THE ROLE OF Ca^{2+} IN THE SPROUT GROWTH REGULATION OF GERMINATING PUMPKIN PLANTS (*CUCURBITA PEPO* L.) AS TESTED BY MATHEMATICAL ANALYSIS

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Mathematical analysis of the rate of growth and the total growth enables us to draw conclusions about the significance of calcium for the growth of pumpkin sprouts. The evaluation is based on our knowledge concerning the role of calcium in the permeability of membranes which affects the volume increase in plant parts. In the early stages of sprout growth, the length and width of the organs were measured using non-destructive methods. From these values, using non-linear regression analysis, we were able to establish the growth parameters. The latter then helped us to make to draw the conclusion concerning the dynamics of the movement of Ca^{2+} from reserve (var. '–Ca') or external ('NS') sources, respectively. As far as var. '–Ca' was concerned, the lack of reserve Ca demonstrated itself already between the third and the fifth day of cultivation by a significant decrease in growth of all parts of the sprout; electrometric tests on hypocotyls indicated a significant lowering of capacitance and increased permeability of membranes of the tissue in comparison to externally supplied control ('NS').

growth analysis; calcium; nonlinear regression

INTRODUCTION

We studied the impact of Ca^{2+} on plant growth and on some biochemical relationships (particularly amylases) in a number of previous works (Dvořák, 1973; Dvořák, Sakařová-Ledinská, 1970). *Cucurbita pepo* L. is very sensitive to Ca^{2+}, especially during germination. The volume growth of cells is associated with the accumulation of osmotically active substances flowing through the plasma membrane to the protoplast. Ca^{2+} plays a decisive role in regulating the permeability of plasmalemma and other membranes and in the active transport processes in plant tissues (Ferguson, Drobak, 1988; Elliot, Skinner, 1987; Evans et al.,

1991; Tretyn et al., 1991; Chanson, 1993 etc.). As a result, the lack of calcium in a plant results in an inhibition of growth, the loss of turgidity of tissues, and, electrometrically, in a loss of capacity current on the membranes (Dvořák et al., 1981; Černohorská et al., 1989), etc. In view of the limited mobility of Ca^{2+} through the symplast (Dvořák, 1980), the reaction to the calcium deficit tends to be rather steep in time.

This enables us to use mathematical analysis of growth as a suitable non-destructive mathematical method of following the function and movement of Ca^{2+} within a plant rather well. We have followed its dynamics using the parameters of growth of a young pumpkin sprout, and the state of the membranes in the tissue.

MATERIAL AND METHODS

Cultivation: The experimental plant was pumpkin (*Cucurbita pepo* L., cv. Kveta) grown in Knop's nutrient solution (both full and Ca^{2+}-deficient) with three plants each in three glass vessels in each treatment („NS", „–Ca"), in a laboratory using natural light.

Measurements: The changes in the growth of the pumpkin sprouts (length of hypocotyl, paired cotyledons, differentiating petioles, maximum width of the cotyledons) were measured daily in the course of the ten days of germination. The plants were numbered and evaluated individually without being damaged in any way. Contact measure was being used – or a paper stripe, to the extent the organs of the sprout were curved.

At the end of the cultivation, the quality of the membranes of the hypocotyls was tested electrometrically using current-response to saw-tooth voltage (CR-function, see Dvořák et al., 1981).

Evaluation of the data: Computer programmes 'NonLin' and 'CrRegres' were used for the statistical and regression methods of growth quantification and for the electrometrically obtained current-responses. Both programs were devised by our working group.

