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Effects of some Growth Regulators on Pre- and After-storage Quality of 'Red Chief Delicious' Apples

M.A. Koukourikou-Petridou[1)], D.G. Voyiatzis[1)], D.K. Stylianidis[1)], T.E. Sotiropoulos[1,2)] and I.N. Therios[1)]
([1)]Department of Horticulture, Aristotle University of Thessaloniki, Thessaloniki, Greece and [2)]National Agricultural Research Foundation (NAGREF), Institute of Decidious Trees, Naousa, Greece)

Summary

The effects of gibberellic acid (GA_3), kinetin, the commercial preparations Promalin, Perlan (mixtures of $GA_{4/7}$ and benzyladenine) and Novagib ($GA_{4/7}$), and some of their combinations on fruit shape and quality of 'Red Chief Delicious' apples before and after cold storage were studied. Sprays with the chemicals were done once at petal fall. Determinations of fruit shape, expressed as the height/diameter ratio (H/D), fresh weight, seed number were made at harvest, and flesh firmness and the content in soluble solids, total sugars and L-malic acid were done at harvest and after four months in cold storage. Promalin, Perlan, Novagib+kinetin, kinetin+GA_3 and kinetin improved fruit shape, by increasing the H/D ratio, and fruit weight in some cases. Total sugars and L-malic acid content of fruits were higher in all treatments compared with the control, at harvest. After four months in cold storage the content of total sugars increased in all treatments, the increase in the control being three times that of all other treatments. Soluble solids followed a similar pattern of change before and after storage. L-malic acid concentration decreased ten times in all treatments after storage, compared with the initial values at harvest, but remained higher than in the control. GA_3 did not affect fruit shape but significantly improved all other quality parameters and the overall storability of apples. Flesh firmness before and after storage and the number of seeds were not affected. It was also shown that kinetin could replace successfully benzyladenine, which may present a potential health hazard.

Key words. apple shape – cytokinins – flesh firmness – gibberellins – L-malic acid – soluble solids – sugars

Introduction

The most important characteristics that determine fruit quality in apples and therefore have an impact on consumer's preference are appearance, shape, size, characteristic aroma and pleasant taste, the latter depending on the sugars/acids balance.

The typical shape of the 'Red Delicious' apple is the elongated fruit with well-developed calyx lobes. However, a deviation from the typical shape has been observed occasionally, depending on local climatic conditions, in certain areas including apple-producing regions of northern Greece (Stylianidis et al. 2000).

Shape aberrations can be avoided by sprays with gibberellins (Eccher 1986) or cytokinins at or shortly after petal fall. The latter combined with gibberellins enhance the formation of elongated fruit with well-developed calyx lobes (Arteca 1996). A mixture of gibberellins 4/7 and the synthetic cytokinin benzyladenine (BA) has been recommended for this purpose (Unrath 1974). Such a mixture, under the trade name of Promalin, increased fruit size and height/diameter (H/D) ratio (Curry and Williams 1983) without affecting significantly the titratable acidity and soluble solids content of 'Golden Delicious' apples (Eccher and Maffi 1986). In addition, promalin has been reported to exert a beneficial effect on the overall vegetative growth of the apple tree (Youn et al. 2004). At present it is used for the improvement of the shape, size, weight and yield of 'Delicious' apples.

Similar beneficial effects on apple fruit quality have also some other substances commonly regarded as growth regulators. Thidiazuron (THI) and especially forchlorfenuron (CPPU), structurally related compounds with similar and strong cytokinin-like activity in tissue culture, improved the shape of 'McIntosh' apples and increased flesh firmness at harvest and after storage more than Promalin (Greene 1995).

Due to the benzene ring in its moiety, benzyladenine, which is an ingredient of Promalin may present a potential health hazard for the grower at the time of application and a risk to the environment (Us Epa). It would be desirable, therefore, for benzyladenine to be replaced by other cytokinins lacking the benzene ring, such as kinetin, followed by an assessment of such a substitution.

The aim of this work was to study the effects of gibberellic acid (GA_3), of kinetin, of the commercial preparations under the trade names of Promalin, Perlan ($GA_{4/7}$ + BA) and Novagib ($GA_{4/7}$) and of some of their combinations, applied at petal fall, on the quality characteristics of 'Red Chief Delicious' apples at harvest and after cold storage. Their effect on the latter case is of particular im-

portance since apples reach the consumer usually after various periods in storage. In addition, special attention has been given to the effect of kinetin as a potential replacement of BA.

Materials and Methods

The experiments were conducted on 5–6-year old trees of the 'Red Chief Delicious' clone, which belongs to the 'Red Delicious' group, in apple orchards of Northern Greece (lat. 40 ° 37 ' N), in the years 2001 and 2002.

