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**EFFECT OF EXTERNAL FACTORS ON GROWTH AND  
MORPHOGENESIS IN LEMNA TRISULCA L. IN  
AXENIC CULTURE. I. PLANT HORMONES**

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INTRODUCTION

Plants of the family *Lemnaceae* represent the smallest higher plants, which can be easily grown under laboratory conditions, in axenic culture and in controlled nutrient media. They have been used, therefore, as suitable objects to study different aspects of plant metabolism and development. *Lemna trisulca* is a submerse plant, which floats on water only during flowering. It is an exceptional species among *Lemnaceae*, because it forms colonies, in which the individual plants of several generations remain connected by long petioles. Owing to that feature, the individual fronds are interdependent in their development, much more than in any other species of the *Lemnaceae*. Since plant hormones have a dramatic effect on growth and morphogenesis of all higher plants, it seemed interesting to investigate their effect in *L. trisulca*. Data on the effect of GA<sub>3</sub> on chlorophyll retention (Bata and Nešković, 1974) and on the effect of kinetin on branching pattern (Bata, 1973) were previously published. Guern (1965) has extensively studied the correlative inhibition in *L. trisulca* and the role of growth substances in this process.

MATERIAL AND METHODS

The isolation and establishment of axenic cultures of *L. trisulca* was described in details in a previous paper (Bata and Nešković, 1974). The cultures were maintained in modified Bonner-Devirian liquid medium (Gupta and Maheshwari, 1969), supplemented with 1% sucrose, in 100 ml erlenmayer flasks, containing 50 ml of the medium. The pH was adjusted to 5.4-5.6 prior to autoclaving.

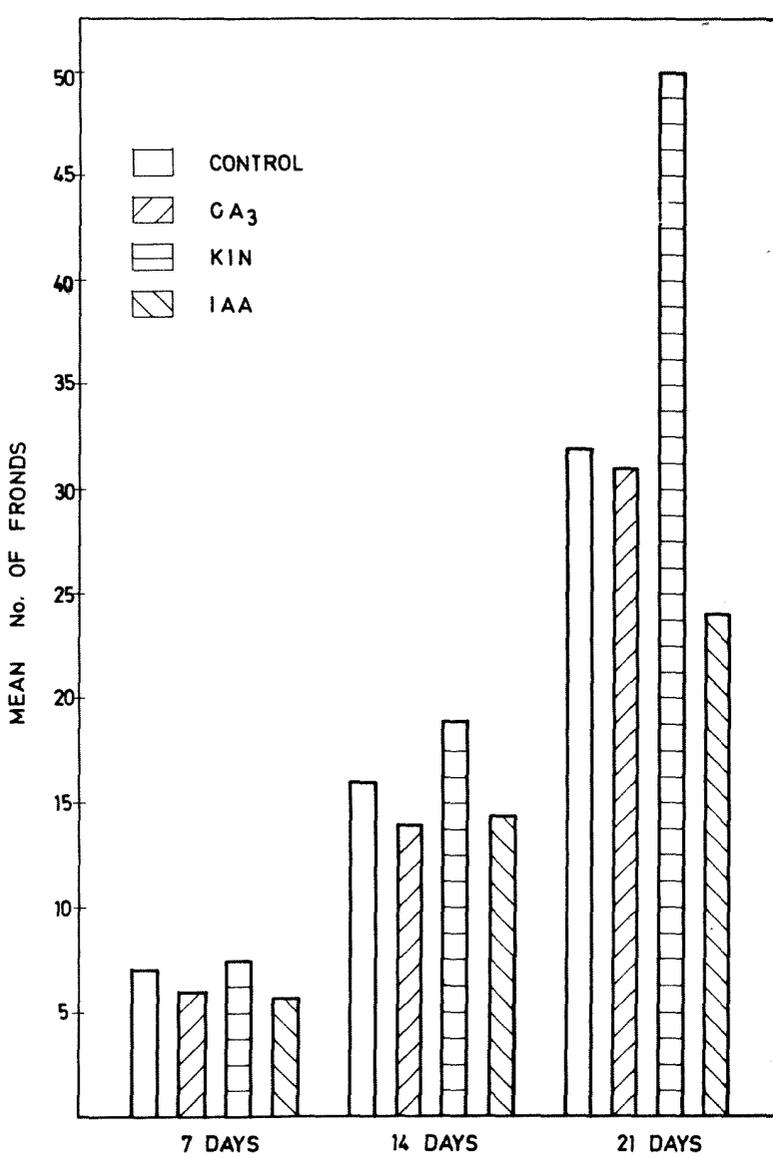


Fig. 1. — Effect of kinetin on the number of fronds in colonies; mean values of 10 colonies; concentrations of kinetin (in  $\text{mg l}^{-1}$ ) indicated in the graph.

