Pak. J. Bot., 12(2): 189-194, 1980.

THE HORMONAL STATUS DURING LEAF DEVELOPMENT OF FRUIT-BEARING AND NON FRUIT-BEARING *CERATONIA SILIQUA* L. PLANTS.

IHSAN ILAHI

Department of Botany, University of Peshawar, Pakistan.

Abstract

Buds and leaves of Carob (*Ceratonia siliqua* L.) contained four growth promoting substances (P_1-P_4) and one inhibitor (I_1) at various stages of development. Buds from trees with fruit contained higher concentration of P_2 (gibberellin-like substance) compared to that of P_3 (auxin-like). The concentration decreased as the leaves expanded and mature leaves had an insignificant amount of the promotors. Buds from trees with fruit contained insignificant amount of growth promoting substances. Their concentration increased after about a month when buds started to sprout and then exhibited a sudden decrease in mature leaves. This decrease was consistent with their counterparts from the non fruit-bearing trees.

Concentration of the inhibitor was higher in buds and leaves from trees with fruit compared to those without. Leaves from both types of trees exhibited a gradual increase in the inhibitor content with leaf expansion. Mature and fully expanded leaves contained a significant amount of the inhibitor. The delay in active growth and high inhibitor content in these vegetative organs from fruit-bearing and non fruit-bearing trees is explained.

Introduction

Auxins and gibberellins are of wide occurrence in buds and leaves at different developmental stages (Bentley, 1958; Humphries & Wheeler, 1963) and their production is correlated with leaf expansion (Goodwin, 1937; Wetmore & Jacobs, 1953; Wheeler, 1960). Majority of these reports established the presence of indoleacetic acid as the principal naturally occurring auxin. In addition gibberellin-like substances have also been reported from different species, either alone or in combination with the auxins.

The nature of growth regulating substances has been reported from reproductive organs (Ilahi, 1979) and fruit of carob (*Ceratonia siliqua* L.) at different stages of development (Ilahi & Vardar, 1974, 1976 a & b). In the present studies the nature and level of growth regulatory substances from the leaves at different stages of development is described. Since carob does not fruit after some years, a comparison has also been made of the origin, behaviour and level of the regulatory substances in the leaves of fruit-bearing and non fruit-bearing trees.

Materials and Methods

Collection of Plant Meterial: Buds and leaves were collected at different developmental stages from carob (*Ceratonia siliqua* L.) growing in the campus of the Ege University, Izmir, Turkey. The weather is usually conducive for growth around the first week of April and many pale coloured apical buds could be seen on the first day of April in non-fruiting trees, while those on fruit-bearing are still dormant. The non-dormant and dark green dormant buds could be differentiated from each other because the former exhibited the linear growth and expansion of the accompanying leaves, while the later did not. Five collections were made at different time intervals viz., (a) The apical buds; those from non fruit-bearing trees were collected on first of April, while those from fruit-bearing on April 1 and 15, respectively; 20 buds weighed 1g. (b) Very young leaves with small indistinguishable leaflets, about 1-2 cm in length were collected on April 7 from non fruit-bearing trees and 10 leaves weighed 1g. (c) Pale brown leaves with distinguishable leaflets, about ¼th their final size (cf 5-6 cm) were collected on April 15 and 3.5-4 leaves weighed 1g. (d) Leaves almost ¾th (cf 15-18 cm) or more of their final size and bright green in colour, collected on April 21. A single leaf weighed 3g.(e) The yellow almost senesced leaves and ready to fall were collected towards the end of September and probably were the leaves from the previous season. A single leaf weighed more than 4g. The various developmental stages described could also be detected concurrently on the same tree. The difference between the leaves from fruit-bearing and non fruit-bearing trees was that the former attained a particular developmental stage somewhat later compared to their counterparts from the non fruit-bearing trees. Moreover, the leaves in the former case exhibited a relatively slow expansion.

