

IDENTIFICATION OF CYTO- AND GENOTOXIC EFFECTS OF LUNULARIC ACID IN *ALLIUM CEPA* L. ROOT TIP MERISTEM CELLS

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In this study, dose-dependent effects of lunularic acid (LA) on some physiological, cytogenetic, biochemical and anatomical parameters were investigated in Allium cepa L. bulbs. For this purpose, physiological parameters to be analyzed experimentally: germination percentage, root length, root number and fresh weight; cytogenetic parameters: micronucleus (MN) frequency, chromosomal aberrations (CAs) and mitotic index (MI); biochemical parameters were determined as catalase (CAT), superoxide dismutase (SOD) activities, malondialdehyde (MDA) level and free proline (Pr) content. In addition, cross-sections were taken from the roots and structural changes in meristem cells were examined. Onion bulbs were divided into four groups as one control and three treatments. The bulbs of the control group were kept in cuvettes containing tap water and the bulbs of the treatment group were kept in cuvettes containing 1 mM, 5 mM and 10 mM LA for 7 days. LA administrations caused a decrease in all investigated physiological parameter values, an increase in the frequency of MN and CAs, and reduce in MI compared to control group. In addition, LA application caused dose-related increases in CAT and SOD activities and MDA and Pr levels compared to control group. LA application promoted CAs such as sticky chromosome, spindle fiber damage, vagrant chromosome, reverse polarization in root meristem cells. After all LA applications, root anatomical structure changes such as epidermis cell deformations, flattened cell nucleus and unclear transmission tissue were observed and it was determined that these changes reached a maximum at 10 mM LA dose. As a result, it has been understood that high doses of LA promote multi-directional toxicity and the Allium test is a very reliable test in determining this toxicity.

Key words: *Allium cepa*, Antioxidant defense system, Bulb

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germination, Genotoxicity, Lunularic acid, Meristematic cell damage.

ВИЗНАЧЕННЯ ЦИТО- І ГЕНОТОКСИЧНОГО ВПЛИВУ ЛУНУЛЯРОВОЇ КИСЛОТИ В КЛІТИНАХ КОРЕНЕВОЇ ВЕРХІВКОВОЇ МЕРИСТЕМИ *ALLIUM CEPA* L.

У цьому дослідженні було вивчено дозозалежний вплив лунолярової кислоти (LA) на деякі фізіологічні, цитогенетичні, біохімічні та анатомічні параметри цибулин *Allium cepa* L. Експериментальним шляхом було проаналізовано такі фізіологічні параметри: відсоток проростання, довжина кореня, кількість коренів та свіжа маса; цитогенетичні параметри: частота мікроядер (MN), хромосомні аберації (CA) та мітотичний індекс (MI); біохімічні параметри визначали як рівні активності каталази (CAT), супероксиддисмутази (SOD), малондіальдегіду (MDA) та вміст вільного проліну (Pr). Крім того, було зроблено поперечні розрізи коренів і вивчено структурні зміни в клітинах меристеми. Цибулини розділили на чотири групи – контрольну та три групи для обробки. Цибулини з контрольної групи зберігали в кюветах, які містили водопровідну воду, цибулини груп обробки зберігали в кюветах, які містили 1 mM, 5 mM та 10 mM LA, протягом 7 днів. Використання LA спричинило зниження всіх показників досліджуваних параметрів, збільшення частоти MN та CA і зниження мітотичного індексу порівняно з контрольною групою. Крім того, застосування LA спричинило дозозалежні підвищення активності CAT і SOD та рівнів MDA і Pr порівняно з контрольною групою. Застосування LA сприяло розвитку таких хромосомних аберацій, як «липка» хромосома, пошкодження ниток веретена, вагрантна хромосома, зворотна поляризація в клітинах кореневої меристеми. Після застосування LA спостерігали такі зміни анатомічної структури кореня, як деформація клітин епідермісу, сплюснуте ядро клітини та нечітка трансмісійна тканина; було встановлено, що ці зміни досягали свого максимуму при дозуванні LA в 10 mM. Було визначено, що високі дози LA сприяють виникненню токсичності мультинаправленої дії і тест з використанням *Allium* дуже надійно визначає її токсичність.

Ключові слова: *Allium cepa*, система антиоксидантного захисту, проростання цибулин, генотоксичність, лунолярова кислота, пошкодження клітин меристеми.

