GLASNIK INSTITUTA ZA BOTANIKU I BOTANIČKE BAŠTE UNIVERZITETA U BEOGRADU
BULLETIN DE L'INSTITUT ET DU JARDIN BOTANIQUES DE L'UNIVERSITE DE BEOGRAD
Tom XXIII, 1989. (1991), Beograd

UDC 581.144 : 582.738(497.1)
Original scientific paper

GORDANA NAUNOVIĆ and MIRJANA NEŠKOVIĆ

EFFECT OF SUBSTANCES AFFECTING APOPLASTIC AND CYTOPLASMIC CALCIUM CONCENTRATION ON RAPID PEA STEM GROWTH RESPONSES

Institute of Botany and Institute for Biological Research
„S. Stanković", University of Beograd

Naunović, G. and Nešković, M. (1989): *Effect of substances affecting apoplastic and cytoplasmic calcium concentration on rapid pea stem growth responses.* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIII, 27–34.

Rapid changes in growth of decapitated dark–grown pea *(Pisum sativum* L. cv. Meteor) stems were measured in the presence of various substances which affect concentration of Ca^{2+} ions in the apoplast or cytoplasm. In darkness, the addition of $CaCl_2$ decreased the growth rate within 40 min, and a simultaneous 3 min RL irradiation enhanced this response. Gibberellic acid or EGTA reversed the effect of $CaCl_2$ and RL. However, GA_3 had no additional effect when growth had been stimulated with EGTA. The ionophore A 23187 and the herbicide amiprophosmethyl induced a very rapid acceleration of growth, regardless of light conditions. Similar increase in growth was produced when these substances were applied 40 min after EGTA, suggesting thus different sites of action. The results demonstrate that the rapid acceleration of growth rate in pea stems may be elicited by agents which decrease Ca^{2+} concentration in the cell wall, as well as those which increase Ca^{2+} concentration in the cytoplasm.

Key words: *Pisum sativum* L. cv. Meteor, growth rate, red light, Ca^{2+} ions, EGTA, gibberellic acid, amiprophosmethyl, A 23187.

Ključne reči: *Pisum sativum* L. cv. Meteor, brzina rastenja, crvena svetlost, Ca^{2+} joni, EGTA, giberelna kiselina, amiprofosmetil, A 23187.

INTRODUCTION

It has been establihsed in recent studies that calcium serves as a second messenger in many hormone–stimulated and light–induced processes in plants (H e p l e r and W a y n e, 1985; P o o v a i a h and R e d d y, 1987). As far as cell elongation is concerned, both apoplastic and cytoplasmic calcium levels have prominent effects although the underlying processes seem to be different (H a n s o n, 1984). The rise in apoplastic calcium concentration leads to decreased cell extension either by mechanical stiffening of the cell wall (N a k a j i m a et al., 1981; B a y d o u n and B r e t t, 1984), or by inhibiting metabolic processes required for an increase in wall extensibility (C l e l a n d and R a y l e, 1977). The role of cytoplasmic calcium in cell extension is less well known. According to current concepts, external stimuli affect calcium transport and bring about a release and subsequent sequestration of ions in different cell compartments. A transient increase in cytoplasmic calcium concentration results in the formation of calcium–calmodulin complex and activation of several enzymatic systems (D i e t e r, 1984). The specific enzymes possibly involved in growth are not known.

In the present work we have administered substances which modify Ca^{2+} activity in the cell wall, or artificially increase Ca^{2+} accumualtion in the cytoplasm, to study their possible effects on rapid changes in pea stem growth rate. Calcium removal from, or addition to the apoplast produce changes in elongation which are measurable after very short latent periods (M o l l and J o n e s, 1981b; P r a t et al.,1984). Substances that increase intracellular calcium concentration were shown to mimic effects of hormones (S a u n d e r s and H e p l e r, 1982), or light (W a y n e and H e p l e r, 1984). The effects of these substances on rapid growth responses have apparently not been studied.

MATERIAL AND METHODS

Pea seeds *(Pisum sativum* L. cv. Meteor) were germinated in complete darkness for 8 days and seedlings with 1.5–2.5 mm long internodes were selected. The seedlings were decapitated below the hook region and their growth rate was measured using an angular posiiton sensing transducer. In order to avoid spontaneous oscillations in growth rate which were observed after decapitation, experiments were begun after 120 min, by which time a constant growth rate had been established. The elongation was registered continuously and presentation of growth rate was essentially the same as in previous experiments (N a u n o v i ć and N e š k o v i ć, 1979).

