33						0	0	65
Szabolcs	34						0	0	40
Szabolcs	35						0	0	39
Vas	36			0	0	0		0	116
Vas	37					0	0	0	80
Vas	38						5.0	5.0	20

– In 1969, during industrial storage in air, at a temperature of 0 °C it happened that 500 tons of Bosc pear from the earliest harvest lost their aptitude for softening, while fruits harvested few days later ripened perfectly after storage.

– In the 1972 growing season carbon dioxide injury, so called “Brown heart” disease, occurred in Jonathan apples in controlled atmosphere stores at 3 °C, 3% CO_2, 3% O_2. Apples in six CA rooms represented all growing regions of the country, with big differences in quantity according to different orchards; the total quantity was 2800 tons. Incidence of the disease was detected with cutting test; the number of samples was related to the quantities transported from each orchard.

Rate of the disease was lower than 1% in apples of 20 suppliers, from the total 39, while the disease occurred in different rates within items originating from 19 orchards. In one of the stored items, the loss was higher than 40%. Incidence of the disease in correlation with the term of storage is detailed in Table 1. Our investigation for the reasons of the disease on the field of storage technology ended with failure. We noted that in spring of 1972 full bloom stage of Jonathan apple trees had begun before 20 April in the southern regions of the country, but it had been interrupted for 14 days with a steady fall in temperature, in some places even frost occurred. Fertilization of flowers of Jonathan apple trees ended in the beginning of May in the north-eastern parts of the country. Incidence of the disorder was higher in the regions where fertilization occurred earlier than in the later ones (KÁLLAY, 1973).

– In 1988 pre-cooled Conference pears and freshly harvested mature Bosc pears were treated with 1% Semperfresh emulsion on the same day, and stored at 2 °C in a cold chamber full of apples (KÁLLAY & SCHUSTER, 1989). This coating produces temporary CO_2 stress in the tissue by blocking gas-transfer through the skin. Neither in post-climacteric, pre-cooled Conference pears, nor in pre-climacteric Bosc pears, served as control, ripening inhibition occurred, while treated Bosc pears remained green for 5 months, their softening was inhibited, with typical signs of CO_2 injury in the flesh of fruits.

Differences in total protein distribution pattern have been demonstrated with two-dimensional polyacrylamide gel electrophoresis (Fig. 1).

Conclusions

Pre-climacteric pears can loose their aptitude for mellowing solely because of cooling (PRATELLA & BIONDI, 1965).

Apples harvested earlier seem to be much less tolerant towards carbon dioxide stress than apples that seem to be riper on the basis of a better ground colour. Degree of softening decreased as a result of CO_2 stress applied for the inhibition of softening in Golden Delicious, though symptoms of CO_2 injury occurred both inside (Brown heart) and on the surface of fruits (LAU & LOONEY, 1978). Proportions of the disease were between 0% and 40% in the 18 items transported from different orchards. According to domestic investigations, incidence of CO_2 injury was at the same magnitude, though at much lower concentrations of CO_2. Variability in occurrence of damages might be based on significant differences in dates of fertilization.

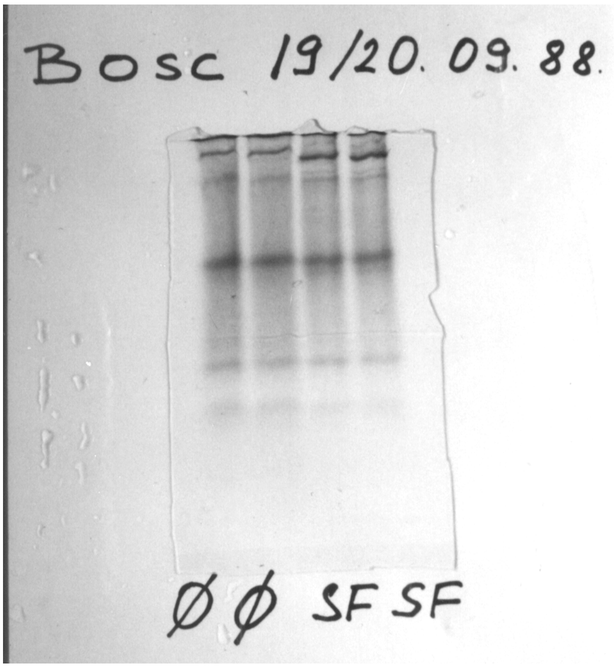

Fig. 1. Total protein distribution pattern of Bosc pears coated with Semperfresh (SF) and untreated Bosc (ϕ) pears after three months

Conference pears stored at 0 °C seemed to be very sensitive to CO_2 injury at high (6%) carbon dioxide and low (0.5%) oxygen concentration (SAQUET et al., 2000). Synergism gave decreasing tendency at the same CO_2 level at higher (3%) O_2 concentration, cooling solely caused no damage. In our experiment (KÁLLAY & SCHUSTER, 1989) post-climacteric Conference pears seemed to be immune from carbon dioxide stress in storage with high ethylene content in the storing environment, while mature Bosc pears suffered irreversible damages.

Defects show the existence of a short, but critical phase of fruit's life in correlation with the date of harvest. Fruits can suffer irreversible changes during this phase as a result of the effects of cooling, cooling and CO_2 stress, or because of the complex effects of cooling, CO_2 stress and O_2 removal. Irreversible disturbances in the first days of controlled atmosphere storage may be manifested later by non-parasitic disorders of fruits.

Experiments performed in storage show that carbon dioxide – ethylene antagonism might be expressed in the inactivation of ethylene and/or ethylene-related processes. There might be circumstances where CO_2 stress can possibly damage normal run of genetic processes and as a consequence the fruit may loose its aptitude for normal ripening. However, damages of the manner described are exceptional in storage practice. Stress caused by harvesting and cooling, exogenous ethylene originated from more ripened fruits have a definitely positive effect on the autocatalytic synthesis of ethylene, so homogenisation of different maturation states of fruits proceeds, which can finally promote the preparation of fruits to become more tolerant to storage conditions.

In fact, there is little but not negligible evidence to consider that the susceptible transient phase of maturity is related, to some extent, to the genetically encoded timing in changes of gene transcription that leads towards fruit ripening.

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