Effects Produced on Tomato Plants, *Lycopersicum esculentum*, by Seed or Root Treatment with Gibberellic Acid and Indol-3yl-Acetic Acid

MARGARET E. BROWN, R. M. JACKSON,[1] AND THE LATE SUSAN K. BURLINGHAM

Soil Microbiology Department, Rothamsted Experimental Station, Harpenden, Herts.

Received 20 January 1968

ABSTRACT

Growth in lengths of tomato stems and leaves was accelerated by 5·0 μg gibberellic acid (GA_3) applied to the seed, or by 5·0, 0·5, and 0·05 μg given to the roots. Treatment with 5·0 μg also decreased bud number and lengthened the time between bud appearance and fruit formation on the first truss by 1–8 days. Smaller amounts applied to roots shortened this time by 1–4 days. Indol-3yl-acetic acid at 0·5 μg had no effect, nor was simultaneous application of GA_3 and IAA to the roots more effective than GA_3 alone. Single applications of very small amounts of GA_3 to seeds or seedling roots thus proved capable of changing growth-rates of stems, leaves, and trusses.

The effects of treating tomatoes with GA_3 and with cultures of *Azotobacter chroococcum*, which contain small amounts of GA_3 and IAA, are compared.

INTRODUCTION

GIBBERELLIC acid, GA_3, when placed on growing points or leaf axils, or when sprayed on leaves accelerates the growth of plants; when applied to flowering shoots it frequently hastens flowering. Treating ungerminated seeds with GA_3 (1–1000 μg/ml) usually results in increased shoot growth, for example in peas and wheat (Agapova and Bynov, 1961; Corns, 1959), in cotton (Bradford and Ewing, 1958), and in broad beans (Yakar-Olgun, 1962). Sometimes total yield is increased, for example in maize (Chirilei, Curticapeanu, and Dorobantu, 1963), or yield is decreased in wheat (Corns, 1959) and cotton (Bradford and Ewing, 1958). Flowering can also be delayed, for example in peas (Barber, Jackson, Murfet, and Sprent, 1958). The magnitude of the effects usually increases with increasing concentration. Tomato seeds treated with solutions containing more than 10 μg/ml GA_3 germinate quicker, plant growth is accelerated, and parthenocarpic fruits are formed larger and heavier than controls (Gray, 1957; Mazzani and Gonzalez, 1958; Srivastava, 1963). In these experiments seeds were soaked in

[1] Present address: Department of Biological Sciences, University of Surrey, 14–16 Falcon Road, London, S.W. 11.

GA_3 solutions for differing periods and the amounts taken up were not known. Persson and Rappaport (1958) found that 100 mg applied to soil, in which mature plants bearing two open flower trusses were growing, promoted tomato fruit set. Wittwer and Tolbert (1960) found that single or repeated applications of 5 mg to 4-in pots of soil in which the plants were growing caused accelerated stem growth of tomatoes. The first application was made when the cotyledons were expanding. To the authors' knowledge there are no reports of treating tomato roots at transplanting with GA_3 solutions.

Gibberellic acid and indol-3yl-acetic acid can interact in their effects on plant growth. Applying GA_3 and IAA together to intact pea or rice seedlings has a greater effect on growth than either substance alone (Brian, 1959). Luckwill (1958) showed that the stimulus responsible for the initiation of tomato fruit growth through to maturity is probably an auxin. With tomato the natural stimulus can be replaced by a single application of a wide range of synthetic auxins to the ovary, but IAA is relatively inactive except when applied with GA_3 when there is marked synergism.

Interest in the effects of GA_3 and IAA on tomatoes arose from the treatment of tomato seeds or roots with cultures of *Azotobacter chroococcum*. This increased the length of stems and leaves and shortened the time between bud appearance and petal fall. The number and final yield of ripe fruit was unaffected by treatment with *Azotobacter* (Jackson, Brown, and Burlingham, 1965). These effects were the same as those produced by gibberellins and suggested that *Azotobacter* might alter plant growth by producing gibberellin-like substances. Cultures were found to contain 0·01 to 0·001 μg/ml of GA_3 (Brown and Burlingham, in preparation). Cultures also contained IAA, the amount increasing from 0·05 μg/ml in a 2-day-old culture to 1·0 μg/ml in a 30-day-old culture (Burlingham, 1964). Comparisons were therefore made between treatments of tomato seeds and roots with *Azotobacter* culture and with different amounts of GA_3 and IAA, applied either separately or together. This paper reports the experimental work with pure growth substances.