All manipulations were performed under a weak green safe light. The red light source (RL) was a Philips TL 20/50 fluorescent tube, equipped with 3–mm plastic Rohm & Haas (Darmstadt, FRG) filter No. 501, with maximum emission at 660 nm and irradiation 1.6 W m^{-2}.

Various substances were dissolved in a buffer solution consisting of 2.5 mM HEPES, 2.5 mM MES and 1 mM succinic acid, adjusted to pH 5.0 with Tris base (M o l l and J o n e s, 1981a). Test solutions included: $CaCl_2$, the chelating agent EGTA, GA_3, the antimicrotubular agent APM, and the ionophore A 23187. The latter was dissolved in buffer containing 0.25% DMSO, which alone had no effect on growth. The epicotyl of experimental plants was inserted into a glass tube and all substances were applied as 10 μl microdrops to the cut epicotyl surface.

At least ten plant were used per treatment, and experiments were repeated three times.

Abbreviations used: RL = red light; GA_3 = gibberelic acid; EGTA = ethyleneglycol –bis(2–aminoethylether)– tetraacetic acid; APM = amiprophosmethyl; DMSO = dimethylsulfoxid; HEPES = N–2–hydroxyethylpiperazine–N'–2– ethanesulfonic acid; MES = 2–(N–morpholino)ethanesulfonic acid; Tris = tris–(hydroxymethyl)–aminomethane base.

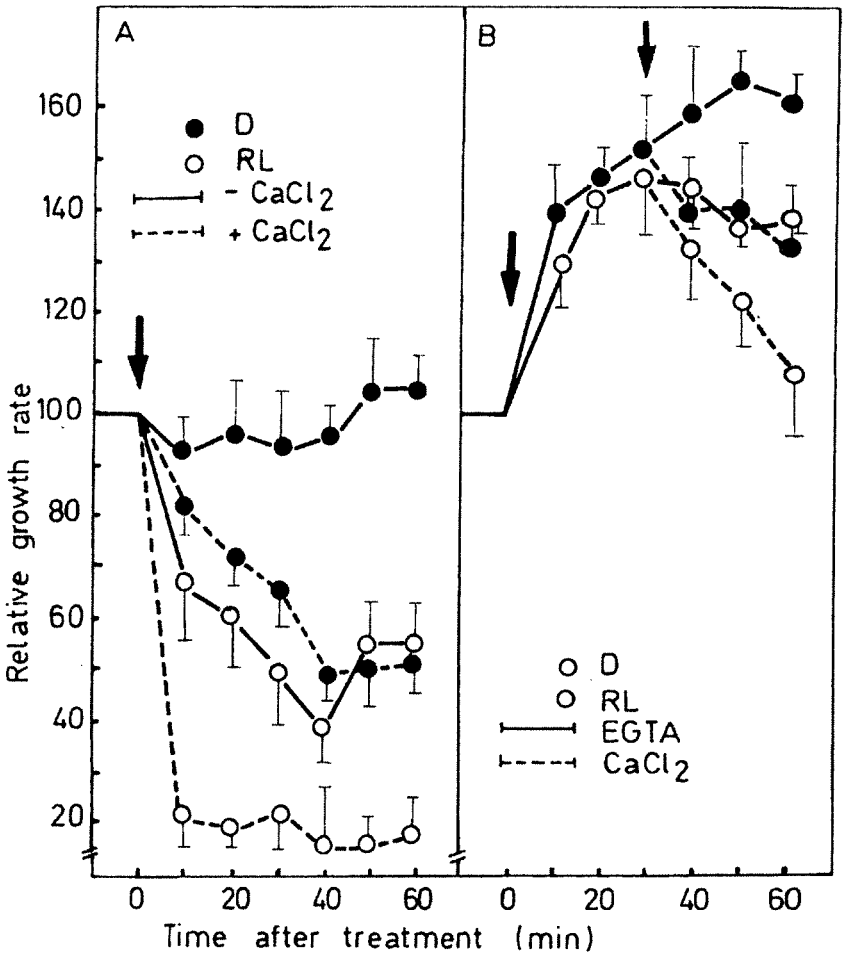

Fig. 1. – Effect of $CaCl_2$ (A) and EGTA (B) on growth rate in darkness, or after 3 min RL. A. $CaCl_2$ (10 mM) and irradiation applied simultaneously at zero time (arrow). B. EGTA (1 mM) and irradiation applied at zero time (first arrow), $CaCl_2$ (10 mM) added after 30 min (second arrow). All values represent the average growth rate of 10 plants, normalized at the initial growth rate. Vertical bars represent SE.

