GLASNIK INSTITUTA ZA BOTANIKU I BOTANIČKE BAŠTE UNIVERZITETA U BEOGRADU
BULLETIN DE L'INSTITUT ET DU JARDIN BOTANIQUES DE L' UNIVERSITÉ DE BEOGRAD
Tom (XIII) XV, 1–3, (1978, 1979, 1980) 1982, Beograd

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ENDOGENOUS GIBBERELLIN–LIKE SUBSTANCES AND INHIBITORS IN CALLUS TISSUE OF SPINACIA OLERACEA L.

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INTRODUCTION

It has been known that optimal growth of callus tissues in culture requires some definite combination of nutritive and hormonal factors, which may be different for various plant species. In most cases there is an absolute requirement for endogenous auxins and cytokinins, while such a need has not been established for gibberellins or abscisic acid (Gresshoff, 1978). However, an interaction of the latter with auxins and cytokinins has been noticed in some cultivated tissues. The growth of tobacco callus is stimulated by gibberellins (Helgeson and Upper, 1970; Lance *et al.*, 1976), while olive callus tissue responds to both ABA and GA_3 (Lavee and Adiri, 1974). Abscisic acid and kinetin have a synergistic effect in stimulating the growth of soya (Blumenfeld and Gazit, 1970) and spinach (Nešković *et al.*, 1977) callus tissue. It is possible that at least in some tissues, not requiring exogenous gibberellins, the need for these hormones were actually satisfied by endogenous substances, if the capacity for their synthesis is present.

The content of endogenous hormones in callus tissues has been less investigated, than the effects of their application. Nickell (1958) has found gibberellin–like activity in callus tissues of different origin. Since the tissues of 25 plant species show various responses to exogenous gibberellins, Nickell and Tulecke (1959) suggest that this variability may be due to the unequal content of endogenous substances.

In a previous paper (Ćulafić and Nešković, 1975) we described the content of gibberellins in staminate and pistillate spinach plants. Since the callus tissue cultures were obtained from these plants, it seemed interesting to find out whether cultures had a similar capacity for gibberellin synthesis, as the intact plants. The results are described in the present paper.

MATERIAL AND METHODS

Plant material

Seeds of spinach *(Spinacia oleracea* L. cv. Matador) were sterilized with 5% calcium hypochlorite for 1 h, washed with sterile water and sown in test tubes, filled with sterile vermiculite. After 5–7 days the seedlings had a well developed pair of cotyledons and the hypocotyl was 2–3 cm long. Apical parts of the hypocotyls (5–10

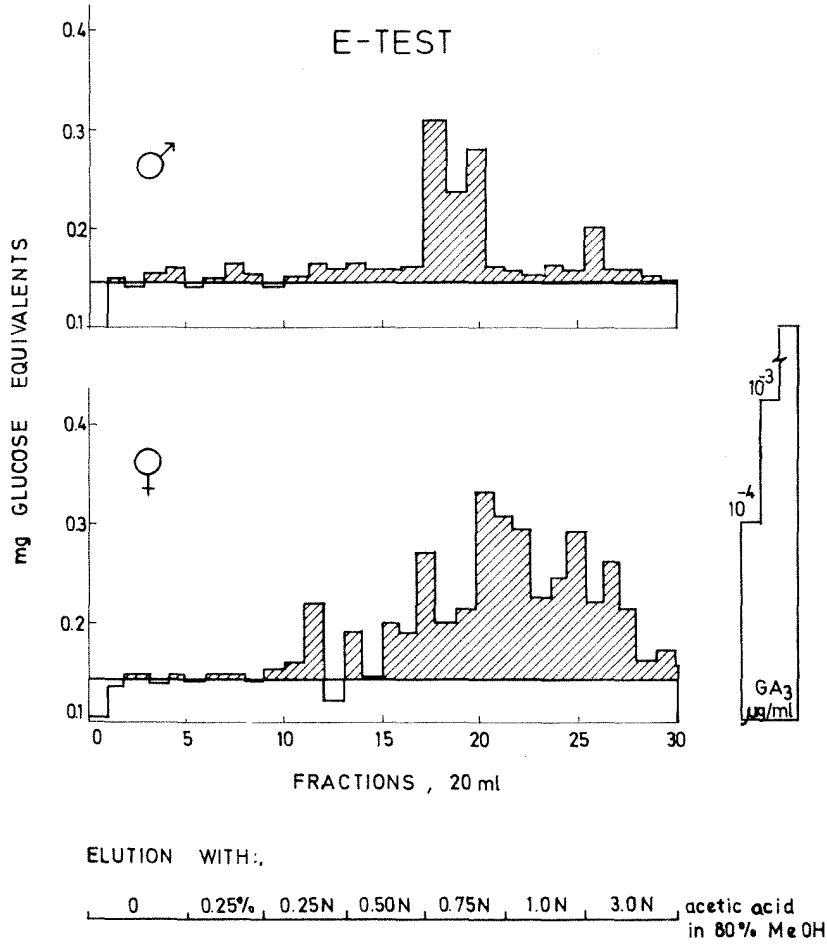

Fig. 1. – Chromatography of the methanol extract of callus tissues (10 g fresh weight) on DEAE–Sephadex A–25 column; the gibberellin–like activity was determined by endosperm bioassay (E–test).

mm long) were cut off and transferred onto an agar medium, containing Murashige and Skoog (1962) mineral solution and 2% sucrose. The explants were kept, for at least two weeks, under an inductive light regime (16 h light), at 20 ± 2°C, to induce flowering. When the sex of flowers was established, the hypocotyl was cut into segments of 2–3 mm and transferred to another medium, having the same mineral solution and sucrose, but also supplemented with (in mg l^{-1}) thiamine 2, nicotinic acid 5, adenin 2, 2,4–D 1 and kinetin 1. All hypocotyls developed calluses, which were maintained in permanent culture, with subcultures every 4 weeks.

Extraction and purification of hormones

Callus tissues of male and female plants were extracted during the 14th, 15th and 16th passages, three weeks after subculturing. The tissue (10 g fresh weight) was grinded in cold (– 20°C) methanol and extracted for 24 h at 4°C. The extraction was repeated for 30 min with fresh methanol, and after filtration the solvent was evaporated off using a flash evaporator, at 37°C. The residue was dissolved in a small amount of methanol, applied on the top of a DEAE Sephadex A–25 column (30 x 1.8 cm) and eluted with increasing gradients of acetic acid in 80% methanol (Gräbner *et al.*, 1975), collecting fractions of 10 ml.

TLC and biological tests

To determine biological activity in the extracts, 1 ml of each fraction was used. Gibberellin–like substances were detected using barley endosperm test (E–test) (Coombe *et al.*, 1967). The same test, with internal standard (10^{-3} μg ml^{-1} GA_3) was used to locate inhibitors, like abscisic acid. Inhibitors were also detected using the oat first internode test (M–test) (Nitsch and Nitsch, 1956). Fractions containing gibberellins or inhibitors were pooled, evaporated and chromatographed on silica gel G thin layers, in various solvent systems. After elution of the silica gel sections, the eluates were again tested with biological tests.

RESULTS AND DISCUSSION

The separation of active substances on the Sephadex column is shown in Fig. 1. As can be seen, tissues derived from both male and female plants contain substances corresponding in the elution profile to GA_3 and ABA markers. The gibberellin–like substances were rather abundant. Unfortunately, some of the fractions with gibberellins contained also an inhibitor, which prevented the response of barley half–seeds to gibberellins, which was established using the internal standard. Therefore, a correct quantitative comparison of the gibberellin content in tissues derived from male and female plants was not possible. In TLC, three zones of activity were found, corresponding to GA_3, GA_{4+7} and GA_5 marker spots. The most polar zone was usually most abundant. The pattern of histograms was very similar to those obtained previously from intact male and female spinach plants (Ćulafić and Nešković, 1974)

The ABA–like inhibitors were detected in fractions from the column with both biological tests, although this zone probably contained more than one substance (Figs. 2 3).

