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Relationship between Dormancy and Zeatin Level during Pre- and Postharvest Storage Period of Carrots (*Daucus carota* L.)

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Summary

The cytokinin zeatin level was determined in carrots during pre- and postharvest period to assess a potential relationship with dormancy. Samples of the leaves and roots of the F_1 carrot cv. 'Nandor' were obtained approximately two months before harvest for hormone analysis. After harvest, carrots were stored in 5 kg perforated polyethylene (PE) bags, and placed into the cold stores set at 0 ° and 5 °C, and 85 % to 90 % relative humidity (RH). At monthly intervals in the post-harvest period, all samples were evaluated for their content of the zeatin, rooting ratio and degree and sprouting ratio and degree. Leaves possess a higher zeatin content than roots in the pre harvest period. During growth and development, zeatin content decreased in leaves, but increased in the root tissues. In the post-harvest period, zeatin levels of roots stored at 5 °C increase and then subsequently decline. Rooting and sprouting are observed at the same time when the level of cytokinin reaches its highest value. These data show that increasing levels of zeatin cause dormancy break in carrot tissues, and that levels of cytokinin fall with aging. Zeatin increases in 0 °C treatment beginning of month 4.

Zusammenfassung

Beziehung zwischen dem Zeatingehalt von Möhren vor der Ernte und während der Ruhephase im Kühllager. Der Zeatingehalt von Möhren wurde vor und nach der Ernte ermittelt und seine Beziehung zum Lagerverhalten untersucht. Zu diesem Zweck wurden in den letzten Monaten vor der Ernte fünf bzw. sechs Wurzel- und Blattproben von Karotten der F_1-Sorte 'Nandor' zur Hormonanalyse entnommen. Nach der Ernte wurden die Möhren in Polyethylensäcke (PE) mit 5 kg Fassungsvermögen gepackt und bei Temperaturen von 0 ° und 5 ° C bei 85–90 % relativer Luftfeuchtigkeit in Kühlräumen eingelagert. Die Zeatinkonzentration sowie Grad und Ausmaß der Wurzelbildung und des Austriebs wurden bei allen Proben in monatlichen Intervallen festgestellt. Vor der Ernte war der Zeatinpegel in den Blättern höher als in den Wurzeln. Während die Möhren heranwuchsen und sich entwickelten, nahm der Zeatingehalt der Blättern ab, während der Wert in den Wurzeln anstieg. Nach der Ernte stieg der Zeatingehalt der bei 5 °C gelagerten Möhre an, um dann wieder abzunehmen. Dieser Anstieg war zeitgleich mit dem Beginn der Bewurzelung und des Austriebs zu beobachten. Die Daten belegen, dass die Ruhephase mit Anstieg des Zeatins endet und dass der Cytokiningehalt während des Alterungsprozesses sinkt. Bei der 0 °C-Variante begann der Zeatinanstieg im vierten Monat nach der Ernte.

Key words. carrot (*Daucus carota* L.) – dormancy – zeatin – growth – postharvest – sprouting – rooting

Introduction

One of the most important changes in the quality of root vegetables occurring during the post-harvest storage period is rooting and sprouting. While environmental conditions can induce rooting and sprouting, endogenous factors such as hormones, exert this effect as well as numerous studies show particularly of onion, garlic and potatoes. Rooting and sprouting may be side-effects of the action of several interacting hormones to regulate physiological events in the pre-harvest stages of growth and development (KAMERBEEK et al. 1970; KEFELI and KADYROV 1971).

Onions at harvest show high levels of substances inhibitory to the action of auxin, gibberellins, and cytokinins, whose levels are low (WAREING and SAUNDERS 1971; ISENBERG et al. 1987). After harvest of onion, the levels of these inhibitory substances decrease during storage, and this is matched by increases in the levels of promoter hormones. Increases in levels of gibberellins result in the development of sprouting, and increases in cytokinins stimulate cell proliferation in the meristem zones. Dormancy observed in onions for the first three months of storage is attributed to a high abscisic acid (ABA) content. Following this dormant period, the content of promoter hormones increases and breaks down the state of dormancy. This leads to sprouting and results in the loss of stored onions. Storage at high temperatures has also been identified as leading to sprouting in onions (MIEDEMA and KAMMINGA 1994).