REFERENCES

Akgündüz MC, Çavuşoğlu K, Yalçın E (2020) The potential risk assessment of phenoxyethanol with a versatile model system. *Sci Rep* 10:1209–1218. <https://doi.org/10.1038/s41598-020-58170-9>

- Andrade LF, Davide LC, Gedraite LS (2010) The effect of cyanide compounds, fluorides, aluminum, and inorganic oxides present in spent pot liner on germination and root tip cells of *Lactuca sativa*. *Ecotoxicol Environ Saf* 73:626–631. <https://doi.org/10.1016/j.ecoenv.2009.12.012>
- Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ Exp Bot* 59:206–216. <https://doi.org/10.1016/j.envexpbot.2005.12.006>
- Aydın D, Yalçın E, Çavuşoğlu K (2022) Metal chelating and antiradical activity of *Salvia officinalis* in the ameliorative effects against uranium toxicity. *Sci Rep* 12:15845. <https://doi.org/10.1038/s41598-022-20115-9>
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. *Plant Soil* 39:205–207. <https://doi.org/10.1007/BF00018060>
- Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal Biochem* 44:276–287. [https://doi.org/10.1016/0003-2697\(71\)90370-8](https://doi.org/10.1016/0003-2697(71)90370-8)
- Beers RF, Sizer IW (1952) Colorimetric method for estimation of catalase. *J Biol Chem* 195:133–139
- Boughalleb F, Abdellaoui R, Mahmoudi M, Bakhshandeh E (2020) Changes in phenolic profile, soluble sugar, proline, and antioxidant enzyme activities of *Polygonum equisetiforme* in response to salinity. *Turk J Bot* 44:25–35
- Chaparro TR, Botta CM, Pires EC (2010) Biodegradability and toxicity assessment of bleach plant effluents treated anaerobically. *Water Sci Technol* 62:1312–1319. <https://doi.org/10.2166/wst.2010.944>
- Çavuşoğlu D, Yalçın E, Çavuşoğlu K, Acar A, Yapar K (2021) Molecular docking and toxicity assessment of spirodiclofen: protective role of lycopene. *Environ Sci Pollut Res* 28(40):57372–57385. <https://doi.org/10.1007/s11356-021-14748-y>
- Dahl KN, Ribeiro AJ, Lammerding J (2008) Nuclear shape, mechanics, and mechanotransduction. *Circ Res* 102:1307–1318. <https://doi.org/10.1161/CIRCRESAHA.108.173989>
- Dauer WT, Worman HJ (2009) The nuclear envelope as a signaling node in development and disease. *Dev Cell* 17:626–638. <https://doi.org/10.1016/j.devcel.2009.10.016>
- Davey MW, Stals E, Panis B, Keulemans J, Swennen RL (2005) High-throughput determination of malondialdehyde in plant tissues. *Anal Biochem* 347(2):201–207. <https://doi.org/10.1016/j.ab.2005.09.041>
- Devireddy AR, Tschaplinski TJ, Tuskan GA, Muchero W, Chen JG (2021) Role of reactive oxygen species and hormones in plant responses to temperature changes. *Int J Mol Sci* 22(16):8843. <https://doi.org/10.3390/ijms22168843>
- Dikker O, Şahin M, Atar S, Bekpınar S (2018) Examination of oxidative stress markers in women with postmenopausal osteoporosis. *Turk J Osteoporos* 24(1):15–20. <https://doi.org/10.4274/tod.71501>
- Fan X, Zhou X, Chen H, Tang M, Xie X (2021) Cross-talks between macro- and micronutrient uptake and signaling in plants. *Front Plant Sci* 12:663477. <https://doi.org/10.3389/fpls.2021.663477>
- Fedina IS, Benderliev KM (2000) Response of *Scenedesmus incrassatulus* to salt stress as affected by methyl jasmonate. *Biol Plant* 43:625–627. <https://doi.org/10.1023/A:1002816502941>
- Gorham J (1990) Phenolic compounds other than flavonoids from bryophytes, in *Bryophytes: their chemistry and chemical taxonomy*, Zimsmeister HD, Mues R, eds, Oxford 171–200 p.
- Grant WF (1999) Higher plant assays for the detection of chromosomal aberrations and gene mutations—a brief historical background on their use for screening and monitoring environmental chemicals. *Mutat Res* 426:107–112. [https://doi.org/10.1016/s0027-5107\(99\)00050-0](https://doi.org/10.1016/s0027-5107(99)00050-0)
- Harashima H, Schnittger A (2010) The integration of cell division, growth and differentiation. *Curr Opin Plant Biol* 13(1):66–74. <https://doi.org/10.1016/j.pbi.2009.11.001>
- Hashimoto T, Tori M, Asakawa Y (1988) A highly efficient preparation of lunularic acid and some biological activities of stilbene and dihydrostilbene derivatives. *Phytochem* 27:109–113. [https://doi.org/10.1016/0031-9422\(88\)80599-5](https://doi.org/10.1016/0031-9422(88)80599-5)
- Ighodaro OM, Akinloye OA (2018) First line defence antioxidants—superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. *Alexandria J Med* 54(4):287–293. <https://doi.org/10.1016/j.ajme.2017.09.001>
- Imoto SA, Ohta Y (1985) Intracellular localization of lunularic acid and prelunularic acid in suspension cultured cells of *Marchantia polymorpha*. *Plant Physiol* 79:751–755. <https://doi.org/10.1104/pp.79.3.751>
- Kalefetoğlu Macar T, Macar O, Çavuşoğlu K, Yalçın E, Yapar K (2022) Turmeric (*Curcuma longa* L.) tends to reduce the toxic effects of nickel (II) chloride in *Allium cepa* L. roots. *Environ Sci Pollut Res* 29(40):60508–60518. <https://doi.org/10.1007/s11356-022-20171-8>
- Küplemez H, Yıldırım MU (2020) Effects of cytokinin and auxin on plant development and vascular tissues in *Lens culinaris*. *Commagene J Biol* 4(1):16–21. <https://doi.org/10.31594/commagene.704271>
- Kwak JM, Nguyen V, Schroeder JI (2006) The role of reactive oxygen species in hormonal responses. *Plant*