METHODS

Application of GA_3 and IAA to seeds or roots

Seed treatment. Seeds of tomato, *Lycopersicum esculentum*, cultivar Money Maker, when allowed to imbibe in water for 1 h, take up on average 1·5 μl per seed. What proportion of water was absorbed by the seed and what held on the hairy seed coat could not be determined. Seeds were placed for 1 h in solutions of GA_3, 1·5 μl containing either 5·0, 0·5, 0·05, 0·005, or 0·0005 μg. Seeds were then sown in pots of unsterile compost (2-kg capacity).

Root treatment. Seeds of tomato were germinated in boxes of compost and when the cotyledons were expanding seedlings were transplanted to pots of unsterile compost (2-kg capacity). Aqueous GA_3 solutions contained either 5·0, 0·5, 0·05, 0·005, or 0·0005 μg per 0·25 ml and at transplanting each seedling root was wetted with this quantity of solution at the required dilution. All the liquid was usually held on the root system but any excess dripped into the planting hole.

In the interaction experiments roots of tomato seedlings were treated at transplanting with aqueous solutions containing either 0·01 μg GA_3 or 0·5 μg IAA per

0·25 ml, or with solutions containing 0·01 μg GA_3 and 0·5 μg IAA per 0·25 ml. All control seeds or seedlings were treated with distilled water.

Plant culture. Plants were grown in the greenhouse kept at a mean day temperature of 21 °C and night temperature of 13 °C until three fruit trusses had developed. In winter supplementary incandescent light was provided to give a 16-h day. Experiments on seed treatments were begun in February, April, August, October, and November, and those on root treatments in January, April, June, July, October, and November. At fortnightly intervals plants received 100 mg N, 10 mg P, and 30 mg K supplied as $NaNO_3$, $Ca(H_2PO_4)$, H_2O, and K_2SO_4 respectively.

There were 10 replicate plants for each treatment and all pots were randomized. Significant differences between treatments and controls were tested at the 5 per cent level of probability.

Grading. Observations on the plants were made at weekly intervals. Stem height from the point of insertion of the cotyledons to the growing point, internode length, and lengths of all leaves added together (including petioles) were measured until the flower buds of the first truss showed. Total leaf area was shown to be directly correlated with total leaf length which was therefore used as the indicator for leaf size.

For each truss from the time of bud appearance until all the fruits were formed (hereafter called 'truss development time') the total number of closed buds, opening buds, fully open flowers, flowers with petals withered, and fruits were counted. The averages per plant for each developmental stage were multiplied by a representative factor, i.e. 1, 2, 3, 4, or 5 respectively. The total score was plotted against time to give a curve representing the development of buds into fruits.

RESULTS

Effects of treatment with GA_3

Seed treatment. There were six similar experiments, started at different dates, on treating tomato seeds with the five doses of GA_3. The germination rate of the seeds was unaltered by any treatment. Table 1 shows all the effects on plant growth measured in one experiment started in November. The effects produced in the other experiments were similar; for example, at the time when four true leaves were measurable (i.e. greater than 5·0 mm long) the mean height of control stems was 14 ± 1 mm and after seed treatment with 5·0 μg GA_3 the mean height was 18 ± 2 mm (measurements from six experiments). Seed treatment with 5·0 μg GA_3 always significantly accelerated stem and leaf growth. Accelerated stem growth occurred in young internodes; mature internodes of treated and control plants were approximately the same length. In three experiments started in February and October accelerated stem growth continued until flower buds of the first truss showed and in three experiments started in April, August, and November it continued until four true leaves had formed. The number of nodes preceding the first truss was the same on treated and control plants. All leaves of treated plants were larger than control leaves, significantly so in the early stages of growth. Leaves were of normal shape and size and were not chlorotic.

Amounts of GA_3 less than 5·0 μg did not significantly alter stem and leaf growth and a mean value for all these measurements is given in Table 1. In all experiments 5·0 μg GA_3 decreased the mean number of flower buds on the first truss from nine to seven, and in one experiment caused bud

abscission. On treated plants 'truss development time' was lengthened by 1 to 4 days. Fig. 1 from the experiment started in October shows the effects of seed treatment with 5·0, 0·5, and 0·05 μg GA_3 on the development of the first truss. Using less than 5·0 μg of GA_3 did not alter bud number but could lengthen 'truss development time' by 1 to 2 days.

