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# Further biological characteristics of galactoglucomannan oligosaccharides

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## Abstract

The biological activity of cell wall-derived galactoglucomannan oligosaccharides (GGMOs) was dependent on their chemical structure. Galactosyl side chains linked to the glucomanno-core influenced their inhibition of elongation growth of pea (*Pisum sativum* L. cv. Tyrkys) stem segments induced by 2,4-dichlorophenoxyacetic acid (2,4-D). Reduction of the number of galactosyl side chains in GGMOs caused stimulation of the endogenous growth. Modification on the glucomanno-reducing end did not affect significantly the activity of these oligosaccharides. GGMOs inhibited also the elongation induced by indole-3-acetic acid (IAA) and gibberellic acid ($GA_3$). In the presence of IAA the elongation growth was inhibited to 20 - 35 % after 24 h of incubation depending on GGMOs concentrations (1 μM, 10 nM, 0.1 nM), similarly as in the presence of 2,4-D, which confirms the hypothesis of GGMOs antiauxin properties. The elongation induced by $GA_3$ was inhibited to 25 - 60 %, however, the time course of inhibition was different compared with IAA and 2,4-D. The highest inhibition was determined already after 6 h of incubation with a significant decrease after this time. The results indicated a competition between GGMOs and growth regulators.

*Additional key words*: biologically active oligosaccharides, 2,4-D, elongation growth, $GA_3$, IAA, *Pisum sativum.*

## Introduction

Various substances isolated from plants influence in different way plant growth and development (Kumaravelu *et al.* 2000, Kato-Noguchi and Tanaka 2003/4, Terzi *et al.* 2003/4). The plant cell wall is a source of polysaccharide fragments, oligosaccharides, showing several biological activities – elongation growth, morphogenic aspects and differentiation, defense responses – when applied to plant tissues (Bellincampi *et al.* 1993, Fry *et al.* 1993, John *et al.* 1997, Altamura *et al.* 1998, Esquerré-Tugayé *et al.* 2000, Takeda *et al.* 2002, Kaku *et al.* 2004). The activities of oligosaccharides are dependent on their chemical structure (Fry 1989, McDougall and Fry 1989, Zablackis *et al.* 1996). Xyloglucan oligosaccharides and cello-oligosaccharides inhibited the 2,4-dichlorophenoxyacetic acid (2,4-D) induced elongation growth of pea stem segments (York *et al.* 1984, Lorences *et al.* 1990). Likewise xylomanno-oligosaccharides (Priem *et al.* 1990) and oligogalacturonides (Branca *et al.* 1988, Zabotina *et al.* 1995) influenced this process as well. The inhibition of the elongation growth by xyloglucan oligosaccharides in the presence of various types of auxins has been proved (Hoson and Masuda 1991, Warneck 1994) and different opinions on their antiauxin activity are presented. It was ascertained that these oligosaccharides inhibit also the $GA_3$-promoted elongation of pea epicotyls (Warneck and Seitz 1993).

Galactoglucomannans are structural constituents of both primary and secondary cell walls of higher plants (Dey 1980, Akiyama *et al.* 1983, Schröder *et al.* 2004). It was found that galactoglucomannan oligosaccharides (GGMOs) isolated from poplar wood (Auxtová *et al.* 1995), as well as from spruce sawdust (Capek *et al.* 2000) are inhibitors of the 2,4-D-induced elongation growth of pea and spruce stem segments at very low concentrations. This effect was determined in long-term as well as in short-term experiments (Auxtová *et al.* 1995, Auxtová-Šamajová *et al.* 1996). The relation of indirect dependency has been postulated between 2,4-D and GGMOs by Auxtová-Šamajová *et al.* (1996). Our data show also that GGMOs may alter some glycosidase activities during elongation growth in different compartments of the cell (Bilisics *et al.* 2004). However, besides elongation growth GGMOs are able to influence the development of zygotic spruce embryos, viability and regeneration of spruce protoplasts (Lišková *et al.* 1995),

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*Abbreviations*: 2,4-D - 2,4-dichlorophenoxyacetic acid, GGM - galactoglucomannan, GGMOs - galactoglucomannan oligosaccharides, $GA_3$ - gibberellic acid, IAA - indole-3-acetic acid.
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and they seem to be effective factors in the induction of defense reactions in plant cells as well (Slováková *et al*. 2000).

