Pak. J. Bot., 8 (2): 181—187, 1976.

STUDIES IN THE TURKISH CAROB (*CERATONIA SILIQUA L.*) V. THE LEVEL OF GIBBERELLIN-LIKE SUBSTANCES DURING FRUIT DEVELOPMENT.

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Abstract

Carob, during its development, contained at least two gibberellin-like substances, P_2 and P_4, with the former restricted to GA_3 Rf. Fruit exhibited active growth only after P_2 had achieved its maximum level, so it was a cause and not its result. It exhibited a single peak, while P_4 had two. One of the peak was during the dormant period of fruit growth and the other when the fruit had advanced well into its development. Gibberellins were not the main cause of fruit growth but complemented by auxins as well.

Introduction

Gibberellin-like substances have been demonstrated in a variety of fruit and seeds (Crane, 1964). Coombe (1960), after having extracted gibberellin-like substances in considerable amounts from seedless grape berries, suggested their role in the parthenocarpic development of such fruit. However, later sutdies showed their importance in fruit and seed development and that gibberellins were found to be present mainly and in higher concentrations in the seeds compared to fruit (Corcoran & Phinney, 1962; Ogawa, 1963). More recent studies (Jackson & Coombe, 1966; Iwahori *et al.*, 1968; Lodhi *et al.*, 1969) indicated the involvement of gibberellins in fruit growth. As we have reported the nature of auxin-like substances during carob fruit morphogenesis, it was thought worthwhile to undertake the study of nature and level of gibberellin-like substances as well. During the present communication, the nature and level of gibberellins from the developing fruit will be reported and compared with those of seeds in a later communication.

Materials and Methods

Carob (*Ceratonia siliqua* L.) "wild" type fruit were collected at two week during slow growth and one week intervals during active period from trees growing on the campus. The fruit were extracted immediately with ice cold methanol. The extraction period usually lasted for about 16 hrs at 0°C. The residue, after filtration, was further extracted twice with fresh methanol for a period of 2 hrs each and all the extracts combined together. Methanol was evaporated under vacuum at 30°C and the aqueous residue adjusted to pH 3.0 with N HCl. It was then partitioned with ether and the latter fraction again with 5% $NaHCO_3$. The $NaHCO_3$ fraction was adjusted to pH 3.0 with N HCl and then extracted with eher. This acidic ether fraction was evaporated in vacuo and the residue left was either applied to a Whatmann No. 2 chromatographic paper (45×45 m) or silica gel thin layer (Kiesegel G 60) accordingly. The following solvents in that order, unless otherwise stated, were employed during the present work. Solvent 1: isopropanol-NH_4OH-H_2O, 80:10:10 (v/v) and solvent 2: Benzene-acetic acid-H_2O, 8:3:5 (v/v). Chromatography was carried out in a dark room with the temperature regulated at 25±1°C and the chromatograms equiliberated before actual chromatography. For comparison, fruit extracts were also purified according to the method described by Badr *et al.* (1972).

The following Bioassay systems were employed :

1. Wheat coleoptile bioassay of Nitsch & Nitsch (1956).

2. Cucumber assay. Cucumber seeds (*Cucumis sativus* L.). after being soaked for 2 hrs in running water, were planted in vermiculite. Seedlings were selected for uniformity and treated 24 hrs after the unfolding of cotyledons. A 10 μ ml drop of eluate was applied to the apical buds. Controls were treated with methanol only and seedlings measured after 96 hrs.

3. Bean bioassay. Two tall and one dwarf varieties of beans (*Phaseolus vulgaris* L.) were soaked in running water for about 6 hrs and then planted in vermiculite. Again a 10 μ ml drop of eluate was applied to either the terminal bud or to the upper surface near the base of one of the primary leaf petioles, when they had expanded nearly 50%. Epicotyl elongation was determined 72 hrs following the treatment.

4. Pea bioassay. Seeds of dwarf pea (*Pisum sativum* L. var. Meteor) were soaked for 4 hrs in running water prior to sowing in vermiculite. 10 μ ml drop of eluate was applied to the terminal bud and epicotyl measured 72 hrs after treatment.