The number of flower buds on second and third trusses was unaffected by gibberellic-acid treatment, but 5·0 μg lengthened second 'truss development time' by 3 to 5 days; third 'truss development time' was unaffected. Gibberellic acid did not induce parthenocarpy.

TABLE 1

Effect of seed treatment with gibberellic acid on stem and total leaf length of tomato

(One experiment)

Measurements in mm. Mean of 10 replicates

Time from treatment (weeks)	Total no. leaves measured per plant		Quantity of GA_3 μg applied: 0	5·0	0·5–0·0005	L.S.D. $P = 0·05$
4	3	Height	6	9*	7·2	±2·7
		Internode 1	6	9*	7·2	±2·7
		Leaves	61	82*	71·0	±19·7
5	4	Height	14	18*	16·2	±4·0
		Internode 1	9	12*	11·0	±3·0
		Leaves	103	133*	118·7	±26·8
6	5	Height	28	34*	31·5	±6·0
		Internode 1	17	18	17·7	±4·0
		2	6	8*	6·7	±1·8
		Leaves	187	225*	202·2	±33·5
7	6	Height	40	44	43·0	±8·1
		Internode 1	19	20	21·0	±5·0
		2	10	10	10·2	±5·0
		3	5	6	5·2	±2·0
		Leaves	271	308	281·5	±40·0

* Denotes significantly different from controls.

Root treatment. There were six similar experiments, started at different dates. Table 2 shows all the effects on plant growth in one experiment started in June. Effects in the other experiments were similar. For example, at the time when five true leaves were measurable, the mean height of control stems was 19±3 mm; mean stem height after root treatment with 5·0 μg GA_3 was 27±4 mm. As with seed treatment, extending internodes and expanding leaves were longer than those of control plants; stimulation was directly related to the amount of GA_3 applied down to 0·05 μg. The number of nodes preceding the first truss was the same in treated and control plants. Leaf morphology was changed only by 5·0 μg GA_3; the margins of the leaflets became entire and resembled those of a potato leaf; there was slight chlorosis.

In all experiments 5·0 μg GA_3 significantly decreased the mean number of flower buds on the first truss from seven to five and 'truss development time'

was longer by 1 to 8 days; bud abscission occurred in one experiment. Amounts of GA_3 less than 5·0 μg had no effect on bud number and usually shortened 'truss development time' by 1 to 4 days. Fig. 2 shows the effects of 5·0, 0·5 and 0·05 μg GA_3 on the development of the first truss in the experiment started in June. The number of flower buds on second and third

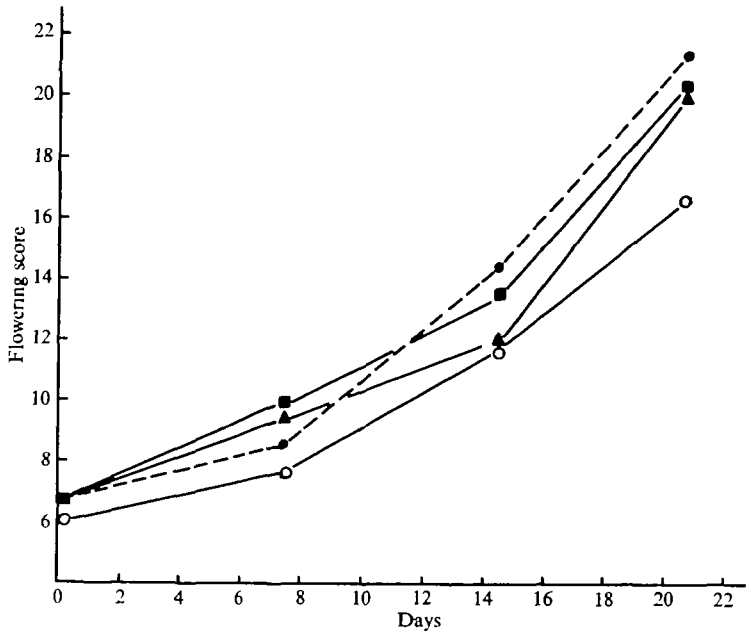

FIG. 1. Effects on truss development of different amounts of gibberellic acid applied to seeds. Control ●- - - -●, 5·0 μg ○——○, 0·5 μg ▲——▲, 0·05 μg ■——■. 5·0 μg GA_3 significantly lengthens truss development time by 5 days, 0·5 μg by 2 days, and 0·05 μg by 1 day.

trusses was unaltered by any treatment and all amounts except 0·0005 μg usually shortened 'truss development time' by 2 to 5 days.