In this paper we were interested in the GGMOs structure-function relationship, and in their interactions with various growth regulators.

## Materials and methods

**Source of oligosaccharides:** Galactoglucomannan (GGM) was isolated from spruce sawdust (*Picea abies* L. Karst) by the fractionation procedure described previously (Capek *et al*. 2000). GGMOs were obtained by partial acid hydrolysis of GGM with 0.4 M trifluoro-acetic acid for 70 min at 100 °C. Trifluoroacetic acid was evaporated and a mixture of mono- and oligosaccharides was separated on a column (200 × 2.5 cm) of *Bio-Gel P-2* (*Serva*, Heidelberg, Germany) by water elution. Fractions of 5 $cm^3$ were collected and analyzed for the sugar content by phenol-sulfuric acid assay (Dubois *et al*. 1956). Their degree of polymerization (d.p. 4 - 8) was identified by comparison with the elution volumes of malto-oligosaccharides (*Serva*) used as reference standard (Capek *et al*. 2000). Galactoglucomannan oligomers consisted of galactose, glucose and mannose in the molar ratio 1:8:33. GGMOs-g, with reduced number of D-galactose units to about 50 % were prepared by treatment of GGMOs with α-galactosidase, and GGMOs-r, with reduced reducing ends were prepared by treatment of GGMOs with $NaBH_4$ (Bilisics and Kubačková 1989).

**Pea stem bioassays:** Seeds of pea (*Pisum sativum* L. cv. Tyrkys) from *Selgen Slovakia* (Bratislava, Slovakia) were germinated in wet *Perlite* in darkness at 24 °C. The bioassays were performed as described by McDougall and Fry (1988) with some modifications. Segments (6 mm long) were cut from the third internode of 8-d-old pea seedlings, 3 mm below the maximum curvature of apical hook. Then the segments were incubated in a solution containing 1 % sucrose, and 5 mM K-phosphate buffer (pH 6.1), plant growth regulators [2,4-D, indole-3-acetic acid (IAA), or gibberellic acid ($GA_3$)] in 0.9 μM concentration added after 90 min of pre-incubation with GGMOs (0.1 nM to 1 μM concentrations), or growth regulators and GGMOs added simultaneously at the beginning of the experiment. In short-term experiments growth regulators were added in 1, 5, 10, and 50 μM concentrations after 2 h of pre-incubation with GGMOs. In experiments aimed on the oligosaccharides structure GGMOs, GGMOs-g, and GGMOs-r from 0.1 nM to 1 μM concentrations were used. Control contained 1 % sucrose, and 5 mM K-phosphate buffer (pH 6.1). GGMOs-free control contained 0.9 μM 2,4-D, IAA, or $GA_3$, 1 % sucrose, and 5 mM K-phosphate buffer (pH 6.1). All solutions were filter-sterilized before use. The incubation was performed in the dark at 24 °C on a rotatory shaker (110 rpm). The length of each segment was determined either after 4 and 24 h, or after 6, 12, 18, and 24 h of incubation.

**Statistics:** Data are presented as elongation (difference of final and initial length measured by use of a projector at 2.5-fold magnification), and percentage of inhibition of 2,4-D, IAA or $GA_3$-induced elongation growth calculated according to McDougall and Fry (1988). The experiments were repeated at least three times with 20 samples for each type of incubation solution. The significance of elongation differences was calculated by Student's *t*-test.