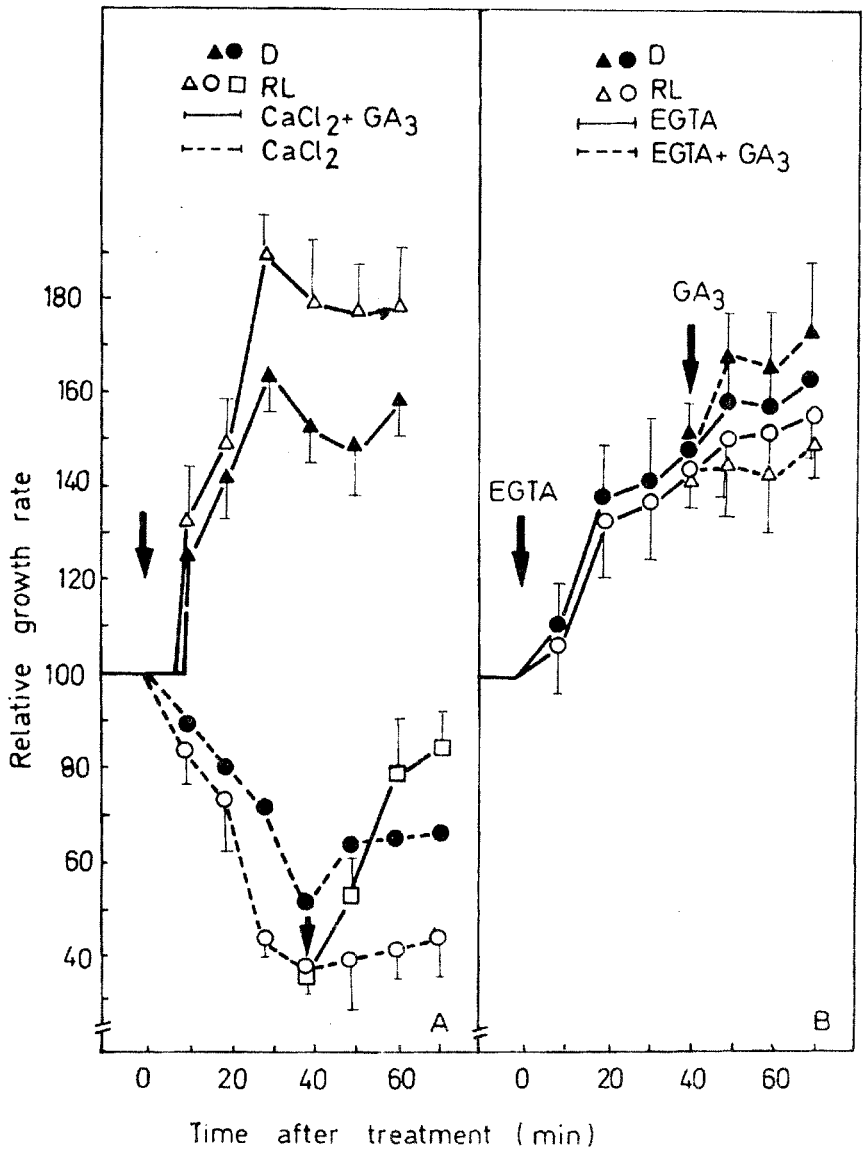

Fig. 2. – Effect of simultaneous and successive application of GA_3 and $CaCl_2$ (A), and of EGTA and GA_3 (B). A. The response to $CaCl_2$ (10 mM) alone, or $CaCl_2$ + GA_3 (10 μg), added at zero time (first arrow); irradiation (3 min RL) at zero time, where indicated; GA_3 alone added after 40 min where indicated (second arrow). B. Response to EGTA (1 mM) added at zero time (first arrow) in darkness, or following 3 min RL, with or without subsequent addition of GA_3 after 40 min (second arrow). Presentation of the results same as in Fig. 1.

