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THE INFLUENCE OF THE PROTEINASE INHIBITOR EP475 ON SOME MORPHOLOGICAL CHARACTERISTICS OF POTATO PLANTS (*Solanum tuberosum* L. cv. Desirée)

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Abstract

EP475 is a synthetic cysteine proteinase inhibitor. The potato plants *Solanum tuberosum* L. cv. Desirée grew in node culture in controlled conditions. We added 10μM or 50μM of EP475 to the media to investigate its influence on some morphological and biochemical characteristics. The medium without EP475 was used as a control.

The number of nodes of the major shoot increased significantly under the influence of 10μM EP475, but the plants shortened under the influence of 50μM EP475. With the increase of EP475 concentration in the media the primary roots shortened. The number of secondary roots on a single primary root decreased significantly by 50μM EP475. Application of EP475 also resulted in an increased number of lateral shoots and in a significant decrease of leaf areas.

The ratio between dry and fresh weights significantly increased in roots, stems and leaves under the influence of EP475. Thus, EP475 decreased the amount of water in plants.

Decreased growth and biosynthesis due to EP475 were probably a consequence of decreased activity of endogenous proteinases.

Introduction

Proteinases and proteinase inhibitors are proteins that are ubiquitous in nature. The majority of work was done on cereals, legumes, the tomato, potato and tobacco

plants (1). Proteinase inhibitors specifically inhibit each of the four known classes of endopeptidases: serine, cysteine, aspartic and metalloproteinases. Among them, cysteine proteinases and their inhibitors are the best characterised in plants.

Proteinases and proteinase inhibitors were isolated and indentified from different plant organs but mostly they were studied in seeds. In leaves, proteinases and proteinase inhibitors are involved in degradation processes that take place in a neutral environment of cytosol, the acidic environment of lysosomes and the alkaline environment of chloroplasts (1). The ubiquitin-dependent proteolytic pathway that has an integral role in the turnover of many intracellular proteins in plants was identified in cytosol (9). Cell walls and intracellular space also exhibit proteolytic activities, mainly from the serine class (1). There is not much known about proteinases and their inhibitors in roots.

The functional role of these inhibitor proteins appears to be either to protect tissues or fluids from proteolysis by foreign proteases or to regulate the levels of proteases that are metabolically active in the tissues or fluids that they are associated with (8). Proteinases and their inhibitors are involved in senescence proceses (2) and in plant responses on stress (9), pathogens and wounding (3).

Much work has been done on cDNA cloning (2, 3, 4, 5, 10) and on the structure and molecular characteristics of proteinases and their inhibitors.

EP475 is synthetic cysteine proteinase inhibitor with a molecular weight of 370 g/mol. In preliminary experiments it was found that EP475 is transported from media into the plant and along the plant itself. To examine the influence of EP475 on growth and development of potato plants and eventual interactions with endogenous proteinases, EP457 was added into the growth media.

Materials and methods

Single-node cuttings of potato plants (*Solanum tuberosum* L. cv. Desirée) were grown *in vitro* on modified Murashige-Skoog medium (6) without EP475 or with a supplement of 10 μM or 50 μM EP475. The plants were kept at a temparature 21±2°C, the light was 50 $\mu Mm^{-2}s^{-1}$ (Osram L18W 20 lamps), with a photoperiod of 16 h.

In 3 and 5-week-old stem node cultures the length of axillary shoots was measured and stem nodes were counted. After 3 weeks the number of primary roots per plant and the number of secondary roots per primary root were determined and the length of primary roots was measured. After 5 weeks lateral shoots were counted and leaf areas were measured with a ΔT areameter.

To determine the fresh weight of plants, the leaves, stems and roots were separated and weighed. After drying at 45°C to a constant weight they were reweighed to determine the dry weight.

Results and discusion

The plants grown on media supplemented with 50 µM EP475 were significantly smaller than the controls. The number of stem nodes increased significantly on medium supplemented with 10 µM EP475 as compared to control.