Gibberellic acid treatment did not induce parthenocarpy.

Effect of root treatment with GA_3 and IAA

The amounts of growth substances tested were based on the amounts found per ml in 14-day-old cultures of *Azotobacter chroococcum*, i.e. 0·01 μg GA_3 and 0·5 μg IAA. Table 3 shows the results of treatment of seedlings with the growth substances applied separately or together.

GA_3 alone promoted stem and leaf growth and IAA alone had no effect. Together the growth substances significantly accelerated stem growth but only to the same extent as when GA_3 was added alone. Leaf growth was not altered.

GA_3 alone shortened 'truss development time' by 2 days, but had no effect when applied with IAA. IAA alone had no effect.

TABLE 2

Effect of root treatment with gibberellic acid on stem and total leaf length of tomato

(One experiment)

Measurements in mm. Mean of 10 replicates

Time from treatment (weeks)	Total no. leaves measured per plant		Quantity of GA_3 μg applied						L.S.D.
			0	5·0	0·5	0·05	0·005	0·0005	$P = 0.05$
2	3	Height	5	12*	8	6	5	5	±1·8
		Internode 1	5	12*	8	6	5	5	±1·8
		Leaves	66	113*	96*	84*	71	57	±16·8
3	5	Height	19	34*	27*	25*	22	17	±4·0
		Internode 1	12	19*	16*	15*	13	11	±2·1
		2	5	8*	7*	6	6	5	±2·0
		3	3	6*	3	3	3	3	±1·6
		Leaves	282	419*	401*	340*	308	250	±56·1
4	7	Height	58	94*	87*	84*	69*	51	±9·9
		Mature internodes 1–2	35	37	37	38	35	29	±6·0
		Expanding internodes 3–5	22	50*	37*	37*	30	21	±11·0
		Leaves	659	976*	884*	802*	727	555	±135·0
5	8	Height	114	167*	150*	151*	126	101	±16·2
		Mature internodes 1–3	64	64	71	69	65	61	±11·1
		Expanding internodes 4–5	31	50*	54*	54*	44	37	±13·1
		Leaves	1175	1610*	1529*	1392*	1292	1040	±152·0

* Denotes significantly different from controls.

DISCUSSION

These experiments have shown that GA_3 applied to seeds or seedling roots of tomatoes at the cotyledon stage of development affected subsequent growth of the plant. Internodes elongated and leaves expanded more rapidly than those of control plants and both bud numbers and the time between bud appearance and petal fall were altered. Root treatment with amounts of GA_3 down to 0·01 μg had significant effects, the resultant increase in plant size being related to concentration. When seeds were treated only the greatest quantity, 5·0 μg, was effective. The increase in plant size was less than that when 5·0 μg was added to roots. These differences might be related to the stage of plant development at which the treatment was given. Wittwer and Teubner (1956) showed in tomato that the number of nodes preceding the first truss and the differentiation of flowers was determined in the 2 weeks immediately after the cotyledons expanded. It was at this stage that GA_3 was applied to seedling roots and might, therefore, be expected to have most effect. In the seed the initials of the organs most susceptible to GA_3 are either not laid down or only incompletely differentiated and the GA_3 applied

to the seed might be partly inactivated in the time interval before the initials were properly differentiated. The greatest quantity of GA_3 used was least likely to be totally inactivated during the differentiation period and some could be left to affect the development of the initials formed.