- Physiol 141(2):323–329. <https://doi.org/10.1104/pp.06.079004>
- Macar O, Macar TK, Çavuşoğlu K, Yalçın E (2020) Protective effects of anthocyanin-rich bilberry (*Vaccinium myrtillus*) extract against copper (II) chloride toxicity. *Environ Sci Pollut Res* 27(2):1428–1435. <https://doi.org/10.1007/s11356-019-06781-9>
- Marrelli M, Amodeo V, Statti G, Conforti F (2019) Biological properties and bioactive components of *Allium cepa* L.: focus on potential benefits in the treatment of obesity and related comorbidities. *Molecules* 24:119–136. <https://doi.org/0.3390/molecules24010119>
- Martinez CA, Maestri M, Lani EG (2003) In vitro salt tolerance and proline accumulation in *Andean potato* (*Solanum* spp.) differing in frost resistance. *Plant Sci* 116:117–184
- Matysik J, Alia-Bhalu B, Mohanty P (2002) Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr Sci* 82:525–532. <https://www.jstor.org/stable/24105959>
- Mithufer A, Maffei ME (2017) General mechanisms of plant defense and plant toxins. *Plant Toxins* 3–24. https://doi.org/10.1007/978-94-007-6464-4_21
- Munns R, Tester M (2008) Mechanisms of salt tolerance. *Ann Rev Plant Biol* 59:651–681. <https://doi.org/10.1146/annurev.arplant.59.032607.092911>
- Novikova GV et al (2020) Coupling of cell division and differentiation in *Arabidopsis thaliana* cultured cells with interaction of ethylene and ABA signaling pathways. *Life* 10(2):15. <https://doi.org/10.3390/life10020015>
- Othman EM, Naseem M, Awad E, Dandekar T, Stopper H (2016) The plant hormone cytokinin confers protection against oxidative stress in mammalian cells. *PLoS One* 11(12):e0168386. <https://doi.org/10.1371/journal.pone.0168386>
- Per TS et al (2018) Jasmonates in plants under abiotic stresses: crosstalk with other phytohormones matters. *Environ Exp Bot* 145:104–120
- Peruzzi L, Carta A, Altinordu F (2017) Chromosome diversity and evolution in *Allium* (Allioideae, Amaryllidaceae). *Plant Biosyst* 151:212–220. <https://doi.org/10.1080/11263504.2016.1149123>
- Pryce RJ, Kent UK (1971) Lunularic acid, a common endogenous growth inhibitor of liverworts. *Planta* 97:354–357. <https://www.jstor.org/stable/23369226>
- Rademacher W (2015) Plant growth regulators: back-grounds and uses in plant production. *J Plant Growth Reg* 34:845–872. <https://doi.org/10.1007/s00344-015-9541-6>
- Rajaei P, Mohamad N (2013) Effect of beta aminobutyric acid (BABA), ABA and ethylene synthesis inhibitor (CoCl₂) on seed germination and seedling growth of *Brassica napus* L. *Eur J Exp Biol* 3:437–440.
- Sagi M, Fluhr R (2006) Production of reactive oxygen species by plant NADPH oxidases. *Plant Physiol* 141:336–340. <https://doi.org/10.1104/pp.106.078089>
- Schwabe WW (1990) Lunularic acid in growth and dormancy of liverworts. in: *Bryophyte development in physiology and Biochemistry*, Chopra RN, Bhatla SC, eds, Boca Raton 245–257 p
- Sharma PC, Gupta PK (1982) Karyotypes in some pulse crops. *Nucleus* 25:181–185.
- Sipahi Kuloğlu S, Yalçın E, Çavuşoğlu K, Acar A (2022) Dose-dependent toxicity profile and genotoxicity mechanism of lithium carbonate. *Sci Rep* 12:13504. <https://doi.org/10.1038/s41598-022-17838-0>
- Soares AMS, Souza TF, Jacinto T, Machado OLT (2010) Effect of methyl jasmonate on antioxidative enzyme activities and on the contents of ROS and H₂O₂ in *Ricinus communis* leaves. *Braz J Plant Physiol* 22:151–158. <https://doi.org/10.1590/S1677-04202010000300001>
- Spann TL, Ferguson L (2014) Commercial production of container grown Citrus trees, in: *Citrus production manual*. University of California Agriculture and Natural Resources Press Ferguson L, Grafton Cardwell E, eds 433 p
- Srivastava AK, Singh D (2020) Assessment of malathion toxicity on cytophysiological activity, DNA damage and antioxidant enzymes in root of *Allium cepa* model. *Sci Rep* 10:1–10. <https://doi.org/10.1038/s41598-020-57840-y>
- Tedesco SB, Laughinghouse IVHD (2012) