Asian Journal of Plant Sciences

ISSN 1682-3974

Asian Journal of Plant Sciences 7 (3): 319-322, 2008
ISSN 1682-3974

The Seasonal Changes in Endogenous Levels of Indole-3-Acetic Acid, Gibberellic Acid, Zeatin and Abscisic Acid in Stems of Some Apple Varieties (*Malus sylvestris* Miller)

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Abstract: Seasonal changes of total indole-3-acetic acid (IAA), gibberellic acid (GA_3), zeatin and abscisic acid (ABA) levels were investigated in stem cuttings of apple (*Malus sylvestris* Miller) varieties, Golden delicious, Starkrimson delicious and Misket delicious' at different times of the year. All of the apple varieties exhibited seasonal differences in hormone levels. The total IAA, GA_3 and zeatin contents of these apple varieties increased to the highest value in May but it was the lowest in February. Whereas ABA levels observed in May were lower in the studied apple varieties, but it was the highest level in February. Except for ABA other hormones, IAA, GA_3 and zeatin levels, were the lowest in 'Misket delicious' among the all apple varieties in all sampling periods (August-May).

Key words: Abscisic acid, apple, gibberellic acid, indole-3-acetic acid, seasonal changes, zeatin

INTRODUCTION

It is known that the plant hormone levels are varied relating to seasons in some plants (Funada *et al*., 2001; Suzuki *et al*., 2004). The plant hormones are organic substances which occur naturally and influence the physiological processes at low concentrations. The main influence is growth differentiation and development, although other processes, such as stomatal movement, may also be affected. Plant hormones have also been referred to as phytohormones (Davies, 1995; Iten *et al*., 1999). Complex physico-biochemical changes including the balance between endogenous phytohormones, lowering the levels of some and raising others, are the result of different environmental variables. An increasing number of reports indicate that environmental variables have a great influence on the endogenous levels of several types of plant hormones. For instance, constituent of the plant hormones change under low and high temperature, light, drought and salinity (Daie and Campbell, 1981; Walker and Dumbroff, 1981; Chen *et al*., 1983; Srivastava, 2002).

Auxin is the mobile signal controlling the rate of growth and specific aspects of the development of plants. It has been known for over a century that auxins act as a messenger linking in the plant development at specific environmental changes (Rapparini *et al*., 2002). Abscisic acid (ABA) plays an important role in synthesis of protein and nucleic acid and can reverse the effect of growth promoters such as gibberellic acid (GA_3), indole-3-acetic acid (IAA) and cytokinins (Fosket, 1994).

Blakesley *et al*. (1991) reported the results about of the seasonal changes of IAA and ABA levels in the leaf, the upper stem and the lower stem of in *Cotinus coggygria*. In cuttings taken in early June, the level of IAA was much higher than that of conjugated IAA. In late July, the opposite was found. No significant differences in ABA levels were found although the ABA/IAA ratio changed dramatically.

Funada *et al*. (2001) showed the seasonal variations in endogenous indole-3-acetic acid and abscisic acid in the cambial region of *Pinus densiflora* stems in relation to earlywood-latewood transition and cessation of tracheid production. The total amount of IAA changed seasonally in all trees and at all stem positions, being maximal in early summer (May or July). The total amount of ABA was lower than that of IAA in all trees and at all stem heights and the changes were not correlated with specific changes during the annual cycle of cambial activity and dormancy.

The aim of this study was to investigate the endogenous IAA, GA_3, ABA and zeatin levels in the stems of these three apple varieties (Golden delicious, Starkrimson delicious and Misket delicious) and determine its the seasonal variations.