For the growth evaluation, we used the simple sigmoidal functions (logistical, Richards' and logistical which may be shifted along the ordinate). For the current-response, we used the function derived by K. Janáček:

$$I = U_{max}/T \,.\, (C \,.\, (2 - e^{(-a1 \,.\, t)} - e^{(-a2 \,.\, t)}) + R^{-1} \,.\, t))$$

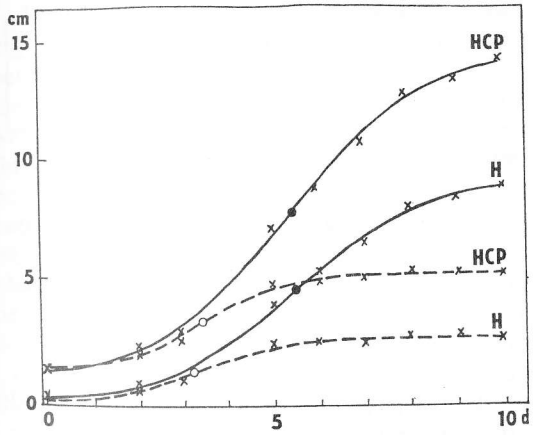
where: a_1, a_2 – rate of saturation of the largest and the smallest capacitor
U_{max} – maximum voltage (8 V)
T – duration of the pulse (2 ms)
t – actual time; for $t = T$ the current corresponds to I_{max} (μA)

The distance of the electrodes = 2.5 mm, C = relative capacitance.

Furthermore, in order to determine the significance (P, zero hypothesis), we used the t-test of differences between the values measured simultaneously and in sequence, as well as the F-test of the significance of the selected function for regression of the measured data. The basic values calculated in this manner are indicated in the tables and figures given below.

RESULTS

Plants of both treatments grew under the same conditions. In both cases the increases of all tested sprouts were highly significant in all parameters between the 2nd and 10th day ($P < 0.1\%$). Thus included the value of the sum total of all the lengths of all parts of the sprouts (Fig. 1). The F-test of regression calculation of the functions of all growth parameters was quite significant ($P < 1\%$, see Tab. I).



1. Dynamics of hypocotyl growth by length (H), and the sum total of the lengths of hypocotyl + petiole + cotyledons (HCP). The full line denotes 'NS', broken line refers to '–Ca'. Positions of the inflexion points are indicated by circles; x-axis = time (days), y-axis = length (cm)

I. The significance of the F-test for the functions calculated from the averages of all plants measured (P in %)

Var.	Hypocotyl (l)	Cotyledons + petiole (l)	Cotyledons (l)	Petiole (l)	Cotyledons (w)
NS	$9.4 \,.\, 10^{-5}$	$2.9 \,.\, 10^{-3}$	$3.4 \,.\, 10^{-4}$	$1.5 \,.\, 10^{-1}$	$3.0 \,.\, 10^{-3}$
–Ca	$1.4 \,.\, 10^{-3}$	$7.5 \,.\, 10^{-4}$	$7.3 \,.\, 10^{-3}$	$3.6 \,.\, 10^{-4}$	$6.7 \,.\, 10^{-3}$

(l) = length, (w) = width, P-limits for values < [5, 1, 0.1]%

The differences between the growth of the hypocotyls of the two treatments are highly significant from 5th day. The differences in the timing of the maximum rate of growth (in reference to the inflexion point) of both treatments are indicated in Tab. II, the rate of growth in Tab. III. The growth

II. Time position of inflexion points (T_i) for growth (days)

Var.	Hypocotyl (l)	Cotyledons + petiole (l)	Cotyledons (l)	Petiole (l)	Cotyledons (w)
NS	5.49	5.53	5.35	6.51	4.82
–Ca	3.36	4.05	3.64	5.03	3.79
δ	2.12	1.48	1.71	1.47	1.03

(l) = length, (w) = width, δ = time difference

III. Maximum rates of growth (length, cm/d)*

Var.	Hypocotyl	Cotyledons + petiole	Cotyledons	Petiole
NS	1.54	0.743	0.603	0.227
–Ca	0.658	0.420	0.391	0.342