Seedlings in bioassays 2 through 4 were grown in a growth chamber with temperature regulated at 25 $\pm$1° C and 16 hrs light during every 24 hrs cycle. The light intensity was at 5000 lux at 75 cm from lamps. For comparison, each test organism was treated with various concentrations of synthetic gibberellic acid (GA_3) using the same quantity as of eluates. The wetting agent used was Tween 20 at @ .05% concentration.

Results

As demonstrated earlier, (Ilahi & Vardar, 1976 b), an examination of Fig. 1

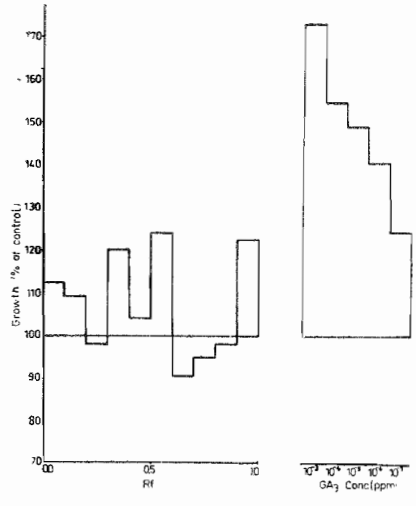

Fig. 1 Growth regulatory substances from carob. Wheat celeoptile bioassay.

revealed the presence of four growth promoting substances in the young carob. They were named as P_1 through P_4 according to their Rf. Thus P_1 was located at Rf 0.00-0.20, P_2 at 0.30-0.40, P_3 at 0.50-0.60 and P_4 at Rf 0.90-1.00. Both P_2 and possibly P_4 were not auxin-like substances as determined by our previous studies (Ilahi and Vardar, 1976 b). The following methods were adopted to determine the nature of these substances in detail. The Rfs regions belonging to P_2 and P_4 were eluted with methanol from a paper loaded with 20 gm equivalent of the fresh fruit and streaked separately on silica gel thin layer. Concurrently the residue left after the evaporation of acidic ether, again equivalent to 20 gm fresh fruit weight, was separately applied on a thin plate and all of them developed in solvent 2. These plates were examined under UV-light after being sprayed with the following reagent: ethanol/Conc H_2SO_4, 95:5 (v/v). No fluorescence could be detected. The plates

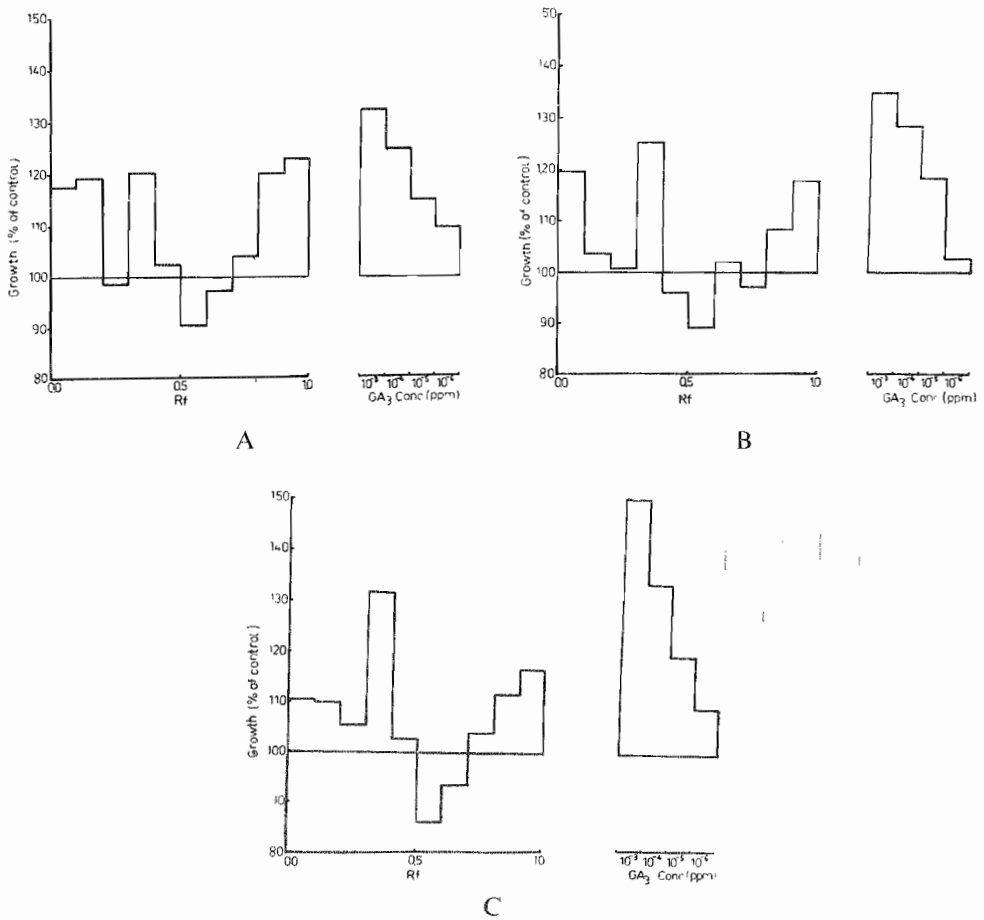

Fig. 2 Response of a) cucumber hypocotyl, b) and c) been epicotyl bioassays to various growth regulatory substances from carob.

were then heated at 120°C for 10 min and again examined under UV-light. This time two yellowish-blue spots developed at the Rfs of P_2 and P_4. The Rf of P_2 was slightly higher on the paper chromatogram developed in solvent 1 and slightly lower on thin

plate in solvent 2 to that of chromatographed authentic GA_3. The same thing happened when propionic acid was substituted for acetic acid. P_2 and GA_3 spots were close to one another when the plates were developed in solvent: n-butanol-acetic acid-H_2O, 5:1:2.5 (v/v). However, GA_3 developed a similar fluorescence as the carob substances when sprayed with the chromogenic reagent.

To determine whether these substances were gibberellin-like in nature, some bioassays specific to gibberellins were carried out. For this purpose, paper chromatograms loaded with 50 gm fresh fruit were used. They were then developed in solvent 1 and each Rf eluted with 5 ml of methanol. Various bioassays exhibited three zones of activities (Fig. 2 a, b, and c), corresponding to the Rfs of P_1, P_2 and P_4. The activity of P_3, as demonstrated earlier, was exclusively due to an auxin-like substance, therefore it did not express itself in these bioassays. Same results were obtained when eluates, equivalent to 10 gm fruit, from thin plates developed in solvent 2 were employed. To confirm whether this activity was due to gibberellin-like substances only, two bioassays with dwarf beans (*P. vulgaris* L. cv. Burdur) and pea (*P. stivum* L. cv. Meteor) were carried out. This time the activity got restricted mainly to P_2 and P_4

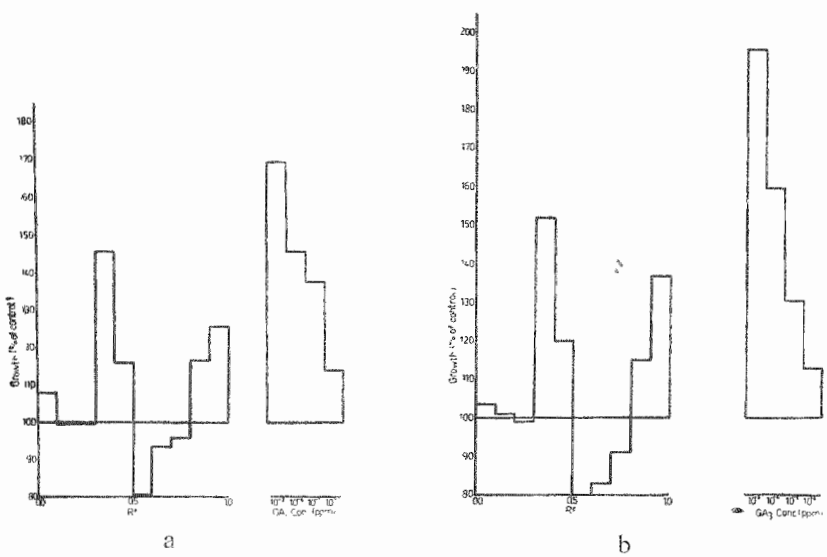

Fig. 3 Response of a) dwarf bean and b) dwarf pea to gibberellin-like substances from carob.

zones (Fig. 3 a and b). Studies with dwarf bean were further extended by the application of either 10 μ ml of the carob substances or GA_3 to the terminal buds once every week for a total period of 5 weeks and measurements of the first internode taken at the end of 6th week. There was a tremendous increase in the internode length induced by P_2 compared to that of either P_4 or control (Fig. 4). When the plants were allowed to grow in these conditions for a further period of two weeks, they assumed a vine habit like those brought about by GA_3 applications.

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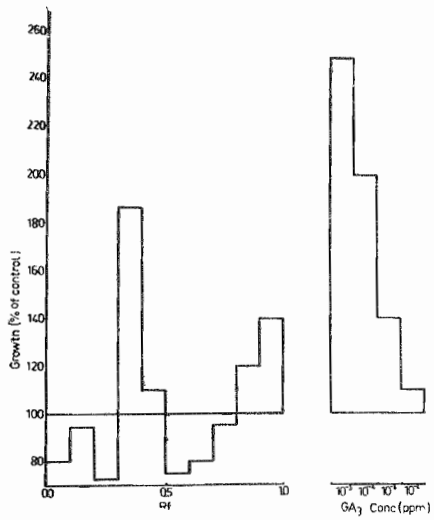

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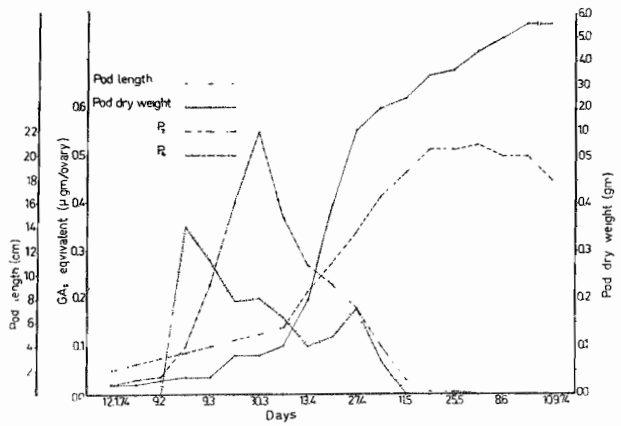

Fig. 4 Response of dwarf bean first internode to gibberellin-like substances from carob.

Fig. 5 The level of gibberellin-like substances at various stages of fruit development.

Carob pods exhibited a sigmoid type of growth curve, whether measured on the scale of length or dry weight/ovary (Fig. 5). During fruit development, P_4 had a sudden origin and maximum peak, while the pods were still immature and showed little growth. Then this substance had a gradual decline but attained another maximum when the pods had attained more than half of their length or dry weight. P_2, on the contrary, had only one maximum peak at a time when the fruit entered into active period of growth. Then it had a gradual decline and nearly absent from pods which had attained more than 3/4th of their length or dry weight. P_2 did not attain as much level, when expressed in μ gm quantities/ovary, as that attained by the acidic auxin-like substances.

Discussion

Carob exhibited a sigmoid growth curve and this held true for the increase in either length, fresh or dry weights. During this process a number of growth substances were involved. The dormant or slow growth period was characterised by a number of neutral auxin-like substances, which gradually vanished as the fruit entered active growth at the departure of severe cold conditions (Ilahi and Vardar, 1976 a, in press). Out of the four acidic promoting substances, two of them, as proved by the results of present communication, could be of gibberrellin nature. P_2 could be considered to be the main substance responsible for fruit morphogenesis as it was present in highest concentration compared to P_4. The former exhibited a single peak and attained its highest level before the fruit entered into active growth, then had a gradual decline and was totally absent from mature pods. Gibberellin-like substances are apparently present in largest quantities in rapidly growing tissue (Crane, 1964). Carob substances (viz. P_2 & P_4), as well, were present at highest concentration at this stage and these studies were further in agreement with those of Ogawa (1963) for *Lupinus luteus*. P_4, on the cotnrry, had two peaks, the first even prior to that of P_2, but could not attain the level of the latter. The second peak was when the fruit had advanced well into active growth. Our results differed from those of Nitsch (1965) as the gibberellin peak reached one week after fertilization in *Phaseolus vulgaris* and 13 days in *Pharbitis nil*. Such an activity in carob could only be observed after the intervention of a long cold period. P_2, however, attained its highest peak at the same stage of fruit development as the auxin-like substance at IAA Rf reported earlier (Ilahi & Vardar, 1976 b).