## Results and discussion

**Structure-function relationship:** The importance of oligosaccharides structure for their biological activity has been ascertained. GGMOs and their modified forms – GGMOs-r oligosaccharides with reduced reducing ends and degalactosylated oligosaccharides (GGMOs-g) – inhibited the elongation growth of pea stem segments induced by 2,4-D in a similar course (Fig. 1), but with different intensity dependent on the structural modification of oligosaccharides. GGMOs were used as control sample. Concentrations 0.1 nM of GGMOs (about 40 %), GGMOs-r (about 35 %) and 10 nM of GGMOs-g (about 18 %) caused the highest inhibition of elongation growth after 24 h of incubation. GGMOs-r inhibited the elongation growth in the same range like GGMOs; no significant differences were determined. The reduction of the reducing end of GGMOs was thus without significant effect on the biological activity of these oligosaccharides. Cheong *et al*. (1991), Shibuya *et al*. (1993) and Baureithel *et al*. (1994) had observed similarly that the reducing ends of glucan and chitin oligosaccharides did not affect their biological activity. On the other hand, Spiro *et al*. (1998) observed that the modification at the reducing end of oligosaccharides inhibited and/or influenced in different way the biological activity of oligogalacturonides in morphogenic bioassays. On the other hand, the inhibition of elongation growth by GGMOs-g was significantly lower (about 10 - 18 %, $P < 0.01$) compared with GGMOs. It is probable that galactosyl side chains in GGMOs play an important role in their biological activity examined in this growth process, likewise the fucosyl side chains of xyloglucan oligosaccharides (McDougall and Fry 1989, Dunand *et al*. 2000). GGMOs degalactosylation for these studies was successful only to about 50 %. Not complete splitting

of side chains is a phenomenon which may occur by exoenzymes digestion, in this case by the cleavage of α-linked galactose residues with α-galactosidase. The most plausible causes for this state are inhibition of the reaction by end-product, or steric reasons of the molecule. Further studies are aimed to the preparation of fully degalactosylated oligomers, which could confirm the indispensable role of galactose units for GGMOs inhibition activity in elongation growth.

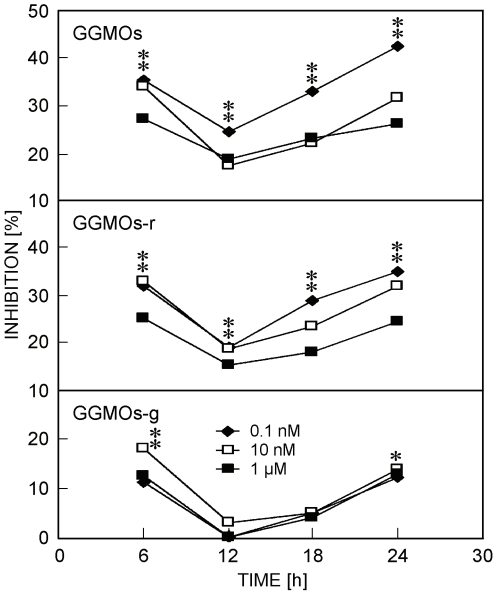


Fig. 1. Effect of different concentrations of GGMOs, GGMOs-r, and GGMOs-g on 2,4-D-induced elongation growth of pea stem segments. Segments were measured after 6, 12, 18 and 24 h. Data are presented as the inhibition [%] of 2,4-D-induced growth. * - values significantly different at $P < 0.05$ from GGMOs-free control, ** - values significantly different at $P < 0.01$ from GGMOs-free control. The degree of significance is at given times the same for all 3 concentrations of GGMOs.

