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The effect of different concentrations on gibberellin of growth response of *Zea mays* L. and an attempt for the detection of naturally occurring gibberellins in control plants

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The phenomenon of heterosis is usually connected with the increase of the intensity of metabolic processes. In the case of heterosis plants exhibit a stronger development of the root system and a more pronounced growth of stems and leaves. It is possible that due to a complementary effect of genes, that give rise to a specific growth substance in plants exhibiting heterosis — an activation in cell division as well as an activation of cell elongation occure.

It has been generally known that plants increase their height by treatment with gibberellin (Brian 1960; Phinney 1956).

In our earlier experiments (Bańkowska 1964a, 1964b, 1966) the possibility was stated of isolation lines with fixed vigorous growth in maize. It was of great interest to investigate the lines WD, W9, F_1 (WD × W9) and the line No. 10 with a hereditary fixed vigorous growth in connection with their response to gibberellin treatment.

The purpose of this work was to compare the response of these plants to a different concentration of aqueous solutions of gibberellin and, moreover, to attempt the detection of naturally occurring gibberellins in the control plants.

MATERIAL AND METHODS

The experimental material included four series of plants: inbred lines WD, W9, F_1 (WD×W9) and the fixed vigorous line No. 10. The seed was sown on 14 May 1965 in the experimental garden of the Departament of Genetics at Ursynów. The plants grew in 80×70 cm rows in rich soil. Each series consisted of four rows (ten plants per row). The first row included the controls, the second, third and fourth rows represented plants treated with a water solution of gibberellin of the con-

centration of 20 ppm, 50 ppm and 100 ppm respectively. In the experiment „Gibrescol" (Adamiec, Paśś, Wierzchowski 1962) was used, this compound contained mainly gibberellin A_3. Gibberellin was added in aqueous solution (0.1 ml per leaf) to the uppermost unfolding leaves at three-day intervals. The first treatment was applied on 17 July and the last one on 16 August. The length of the treated leaf and the length of the stem growing above it were measured before the treatment and after a three-day interval. For each series of plants the arithmetic mean for ten plants was computed.

Samples (100 grams of shoot and leaf tissue, fresh weight) were taken from controls, growing apart, for the assay for detection of naturally occurring gibberellins. Each sample was ground with 100 grams of ice, obtained from distilled water, and then extracted with acetone (125 ml) and kept for 24 hours at zero temperature. The extracts were distilled from acetone at 42°C. Gibberellins were extracted with ethyl acetate. The extracts were spotted on Whatman No. 1 filter paper and also on glass plates with a thin layer of silica-gel G. The paper chromatograms were developed by the descending method with two solvent systems of: 1 Chloroform / ether (7 : 3) saturated with 0.2 molar solution of citric acide and 2 toluen / acetic acid / water (6.5 : 3.5 : 10). The solvents were run at room temperature. The chromatograms were sprayed with 5% ethanol solution of concentrated sulphuric acid. The gibberellins are only visible in ultra-violet light, when a stream of heated air in controlled conditions is used. An ethanol solution of „Gibrescol" (100 mg in 5 ml of ethanol) was used as standard.

For the ascending thin-layer chromatography two solvent systems, solvent 2 and 3 were used after MacMillan and Suter (1963): 2 benzen / acetic acid / water (8 : 3 : 5) and 3 benzen / propionic acid / / water (8 : 3 : 5). Two sprays were used: "a" ethanol / concentrated sulphuric acid (95: 5) and "b" water / concentrated sulphuric acid (30 : 70). The R_f value for each spot was estimated and its colour described.

RESULTS

The experimental data are given in table 1 (I to II). Table 1 (I) presents the differences between the arithmetic mean values of stem and leaf length of treated plants and respective controls in the line WD, the treated plants showed an increase in differences of stem length according to the height of ppm value of the gibberellin solution. The greatest differences were obtained at 100 ppm, 9 days after treatment. The next great differences were obtained with the solution of 50 ppm, 18 days after treatment. As concerns the leaf length the greatest differences were observed at 50 ppm, 18 days after treatment.