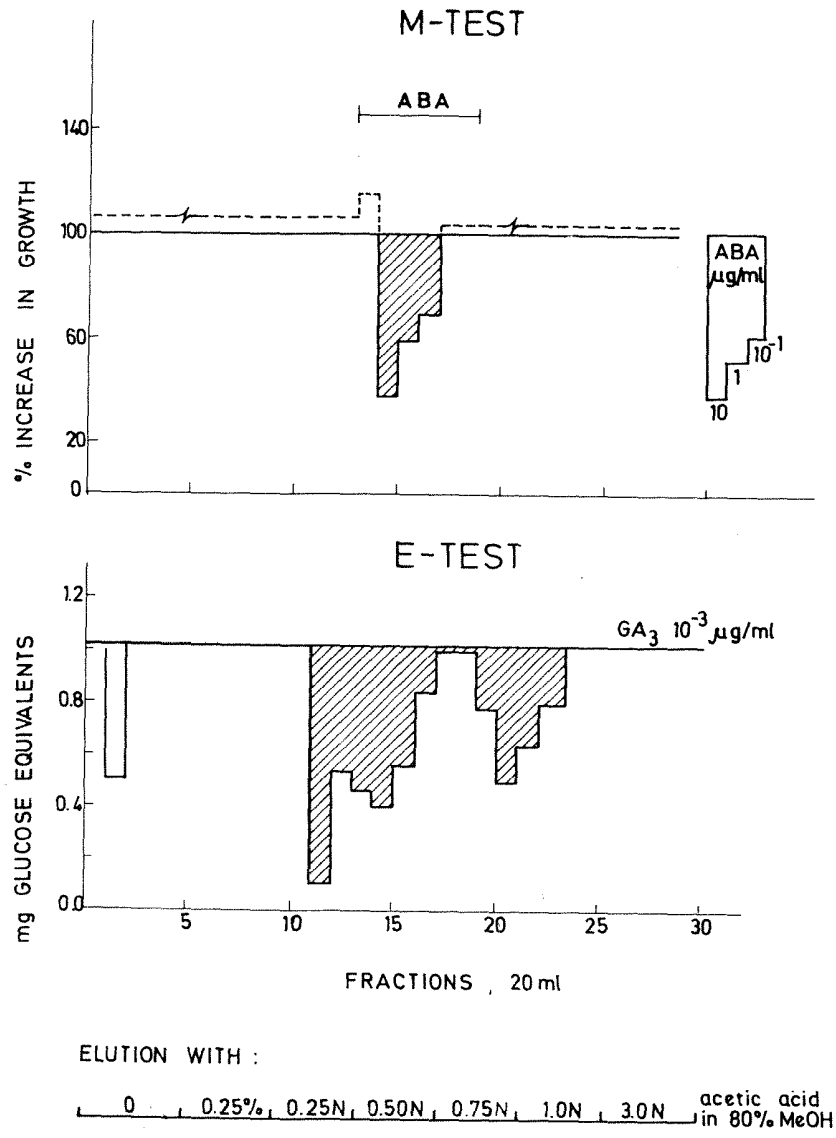

Fig. 2. – Extracts of callus tissue derived from male plants; chromatography on DEAE–Sephadex A–25 column; inhibitory activity detected by E–test and M–test.

Endogenous gibberellins in spinach plants have recently been analyzed by combined GC–MS (Metzger and Zeevaart, 1980 a, b) and the presence of six substances has been proved. Some gibberellins present in our extracts could be