α-D-oligogalacturonides used by Branca *et al*. (1988) and poplar GGMOs used by Auxtová *et al*. (1995) did not significantly affect the endogenous growth; it means the growth without exogenous application of growth regulators. Spruce GGMOs and their reduced form GGMOs-r showed similar effect on endogenous growth ($P > 0.05$) (Fig. 2) unlike of xyloglucan nonasaccharide (in the range between 0.1 nM to 10 nM) and synthetic xyloglucan pentasaccharide which significantly inhibited the endogenous growth between 20 and 40 % (Warneck and Seitz 1993). However, GGMOs-g in this case significantly ($P < 0.01$) stimulated pea stem elongation, which again indicates the important role of galactosyl side chains in the activity of GGM-derived oligosaccharides. The most effective concentration was 0.1 nM except of the values after 12 h of incubation with the most effective concentration 10 nM.

It is known that the timing of GGMOs action in pea-stem bioassay is a very important factor affecting the elongation growth in the presence of 2,4-D (Auxtová *et al*. 1995). The two modified forms of GGM-derived oligosaccharides showed similar picture in experiments when 2,4-D and oligosaccharides were added simultaneously at the beginning of the experiment (Expt. 1), or in the case when 2,4-D was supplied after 90 min of preincubation in the presence of oligosaccharides (Expt. 2, Fig. 3). The only difference was in the percentage of inhibition, again with high similarity between GGMOs and GGMOs-r, and lower or non-significant effect of GGMOs-g. Differences in the effects of GGMOs and GGMOs-r between Expt. 1 and 2 were significant (GGMOs $P < 0.05$ at 10 nM; $P < 0.01$ at 0.1 nM, and 1 μM; GGMOs-r $P < 0.05$ at 1 μM; $P < 0.01$ at 0.1 nM and 10 nM), for GGMOs-g the differences were non-significant ($P > 0.05$).

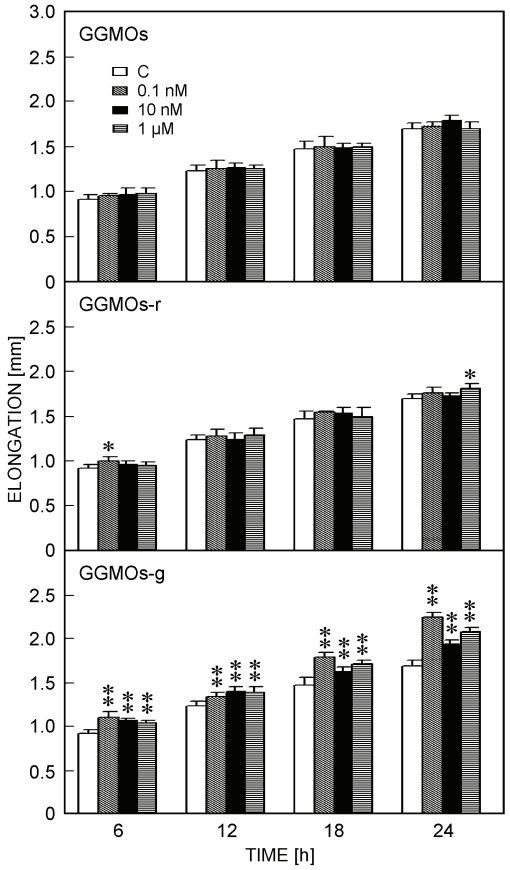


Fig. 2. Effect of different concentrations of GGMOs, GGMOs-r and GGMOs-g on endogenous growth of pea stem segments in the absence of exogenously applied 2,4-D. Segments were measured after 6, 12, 18 and 24 h. Data are presented as the elongation of pea stem segments ± SE, $n = 3$. C - control. Values significantly different from control at * - $P < 0.05$, ** - $P < 0.01$.