Table 1

Deviations from the controls in the average values of stem and leaf increases of plants treated with gibberellin, in cm (mean of ten plants) in the line WD, W9, No. 10 and F_1

Lines and F_1		Concentration	Number of days after the first treatment										
			3	6	9	12	15	18	21	24	27	30	Total
I Line WD	Stems	20 ppm	+ 1,3	+ 0,4	+ 2,5	+ 0,7	+ 3,1	− 0,1	+ 1,6	+ 0,6	+ 4,7	+ 2,1	16,9
		50 ppm	+ 0,65	+ 1,0	+ 3,8	− 0,2	+ 4,6	+ 1,0	+ 1,0	+ 0,3	+ 3,2	+ 5,4	20,75
		100 ppm	− 0,77	+ 0,4	+ 6,57	+ 2,8	+ 5,1	+ 1,7	+ 1,7	+ 1,7	+ 2,4	+ 2,3	23,9
	Leaves	20 ppm	+ 1,4	+ 1,2	+ 1,8	0	+ 1,1	+ 0,3	+ 2,1	+ 2,8	+ 0,5	− 0,1	11,1
		50 ppm	− 0,2	+ 1,1	+ 0,9	+ 0,3	+ 1,3	+ 4,0	+ 2,9	+ 2,6	+ 1,4	+ 0,2	14,5
		100 ppm	− 0,9	+ 2,2	0	− 1,4	+ 3,5	+ 0,8	+ 1,4	0	+ 1,7	0	7,3
II Line W9	Stems	20 ppm	+ 1,6	+ 0,3	+ 0,9	+ 1,5	+ 0,2	− 1,3	− 0,1	+ 2,2	+ 1,1	− 2,2	4,2
		50 ppm	+ 2,4	− 0,5	+ 1,7	+ 1,8	+ 3,1	− 0,9	− 0,1	+ 0,2	+ 5,3	− 3,3	9,7
		100 ppm	+ 1,5	+ 4,7	+ 4,1	− 0,6	+ 1,3	− 0,6	+ 0,3	+ 3,1	+ 0,3	− 4,3	9,8
	Leaves	20 ppm	− 1,6	− 0,5	+ 3,1	− 1,3	+ 1,6	+ 0,4	− 3,3	+ 0,3	+ 0,1	+ 1,3	0,1
		50 ppm	− 1,3	− 3,0	+ 0,4	− 0,8	+ 3,3	+ 0,6	+ 0,3	+ 1,9	+ 0,2	+ 0,2	1,8
		100 ppm	+ 0,9	− 2,7	+ 1,8	− 0,1	+ 1,4	+ 0,7	+ 1,8	+ 2,3	+ 0,3	+ 1,0	7,4
III F_1	Stems	20 ppm	+ 3,5	+ 0,2	+ 2,3	+ 2,8	− 1,7	+ 2,4	− 2,8	+ 2,3	+ 2,7	+ 0,1	11,8
		50 ppm	+ 3,7	− 0,8	+ 3,3	− 0,6	+ 0,8	+ 1,0	− 0,9	+ 8,5	+ 1,9	− 2,0	14,9
		100 ppm	+ 3,9	+ 2,6	+ 1,7	+ 1,5	+ 4,5	− 0,9	− 2,5	+ 2.4	+ 3,6	− 1,5	15,3
	Leaves	20 ppm	+ 0,6	+ 1,5	+ 0,2	+ 2,6	+ 0,8	+ 0,1	+ 0,5	− 0,7	+ 1,0	+ 1,1	7,7
		50 ppm	+ 0,4	+ 3,7	+ 2,2	+ 2,4	+ 1,6	− 0,8	+ 0,3	− 1,4	+ 1,5	+ 0,2	10,1
		100 ppm	+ 1,6	+ 2,6	+ 1,0	+ 1,4	+ 2,2	+ 0,8	+ 1,2	+ 3,2	+ 3,8	+ 2,2	20,0
IV Line No. 10	Stems	20 ppm	− 1,6	− 0,7	+ 1,1	− 0,1	+ 2,1	− 0,1	+ 2,8	− 2,0	+ 0,8	+ 2,0	4,3
		50 ppm	+ 0,2	− 3,3	+ 1,4	+ 1,1	+ 1,3	+ 0,3	− 1,0	− 2,4	+ 0,2	+ 7,4	5,2
		100 ppm	+ 0,8	− 3,3	+ 3,4	+ 1,3	+ 1,8	− 2,5	+ 0,4	− 2,2	+ 1,1	+ 7,8	8,6
	Leaves	20 ppm	+ 4,1	− 0,6	+ 2,8	− 4,2	+ 0,8	− 1,0	+ 5,2	− 0.2	0	+ 0,3	7,2
		50 ppm	+ 4,5	− 1,1	+ 4,5	− 0,6	+ 2,3	− 0,5	+ 3,3	− 0,7	0	+ 1,1	12,8
		100 ppm	+ 5,6	− 0,4	+ 3,2	− 2,4	+ 2,5	− 2,3	+ 2,4	− 0,6	+ 1,3	+ 0,1	9,4

The differences in line W9 are presented in the Table 1 (II). In general they are not so great as in line WD. In the first period 100 ppm gave the best results and 50 ppm in the last. As concerns the leaf length the greatest differences are observed at 50 and 100 ppm.