The flasks were kept under white light of fluorescent tubes, at an intensity of 550 lx at the plant level, in a day of 16 h and temperature of 26°C during the day and 22°C during the night.

The inoculation of cultures was done by cutting off a unit of 3 fronds, consisting of mother frond and two daughter fronds, just emerging from the pockets on both sides. The mother frond was designated as zero generation ( $G_0$ ), daughter fronds as 1st generation ( $G_1$ ), their daughters as 2nd generation ( $G_2$ ), etc.

Growth of the colonies was evaluated during the logarithmic phase, 7, 14 and 21 days after inoculation. Following parameters were used for evaluation: fresh and dry weight of colonies, number of fronds in each generation, length and width of the fronds and length of roots and petioles.

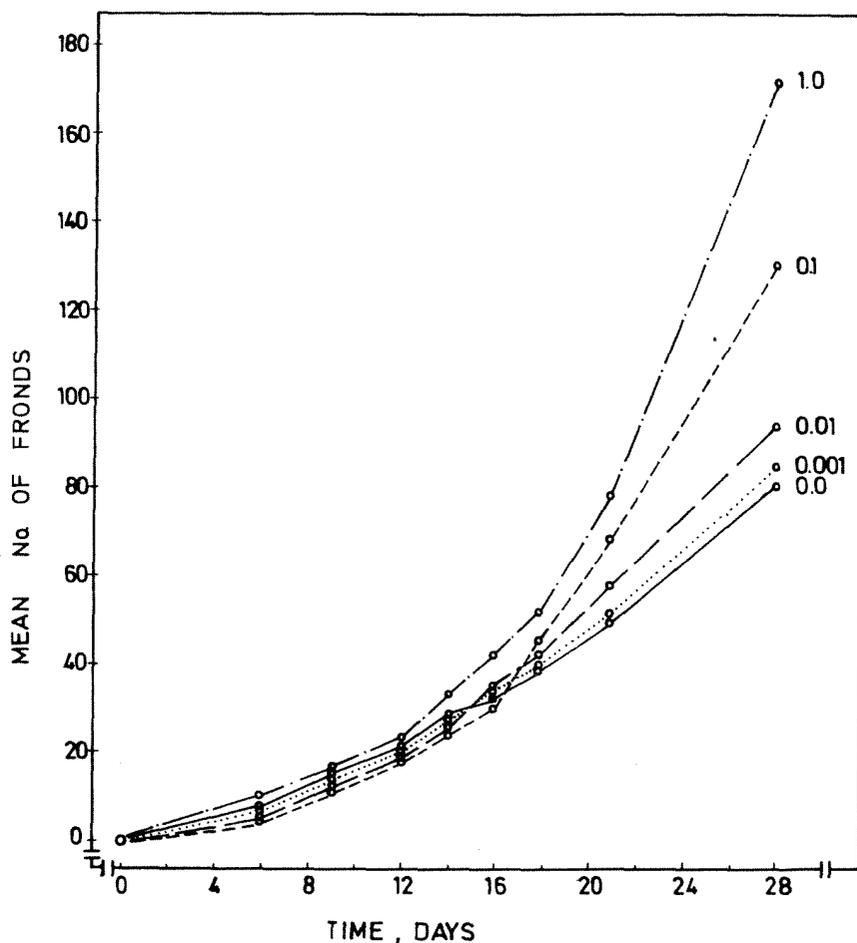


Fig. 2. — Effect of  $GA_3$ , kinetin and IAA on the number of fronds, after 7, 14 and 21 days; concentrations of hormones  $1.0 \text{ mg l}^{-1}$ ; mean values of 10 colonies.

Growth substances were added to the medium prior to autoclaving. Indolyl-3-acetic acid (IAA), kinetin (Kin) and gibberellic acid ( $GA_3$ ) were used in serial dilutions, as indicated in the text.