The trees were sprayed once, at petal fall, with aqueous solutions of the following chemicals:

Control (water)
GA_3 (10 mg l^{-1})
Kinetin (6-furfurylamino-purine) (150 and 200 mg l^{-1})
Promalin ($GA_{4/7}$ 1.8 % w/w + BA 1.8 % w/w) (0.5, 1.0 and 1.5 ml l^{-1})
Perlan ($GA_{4/7}$ 19.2 g l^{-1} + BA 19.2 g l^{-1}) (0.5, 1.0 and 1.5 ml l^{-1})
Novagib [$GA_{4/7}$ 19.2 g l^{-1} (0.5 ml l^{-1})] + kinetin (150 mg l^{-1})
Promalin (0.5 ml l^{-1}) + GA_3 (10 mg l^{-1})
Perlan (0.5 mg l^{-1}) + GA_3 (10 mg l^{-1})
GA_3 (10 mg l^{-1}) + kinetin (200 mg l^{-1})

The fruits were harvested according to the acceptable commercial harvesting criteria and the fruit fresh weight, height (along the longitudinal axis of the fruit from shoulder to the highest calyx lobe), diameter (the mean of two measurements at right angles at the equatorial plane of the fruit) and seed number per fruit were determined. Flesh firmness, soluble solids, total sugars and the L-malic acid content of mature fruit were also determined at the time of harvest and again after four months in cold storage (0–2 °C, 80–85 % RH). All measurements were made in a 5-fruit sample from each replicate (5 fruits x 5 replicates = 25 fruits treatment^{-1}), and were repeated once, with a fresh sample. Mean values for each sample and for the five replicates were calculated accordingly.

Flesh firmness and soluble solids determination

Flesh firmness was determined with a Magness-Taylor penetrometer and expressed in newtons (1 N=4.446222 pounds). Measurements were taken at two points located at the equatorial plane of the fruit, at an angle of 90 °, and their mean was calculated for each fruit of the sample.

For soluble solids determination, each sample consisting of 5 fruits was macerated in a blender and the juice was extracted after filtration. Soluble solids content of the juice, expressed in ºBrix, was determined with a Zeiss portable refractometer.

Determination of total sugars

Total sugars were determined by means of the phenol/sulfuric acid reaction and quantified with the help of a reference curve of known sugar concentrations. The juice of each sample was extracted as described above and was further processed as follows: one ml of an aqueous solution of phenol (5 % w/v) was added in a test tube containing 1 ml of diluted apple juice (1/1000). The mixture was stirred in a vortex stirrer and 5 ml of concentrated H_2SO_4 were added and stirred again. Stirring was repeated after 10 min. The tubes were placed in a water bath (20–25 °C) until the samples had acquired the ambient temperature. The absorbance of the solution at 490 nm was measured in a spectrophotometer and its values were transformed to g l^{-1} of juice.

Determination of L-malic acid

The determination of L-malic acid was done according to the protocol of Boehringer Mannheim/R-Biopharm for the determination of L-malic acid in foodstuffs and other materials (undated). One ml of a solution consisting of a glycylglycine buffer (pH 10.0) and L-glutamic acid (440 mg), 0.2 ml of a freshly prepared solution of lyophilized NAD (210 mg in 6 ml redistilled water), 0.01 ml of a glutamate-oxaloacetate transaminase suspension and, finally, 0.1 ml of diluted apple juice (1/1000 or 1/10) were added, in this order, in a small test tube. The mixture was stirred for 3 min and its absorbance was measured at 340 nm in a spectrophotometer (measurement M1). After measurement, 0.01 ml of L-malate dehydrogenase solution (about 2400 U) was added, the mixture was stirred and after 5–10 min the absorbance of the mixture was measured again at 340 nm (measurement M2). With each measurement the absorbance of an appropriate blank was also determined (B1 and B2). The L-malic acid content was determined from the mathematical equation:

L-malic acid (g $l^{-1}$of juice) = 2.977 x [(M2 − M1) − (B2 − B1)] x DF/6.3

where DF= Dilution Factor

Statistical analysis

The experiments were arranged according to a randomized complete-block design with fourteen treatments and five blocks (replicates), each block consisting of fourteen trees, one for each treatment, arranged in a row. The experimental data were subjected to ANOVA, and statistical differences were determined with the LSD test ($P<0.05$). For the statistical analysis, the software StatView for Macintosh was used.