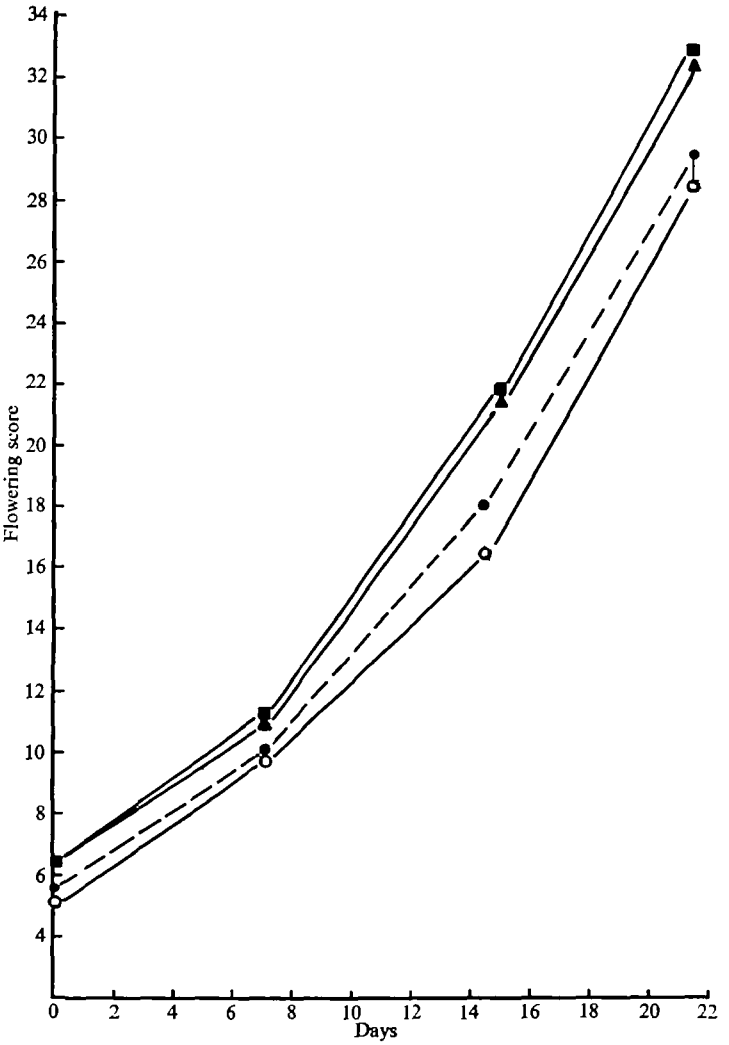

FIG. 2. Effects on truss development of different amounts of gibberellic acid applied to seedling roots. Control ●- - - -●, 5·0 μg ○——○, 0·5 μg ▲——▲, 0·05 μg ■——■. 5·0 μg GA_3 significantly lengthens truss development time by 2 days. 0·5 μg and 0·05 μg GA_3 significantly shorten truss development time by 3 days.

In calculating the amounts given to the plants two assumptions were made. Firstly that the gibberellic acid molecule passed into the seed at the same rate as that of water. Owing to the limitations of assay methods and the small quantities used it was not possible to estimate the amount of GA_3 imbibed by

the seed. With root treatment it was assumed that all the GA_3 applied was taken up by the plant. However, what proportion was absorbed immediately by the root and what was adsorbed on to the soil particles thus becoming unavailable to the plant were not known. With both methods of application the effective dose may have been less than the amount applied.

The amounts of GA_3 used were much less than those for most experiments described in the literature. Generally, repeated treatments of plants, usually by sprays, were necessary to produce effects on stems, leaves, flowers, and fruits (Rappaport, 1957; Gray, 1957; Bukovac, Wittwer, and Teubner, 1957), whereas in the present work one application was sufficient to alter the course of plant development for several weeks.

TABLE 3

Effect of root treatment with gibberellic acid and indol-3yl-acetic acid on stem and total leaf length of tomato

Measurements in mm. Mean of 10 replicates

Time from treatment (weeks)	No. leaves measured per plant		Quantity of growth substances μg. 0	GA_3 0·01	IAA 0·5	GA_3 0·01 + IAA 0·5	L.S.D. $P = 0·05$
2	2	Height	4	5*	4	5*	±0·9
		Leaves	44	54*	46	52	±8·5
3	5	Height	24	28*	23	29*	±2·7
		Leaves	274	309	284	311	±38·0
4	7	Height	81	85	77	88	±7·9
		Leaves	812	810	782	841	±92·2

* Denotes significantly different from controls.

Unlike the results of Gray (1957), seed treatment did not alter the germination rate; neither did seed or root treatment alter the number of nodes preceding the first truss, as Bukovac, *et al.* (1957) found after using foliar sprays.

When GA_3 and IAA were added together to seedling roots in very small amounts there was no greater effect on plant development than when gibberellic acid was added alone. Thus the two growth substances did not interact.