**Interaction of GGMOs and growth regulators:** From previous results it is evident that GGMOs are able to inhibit 2,4-D-induced elongation growth. This effect was observed in long-term, as well as in short-term experiments (Auxtová *et al*. 1995, Auxtová-Šamajová

*et al.* 1996). The aim of these studies was to confirm that the inhibitory effect of GGMOs is not restricted only to 2,4-D. The inhibition activity of GGMOs was examined in the presence of further growth regulators (IAA, $GA_3$) and compared with 2,4-D. It was shown that IAA induced elongation growth is inhibited in similar way as by 2,4-D (Fig. 4). The maximal inhibition values were achieved after 24 h of incubation (about 37 % for 2,4-D and 34 % for IAA). The most effective concentration of GGMOs was 0.1 nM in the presence of both substances. Unlike of these results Hoson and Masuda (1991) had observed that xyloglucan oligosaccharides influence 2,4-D-induced elongation of stem segments only in short-term (4 h), however, not in long-term experiments (6 - 24 h) and did not affect the IAA-induced elongation of these segments. GGMOs inhibited the elongation growth also in the presence of $GA_3$, however, in different way compared with 2,4-D and IAA (Fig. 4). The maximum of inhibition has been observed already after 6 h (about 48 - 65 %) of incubation and the inhibition decreased till the end of incubation (23 - 34 %). The most effective inhibitory concentration of GGMOs in the presence of $GA_3$ was 10 nM. Xyloglucan oligosaccharides inhibited $GA_3$-induced elongation growth in the range of 0.01 to 1 nM concentrations (Warneck and Seitz 1993). On the other hand oligogalacturonides were without any effect on elongation growth in the presence of gibberellin (Branca *et al.* 1988). The different course of inhibition of auxin and 2,4-D-induced elongation compared with gibberellin connects probably with the mechanism of GA-induced growth differing from that of auxin-induced growth (Barratt and Davies 1997).

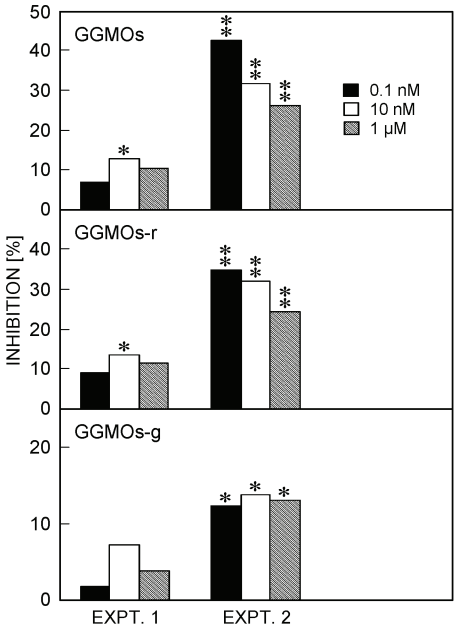


Fig. 3. Timing of GGMOs, GGMOs-r, and GGMOs-g action in the elongation growth of pea segments induced by auxin. Expt. 1, GGMOs (at the indicated concentrations) and 2,4-D were added simultaneously at the beginning of the experiment. Expt. 2, 2,4-D were added in the 90$^{th}$ min and GGMOs at the beginning of the experiment. Segments were measured after 24 h. Data are presented as the inhibition [%] of 2,4-D-stimulated growth. Values significantly different at * - $P < 0.05$ and ** - $P < 0.01$ from GGMOs-free control.

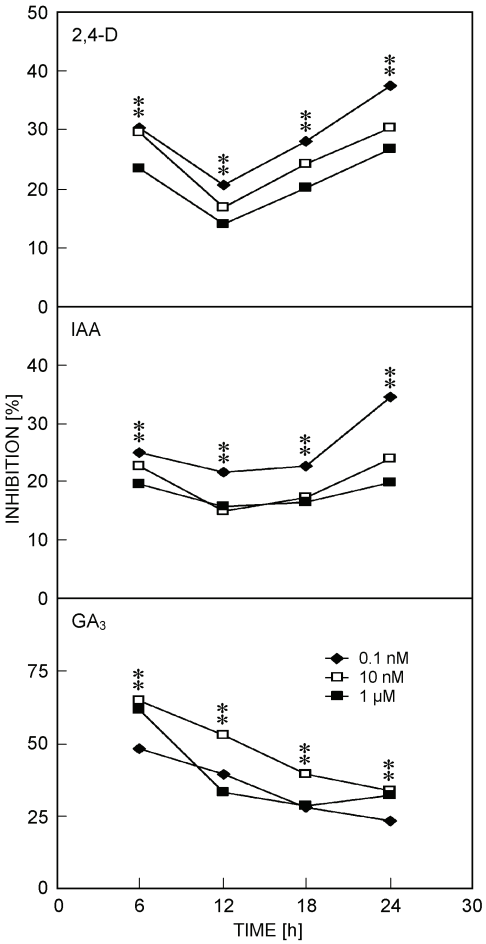


Fig. 4. Effect of different concentrations of GGMOs on the 2,4-D, IAA or $GA_3$-induced elongation growth of pea stem segments. Segments were measured after 6, 12, 18 and 24 h. All values of the significance were $P < 0.01$, the only exception was in the 12$^{th}$ hour for $GA_3$-induced elongation growth at the concentration of GGMOs 1 μM ($P < 0.05$).

The timing of GGMOs and various growth regulators (2,4-D, IAA, $GA_3$) action in the pea stem segment bioassay affected the course of elongation growth. When GGMOs and growth regulators (2,4-D, IAA or $GA_3$) were applied simultaneously at the beginning of incubation (Expt. 1), the inhibition effect was significantly lower (8, 9 and 23 %) compared with Expt. 2 (Fig. 5), when the growth regulators were added after 90 min of preincubation with GGMOs. Differences for 2,4-D between Expt. 1 and 2 were highly significant ($P < 0.01$ at all used concentrations of GGMOs), for IAA these values were significant or highly significant depending on GGMOs concentration ($P < 0.01$ at 0.1 nM and 10 nM; $P > 0.05$ at 1 μM), and significant or highly

significant for $GA_3$ depending on GGMOs concentration ($P < 0.01$ at 10 nM and 1 μM; $P > 0.05$ at 0.1 nM). These results indicate an interaction between GGMOs and growth regulators.

To elucidate the interaction of GGMOs and growth regulators the inhibition effect of GGMOs (0.1 nM, or 10 nM) in the elongation growth in the presence of different concentrations (1 to 50 μM) of growth regulators (2,4-D, IAA or $GA_3$) has been proved (Fig. 6). The addition of GGMOs caused a parallel shift to smaller values of all growth regulators dose-response curves. The results suggest that GGMOs behave as competitive antagonists of growth regulators used (Ariëns *et al.* 1964a,b). This characteristic was determined also for oligogalacturonides by Branca *et al*. (1988). The inhibitory effect of GGMOs was the highest when the growth regulators were used in physiological concentrations (1 and 10 μM) required for elongation growth of plant cells (Taiz and Zeiger 1991).

From results obtained it can be concluded that galactose side chains attached to the β-(1→4) glucopyranosyl-mannopyranosyl backbone are probably responsible for the biological activity of GGMOs in the process of elongation growth. Besides, the modification of the reducing end of the glucopyranosyl-mannopyranosyl backbone caused only non-significant changes in the activity of oligosaccharides tested. GGMOs prepared from spruce sawdust inhibit, similarly as GGMOs-derived from poplar wood, the elongation growth induced by 2,4-D. However, the inhibition effect of GGMOs is not restricted only to one growth regulator (IAA, 2,4-D, also $GA_3$). The timing of GGMOs action significantly influences the inhibition value. The results indicate competitive antagonism of GGMOs against growth regulators used.