Data concerning F_1 are given in Table 1 (III). The plonts showed an increase of differences according to the height of ppm value of the gibberellin solution at the beginning of treatment. The greatest differences in stem length were observed at 50 ppm, 24 days after treatment. The greatest differences in leaf length were observed at 100 ppm at the beginning of treatment but at the end of the experiment 50 ppm was more effective.

Table 1 (IV) presents data concerning the differences in line No. 10. In comparison with F_1 and the line WD they are rather small, especially at the beginning of treatment, later, at the end of the experiment they are much greater. The effect of gibberellin in promoting leaf elongation in comparison with stem elongation is better pronounced in this line.

The Figurs 1 and 2 present the deviations of the total sum of the arithmetic mean differences in stem and leaf elongation respectively of the treated plants from the appropriate controls in each series. The values of control plants are expressed by zero. The data represent the whole period of gibberellin treatment. As concerns the stem (Fig. 1) the

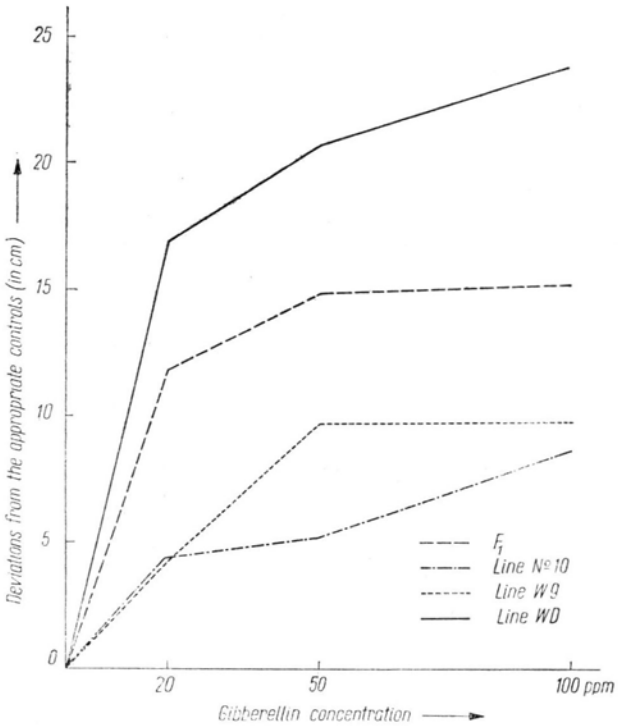

Fig. 1. Deviations from the appropriate controls of the total sum of mean deviations in stem elongation

deviation rises proportionally to the increase of the gibberellin concentration. The greatest deviations are obtained in line WD. This line shows the most pronounced response to gibberellin treatment. The effect of gibberellin on promoting growth in F_1 is distinctly expressed. Line W9 gave a worse response and the smallest effect was observed in line No. 10. Differences between individual series of plants (Fig. 1) are statistically proved, except the differences between the line W9 and F_1 at 20 ppm and at 100 ppm.

The data in Figure 2 show that only F_1 and line W9 react proportionally to the height of the gibberellin concentration, but the deviations from the control in line W9 are the lowest. Other lines demonstrated the highest deviations at 50 ppm and at 100 ppm — a distinct decline. As concerns the leaves (Fig. 2) all differences at 20 ppm are significant.

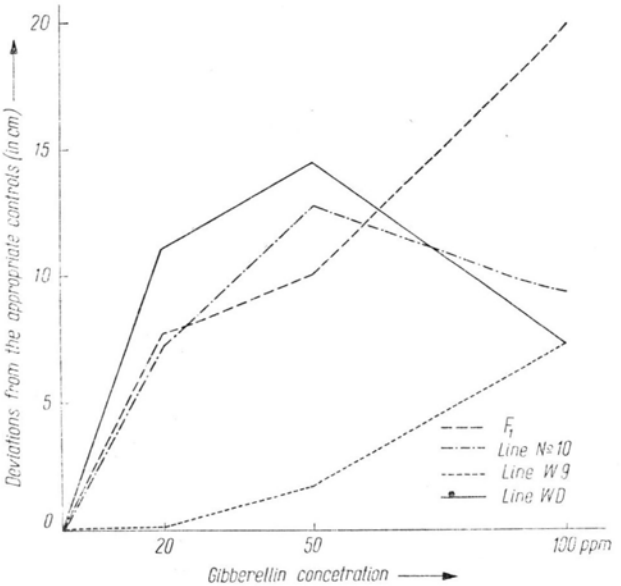

Fig. 2. Deviations from the appropriate controls of the total sum of mean deviations in leaf elongation

The difference between F_1 and line No. 10 is an exception it amounts to only 0,5 cm and is not significant. The differences at 50 ppm are significant, except the difference between line WD and line No. 10. The differences at 100 ppm are statistically proved with the exception for the differences between the line No. 10 and line W9 and between line No. 10 and line WD as well as between line W9 and line WD.