Results

All treatments, except GA_3, increased the H/D ratio compared to the control and kept it well over 0.95, which is considered as the lower limit for the acceptable shape typical for the cultivar. In addition, kinetin and most treatments with Perlan and Promalin increased fruit weight significantly. On the other hand, the sprayed chemicals did not affect the number of seeds per fruit (Table 1).

All treatments raised significantly the level of total sugars at harvest compared with the control (Table 1). After four months in cold storage, an increase in the content of total sugars was observed in all treatments including the control, compared to the corresponding values at harvest. However, the increase of the control was almost three times that of all other treatments resulting in significantly higher sugar content. A similar pattern was observed for soluble solids, which values fluctuated in different treatments between 10.3 and12.3 °Brix at harvest and between 12.8 and

Table 1. Fruit height/diameter ratio (H/D), fruit fresh weight and number of seeds per fruit of 'Red Chief Delicious' apples, at harvest (September 2001), concentration of total sugars, L-malic acid, soluble solids content and flesh firmness in 'Red Chief Delicious' apples, at harvest (September 2001) and after four months in cold storage (January 2002).

Treatments (mg l^{-1} or ml l^{-1})[2]	H/D	Fresh weight $fruit^{-1}$ (g)	Number of seeds $fruit^{-1}$	Total sugars (g l^{-1} of juice)		L-malic acid (g l^{-1} of juice)		Soluble solids (°Brix)		Flesh firmness (newtons)	
	Sep.	Sep.	Sep.	Jan.	Sep.	Jan.	Sep.	Jan.	Sep.	Jan.	Sep.
Control	0.93 c[1]	230 bc	5.4 a	97.5 c	130.0 a	3.55 d	0.35 d	10.3 c	15.1 a	70.3 b	42.3 b
GA_3 (10)	0.93 c	228 bc	5.3 a	108.0 b	116.5 b	6.05 a	1.10 a	12.1 a	13.5 bc	71.6 b	48.0 a
Kinetin (150)	0.97 bc	254 a	5.3 a	107.5 b	116.0 b	4.20 c	0.49 c	11.8 a	13.5 bc	70.3 b	44.5 ab
Kinetin (200)	0.97 bc	255 a	4.3 a	110.0 ab	119.5 b	4.25 c	0.48 c	11.8 a	14.0 b	72.5 b	45.8 ab
Kinetin (200) +GA_3 (10)	0.98 abc	255 a	3.9 a	109.5 ab	120.0 b	5.55 c	0.78 b	12.3 a	14.0 b	69.8 b	45.8 ab
Promalin (0.5)	1.02 ab	248 ab	5.5 a	112.0 a	118.5 b	4.35 c	0.44 cd	12.1 a	13.4 bc	71.2 b	44.5 ab
Promalin (1.0)	1.00 ab	235 bc	5.0 a	112.0 a	120.5 b	4.20 c	0.43 cd	11.9 a	13.5 bc	70.7 b	44.5 ab
Promalin (1.5)	1.02 ab	240 b	4.6 a	107.5 b	119.0 b	4.40 c	0.41 cd	12.1 a	12.8d	79.6 a	45.8 ab
Promalin (0.5) +GA_3 (10)	1.02 ab	247 ab	4.6 a	107.0 b	115.5 b	5.30 b	0.80 b	12.0 a	13.0 cd	68.9 b	43.6 ab
Perlan (0.5)	1.07 a	230 bc	4.4 a	112.5 a	118.0 b	4.10 c	0.42 cd	12.0 a	13.9 b	68.9 b	43.6 ab
Perlan (1.0)	1.07 a	254 a	4.8 a	110.0 ab	119.0 b	4.25 c	0.43 cd	11.8 a	13.9 b	71.2 b	44.9 ab
Perlan (1.5)	1.02 ab	240 b	4.6 a	108.5 b	116.0 b	4.50 c	0.44 cd	11.4 b	13.5 bc	70.3 b	44.0 ab
Perlan (0.5) +GA_3 (10)	1.02 ab	225 c	5.5 a	110.5 ab	117.0 b	6.15 a	0.80 b	11.9 a	13.7 bc	72.5 b	44.5 ab
Novagib (0.5) +Kinetin (200)	1.01 ab	237 bc	4.8 a	112.0 a	118.5 b	5.85 a	0.55 c	11.8 a	14.1 b	70.7 b	43.6 ab

[1]Means within the same column accompanied by different letters differ significantly at P<0.05 level.
[2]GA_3 and Kinetin: mg l^{-1}; Promalin, Perlan and Novagib: ml l^{-1}.

15.1 °Brix after storage with the control having the lowest value at harvest and the highest after storage (Table 1).