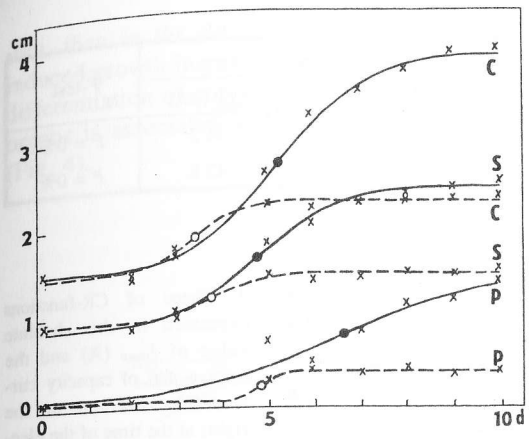
* higher velocity values are underlined

of hypocotyls of the '–Ca' treatment was reduced already by the third day, whereas the treatment supplied with calcium reached maximum growth only by the fifth day. The average total length of hypocotyls was 8.73 cm in case of the control, but only 2.54 cm in the '–Ca' treatment ($P < 10^{-7}$%), i.e. less than a third of the control treatment.

The maximum rate of growth (cm . d^{-1}) in the inflexion point is only 43 % of the value of the full 'NS'. That corresponds both to the reduced transport of Ca^{2+} from the cotyledons in the Ca-deficient, as well as to the two third deficit of the Ca^{2+}.

A significant difference between the two treatments is also in that whereas all plants of the control treatment continued to grow beyond 10th day, no plants of the '–Ca' treatment grew after 5th day, one plant died on day 8, and two more on day 9. Previous experiments have shown that many Ca-deficient pumpkin seedlings die after the 7th day of cultivation (D v o ř á k , 1973).

The cotyledon blade reaches its maximum growth rate after 5th day in the case of the 'NS', whereas in the case of the '–Ca' treatment this happens almost two days earlier (similarly as in the case of the hypocotyls, see Tab. II). The rates for the width are similar (Fig. 2).



2. Dynamics of cotyledon growth by length (C) and by width (S) and the length of petioles (P). Other symbols as in Fig. 1

The petioles of the cotyledons begin to lengthen after the third or fourth day, they are not measurable until then. The differentiation and growth of the petioles are highly significant already on day 5 with the 'NS' treatment, but they only become detectable after day 6 with the '–Ca' treatment ($P < 5$%). Moreover, the steep character of the petiole growth curve is quite evident – this corresponds to the 50% higher rate of growth (Tab. III), which actually occurs between 4th day and 5th day. Compared to that the growth of the control petiole was not completed till day 10 (Fig. 2). The continuing growth of the petiole is also the reason why the development average of all nine plants rendered the relatively least significant values (Tab. I): This seems to be the result of continual Ca^{2+} input from the nutrient solution and individual differences; the cotyledon's Ca^{2+} seems to be used up by day 4.

The plant dimensions on harvest day are listed in Tab. IV; it surveys both the value differences, as well as the relative values for the Ca-deficient treat-

IV. Final dimensions of plant organs (day 10)

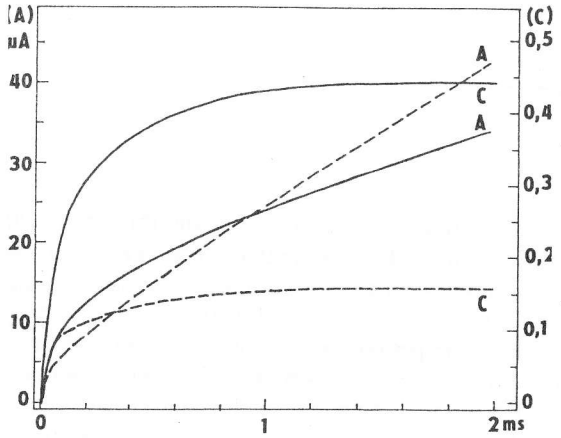
Var.	Hypocotyl (l)	Cotyledons + petiole (l)	Cotyledons (l)	Petiole (l)	Cotyledons (w)
NS	8.73	5.19	3.86	1.33	2.41
–Ca	2.54	2.61	2.27	0.33	1.46
δ	6.15	2.58	1.59	1.00	0.95
% NS	29	50	59	25	61

(l) length, (w) = width, δ = difference (cm), % NS for '–Ca'

V. Evaluation of current-response of hypocotyls

Var.	Parameters of CR-functions				*F*-test*
	a_1	a_2	c_{rel}	I_{max} (μA)	
NS	40.85	5.21	0.450	34.2	P = 0%
–Ca	71.24	4.92	0.163	42.8	P = 0%