In connection with a different degree of response to gibberellin treatment in individual series it was of great interest to examine the interrelationship between the degree of response and the differences in the amount of naturally occurring gibberellins in the investigated material. The chromatographic technique play a particular role in the detection for growth promoting substances.

The first description of the occurrence of gibberellin-like substances in higher plants was reported by Mitchell, Skaggs and Anderson (1951). Since then a number of reports concerning this problem have been published. MacMillan and Suter (1958) succeeded

in isolation of gibberellin A_1 from the seed of runner bean Sumiki (1961) isolated it in pure crystalline form. West and Phinney (1957) reported the occurrence of gibberellin from different species of flowering plants.

It was of great interest to investigate the experimental material of maize in connection with the occurrence of native gibberellins. The extracts of each series of control plants as well as the standard solution were spotted on paper chromatograms and on silica-gel plates.

On the chromatogram developed in the toluen solvent on the starting-line spots of yellow fluorescence were observed: very intensive in line No. 10, medium intensive in line W9, in line WD and in F_1 of low intensity. The standard spot was of blue fluorescence. These spots indicate the presence of gibberellin A_1 (which gives a yellow fluorescence when the chromatogram is not heated too long) and A_3 (which gives a blue-greenish fluorescence when the chromatogram is heated for a short time). Very close to the starting-line remained the spots with blue fluorescence, which run at an *Rf* of 0.08 to 0.14.

The next spots of the *Rf* value of 0.23 showed a greenish-blue fluorescence in all extracts. After applying the technique of heating in two steps (Adamiec, Paśś, Wierzchowski 1964) it was found that spots of *Rf* value = 0.33, which gave an yellow fluorescence after heating for a short time disappeared when a strong heating was applied and then new spots arose with an *Rf* value of 0.40, they gave a blue-purple fluorescence (very strong in line No. 10). This suggests that the spots of *Rf* value = 0.33 may be gibberellin A_7 and those of *Rf* = 0.40 could represent gibberellin A_4. This spot was absent in F_1 extract. Moreover spots were observed of blue distinct fluorescence of *Rf* value = 0.72 in all series except line WD. These spots could not be identified for lack of appropriate standard.

On the chromatogram developed with the chloroform solvent on the starting-line spots with yellow-greenish fluorescence were observed in all extracts. Very close to the starting-line remained spots with blue fluorescence which run at an *Rf* of 0.03 to 0.04. The next spots, which run at an *Rf* of 0.11 to 0.14, showed a greenish-blue fluorescence they appeared in all extracts. The standard spots of a blue fluorescence run at *Rf* = 0.12 and at *Rf* = 0.19, corresponding spots appeared in all extracts (at *Rf* of 0.20 to 0.21) with also blue fluorescence. Moreover spots were observed with blue fluorescence in all extracts (very intensive in line No. 10) and in the standard, which run at an *Rf* of 0.33. The most distant spots at *Rf* of 0.72 to 0.74 with greenish-blue fluorescence appeared in all extracts.

The assay for gibberellin detection by the thin-layer chromatography technique was performed after MacMillan (1963). For this purpose

solvent systems 2 and 3, with spraying "a" and "b" were used on the plates with silica-gel.

The plate developed in solvent system 2 and sprayed with "a" revealed on the starting-line in the standard a very clear blue spot and in all extracts spots with brown fluorescence, which would appear to be gibberellins A_1, A_3 and perhaps A_8. The spot in line WD with purple fluorescence, which from its $Rf = 0.22$ could represent gibberellin A_6 had no corresponding spots in other extracts. The next spots with yellowish fluorescence in line WD (at $Rf = 0.26$) and in line No. 10 (at $Rf = 0.29$) revealed the presence of gibberellin A_5. Moreover in these lines spots with yellow fluorescence at Rf of 0.38 (line WD) and at Rf of 0.40 (line No. 10) appeared. The most distant spots with pink fluorescence running at Rf of 0.52 to 0.55 appeared in all the investigated extracts. A corresponding spot in the standard run at $Rf = 0.53$.