MATERIALS AND METHODS

Plant material: The plant materials (ten to fifteen cm long) were collected from naturally growing stems of three apple (*Malus sylvestris* Miller) varieties (Golden delicious, Starkrimson delicious and Misket delicious), then the

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Table 1: The mean meteorological conditions during the sampling periods in 2000-2002

Meteorological parameters	August	October	February	May
Temperature (°C)	23.1	13.7	3.7	15.5
Humidity (%)	61.4	67.2	67.3	63.9
Total rainfall (mm)	12.4	29.0	35.2	64.2

leaves/flowers were removed. Stems were collected at altitude 623 m in Tokat-Turkey (the latitude of Tokat is at 39°51'-40°55' N and 35°27'- 37°39' E) in August, October, February and May 2000-2002 and analysed. Some parameters of the meteorological conditions during the sampling periods were showed in Table 1.

Hormone analysis: Five gram fresh stems samples of each apple varieties were placed in 100 mL methanol: chloroform: 2 N ammonium hydroxide (12:5:3 v/v/v) and homogenized using a Kinematic Polytron homogenizer. After addition 1 μg/100 mL Butylated Hydroxytoluene (BHT), the samples were frozen at -80°C for one week, for further analysis. Then the extracts were transferred into 250 mL conical flasks and added 22.4 mL bi-distilled water. To obtain a homogenize mixture, the conical flasks were shaken 3 or 4 times. Thus, with the exception of plant growth substances, the other organics in methanol were allowed to pass into the chloroform phase. In all varieties of apple samples, the extraction, purification and quantitative determination of total IAA, GA_3, ABA and zeatin were done according to literature methods of Unyayar *et al.* (1996) and Yureklı *et al.* (1999, 2001). A scanning densitometer (DESAGA CD 60) was used to determine the amounts of IAA, GA_3, ABA and zeatin (Saucedo *et al.*, 1989; Yüreklı *et al.*, 1999, 2001). Results are the mean of three replicates.

Statistical analysis: Data from three replications of all treatments were subjected to analysis of variance using SPSS 8.0 for Windows for all statistical analysis. Differences between means at 5% ($p<0.05$) level were considered as significant.

RESULTS

The total endogenous IAA contents of three apple varieties (Golden delicious, Starkrimson delicious and Misket delicious) related to sampling periods are shown in Fig. 1. IAA concentration showed a major peak in May for all of the apple varieties. The lowest IAA endogenous content in stems of three apple varieties were observed in February (Fig. 1, $p<0.05$). In the all sampling periods (August-May), the highest the total IAA were found in Golden delicious and the lowest values were found in Misket delicious (Fig. 1, $p<0.05$).

The total endogenous GA_3 levels of the apple varieties (Golden delicious, Starkrimson delicious and Misket delicious) exhibited seasonal variations (Fig. 2). Endogenous GA_3 levels of the three apple varieties were the lowest in February and the highest in May ($p<0.05$). In Golden delicious and Starkrimson delicious, the GA_3 content was higher than Misket delicious in all sampling periods (August-May) (Fig. 2, $p<0.05$).

The total endogenous zeatin levels of the three apple varieties were the highest in May (Fig. 3, $p<0.05$). In Starkrimson delicious and Misket delicious, total zeatin levels were found the lowest in February (Fig. 3, $p<0.05$). Very similar variation pattern was also observed in Golden delicious in October and February. In Starkrimson delicious, the zeatin level was higher than other apple varieties in May. In Misket delicious stems of zeatin levels were the lowest at the all of sampling periods (August-May) (Fig. 3).

The total endogenous ABA levels for all of the apple varieties (Golden delicious, Starkrimson delicious and Misket delicious) are shown in Fig. 4. Endogenous ABA

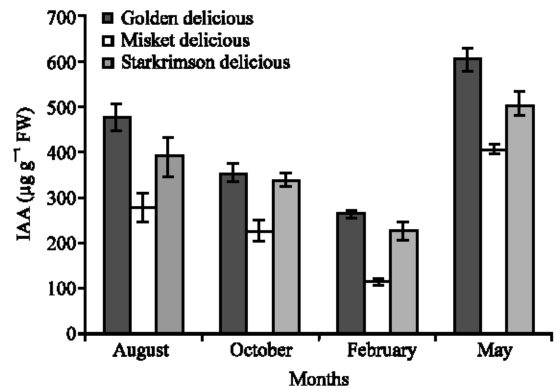

Fig. 1: Seasonal IAA contents of the stems of Golden delicious, Misket delicious and Starkrimson delicious

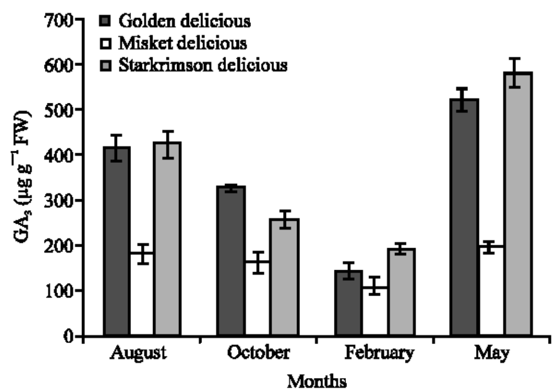

Fig. 2: Seasonal GA_3 contents of the stems of Golden delicious, Misket delicious and Starkrimson delicious

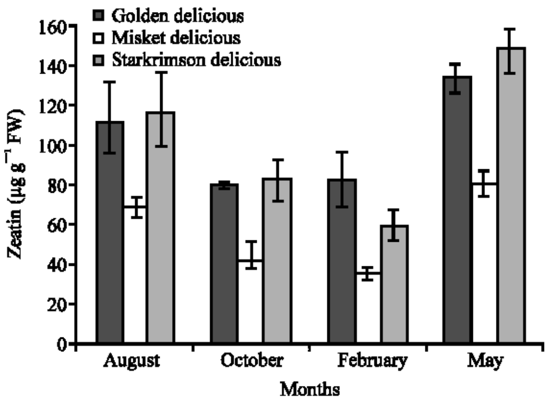

Fig. 3: Seasonal zeatin contents of the stems of Golden delicious, Misket delicious and Starkrimson delicious

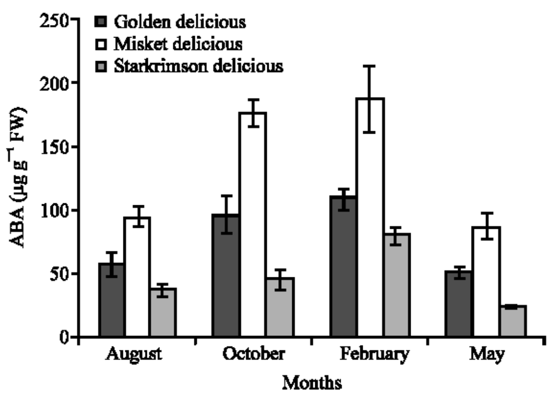

Fig. 4: Seasonal ABA contents of the stems of 'Golden delicious, Misket delicious and Starkrimson delicious

levels of the three apple varieties were the highest in February but the lowest in May (Fig. 4, $p<0.05$). In Misket delicious, ABA content was the highest of all the other apple varieties in all sampling periods (August-May) but the lowest in Starkrimson delicious (Fig. 4, $p<0.05$).