PARAMETER	AGE (weeks)	CONTROL	10 µM EP475	50 µM EP475
shoot height (mm)	3	30.44 ± 0.52	33.44 ± 0.57	18.52 ± 0.44
shoot height (mm)	5	57.00 ± 1.10	70.32 ± 1.87	46. ± 1.11
number of nodes	3	8.24 ± 0.05	9.48 ± 0.08	8.32 ± 0.10
number of nodes	5	11.72 ± 0.08	14.2 ± 0.12	13.32 ± 0.12
primary root length (mm)	3	30.33 ± 1.04	22.66 ± 0.77	15.49 ± 0.55
number of secondary roots	3	3.21 ± 0.31	2.04 ± 0.21	0.65 ± 0.11
number of lateral shoots	5	0.04 ± 0.02	0.48 ± 0.07	0.88 ± 0.11
leaf area (cm^2)	6	7.95 ±0.86	3.76 ± 0.71	1.62 ± 0.09
fresh weight of plant (mg)	6	261.53 ± 15.18	207.57 ± 14.48	124.52 ± 8.69
dry weight of plant (mg)	6	19.96 ± 0.85	18.66 ± 0.89	13.23 ± 0.64
dry/fresh weight of plant	6	0.0771± 0.0018	0.0912± 0.0023	0.1076± 0.0026

Table 1: Various parameters of plants grown on control medium, on medium supplemented with 10 µM EP475 and on medium supplemented with 50 µM EP475. The average ± standard error is shown.