In addition, exogenous application of the cytokinin benzyladenine (BA) strongly inhibits sprouting. Cytokinins are the primary factor involved in the inhibition of sprouting in root tissues (MIEDEMA 1994). Prolongation of dormancy and delay in sprouting and rooting as an effect of temperature have also been demonstrated in garlic. Dormancy is broken more rapidly for garlic stored at 5 ° than at 35 °, with ABA levels increasing slowly at the cold temperature along with both cytokinin and GA_3 levels in the early phase of dormancy, followed by a greater increase in ABA levels later (LI-XIAODONG et al. 1996). There is a large increase in cytokinin levels of onions after dormancy break (ISENBERG et al. 1987). SUKHOVA et al. (1993) has reported that IAA has no significant effect on tuber dormancy, while cytokinins are probably necessary for sprouting initiation. In potato tubers, there is no correlation between the immediate post-harvest endogenous levels of zeatin (Z) and isopentenyladenosine (IP) and the occurrence of dormancy break (SUTTLE 2001).

Although the effects of these hormones on controlling the growth, development and senescence is reported for many horticulturally important crops, little is understood about how changes in endogenous levels of hormone affect the dormancy of carrots during the cold storage period. We report upon the relationship between the level of the cytokinin zeatin and rooting and sprouting in carrot roots in the pre- and post-harvest period.

Materials and Methods

'Nandor' F_1 carrot cultivar was used in this study, and was cultivated in the Ankara-Ayaş district, the area in Turkey where carrots are the most widely grown.

Production, harvest and storage of carrot

Seeds were sown in 15×7 cm areas in the field. Local practices of cultivation were applied during plant production. Plants were allowed to grow until four to five leaves had formed. Thereafter, and starting at 75 days prior to harvest, leaf and root samples were collected for 15-day intervals. Upon completion of root development, and as green tops began to turn yellow, the roots were harvested in November, washed, topped, and finally packed into 5 kg size perforated polyethylene bags, with twelve 5 mm diameter perforations. Relative humidity was kept between 85 and 90 % for both temperatures, 0 ° and 5 °C, in these post-harvest studies.

Ten carrots were used for each data point in an experiment, and three replications of each experiment were performed.

Cytokinin analysis

The method of GUINN and BRUMMETT (1990) was used for the determination of the cytokinin level. Briefly tissues are extracted in methanol containing BHT (Butylated hydroxytoluence) and disodium EDTA, and subsequently extracted in hexane. The residue remaining after evaporation of hexane is acidified slightly and passed through a series of reversed-phase cartridges (C_{18}), strong anion exchange cartridges, and nylon filters. After these extensive preparative steps, the sample is analyzed using a Hewlett-Packard HPLC system equipped with a 0.46×25 cm long SCX column with 5 μm particle, with an initial mobile phase of 0.02 N acetic acid containing 60 % methanol. Detection was performed at 254 nm. 1 mL fractions were collected and methanol was removed with a centrifugal evaporator. For zeatin analysis, the mobile phase was changed to 10 mM potassium phosphate, pH 3, containing 40 % MeOH.

Rooting and sprouting ratio

The method developed by RYLSKI et al. (1974) was used. The procedure involves calculating the percentage of carrots that have sprouts and roots.

Rooting and sprouting degree

The scale in Table 1 was developed by HALLORAN et al. (1997) for evaluation of the degree of rooting and sprouting in carrots. Root and sprout number and length were chiefly considered in the development of this scale. For each carrot, a value for the degree of rooting is assigned based on both the mean length and count of adventitious roots. Also for each carrot, a value for the degree of sprouting is assigned based on both the mean length of sprouts and the count of sprouts. The degree value is determined according to the evaluation matrix given in Table 1. For example, if a carrot has 7 adventitious roots, and the mean length of the 7 roots is 2.45 cm, the rooting degree obtained from the table shows a value of 4 based on the count and mean length. The same process is applied in the evaluation of sprouts to determine sprouting degree.

Table 1. Scale for degree of sprouting and rooting.

Rooting and sprouting degree	Number of roots or sprouts	Length of roots or sprouts (mm)
0	<3	<1
1	≥3	<1
2	1–3	1–3
3	1–3	>3
4	>3	1–3
5	>3	>3

Statistical analysis

A completely randomized model with repeated measures using factorial design was used to evaluate the results of the mean values of three replicates (WINER 1971). Minitab Windows 11 and Mstat computer programs were used for all statistical analyses. Statistical differences were determined with Duncan multiple range test at $p<0.05$ significance level.

Results

Changes in zeatin level during the pre-harvest period

Changes in zeatin levels in carrot leaves and roots during the pre-harvest period are shown in Fig. 1. Zeatin increases steadily in roots close to harvest, but generally declines in leaves. The zeatin level was determined to be 4.12 ng g^{-1} in root tissues and 19.26 ng g^{-1} in leaf tissues two months prior to harvest. The values were 15.42 ng g^{-1} and 12.17 ng g^{-1} at harvest, respectively. The differences between zeatin levels of root and leaf tissues and between evaluation dates were statistically significant.

Effect of different storage temperatures on zeatin content

The zeatin level was about 21 ng g^{-1} at harvest time of carrot root tissues (Fig. 2). At the beginning of storage period, the level of cytokinin is generally low at 0 °C. At 5 °C storage, the zeatin level peaks at a significantly higher level in the first month, but returns quickly to the pre-storage level in subsequent months. At 0 °C, zeatin content shows no difference from pre-storage levels until the 4th month, after which the level increases tremendously. The zeatin level is as low as 14.74 ng g^{-1} in the first month of storage at 0 °C, increasing to 80.83 ng g^{-1} by the end of storage period. In 5 °C storage, the zeatin level is as high as 35.79 ng g^{-1} in the first month, decreasing to 18.54 ng g^{-1} by the last month of storage. Differences between zeatin levels at two temperatures in different months are statistically significant.

Sprouting ratio

Table 2 shows that there are significant differences between the sprouting ratios of carrots stored at 0 ° and

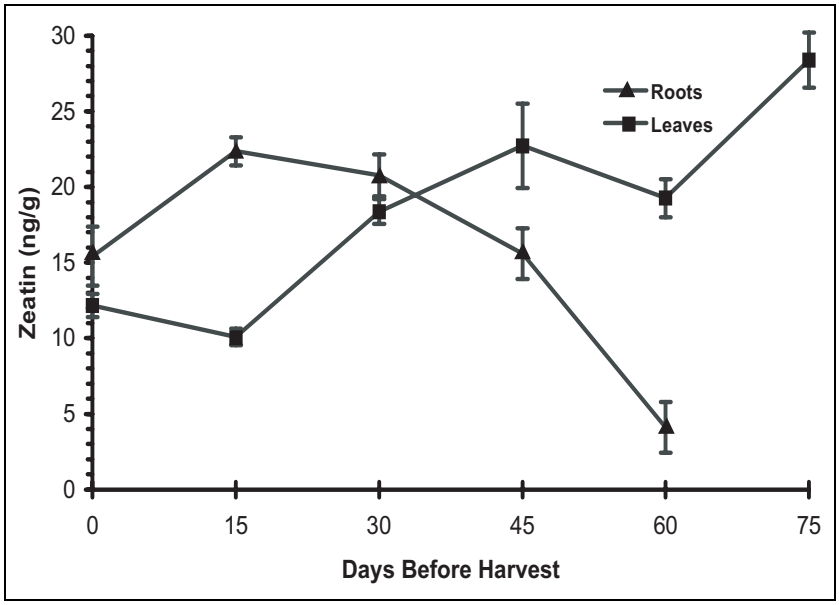


Fig. 1. Changes at zeatin content of carrot leaf and root tissues during pre-harvest period