Extraction:

Extraction procedure has been described by Ilahi & Vardar (1975). The presence of gibberellin-like substances was confirmed by the procedure used by Ilahi & Vardar (1976b). The residue left after acidic ether evaporation, always equivalent to 1g fresh weight in the case of buds and young leaves or a whole leaf, where a single leaf exceeded 1g, was applied to a Whatman No. 2 chromatographic paper. Chromatography was carried out in the dark with controlled temperature never exceeding $25\pm1\,^{\circ}C$, employing the following solvent: isopropanol-25% NH_4OH-H_2O, 80: 10:10 (v/v). The chromatograms were equiliberated for 10h before chromatogram development and the solvent front allowed to travel a distance of about 40 cm from the origin. The chromatograms were then cut into 10 equal pieces for bioassay. Wheat coleoptile test of Nitsch & Nitsch (1956) was employed.

Results:

Growth regulatory substances of leaves from trees without fruit:

Four growth promoting substances viz. P_1 at Rf 0.00-0.20, P_2 at 0.30-0.40, P_3 at 0.50-0.60 and P_4 at 0.90-1.00 and an inhibitor, I_1, restricted to Rf 0.60-0.80 could be observed (Fig. 1). The buds contained a negligible amount of P_1 and P_4, but a significant amount of P_2 and P_3. Especially P_2 was higher compared to all other promoting substances at this developmental stage (Fig. 1a). The level of P_1, P_2 and P_3 decreased (Fig. 1b) in very young leaves (See Materials and Methods for explanation). In the leaves which had attained ¼th their final size, there was no change in the level of P_2. However, there was a decrease in P_3, but an increase in P_1 which was equal to its previous level (Fig. 1c). The level of P_2 decreased in leaves which had attained ¾th their final size, but not that of P_3, which increased considerably over all its previous levels and equalled the concentration of P_2 in the bud condition (Fig. 1d). Like P_3, P_4 also attained a level higher to all its previous levels. All the growth promoting substances exhibited a decline in mature, yellow senescent leaves (Fig. 1e).

The insignificant level of the inhibiting substance, I_1, remained almost constant in buds and leaves on their first and second stage of development. However, the level of the inhibitor increased greatly in the leaves collected at the third developmental stage (Fig. 1d). This level increased further in leaves which had senesced (Fig. 1e).

Growth regulatory substances of leaves from trees with fruit:

The buds and leaves contained the same regulatory substances, having the same Rfs as their counterparts, therefore they were named as P_1-P_4 and I_1. The buds collected on April 1 contained an insignificant amount of P_2 and P_3 as compared to the buds from trees without fruit (Fig. 2a). On the contrary, these buds contained a significant amount of P_1. Incidently the substance P_4 in this case, too, was not present in significant concen-

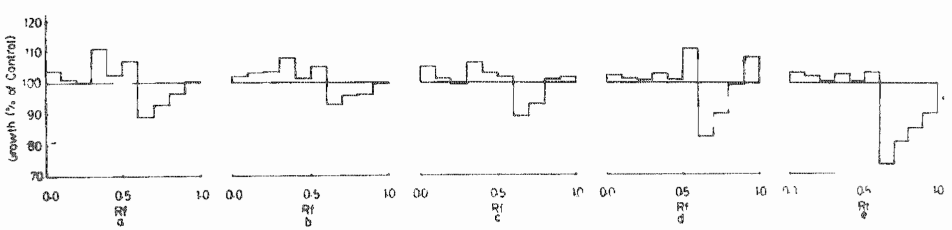

Fig. 1 Growth regulatory substances of leaves from trees without fruit of *Ceratonia siliqua*.