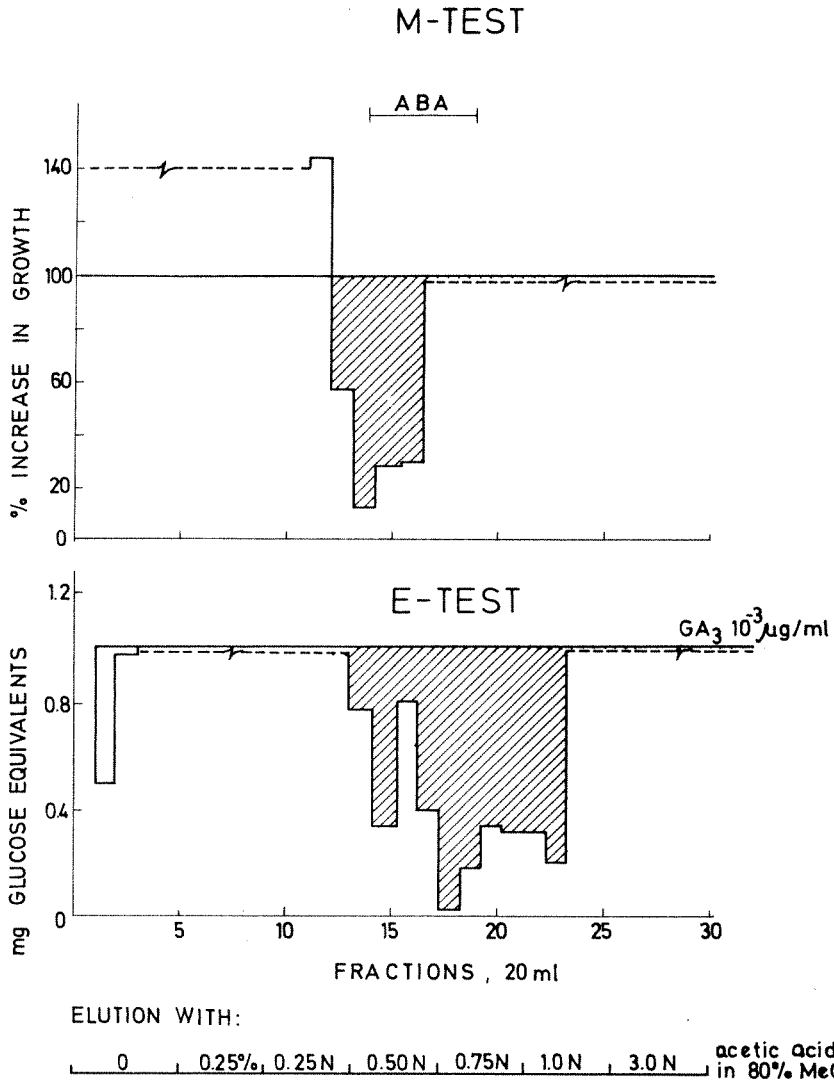

Fig. 3. – Extracts of callus tissues derived from female plants; chromatography on DEAE–Sephadex A–25 column; inhibitory activity detected by E–test and M–test.

identical with those, but closer comparison is not possible without further analytical work. Nevertheless, our results suggest that calluses obtained from hypocotyl tissue, retained the capacity to synthesize a considerable amount of gibberellins apparently, the same ones as those found in intact plants.

Organogenesis in spinach tissue culture is a highly unfrequent phenomenon (Nešković and Radojević, 1973, and later unpublished results). As in many tissues gibberellins inhibit organ induction (Thorpe and Meier 1973), it is possible that the same is valid for spinach. The endogenous gibberellins in spinach tissue, found in the present work, may perhaps be the cause for the lack of organogenic capacity. This possibility could be checked using inhibitors of GA biosynthesis, which is under way in our laboratory.

SUMMARY

The content of endogenous gibberellin–like substances and abscisic acid–like inhibitors was investigated in spinach callus tissue, grown *in vitro* during 14th to 16th passages. It has been found that the extracts of calluses contain substances of both groups in considerable amounts. The active substances are chromatographically similar to those found in intact plants. The technique used did not permit to reveal possible differences between calluses derived from male and female plants.

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Rezime

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ENDOGENI GIBERELINI I INHIBITORI U KALUSNOM TKIVU SPINACIA OLERACEA L.

U ovom radu je ispitivan sadržaj endogenih giberelina i inhibitora u kalusnom tkivu spanaća, gajenom u kulturi *in vitro* u toku 14. do 16. pasaža. Nađeno je da ekstrakti kalusnog tkiva sadrže obe grupe supstanci u merljivim količinama, po aktivnosti u specifičnim biološkim testovima. Po hromatografskim karakteristikama ovi hormoni odgovaraju supstancama nađenim kod intaktnih biljaka.

Na osnovu podataka dobijenih hromatografskim metodama i biološkim testovima pouzdano je potvrđeno prisustvo endogenih hormona, ali nije bilo moguće pokazati da li postoje kvantitativne razlike kod kalusa, koji su poreklom od muških i ženskih biljaka.