DISCUSSION

The plant hormones are organic substances which occur naturally and influence the physiological processes at low concentrations. External factors such as temperature, light, water supply and nutrition affect the internal factors such as carbohydrates and hormones levels (Gur, 1985). Present results also support that the hormone levels are related to the seasonal variations (Fig. 1-4). Day and Loveys (1995) reported that seasonal variation in *Hypocalymma angustifolium* and *Boronia megastigma* propagation success may be mediated through changes in the endogenous plant growth regulator or carbohydrate concentrations. Seasonal variations of IAA and ABA in the leaves of *Citrus sinensis* L. were reported elsewhere. There was a major ABA peak in early spring. ABA levels were low in summer. There was a minor ABA peak in autumn. Endogenous IAA in leaves was the highest in winter/spring (Plummer *et al*., 1991). Kamboj *et al*. (1999) reported the concentrations of ABA and IAA measured by GC-MS-SIM in the shoot bark of clonal apple rootstocks (M27, M9, MM106, MM111) when the rootstocks were growing actively in the UK. Generally the concentrations of ABA in shoot bark from all rootstocks were smallest in May; they then increased until July before declining in August. IAA values from the August samples were slightly lower than other months (May, June and July). In the present study, the ABA levels in the all of apple varieties was the highest in winter (February) but the lowest in spring and in summer (May and August) (Fig. 4). However, endogenous IAA was highest in spring (May) (Fig. 1). The increase of the ABA in February may be related to the decreasing of the growth period (cold, water stress). The increase of the IAA in May may be related to the increasing of the growth period.

Tromp and Ovaa (1990) reported that the total cytokinin concentration was low from mid summer until late in winter xylem sap of mature apple trees. In literature xylem extrudate of Douglas-fir was reported levels of cytokinin-like compounds varied throughout the spring but generally were the highest level from late April to early May (Doumas and Zaerr, 1988). The present study support the above report which explained the zeatin levels of the three apple varieties were the highest in spring (May) but the lowest in winter and autumn (February and October) (Fig. 3).

Nagar and Kumar (2000) reported that the changes in gibberellin activities were detected in tea shoots during winter dormancy and subsequent to dormancy release. Free GA-like activity was extremely low at the initiation of dormancy and remained so during the dormancy period. Conjugated GA-like activity (ficin hydrolyzable and beta-glucosidase hydrolyzable compounds) remained high during the dormancy period. Gibberellins could play a role in the initiation of shoot growth by dormant plants in the spring (Kramer and Kozlowskı, 1979). In the present study, the GA_3 levels of the all apple varieties were the highest in spring (May) but the lowest in winter (February) (Fig. 2). For this reason, the increasing of GA_3 in all apple varieties in spring may be related to the bud activity.

CONCLUSION

IAA, GA_3, zeatin and ABA levels exhibited seasonal differences in stem of the all apple varieties investigated.

Except for ABA, IAA, GA_3 and zeatin levels increased in May and decreased in February. Adversely, ABA levels decreased in May and increased in February. These findings could be taken some evidence for the adaptation processes of three apple varieties have developed against favourable or unfavourable environmental conditions such as temperature variations occurring in different seasons.

ACKNOWLEDGMENT

Financial support from Cumhuriyet University, Turkey is gratefully acknowledged.

REFERENCES

Blakesley, D., G.D. Weston and M.C. Elliot, 1991. Endogeneous levels of indole-3-acetic acid and abscisic acid during the rooting of *Cotinus coggygria* cuttings taken at different times of the year. Plant Growth Regul., 10 (1): 1-12.

Chen, H.H., P.H. Li and M.L. Brenner, 1983. Involvement of abscisic acid in potato cold acclimation. Plant Physiol., 71 (2): 362-365.

Daie, J. and W.F. Campbell, 1981. Response of tomato plants to stressful temperatures. Plant Physiol., 67 (1): 26-29.

Davies, P.J., 1995. Plant Hormones. Kluwer Academic Publishers, Netherlands.

Day, J.S. and B.R. Loveys, 1998. Propagation from cuttings of two woody ornamental Australian shrubs, *Boronia megastigma* Nees and *Hypocalymma angustifolium* Endl. Aust. J. Exp. Agric., 38 (2): 201-206.

Doumas, P. and J.B. Zaerr, 1988. Seasonal changes in levels of cytokinin-like compounds from Douglas-fir xylem extrudate. Tree Physiol., 4 (1): 1-8.

Fosket, D.E., 1994. Plant Growth and Development: A Molecular Approach. Academic Press, San Diego.