## RESULTS AND DISCUSSION

### Number of fronds

Of the three hormones tested, only kinetin affected total number of fronds in a colony. Fig. 1 shows that concentrations of 1.0 and 0.1  $mg\ l^{-1}$  were stimulatory, while the lower ones were within the control limits. Parallel experiments with IAA and  $GA_3$  have shown that these two substances had no stimulative effect on frond multiplication in any of the concentrations used. Fig. 2 shows that the kinetin stimulation becomes very high after 21 days. At the same time, IAA has a slight inhibitory effect, in comparison to the controls.

Each frond has two pockets, bearing 1–3 bud initials. The clone used in the experiments is a left-handed clone, as there is a tendency for the development of left

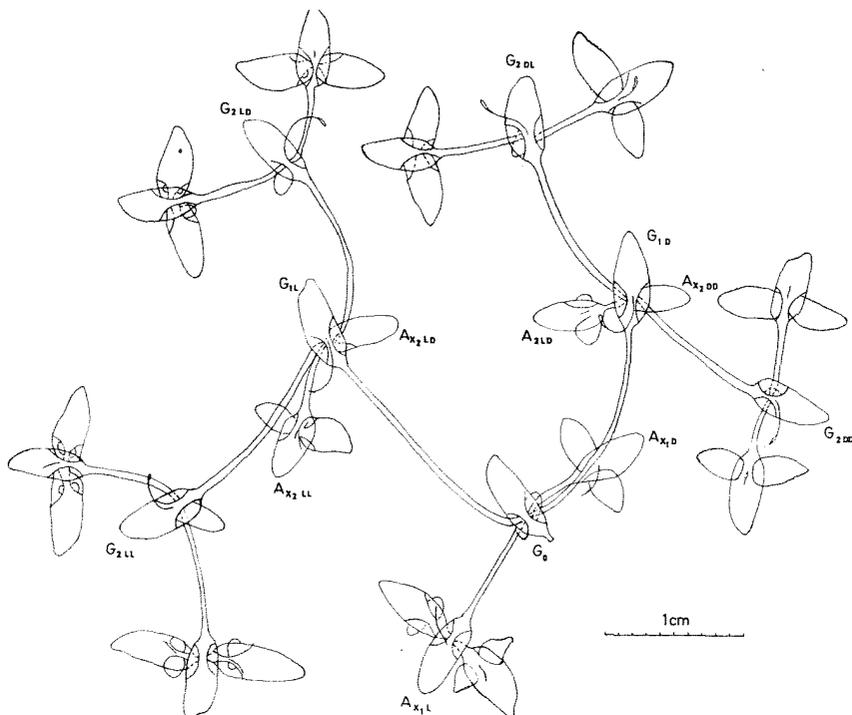


Fig. 3. — Aspect of a colony grown for 21 days in the medium supplemented with 1.0  $mg\ l^{-1}$  kinetin;  $G_0$ – $G_3$  = generations of fronds;  $A_{x1}$  and  $A_{x2}$  = axillary fronds of the first and second generation; L and D = fronds developed in the left and right pocket, respectively.

daughter fronds only, if the conditions are not optimal (B a t a, 1973). However, besides the two daughter fronds, 1–3 axillary fronds sometimes develop from the left or both pockets. It is clear, therefore, that the total number of fronds in a colony may depend either on stimulated branching, or on the shortening of the generation time. This was studied by counting frond number and development in each generation.

After 7 days, the number of 4 fronds in  $G_2$  was attained only in the presence of kinetin ( $1.0 \text{ mg l}^{-1}$ ), but in all cultures  $G_3$  was emerging. After 14 days,  $G_2$  had 4 fronds in the control medium and in those treated with IAA and  $GA_3$ . In the same time,