RESULTS AND DISCUSSION

Effect of the apoplastic calcium on growth rate

The concentration of Ca^{2+} ions in the cell wall may be increased by adding $CaCl_2$, or decreased by EGTA, which presumably removes Ca^{2+} ions from the wall without entering the cytoplasm. A microdrop of 10 mM $CaCl_2$, added to the decapitated seedling in darkness, immediately slowed growth rate. Three min of RL had approximately the same effect, while both factors applied simultaneously produced a roughly additive effect (Fig. 1A). The chelating agent EGTA (1 mM) accelerated growth of both irradiated and control plants for about 30 and 50 min respectively, while the addition of $CaCl_2$ at 30 min again decreased the growth rate (Fig. 1B). Thus the increase of apoplastic calcium concentration produces the expected inhibitory effect on elongation of pea third internodes, as it does in *Avena* coleoptiles (Cleland and Rayle, 1977), lettuce (Moll and Jones, 1981b) and *Vigna radiata* (Prat et al., 1984) hypocotyls.

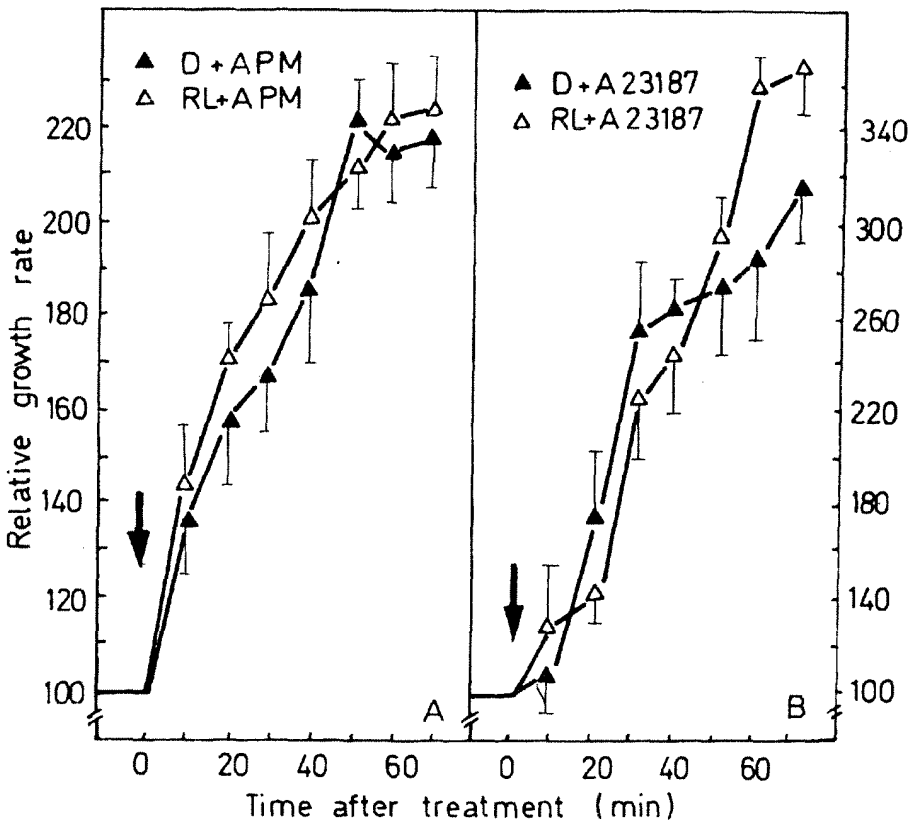

Fig. 3. – Effect of amiprophosmethyl (A) and A 23187 (B) on growth rate in darkenss, or following 3 min RL. A. APM (0.3 μM) and RL applied at zero time (arrow). B. A 23187 (10 μM) and RL applied at zero time (arrow). Presentation of the results same as in Fig. 1.

The effect of $CaCl_2$ alone, and the combined effect of $CaCl_2$ and RL, were both completely counteracted by the application of 10 μg GA_3 either at zero time, or 40 min later (Fig. 2A). However, wehn EGTA was added at zero time, and GA_3 after 40 min, the latter had no additional effect on growth rate (Fig. 2B). Thus these experiments with peas confirm previously published results with lettuce hypocotyls (M o l l and J o n e s, 1981b). Based on the latter findings, it was proposed that GA_3 reduced the calcium activity in the cell wall by promoting its uptake into the cell (M o l l and J o n e s, 1981b). While the removal of Ca^{2+} ions by GA_3 still remains to be proved, W i r k and C l e l a n d (1988) argued that the released Ca^{2+} could simply remain in the wall solution, as long as the pH is kept acidic. There are no experimental data confirming the GA_3 –promoted Ca^{2+} uptake. Effect of substances influencing the cytoplasmic calcium concentration.

We attempted to control the intracellular Ca^{2+} concentration using APM and A 23187, which both maintain a high calcium level in the cytosol. APM (0.3 uM) and A 23187 (10 μM) very dramatically accelerated the growth of pea stems (Fgi. 3A, B). Both substances counteracted the inhibitory RL effect. Their action was nevertheless different from that of EGTA; when EGTA was applied twice, at zero time and after 40 min, the second drop did not further promote growth (Fig. 4A). However, when APM or A 23187

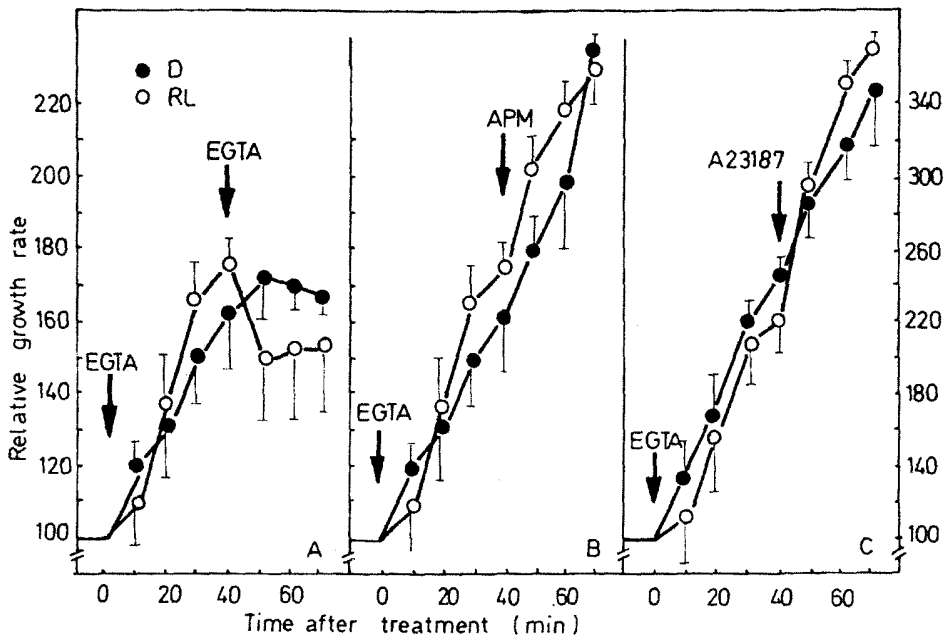

Fig. 4. – Effect of repeated application of EGTA, APM and A 23187 on growth rate in darkenss, or following 3 min RL. A. EGTA (1 mM) added at zero time and 40 min later (arrows). B. EGTA (1 mM) added at zero time, APM (0.3 μM) 40 min later (arrows). C. EGTA (1 mM) added at zero time, A 23187 (10 μM) 40 min later (arrows). Presentation of the results same as in Fig. 1.

were applied 40 min after EGTA, a second growth acceleration was very prominent (Fig. 4B, C). The apoplastic Ca^{2+} ion pool was apparently not essential for that response.

CONCLUSIONS

The results presented here demonstrate that the rapid acceleration of growth rate in decapitated pea stems may be elicited by agents which decrease calcium concentration in the cell wall, such as EGTA. Substances that increase calcium concentration in the cytoplasm, such as APM and A 23187, have similar, but even more pronounced effect on elongation. The inhibitory effect of RL can be counteracted by both groups of agents, as well as by exogenous GA_3 application. While indication of Ca^{2+} involvement in RL and GA_3 effects can be inferred, the precise mechanism of their actions remains to be elucidated in further studies.