* step of freedom = (8, 4)



3. Shape of CR-functions expressed in the absolute value of I_{max} (A) and the relative part of capacity current (C – ordinate on the right) at the time of the electric pulse; x-axis = time (ms), y-axis = current (μA)

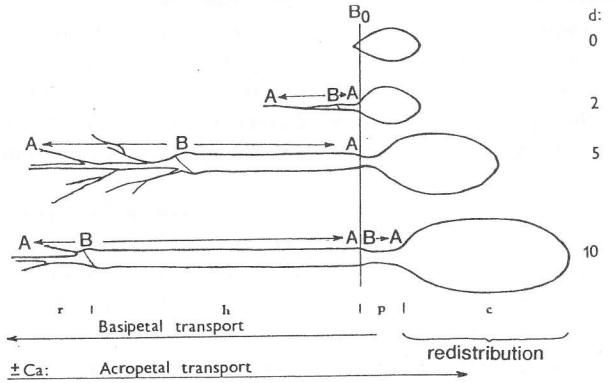
ment. The lack of Ca^{2+} intake from the nutrient solution is reflected in all growth parameters of the '–Ca' treatment. It is demonstrated in a significant shift of the inflexion point towards the beginning of cultivation, as well as by the smaller dimensions of the entire sprout and of all of its parts. By day 7 all differences between the two treatments are quite significant ($P < 1\%$).

In the case of the hypocotyls we were able to establish significant drop of the capacity current (Tab. V, parameter c_{rel}) and a contrasting rise of the Ohmic current at '–Ca' treatment in comparison with the control (Fig. 3). This proves the low resistance and the increased permeability of membranes of Ca-deficient hypocotyls (day 10).

DISCUSSION

The basic supply of Ca^{2+} is stored in the cotyledons of the pumpkin seeds. In the course of germination Ca^{2+} is released as a result of the degradation of the endospermal structures and it is used first in the formation of the primary

root, then in the differentiation of hypocotyls, and in the ontogenetically induced growth increase of the cotyledons. By day 3 it supports the acropetal differentiation of cotyledon petioles. The initial supply of the newly formed organs is associated with a basipetal transport of Ca^{2+} from the cotyledons (Fig. 4).



4. Schematic view of Ca^{2+} transport and growth of *Cucurbita pepo* L. sprout. Direction of growth of the organs is indicated by arrows (sprouts shown according to age, d = days). Directions of Ca^{2+} transport during germination and cultivation indicated by arrows below.

A = apex, B = basis (B_0 = zero growth), r, h, p, c refer to the developing organs = root, hypocotyl, petiole and cotyledon, respectively

Basipetal transport of Ca^{2+} is also associated with the early stages of plant development, primarily as far as the transport from cotyledons is concerned. Once the Ca^{2+} supply is used up, basipetal transport ceases – binding sites in the newly formed tissues are saturated. This was confirmed by, among others, Millikan and Hanger (1966) in their ingenious experiments with ^{45}Ca applied to *Trifolium subterraneum* L. Furthermore, Vaňousová et al. (1973) proved that regenerative processes reactivate the redistribution of Ca^{2+}: wheat and pumpkin were saturated by ^{45}Ca at the beginning of cultivation. Their root parts were removed and the plants were then placed in a non-radioactive solution. The regenerating roots indicated a marked ^{45}Ca presence on autoradiogrammes. Ringoet and DeZeeuw (1968) also confirmed the close connection between growth and redistribution of Ca^{2+}.

Chanson et al. (1993) pointed to the significance of proton transport in Ca^{2+} distribution. It may be argued that the proton activity could be tied to

the higher IAA content within the regenerating zone and that it may be able to release the locally stored ^{45}Ca for exchange reactions with a stable element. Much of Ca^{2+} is fixed in the Donnan free space and on the membranes, from where Ca^{2+} can be released much more easily as a result. This was confirmed by the ionophoretic method (D v o ř á k , 1980).

Fig. 1 indicates that the maximum rate of growth of the hypocotyl and of the entire sprout occurs in parallel in both treatments. As the availability of Ca^{2+} is a limiting factor for the growth of the sprout, its basipetal transport, from cotyledon to petiole to hypocotyl or to the roots, proceeds uniformly. Mathematical analysis can, of course, pinpoint precisely the times at which any part of the sprout reacted to the transported Ca^{2+} (i.e. the gradual sequence of the growth of the cotyledon blade and the differentiation of the petiole).

In the case of the Ca-deficient treatment, slower growth may be partly caused by an imbalance between the synthesis of the protein parts of the α-amylases and calcium content, which is necessary for α-amylase activity in pumpkin cotyledons (D v o ř á k , S a k a ř o v á - L e d i n s k á , 1970): the inability to hydrolyze the stored starch seems to result in a lower metabolism of the reserve saccharides, and hence to a respirational production of protons.

The other transport system is oriented acropetally, it is significant in that it supplies external Ca^{2+} to the newly formed (epicotylar) organs under the influence of the apical dominance. The differences between the maximum total growth of hypocotyls of the 'NS' and '–Ca' treatments (for day 10) quantifies the importance of the transport system supplementing Ca^{2+} over and above its basic supply. Moreover, regression analysis is able to determine when the basic reserve completed its function in supporting growth.

If the external system lacks Ca^{2+}, the latter may be easily retranslocated acropetally from the actively growing basal parts (with a more active H^+-pump) of the sprout (see Fig. 4), in this case from the hypocotyls. Ultimately, Ca^{2+} is released even from the membranes (e.g. plasmalemma). This results in their greatly decreased stability (see Fig. 3) and, in plant, in a habitual loss of turgidity and necrosis of the apical part.

To summarize, as far as we know, Ca^{2+} moves primarily by means of a mass flow system, outside of the symplast, into which it is actively secreted, unless it is bound with the cytoplasmatic system. It can be released from binding systems (matrix) by other cations (Na^+, H^+, K^+) or directly by means of an increased Ca^{2+}-input from outside. If the input ceases, symptoms of Ca shortage may appear in any part of the plant. The apical zones of growth are most likely to be affected.

CONCLUSIONS

The newly obtained findings concerning the dynamics of growth of germinating plants of *Cucurbita pepo* L. and the role played by the availability of Ca^{2+} in that process may be summed up in the following manner:

1) Regression analysis enables us to quantify non-destructively not only the growth dynamics, but also, at the same time, to document the significance of some factors limiting the growth of the plant and of its parts.
2) We analysed the dynamics of Ca^{2+} release from cotyledons according to the growth functions, and we were able to determine the maximum growth rates of the individual organs in the course of the cultivation period.
3) By using two treatments, we were able to quantify the relative proportions of Ca^{2+} transported basipetally and acropetally.
4) Mathematical analysis makes the selection of critical points for further research faster and cheaper.

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Význam Ca^{2+} v regulaci růstu prýtu tykve (*Cucurbita pepo* L.) testovaný matematickou analýzou.

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Metodou matematické analýzy rychlosti růstu a celkového nárostu lze odvodit význam vápníku pro růst klíčních rostlin tykve. Hodnocení se opírá o základní znalosti funkce vápníku v permeabilitě membrán, jejíž důsledky jsou patrné zvláště v objemovém růstu částí rostliny.

V raném období růstu prýtu byly nedestruktivní metodou měřeny délkové, event. šířkové rozměry jeho orgánů a z těchto hodnot byly nelineární regresní analýzou stanoveny parametry růstu. Z nich pak byla odvozena dynamika pohybu vápníku z rezervních (var. '–Ca') či externích ('NS') zdrojů.

U var. '–Ca' se nedostatek zásobního vápníku projevil již mezi třetím a pátým dnem kultivace průkazným snížením nárostu všech částí prýtu; elektrometrický test na hypokotylech prokázal výrazné snížení kapacitance a zvýšení permeability membrán pletiv oproti externě zásobené kontrole.

analýza růstu; vápník; nelineární regrese

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