The L-malic acid concentration at harvest was significantly increased in apples treated with gibberellic acid and its combination with Perlan, Promalin or kinetin as well as with the combination of Novagib with kinetin and with kinetin alone, the latter especially at its higher concentration (200 mg l^{-1}) (Table 1). After cold storage, the content of L-malic acid decreased to about 1/10 of the corresponding values at harvest, but remained significantly higher in almost all treatments compared with the control (Table 1).

Flesh firmness in all treatments and the control, ranged between 68.9 and 79.6 N at harvest but it was reduced to 2/3 of the initial value and ranged between 42.3 and 48.0 N after four months in cold storage (Table 1). Although, generally, there were no significant differences among most treatments either before or after storage, the highest value after cold storage was recorded in GA_3-treated apples and the lowest in the control (Table 1).

Discussion

The elongated fruit shape with distinct calyx lobes is the most easily recognizable feature of cultivars belonging to the 'Red Delicious' group and, therefore, it is the first criterion, along with color and appearance free of blemishes, the consumer will consider in assessing fruit quality. All treatments, except GA_3 when applied alone, improved fruit shape and some of them fruit fresh weight, without affecting ovule fertilization *per se*, as it is reflected by the unaltered seed number per fruit. GA_3 in combination with cytokinins, increased H/D ratio in our experiments, as it did in 'Golden Delicious' and 'Jonagold' when combined with the synthetic cytokinin CPPU (Bangerth 1994).

In all treatments, the concentration of total sugars was higher than that of the control at harvest but after four months in cold storage this concentration was significantly higher in the control than in the treated apples. Considering that all treatments consisted of kinetin or BA, gibberellins and various mixtures of these regulators, the above observation clearly shows that gibberellins and cytokinins retarded the rate of increase in sugar content during storage and consequently fruit senescence. A similar effect of kinetin and gibberellins was recorded on the fluctuations in the soluble solids content before and after storage.

All treatments caused a significant increase in the L-malic acid content at harvest, over the control, and despite its dramatic reduction after storage, it was still kept at significantly higher levels mainly in the GA treatments

than in the control. Malic acid constitutes almost 80 % of total organic acids in apples (MORVAI and MOLNAR-PERL 1990), which in combination with sugars are responsible for the particular taste, a significant characteristic of apple quality. The effect of gibberellins and cytokinins on total sugars and malic acid content acquires a special importance, since the deterioration of apple quality during storage was mainly due to an increase in total sugars and a simultaneous decrease in malic acid concentration.

Apart from keeping malic acid content at a high level after storage, gibberellic acid enhanced the effects of Promalin and Perlan and prevented the appearance of internal browning (data not shown). Since it had also prevented the development of russeting (ECCHER 1986; STEENKAMP and WESTRAAD 1988; GIANFANGA 1995) and botrytis, it seems that it possesses antifungal properties and this may well be an additional benefit to be gained from its usage. According to LOONEY (1993) GAs are used on several crops to improve fruit appearance and quality in addition to their potential to reduce pesticide usage. Our data show that GA_3 kept apples in a better condition before and after storage in respect of flesh firmness, L-malic acid and total sugar content. Similarly, GA_3 has been reported to improve post-storage condition of sweet cherries (LOONEY and LIDSTER 1980).

Other parameters of apple fruit quality such as seed number per fruit and flesh firmness were not affected significantly by most treatments, the latter either at harvest or after four months in cold storage. Even in those cases, where differences from the control in flesh firmness were recorded, they were slight and without practical interest. In this respect, our data agree with observations made previously (ECCHER and MAFFI 1986).

In conclusion, the combination of gibberellins and cytokinins, either as such or as commercial mixtures, may be used for the improvement of fruit shape in cultivars belonging to the 'Red Delicious' group since, in addition, such treatments improved most other quality characteristics and retarded fruit senescence during storage. Kinetin might be a very good substitute for BA, eliminating any potential health hazard for the grower and the environment. Furthermore, kinetin did not enhance the appearance of fruit deformities, such as a narrowing under the calyx of the apple fruit (Voyiatzis unpubl.), and it did not affect negatively the return bloom, as it happened with GA_3, Promalin and Perlan (data not shown). In this respect the use of Novagib + kinetin or GA_3 + kinetin instead of Promalin or Perlan could be preferable.

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Addresses of authors: D.G. Voyiatzis (corresponding author), M.A. Koukourikou-Petridou, D.K. Stylianidis, T.E. Sotiropoulos and I.N. Therios, Department of Horticulture, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece and present address of T.E. Sotiropoulos, National Agricultural Research Foundation (NAGREF), Institute of Decidious Trees, 592 00 Naousa, Greece, e-mail: voyiatzi@agro.auth.gr.