The amounts of growth substances used in these experiments were of the same order as those present in cultures of *Azotobacter chroococcum* used for 'bacterial fertilizer' experiments, where similar effects on tomato growth were found. These results, therefore, support the hypothesis that *Azotobacter* produced growth factors which were in sufficient quantity in the inoculum added to alter plant development.

ACKNOWLEDGEMENTS

The authors would like to thank Mrs. A. Shepherd-Smith for excellent technical assistance and Dr. P. S. Nutman for his interest.

LITERATURE CITED

AGAPOVA, M. V., and BYNOV, F. A., 1961. Effect of gibberellic acid on plant growth. *Uchen. Zap. perm. gos. Univ.* **18**, 53–7.

BARBER, H. N., JACKSON, W. D., MURFET, I. C., and SPRENT, J. I., 1958. Gibberellic acid and the physiological genetics of flowering in peas. *Nature, Lond.* **182**, 1321–2.

BRADFORD, W. W., and EWING, E. C., 1958. Preliminary studies on the application of gibberellic acid to cotton seed and seedlings. *Agron. J.* **50**, 648–50.

BRIAN, P. W., 1959. Effects of gibberellins on plant growth and development. *Biol Rev.* **34**, 37–84.

BROWN, M. E., and the late BURLINGHAM, S. K. Production of plant growth substances by *Azotobacter chroococcum*. (In preparation).

BUKOVAC, M. J., WITTWER, S. M., and TEUBNER, F. G., 1957. Gibberellin and higher plants. VII. Flower formation in the tomato (*Lycopersicon esculentum*). *Q. Bull. Mich. St. Univ. agric. Exp. Stn.* **40**, 207–14.

BURLINGHAM, S. K., 1964. Growth regulators produced by Azotobacter in culture media. *Rep. Rothamsted exp. Stn.* **92**.

CHIRILEI, H., CURTICAPEANU, G., and DOROBANTU, N., 1963. The action of gibberellic acid on the growth and the metabolism in Icar-54 and Vir-42 maize plants. *Lucr. Ses. stiint Inst. agron. Nicolae Balescu Ser.* **B. 6**, 265–72.

CORNS, W. G., 1959. Effects of seed treatment with gibberellin and dates of seedling on winter survival and vegetative yields of Kharkov wheat. *Can. J. Pl. Sci.* **39**, 293–6.

GRAY, R. A., 1957. Alteration of leaf size and shape and other changes caused by gibberellins in plants. *Am. J. Bot.* **44**, 674–82.

JACKSON, R. M., BROWN, M. E., and BURLINGHAM, S. K., 1965. Similar effects on tomato plants of *Azotobacter* inoculation and application of gibberellins. *Nature, Lond.* **203**, 851–2.

LUCKWILL, L. C., 1958. Fruit growth in relation to internal and external chemical stimuli. *17th Symp. Dev. Growth*, 223–51.

MAZZANI, B., and GONZALEZ, O., 1958. Results of preliminary experiments with gibberellic acid on sesame, bean, tomato and papau. *Agron trop. Maracay.* **7**, 175–89.

PERSSON, A., and RAPPAPORT, L., 1958. Gibberellin-induced systemic fruit set in male-sterile tomato. *Science, N.Y.* **127**, 816.

RAPPAPORT, L., 1957. Effect of gibberellin on growth, flowering and fruiting of the Earlypak tomato, *Lycopersicum esculentum*. *Pl. Physiol. Lancaster*, **32**, 440–4.

SRIVASTAVA, R. P., 1963. Effect of presowing treatment with growth regulators and GA on important vegetable crops. 1. *Tomato. Sci. Cult.* **29**, 458–9.

WITTWER, S. H., and TEUBNER, F. G., 1956. Cold exposure of tomato seedlings and flower formation. *Proc. Am. Soc. hort. Sci.* **67**, 369–76.

—— and TOLBERT, N. E., 1960. (2-chloroethyl) trimethylammonium chloride and related compounds as plant growth substances. III. Effect on growth and flowering of the tomato.' *Am. J. Bot.* **47**, 560–5.

YAKAR-OLGUN, N., 1962. Action of gibberellic acid on growth of broad bean (*Vicia faba*). *Istanb. Univ. Fen. Fak. Mecm.* **25**, 193–200.