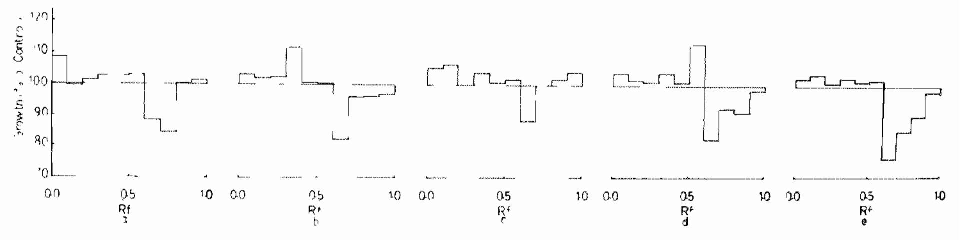

Fig. 2. Growth regulatory substances of leaves from trees with fruit of *Ceratonia siliqua*.

tration. All these substances exhibited the same level in buds collected 15 days later, i.e. April 15. This picture was somewhat different in buds collected after a further lapse of 15 days. There was an increase in P_2 and P_3, but these never attained a level like their counterparts in buds form non fruit-bearing trees. Very young leaves collected on April 28 contained a significant level of P_2 (Fig. 2b). The level of P_2 decreased suddenly in leaves which had attained ¼th their final size (Fig. 2c). On the contrary, both P_1 and P_4 exhibited a significant increase. Except P_3, there was a decrease in the level of all promoting substances in leaves which had attained ¾th their final size, collected on May 10 (Fig. 2d). Like their counterparts in leaves from non fruit-bearing trees, a reappearance of P_3 in significant level was detected at this stage. There was a tremendous loss in the level of P_3 and other promoting substances in mature senescent leaves (Fig. 2e).

The inhibiting substance, I_1, was present at a significant level in buds (Fig. 2a). This level was significantly higher in buds from non fruit-bearing trees. No significant change occurred in the inhibitor level in leaves at various stages of development. However, this level increased in mature senescent leaves (Fig. 2e).

Discussion

Carob leaf development was, like the fruit, regulated by the same substances. Our previous studies (Ilahi & Vardar, 1974; 1976) indicated that P_1 might be accelerator "a", P_2 and P_3 a gibberellin- and auxin-like with the same Rfs as gibberellic acid (GA_3) and indoleacetic acid (IAA), while P_4 possibly a mixture of gibberellin- and auxin-like substances. The inhibitor (I_1) could be the "β-inhibitor" complex.

The level of these substances varied at various stages of development. Usually there is a larger production of auxin during the enlargement of leaves of higher plants which subsequently declines at maturity (Wetmore & Jacobs, 1955; Goodwin, 1937). In carob leaves appreciable amount of the auxin-like substance at IAA Rf was detected at the bud condition, which decreased as the leaves enlarged. P_1 exhibited a continuous increase from a lower level in buds to a maximum in leaves at their ¾th final stage. Even this substance declined in mature leaves. However, P_1 present at a higher level in buds from fruit-bearing trees, showed a decline like their counterparts from non fruit-bearing trees. Similarly, Whightman (1976) demonstrated a close correlation between endogenous levels of auxin-like substances in expanding tobacco leaves and their subsequent growth.

Leaf expansion in *Phaseolus vulgaris* was correlated with their gibberellin content and the time of maximum gibberellin content coincided with the maximum growth rate of primary leaves (Humphries & Wheeler, 1963). The maximum amount of gibberellin-like substance was detected only during the bud stage in carob which gradually decreased to a trace in expanded mature leaves. These results agree with Lang (1960) who detected gibberellins in young but not mature leaves of *Hyoscymus niger.* Humphries & Wheeler (1964) reported production of maximum level of gibberellin-like substances in leaf primordia. Absence of P_2 at an appreciable level in buds from fruit-bearing trees could be explained either due to the mobilizing effect of the young fruit or that these buds could still be dormant under the influence of various internal and external factors. The second possibility can not be considered appropriate as both the plants were growing at the same locality under the same environmental conditions.

The inhibitor, I_1, like the carob fruit development (Ilahi & Vardar, 1975; 1976) increased to its higher level in mature leaves from trees without fruit. The inhibitor was higher and decreased only a little in buds and leaves at various stages of development from trees with fruit. Phillips & Wareing (1958) correlated leaf dormancy of *Sycamore* sp., with maximum inhibitor content. Carob buds and leaves from fruit-bearing trees had a higher level of the inhibitor, which possibly might have induced the dormancy and slow growth rate of these organs.