Jackson & Coombe (1966) and Iwahori *et al.* (1968) found a good correlation between rapid fruit growth and high concentrations of gibberellins in apricot and grapes respectively. Same was true for the carob, but as evident from our previous results, this activity was complemented by acidic auxin-like substances as well. However, Lodhi *et al.* (1969) reported a negative correlation between gibberellins and auxins in fig, 'King' cultivar, development as high gibberellin level coincided with low ones of auxins and vice versa. Carob fruit exhibited active growth only after the gibberellin-like substances had attained their highest concentration, so it could be suggested that they were a cause of growth and not its result as found by Jackson and Coombe (1966) for grapes.

Gibberellins possessing a 7-hydroxyl goup gave a blue fluorescence after being sprayed with ethanol/Conc. H_2SO_4 (95:5) and heated at 120°C (MacMillan & Sutter, 1963). Carob substances gave such a fluorescence as well and possibly one of them, P_2 had an Rf somewhat identical to GA_3. A gibberellin-like substance from both the seeded and seedless Tokay grapes was restricted to Rf 0.40-0.60 (Iwahori *et al.*, 1968). This does not conclude that the grape and carob substance or GA_3 were identical. However further studies are underway to determine their nature exactly.

Acknowledgement

This work was done during the deputation of one of us (I.I.) as an expert under the RCD Technical Cooperaton Programme to the Government of Turkey.

References

Badr, S. A., G. C. Martin and H. T Hartmann. 1971. A modified method for extraction and identification of Abscisic acid and gibberellin-like substances from the olive (*Olea europea* L.) Physiol. Plant., 24: 191-198.

Coombe, B.G. 1960. Relationships of growth and development to changes in sugars, auxins and gibberellins in fruit of seeded and seedless varieties of *Vitis vinifera*. Plant Physiol., 35: 241-250.

Corcoran, M. R. and B.O. Phinney. 1962. Changes in amount of gibberellin in developing seed of *Echinocystis*, *Lupinus* and *Phaseolus*. Physiol. Plant., 15: 252-262.

Crane, J. C. 1964. Growth substances in fruit setting and development. Ann. Rev. Plant Physiol., 15: 303-326.

Ilahi, I. and Y. Vardar. 1976a. Studies in the Turkish Carob (*Ceratonia siliqua* L.) III. Some neutral auxin-like substances in the young fruit. (In press).

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

Iwahori, S., R. J. Weaver and R. M. Pool. 1968. Gibberellin-like activity in berries of seeded and seedless Tokay grapes. Plant Physiol., 43: 333-337.

Jackson, D. I. and B. G. Coombe. 1966. Gibberellin-like substances in the developing apricot fruit. Science, 54: 277-278.

Lodhi, F., M. V. Bradly and J. C. Crane. 1969. Auxins and gibberellin-like substances in parthenocarpic and non-parthenorcapic syconia of *Ficus carica* L. cv. King. Plant Physiol., 44: 555-561.

MacMillian, J. and P. J. Sutter. 1963. Thin layer chromatography of gibberellins. Nature, 197: 790.

Nitsch, J P. 1965. Physiology of flower and fruit development. In Encyclopedia of Plant Physiology W. Ruhland (ed.) Vol. 15/1, pp. 1537-1647. Springer-Verlag. Berlin.

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

Ogawa, Y. 1963. Changes in the content of gibberellin-like substances in ripening seeds and pod of *Lupinus luteus*. Plant and Cell Physiol., 4: 85-94.