On the plate developed in solvent system 2 and sprayed with "b" spots in all extracts with yellowish-green fluorescence are seen on the starting-line before heating, this can suggest the presence of gibberellin A_3. After heating spots with intensive green and in standard with blue fluorescence appeared on the starting-line. The presence of gibberellin A_3 could be then stated and perhaps of gibberellins A_1 and A_8. Close to the starting-line spots appeared in the standard with blue fluorescence (at $Rf = 0.06$) in line WD with purple fluorescence (at $Rf = 0.05$). The spots with purple fluorescence run at Rf of 0.18 to 0.24 (line WD) they may be gibberellin A_6. The next spots with pink fluorescence which run at $Rf = 0.30$ appeared in line WD and in line No. 10 this suggests that it may be gibberellin A_5.

On the plate developed in solvent system 3, with spraying "a" spots with brown fluorescence appeared on the starting-line. Close to the starting-line are seen spots with bluish-purple fluorescence in all extracts and with blue fluorescence in standard, at $Rf = 0.05$. They revealed the presence of gibberelin A_3. The next spots with purple fluorescence appeared in all extracts (except in the line W9) they run at Rf of 0.15 to 0.18, a corresponding spot in the standard runs at $Rf = 0.17$. Further, appeared spots with intensive pink fluorescence at $Rf = 0.50$ in line WD and at $Rf = 0.55$ in line No. 10, moreover, in the last line spots with pink fluorescence at Rf of 0.70 and 0.85 appeared.

The plate developed in solvent-system 3 with spraying "b" revealed before heating spots with intensive yellow fluorescence on the starting-line and with blue fluorescence in the standard. After heating there appeared on the starting-line spots with brown fluorescence in all extracts, this might prove the presence of gibberellin A_3. Close to the starting-line were seen spots in all extracts with purple fluorescence, which run at $Rf = 0.05$ to 0.07 and in the standard at $Rf = 0.05$ with

blue fluorescence. Moreover there appeared spots with purple fluorescence at $Rf = 0.50$ (in the line WD) and at $Rf = 0.57$ (in the line No. 10). In the last line there was seen a more distant spot at $Rf = 0.75$, with purple fluorescence.

These results might be interpreted as presumptive evidence for the presence of native gibberellins in investigated extracts, namely: A_1, A_3, A_4, A_5, A_6 and A_7. In general it was observed that some spots gave more intensive fluorescence in line No. 10 as compared with spots of line WD (for example in the starting-line on chromatograms developed in toluen and chloroform solvents). This can suggest that the amount of naturally occurring gibberellins in line No. 10 is greater than in line WD, this in turn leads to differences in response to gibberellin treatment. The relatively small response to added gibberellin found for line No. 10 would be expected if native gibberellins were less limiting than in line WD.

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Wpływ różnych koncentracji gibereliny na reakcję wzrostową u Zea mays L. *i próba wykrywania naturalnie występujących giberelin w roślinach kontrolnych.*

STRESZCZENIE

W doświadczeniach przeprowadzonych nad heterozją u *Zea mays* L., stwierdzono, że można wyodrębnić w potomstwie heterozyjnych mieszańców linie z dziedzicznie utrwaloną bujnością.

Zadaniem niniejszej pracy było zbadanie linii wsobnych WD, W9, F_1 i bujnej linii Nr 10 w związku z ich reakcją na traktowanie giberelinią. Chodziło też o ustalenie w materiale badanym obecności naturalnie występujących giberelin w roślinach kontrolnych. Reagowanie roślin, wyrażone sumą średnich przyrostów pędów, najsilniej zaznaczyło się w l. WD. Linia Nr 10 reagowała najsłabiej. Przyrosty liści w początkowym okresie zadawania giberelinią były najwyższe w l. WD — najniższe w l. W9. W końcowym okresie najsilniej reagowało F_1.

Przy zastosowaniu chromatografii bibułowej zstępującej i użyciu solwentów toluenowego i chloroformowego stwierdzono obecność giberelin: A_1, A_3, A_4 i A_7, oraz bliżej nie oznaczonej substancji giberelino-podobnej o $Rf = 0{,}72$.

Stosując chromatografię cienkowarstwową wg metody MacMillana i Sutera z 1963 r. wykryto gibereliny A_1, A_3, A_5 i A_6.

Zaobserwowane różnice w intensywności fluorescencji plam, wywołanych obecnością giberelin A_1 i A_3 w poszczególnych porównywanych ekstraktach badanych roślin — sugerują występowanie największej ilości tych substancji w linii Nr 10, a najmniejszych w linii WD. Tłumaczyłoby to z kolei różnice w reagowaniu tych linii na traktowanie giberelinią.