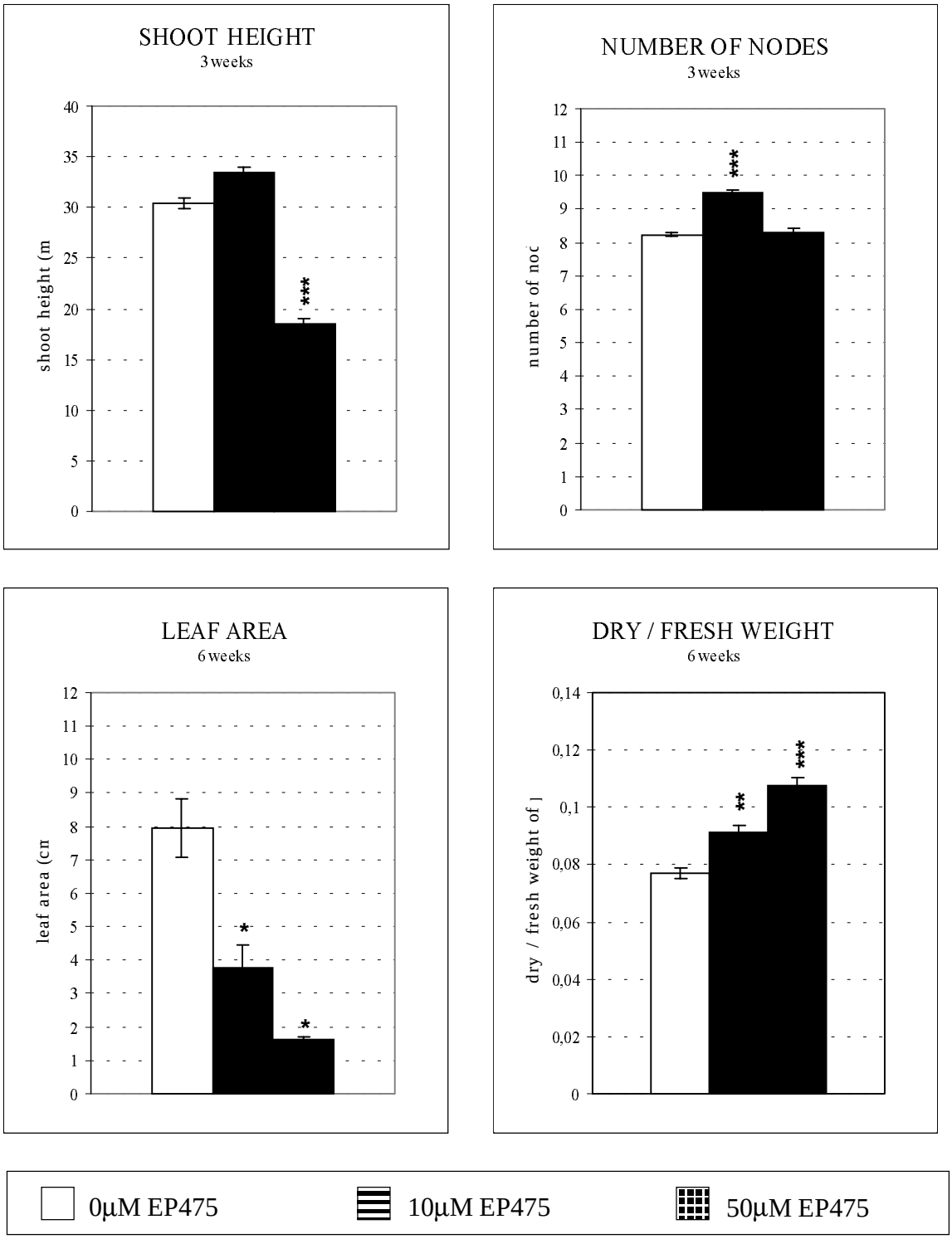

Figure 1: Average shoot height, number of nodes, leaf area per plant and the ratio between dry and fresh weight of plants on control medium without EP475 and on media with 10µM or 50µM of EP475. Statistical comparison is made between plants on media with 10µM of EP475 and plants on control medium and between plants on media with 50µM of EP475 and plants on control medium. Two times the standard error is shown.

With the increase of EP475 concentration in the media were primary roots significantly shorter. The number of secondary roots on a single primary root was
significantly reduced under the influence of 50 μM EP475. Application of EP475 also resulted in an increased number of lateral shoots.

The leaf areas of plants grown on medium supplemented with 10 μM EP475 decreased significantly as compared to controls and the leaf areas of those grown on medium supplemented with 50 μM EP475 were still smaller.

Application of EP475 also resulted in significantly decreased fresh and dry weights of roots and leaves. The fresh weights of stem tended to increase under the influence of 10μM EP475, but decreased significanly with 50 μM EP475. The stem dry weights also increased under the influence of 10 μM EP475. With the increase of EP475 concentration, the fresh weight of whole plants decreased and also the dry weight of whole plants grown on medium with 50 μM EP475 decreased as compared to the controls. The ratio between dry and fresh weights significantly increased in roots, stems, and leaves under the influence of 10 μM EP475 and even more under the influence of 50 μM EP475.

Thus, the decreased size of plants due to EP475 is probably at least in part a result of the decreased amount of plant water and in part a consequence of the decreased activity of endogenous proteinases (T. Popovič, personal communication), though the cell divisions are stimulated as the larger number of nodes and lateral shoots develop.

Following these results, we continue to investigate eventual differences between endogenous activity of proteinases in untreated plants and plants treated with EP475. We are also investigating the immunolocalization of proteinases in tissues of plants grown on medium supplemented with EP475 and on control medium.

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Povzetek

EP475 je sintetični proteinazni inhibitor. Rastline krompirja *Solanum tuberosum* cv. Desirée smo gojili v nodijski kulturi v kontroliranih pogojih. V MS gojišče smo dodali 10µM ali 50µM EP475 in opazovali vpliv na nekatere morfološke značilnosti. Gojišče brez EP475 smo uporabili kot kontrolno gojišče.

Število nodijev glavnega poganjka se je pod vplivom 10µM EP475 statistično značilno povečalo, pri koncentraciji 50µM EP475 pa so bile rastline značilno nižje. Z večanjem koncentracije EP475 se je manjšala dolžina primarnih korenin. Število sekundarnih korenin na posamezni primarni korenini se je statistično značilno zmanjšalo pri rastlinah na gojišču s 50µM EP475. Z dodajanjem EP475 v gojišče se je značilno povečalo tudi število stranskih poganjkov, listna površina pa se je statistično značilno zmanjšala.

Razmerje med suho in svežo težo se je z večanjem koncentracije EP475 v gojišču statistično značilno zvečalo tako v koreninah kot tudi v steblih in listih; EP475 torej vpliva na zmanjšanje količine vode v rastlini. Zmanjšana rast in biosinteza pri 50µM EP475 sta verjetno posledica zmanjšane aktivnosti endogenih proteinaz.