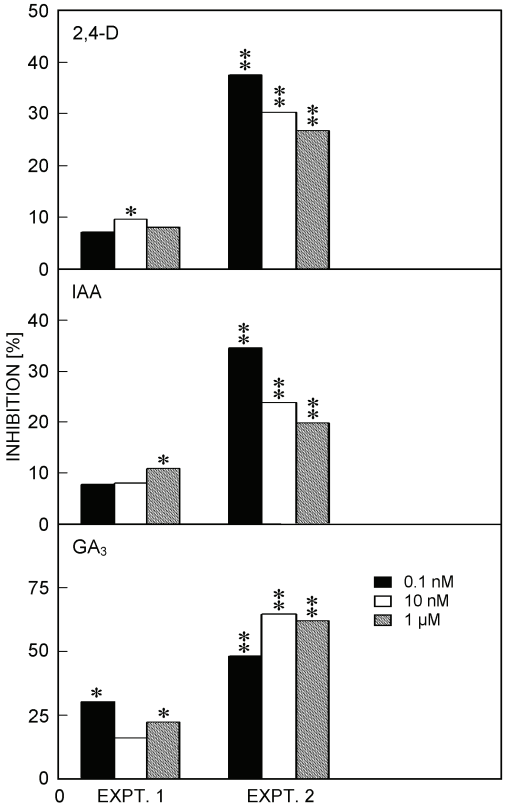


Fig. 5. Timing of the action of GGMOs and growth regulators (2,4-D, IAA or $GA_3$) in the elongation growth of pea stem segments. Expt. 1, GGMOs (at indicated concentrations) and 2,4-D, IAA, or $GA_3$ were added simultaneously at the beginning of the experiment. Expt. 2, 2,4-D, IAA, or $GA_3$ was added in the 90$^{th}$ min and GGMOs at the beginning of incubation. Segments were measured after 24 h of incubation in the presence of 2,4-D and IAA, or after 6 h in the presence of $GA_3$. Data are presented as inhibition [%] of 2,4-D, IAA or $GA_3$-induced growth. Values significantly different at * - $P < 0.05$ and ** - $P < 0.01$ from GGMOs-free control.

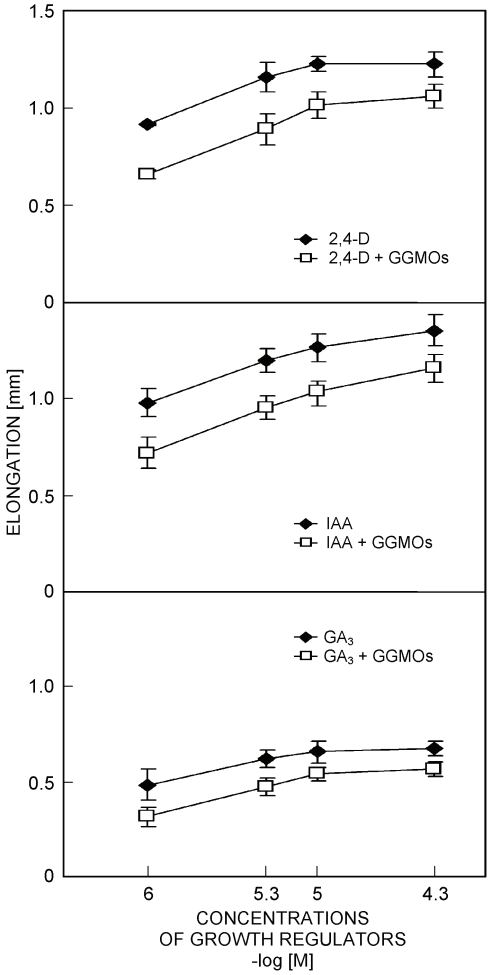


Fig. 6. Effect of GGMOs (0.1 nM in experiments with 2,4-D and IAA, 10 nM in experiments with $GA_3$) on elongation growth of pea stem segments in the presence of 2,4-D, IAA, or $GA_3$ in various concentrations (1, 5, 10, 50 μM). Segments were measured after 4 h of incubation. Data are presented as elongation of pea stem segments ± SE, $n = 3$.

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