Buds and leaves from trees without fruit exhibited active growth at the departure of unfavourable growth conditions. This stage coincided with an increase in the level of growth promoting substances concurrent with decrease in that of inhibitor. However, the leaves from fruit-bearing trees behaved differently. The buds remained dormant for a longer time compared to their counterparts. Furthermore, the growth promoting substances did not attain the level of those from non fruit-bearing trees. In addition, the inhibitor was present at a higher level in leaves and did not decrease appreciably in expanding leaves. Humphries & Wheeler (1963) reported that leaf growth was inhibited by the accompanying young leaves and flowers. This inhibitory influence could either be hormonal or nutritional. Bud and leaf development in fruit-bearing carob trees might have been either delayed by the mobilization effect of the young fruit or the higher level of the inhibitory substance or both the effects combined together. This phenomenon might have been further supplemented by the lower level of growth promoting substances. The leaves and buds from fruit-bearing trees exhibited active growth around the fourth week of April. At this time of the season carob fruit had attained more than ½ its final size and weight (Ilahi & Vardar, 1976b).

Acknowledgements

This work was done at the Department of Botany, Ege University, Bornova-Izmir, Turkey during an assignment under the RCD Technical Cooperation Programme. Thanks are also due to Professors Drs. Y. Vardar and N. Zeybek for their help and cooperation.

References

Bentley, J. A 1958. The naturally occurring auxins and inhibitors. Ann. Rev. Plant Physiol., 9: 47-80.

Goodwin, R.H. 1937. The role of auxin in leaf development in *Solidago* species. Amer. J. Bot., 24: 43-51.

Humphries, E.C. and A. W. Wheeler. 1964. Cell division and growth substances in leaves. In J.P. Nitsch (ed.), "Regulateurs Naturels de la Croissance Vegetable". CNRS, Paris. pp, 505-515.

Ilahi, I. 1979. The nature and balance of growth regulators in the reproductive organs of *Ceratonia siliqua* L. Phyton, 37: 29-34.

Ilahi, I. and Y. Vardar. 1974. Studies in the Turkish Carob *(Ceratonia siliqua* L.*)* I A preliminary survey of the growth regulatory substances of the fruit from various types. *Bitki* 1: 145-153.

Ilahi, I. and Y. Vardar. 1975. II. The level of growth regulatory substances and sugar content at different stages of fruit development. Z. Pflanzenphysiol., 75: 422-426.

Ilahi I. and Y. Vardar. 1976a. IV. Acidic auxin-like and inhibitory substances in fruit morphogenesis. Planta (Berl.) 129: 105-108.

Ilahi I. and Y. Vardar. 1976b. V. The level of gibberellin-like substances during fruit development. Pak. J. Bot., 8: 181-187.

Lang, A. 1960. Gibberellin-like substances in photoinduced and vegetative Hyoscymus plants. Planta, 54: 498-504.

Nitsch, J.P. and C. Nitsch. 1956. Stuides on the growth of coleoptiles and first internode sections. A new straight growth test for auxins. Plant Physiol., 31: 94-111.

Phillips, I.D. J. and P. F. Wareing. 1958. Studies in Dormancy of Sycamore, I. J. Exp. Bot., 9: 350-364.

Wetmore, R. H. and W. P. Jacobs. 1953. Studies on abscission: the inhibitory effect of auxins. Amer. J. Bot., 40: 272-276.

Wheeler, A.W 1960. Changes in leaf growth substances in cotyledons and primary leaves during the growth of dwarf bean seedlings. J. Exp. Bot., 11: 217-226.

Wightman, F. 1976. Gas chromatographic identification and quantitative estimation of natural auxins in developing plant organs. In P.E. Pilet (ed.) "Plant Growth Regulation." Springer-Verlag, Berlin. pp. 77-90.