Funada, R., T. Kubo, M. Tabuchi, T. Sugiyama and M. Fushitani, 2001. Seasonal variations in endogenous indole-3-acetic acid and abscisic acid in the cambial region of *Pinus densiflora* Sieb. et Zucc. Stems in relation to earlywood-latewood transition and cessation of tracheid production. Holzforschung, 55 (2): 128-134.

Gur, A., 1985. Rosaceae-Deciduous Fruit Trees. In: Handbook of Flowering, Halevy, A.H. (Ed.). Vol. 1. Boca Raton: CRC Press, pp: 355-389.

Iten, M., T. Hoffmann and E. Grill, 1999. Receptors and signalling components of plant hormones. J. Recept Sig. Transd. Res., 19 (1-4): 41-48.

Kamboj, J.S., G. Browning, P.S. Blake, J.D. Quinlan and D.A. Baker, 1999. GC-MS-SIM analysis of abscisic acid and indole-3-acetic acid in shoot bark of apple rootstocks. Plant Growth Regul., 28 (1): 21-27.

Kramer, P.J. and T.T. Kozlowskı, 1979. Physiology of Woody Plants. Academic Press. Inc. London, pp: 538.

Nagar, P.K. and A. Kumar, 2000. Changes in endogenous gibberellin activity during winter dormancy in tea (*Camella sinensis* (L.) O. Kuntze). Acta Physiol. Plant., 22 (4): 439-443.

Plummer, J.A., M.G. Mullins and J.H. Vine, 1991. Seasonal changes in endogenous ABA and IAA and the influence of applied ABA and auxin in relation to shoot growth and abscission in Valencia Orange (*Citrus sinensis* (L.) Osbeck). Plant Growth Regul., 10 (2): 139-151.

Rapparini, F., Y.Y. Tam, J.D. Cohen and J.P. Slovin, 2002. Indole-3-acetic acid metabolism in *Lemna gibba* undergoes dynamic changes in response to growth temperature. Plant Physiol., 128 (4): 1410-1416.

Saucedo, J.E.N.S., J.N. Barbotın and D. Thomas, 1989. Continuous production of gibberellic acid in fixed-bed reactor by immobilized mycelia of *Gibberella fujikuroi* in calcium nalginate beads. Appl. Microbiol. Biotechnol., 30 (3): 226-233.

Srivastava, L.M., 2002. Plant Growth and Development: Hormones and Environment. Academic Press, San Diego.

Suzuki, A., M. Watanabe, S. Komori and H. Bessho, 2004. Comparison of endogenous IAA and cytokinins in shoots of columnar and normal type apple trees. J. Jap. Soc. HortSci., 73 (1): 19-24.

Tromp, J. and J.C. Ovaa, 1990. Seasonal changes in the cytokinin composition of xylem sap of apple. J. Plant Physiol., 136 (5): 606-610.

Unyayar, S., S.F. Topcuoglu and A. Unyayar, 1996. A modified method for extraction and identification of Indole-3-Acetic Acid (IAA), Gibberellic Acid (GA_3), Abscisic Acid (ABA) and zeatin produced by *Phanerochaete chrysosporium* ME446. Bulg. J. Plant Physiol., 22 (3-4): 105-110.

Walker, M.A. and E.B. Dumbroff, 1981. Effect of salt stress on abscisic acid and cytokinin levels in tomato. Z. Pflanzen Physiol., 101 (5): 461-470.

Yureklı, F., Ö. Yesılada, M. Yureklı and Ş.F. Topcuoglu, 1999. Plant growth hormone production from olive oil mill and alcoholfactory waste waters by white rot fungi. World J. Microbiol. Biotechnol., 15 (4): 503-505.

Yüreklı, F., İ. Turkan, B.Z. Porgalı and Ş.F. Topcuoglu, 2001. Indoleacetic acid, gibberellic acid, zeatin and abscisic acid levels in NaCl-treated tomato species differing in salt tolerance. Isr. J. Plant Sci., 49 (4): 269-277.