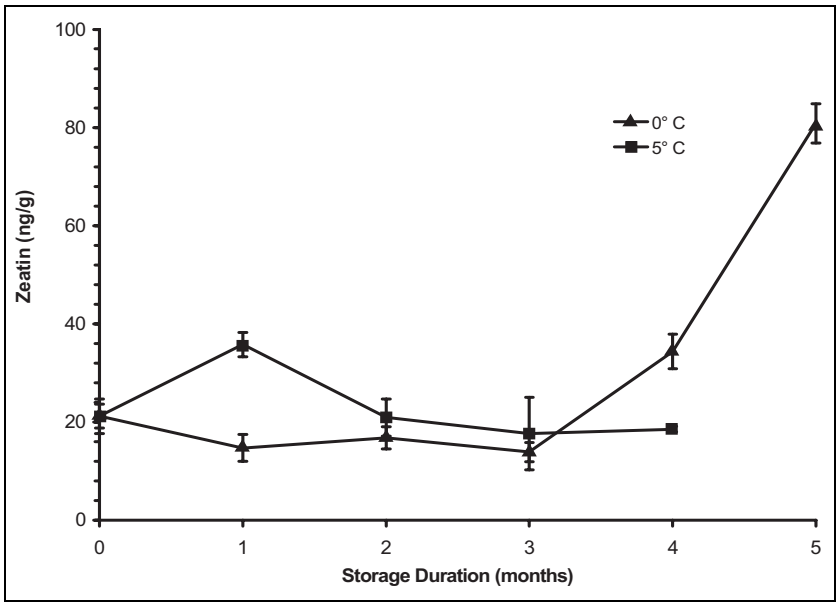


Fig. 2. Changes in zeatin content of root tissues at various storage temperatures

5 °C temperatures. Sprouting at 5 °C begins in the first month of storage, and all carrots show sprouting by the end of the second month. For carrots stored at 0 °C, no sprouting is observed until the end of 4th month. By the end of the 5th month at 0 °C, 66.7 % of roots show sprouting (in the month of May). Differences in sprouting ratios at the two storage temperatures are significant starting from the second month of storage period (in the month of January).

Sprouting degree

Changes in the degree of sprouting of carrots over time at the two storage temperatures are shown in Table 3. Sprouting degree is determined by considering the number and length of the sprouts. For the first month at the 5 °C storage temperature, the sprouting degree was 0.76. Sprouting degree increases with the storage duration, and reaches 5 points by the end of storage period at 5 °C. Sprouting degree is 0.67 at the end of storage at 0 °C temperature. Differences in degree of sprouting between the two storage temperatures and months are statistically significant starting from the second month of storage.

Rooting ratio

Increases in the rooting ratio over time are significantly higher for carrots stored at 5 °C compared to carrots stored at 0 °C (Table 4). In the second month of storage (February) the rooting ratio is 38.1 %, while there is no indication of rooting in carrots stored at 0 °C. For the carrots stored at 5 °C, the ratio values reaches 57.1 % by April and 100 % by May. At the fifth month (May) of 0 °C storage, the ratio value reaches only 23.8 %. Differences between months at 5 °C storage condition are statistically significant.

Rooting degree

The rooting degree of carrots stored at 5 °C reaches 0.37 in the second month of storage (Table 5). By the third month, it nearly doubles, with many carrots having one or two roots featuring lengths under 1 cm. At this storage temperature, there was a very rapid increase in degree of rooting toward the end of the storage period, with a mean value of 3.43. Stored carrots at this time have five or more secondary roots. In contrast to carrots stored at the higher temperature, carrots

Table 2. Sprouting ratio (%) in carrot root tissues during storage at different temperatures*

Storage duration (months)	Storage temperature 0 °C	5 °C
0	0.0 a A	0.0 a A
1	0.0 b A	71.2 a B
2	0.0 b A	100.0 a C
3	0.0 b A	100.0 a C
4	0.0 b A	100.0 a C
5	66.7 B	—

*Small and capital letters indicate significant differences at the $p<0.05$ level between data points for temperatures (small letters) and storage periods (capital letters), respectively.

Table 3. Sprouting degree in carrot root tissues during storage at different temperatures*

Storage duration (months)	Storage temperature 0 °C	5 °C
0	0.00 a A	0.00 a A
1	0.00 a A	0.76 b B
2	0.00 a A	2.81 b C
3	0.00 a A	4.14 b D
4	0.00 a A	5.00 b E
5	0.67 B	—

*Small and capital letters indicate significant differences at the $p<0.05$ level between data points for temperatures (small letters) and storage periods (capital letters), respectively.

Table 4. Rooting ratio (%) in carrot root tissues during storage at different temperatures*

Storage duration (months)	Storage temperature 0 °C	5 °C
0	0.0 a A	0.0 a A
1	0.0 a A	0.0 a A
2	0.0 a A	38.1 b B
3	0.0 a A	57.1 b C
4	0.0 a A	100.0 b D
5	23.8 B	—

*Small and capital letters indicate significant differences at the $p<0.05$ level between data points for temperatures (small letters) and storage periods (capital letters), respectively.

Table 5. Changes at rooting degree in carrot root tissues during storage at different temperatures*

Storage duration (months)	Storage temperature 0 °C	5 °C
0	0.00 a A	0.00 a A
1	0.00 a A	0.00 a A
2	0.00 a A	0.37 a B
3	0.00 a A	0.71 b B
4	0.00 a A	3.43 c C
5	0.24 B	—

*Small and capital letters indicate significant differences at the $p<0.05$ level between data points for temperatures (small letters) and storage periods (capital letters), respectively.

stored at 0 °C show nearly no increase in rooting degree, with a mean value of 0.24 at the end of the storage period. Data points between the two storage temperatures at the start of the third month are found significantly different.

Discussion

An indicator of the effectiveness of good post-harvest storage of carrots is the appearance of unwanted sprouting and rooting. Certain environmental controls, such as low temperature storage, can delay rooting and sprouting, but cannot prevent it entirely. Research efforts have focused on relation between storage conditions and changes in the levels of endogenous hormones in order to understand the regulation mechanism of the physiological processes involved in rooting and sprouting of some root vegetables and tubers (ISENBERG et al. 1987; LUDFORD 1990, 1995).

The zeatin analysis performed during the growth and development period in this research showed that this cytokinin accumulates in root tissues of carrots and the level of cytokinin decreases just before harvest. Completely contrasting results were found in carrot leaf tissues. Our results show that cytokinins are not only synthesized in root tissues as is stated by ARTECA (1995), but they are also translocated from leaf tissues to root during root growth and development. Cytokinin translocation stops as soon as roots have matured.

Post-harvest zeatin measurements showed an increase at different times during storage at 5 ° and 0 °C temperatures. Peak values at both storage temperatures match with the time of the beginning of sprout and root formation. After the peak value, there is a general decrease in cytokinin level at 5 °C. Cytokinins accumulate throughout storage period and reach the peak value when sprouting and rooting occurs at 0 °C. These observations indicate that cytokinins are one of the reasons for breaking down dormancy in carrot tissues. And it is probably related to other growth regulators in the root tissue as previously reported for potatoes by HEMBERG (1970). He reported that cytokinins quickly break down dormancy in potatoes. SUKHOVA et al. (1993) reported similar results. According to their results, cytokinin level increases at the end of dormancy period and decreases with sprouting. They showed an absolute necessity for cytokinins in order to induce sprout formation. In contrast, SUTTLE (2001) has shown that Z (zeatin) and IP (isopentenyladenosine) are ineffective in breaking down tuber dormancy. Cytokinin-related dormancy break has been shown for garlic by LI-XIAODONG et al. (1996), and for onions by WAREING and SAUNDERS (1971), ISENBERG et al. (1987) and MIEDEMA and KAMINGA (1994).

Changes in the levels of other hormones may relate to dormancy, rooting and sprouting. Levels of IAA decrease in potatoes, while sprouting occurs at the end of dormancy period (SUKHOVA et al. 1993). Cytokinin levels are constant until the first appearance of sprouting, after which the sprouting increases are associated with increase of levels of zeatin ribose and isopentenyladenosine. The conclusion was that cytokinin should be present to break dormancy and that IAA has no effect on dormancy. HEMBERG (1970) has supported this finding by showing a relationship with endogenous kinetin and zeatin levels in potatoes.

Temperature significantly affects sprouting and rooting, which are the most important quality criteria for long term stored carrots. Temperatures as low as 0 °C limited the sprout and secondary root formation. A prolonged storage period caused breakdown in carrot attributed to a decrease in zeatin level. Dormancy break occurred in the early period of storage at 5 °C temperature. The effect of temperature on sprouting and rooting of onions and tubers was previously reported by HARDENBURG et al. (1986), MIEDEMA (1994), MIEDEMA and KAMINGA (1994) and SUTTLE (2001).

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