REFERENCES

Baydoun, E. A. H., Brett C. T. (1984): The effect of pH on the binding of calcium to pea epicotyl cell walls and its implications for the control of cell extension. – J. Exper. Bot. *35:* 1820–1831.

Cleland, R. E., Rayle, D. L. (1977): Reevaluation of the effect of calcium ions on auxin–induced elongation. – Plant. Physiol. *60:* 709–712.

Dieter, P. (1984 :) Calmodulin and calmodulin–mediated proceses in plants. – Plant, Cell Envir. *7:* 371–380.

Hanson, J. B. (1984): The functions of calcium in plant nutrition. – In P.B. Tinker and A. Lauchli (eds.): Advances in Plant Nutrition, Vol. *1,* pp. 149–208. Praeger, New York.

Hepler, P. K., Wayne, R. O. (1985): Calcium and plant developemnt. – Ann. Rev. Plant. Physiol. *36:* 397–439.

Moll, C., Jones, R. L. (1981a): Short–term kinetics of elongation growth of gibberellin–responsive lettuce hypocotyl sections. – Planta *152:* 442–449.

Moll, C., Jones, R. L. (1981b): Calcium and gibberellin–induced elongation of lettuce hypocotyl sections. – Planta *152:* 450–456.

Nakajima, N., Morikawa, H., Igarashi, S., Senda, M. (1981): Differential effect of calcium and magnesium on mechanical properties of pea stem cell walls. – Plant Cell Physiol. *22:* 1305–1315.

Naunović, G., Nešković, M. (1979): Rapid responses to light and gibberellic acid in etiolated pea stems. – Photochem. Photobiol. *29:* 1173–1175.

Poovaiah, B. W., Reddy, A. S. N. (1987): Calcium messenger system in plants. – CRC Crit. Rev. Plant Sci. *6:* 47–103.

Prat, R. Gueissaz, M. B., Goldberg, R. (1984): Effects of Ca^{2+} and Mg^{2+} on elongation and H^+ excretion of *Vigna radiata* hypocotyl sections. – Plant Cell Physiol. *25:* 1459–1467.

Saunders, M. J., Hepler, P. K. (1982): Ca^{2+} ionophore A23187 stimulates cytokinin–like mitosis in *Funaria.* – Scinece *217,:* 943–945.

Virk, S. S., Cleland, R.,E. (1988): Calcium and the mechanical properties of soybean hypocotyl cell wills: Possible role of calcium and protons in cell–wall loosening. – Planta *176:* 60–67.

Wayne, R., Hepler, P. K. (1984): The role of calcium ions in phytochrome–mediated germination of spores of *Onoclea sensibilis* L. – Planta *160:* 12–20.

Rezime

GORDANA NAUNOVIĆ i MIRJANA NEŠKOVIĆ

EFEKAT HEMIJSKIH FAKTORA KOJI UTIČU NA KONCENTRACIJU KALCIJUMA U APOPLASTU I CITOPLAZMI NA BRZE PROMENE U RASTENJU STABLA GRAŠKA

Institut za botaniku i Institut za biološka istraživanja
„S. Stanković", Univerzitet u Beogradu

Brze promene u rastenju dekapitovanog etioliranog stabla graška *(Pisum sativum* L. var. Meteor) merene su u prisustvu različitih hemijskih faktora koji utiču na koncentraciju Ca^{2+} jona u apoplastu ili u citoplazmi. Dodavanje $CaCl_2$ u mraku usporava brzinu rastenja u toku 40 min, a istovremeno osvetljavanje crvenom svetlošću od 3 min pojačava ovaj efekat. Giberelna kiselina ili EGTA poništavaju efekat $CaCl_2$ i crvene svetlosti. Međutim, giberelna kiselina nema dopunskog efekta, ako je rastenje prethodno bilo stimulisano pomoću EGTA. Jonofor A 23187 i herbicid amiprofosmetil indukuju vrlo naglo ubrzanje rastenja, bez obrzira na svetlosne uslove. Slično ubrzanje rastenja se javlja ako se ove supstance dodaju 40 min posle EGTA, što ukazuje na različita mesta dejstva. Rezultati pokazuju da se naglo ubrzanje rastenja stabla graška može izazvati pomoću faktora koji smanjuju koncentraciju Ca^{2+} u ćelijskom zidu, kao i faktora koji povećavaju koncentraciju Ca^{2+} u citoplazmi.