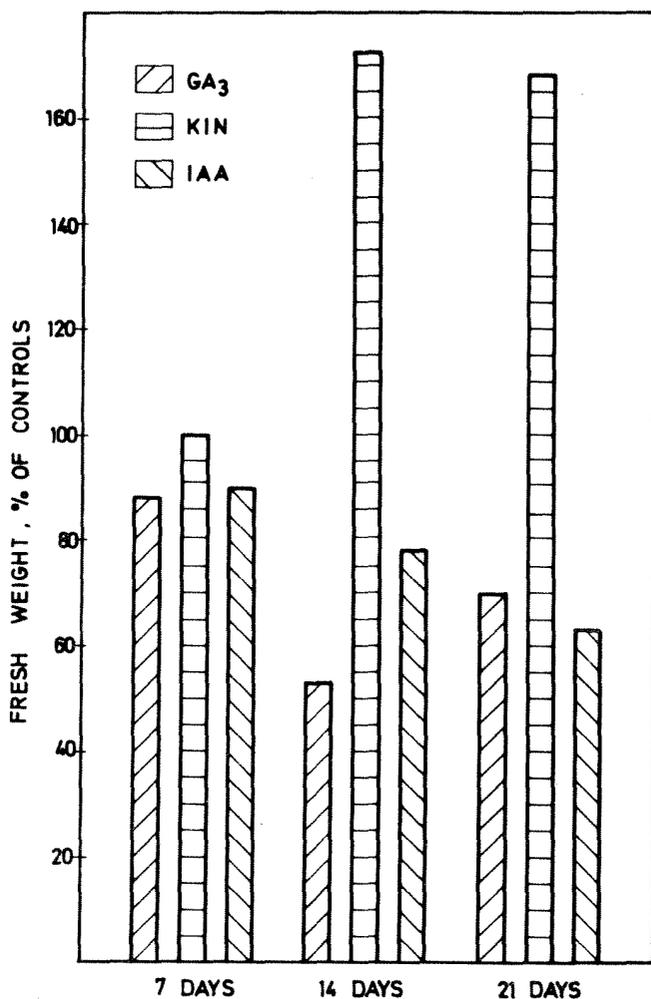


Fig. 4. — Fresh weight of colonies grown in the presence of  $1.0 \text{ mg l}^{-1}$  of  $GA_3$ , kinetin, or IAA; results presented as % of controls; weight of controls (5 cultures) was 36 mg, 84 mg and 178 mg, after 7, 14 and 21 days, respectively.

in cultures containing kinetin  $G_3$  generation already developed maximal number of 8 fronds. However, in control and in IAA and  $GA_3$  treated plants  $G_5$  was emerging, while in kinetin its emergence was delayed and colonies had only 4 generations. Axillary fronds were stimulated by kinetin (0.1 and 1.0 mg l<sup>-1</sup>). They developed out of both pockets of the control  $G_0$  frond, out of the left pocket of  $G_1$  and occasionally in  $G_2$ , too. In all other kinetin concentrations and in controls, axillary fronds developed only in central fronds and in left pockets of  $G_1$ . In  $GA_3$  (1 mg l<sup>-1</sup>) left pocket of the central  $G_0$  frond had axillary fronds, while in IAA axillaries were never observed at that stage.

After 21 days a remarkable increase of total frond number was observed with kinetin, mainly due to the development of axillary fronds in  $G_3$ . In that time, axillary fronds from the central ( $G_0$ ) left pocket developed into small secondary colonies (Fig. 3). Maximal number of fronds in  $G_3$  was obtained in all cultures. In control,  $G_7$  was appearing, while in all cultures with hormones 6 generations were present. Axillary fronds were noticed in both pockets of  $G_2$  with kinetin, while in control and  $GA_3$ -treated colonies  $G_2$  axillary buds were produced in the left pockets only. In IAA, axillaries developed in central frond and in left pockets of  $G_1$ .

It may be concluded, therefore, that kinetin effect on total number of fronds was due to stimulated branching. Daughter fronds appeared earlier and axillary fronds developed on both sides. In  $GA_3$ -treated cultures branching was in the range of controls, while IAA had a retarding effect on that process.

#### Fresh and dry weight

Fresh weight of all cultures after 7 days was near that of the control. After 14 days a remarkable increase was observed in kinetin (1.0 mg l<sup>-1</sup>) and a decrease in  $GA_3$  and IAA. The same trend continued after 21 days (Fig. 4). Dry weight measurements gave the results relatively comparable to those of fresh weight.

#### Length and width of fronds

The size of fronds was measured and expressed as length/width. Table 1 shows that in  $GA_3$  the length is slightly decreased, kinetin had no influence, while in IAA the length of fronds is increased.

Table 1. — Number of fronds in groups of different length/width; concentrations of hormones 1.0 mg l<sup>-1</sup>

Length/width, mm	Control	Kin	$GA_3$	IAA
5.0/2.5	0	0	31	0
6.0/2.5	29	36	79	0
7.0/2.5	62	114	3	41
7.5/2.5	0	29	0	2
8.0/2.5	2	2	0	129
9.0/2.5	0	0	0	24

## Length of roots and petioles

Within 21 days of observation only the fronds of  $G_2$  had attained their final number and size, while all other generations were still growing. Therefore, roots and petioles were measured only in  $G_1$ , supposing that they could represent all roots and petioles in a colony.

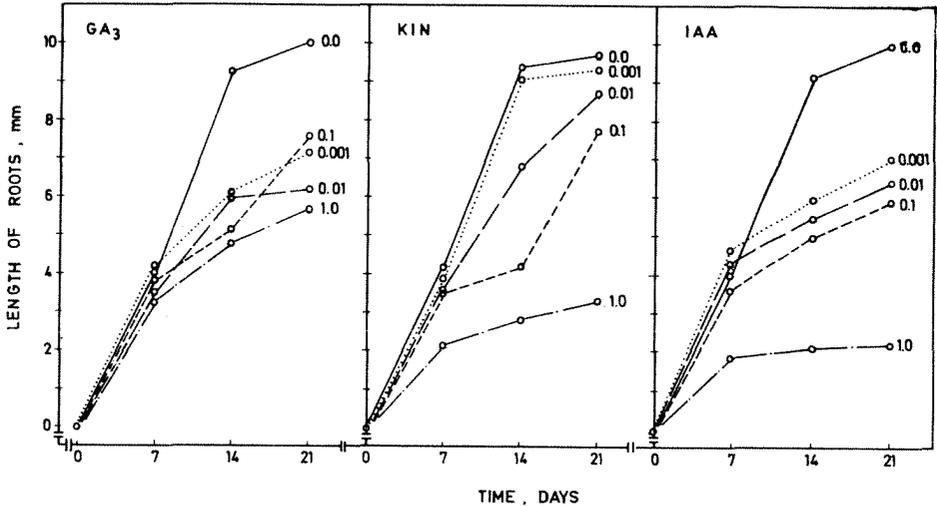


Fig. 5. — Length of roots in the 1st generation, in media supplemented with GA<sub>3</sub>, kinetin or IAA; concentrations of hormones indicated in the graph.

The growth of roots was inhibited by all hormones applied, in all concentrations (Fig. 5), the highest, as could be expected, being the most inhibitory. Petioles, on the contrary, were inhibited only with the highest IAA concentration, and only during the 3rd week. In the beginning of the experiment IAA had some stimulatory effect, which persisted with middle concentration of 0.1 mg l<sup>-1</sup>. Kinetin, and to a lesser extent GA<sub>3</sub>, in concentrations of 0.1 mg l<sup>-1</sup>, also stimulated petiole elongation during the 3rd week. It seems that the growth of petioles of  $G_1$  becomes susceptible to the action of hormones in the 3rd week, when the fronds had finished branching (Fig. 6).

The effect of hormones in *Lemnaceae* has been mostly studied in relation to flowering. Much less attention has been paid to the growth of fronds, petioles and roots, and among the several species of *Lemnaceae*, *L. trisulca* seems to be rather unexplored. The results of the present paper show that the growth substances applied affect some of the parameters measured, while leaving the others unchanged. The most striking effect is the stimulation of frond multiplication by kinetin. Similar effects have also been reported by many authors for other species of *Lemnaceae*. They may be based on the increased cell division rate in meristems, which is a known effect of cytokinins, and also on the release of axillary buds from apical dominance (G u e r n, 1965). This implies that *L. trisulca* responds to cytokinin treatment in a way similar to that of other higher

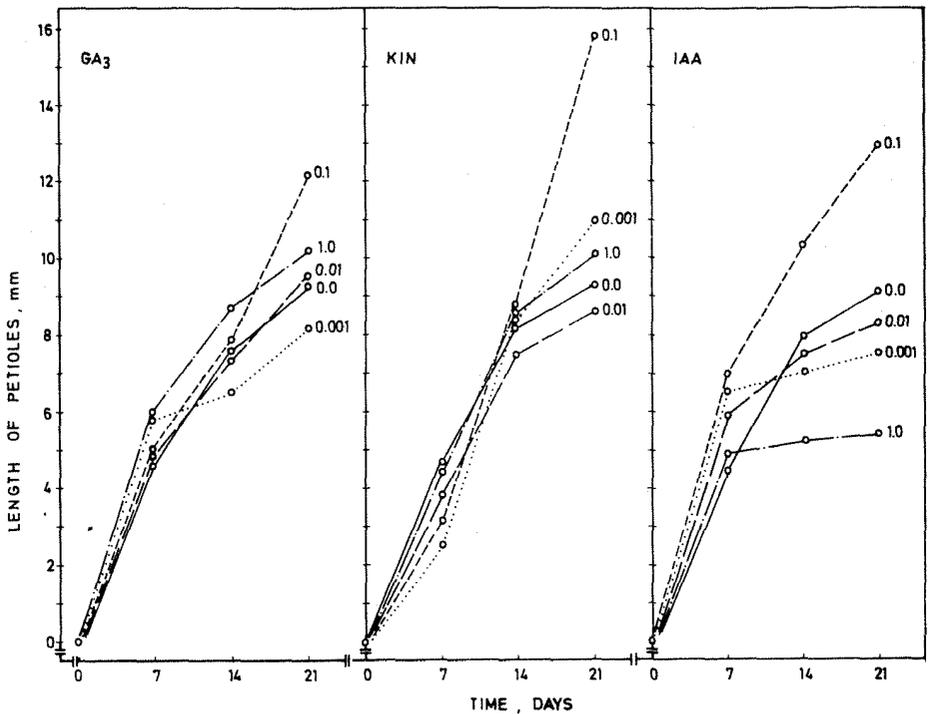


Fig. 6. — Length of petioles in the 1st generation, in media supplemented with GA<sub>3</sub>, kinetin, or IAA; concentrations of hormones indicated in the graph.

plants. A similarity is also found in the reaction of roots towards the hormones applied. As could be expected, roots are highly sensitive to growth substances and are inhibited at all concentrations used. The size of fronds, their length and width, is not significantly affected by any of the hormones used. The greatest part of the frond tissue is considered to be homologous to leaf tissue of other plants and the present results indicate that they are physiologically similar as well. Finally, the elongation of petioles is susceptible to hormones. This is also in accordance with the known effect of GA<sub>3</sub> and IAA on cell elongation in axial organs of higher plants.

#### SUMMARY

*Lemna trisulca* L. was cultivated in axenic culture, in white light, in a day of 16 h. IAA, GA<sub>3</sub> and kinetin were added in various concentrations, and their effect on the growth of colonies was studied. It was found that: (a) the number of fronds was increased by kinetin, which was due to the stimulation of axillary fronds, and not to the increased multiplication rate; (b) fresh and dry weight after 21 days was increased by kinetin, but decreased by IAA and GA<sub>3</sub>; (c) length of the fronds was not changed by

kinetin, but slightly decreased by  $GA_3$  and increased by IAA; (d) elongation of roots was inhibited by all hormones, at all concentrations tested; (e) elongation of petioles was stimulated with moderate concentrations of all three hormones. It is concluded that the organs of *L. trisulca*, although morphologically different from those in other higher plants, respond to the hormones applied in a similar manner.

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#### Re z i m e

JOVANKA BATA i MIRJANA NEŠKOVIĆ

#### EFEKAT SPOLJAŠNJIH FAKTORA NA RASTENJE I MORFOGENEZU LEMNA TRISULCA L. U STERILNOJ KULTURI. I. BILJNI HORMONI

*Lemna trisulca* L. je gajena u sterilnoj kulturi, na beloj svetlosti, pri dužini dana od 16 časova. IAA,  $GA_3$  i kinetin su dodavani u različitim koncentracijama i proučavan je njihov efekat na rastenje kolonija. Rezultati pokazuju da: (a) kinetin povećava broj frondova, putem stimulacije bočnog grananja, a ne putem ubrzanog razmnožavanja; (b) sveža i suva težina su posle 21 dan povećane u prisustvu kinetina, ali smanjene u rastvorima sa IAA i  $GA_3$ ; (c) kinetin ne utiče na dužinu frondova, ali je ona manja u prisustvu  $GA_3$ , a veća na IAA; (d) svi hormoni, u svim primenjenim koncentracijama, inhibiraju izduživanje korenova; (e) izduživanje drški je stimulirano pri srednjim koncentracijama sva tri hormona. Zaključeno je da organi *L. trisulca*, i pored toga što su morfološki vrlo različiti od organa drugih biljaka, imaju sa ovima slične reakcije na hormone koji su ispitivani.

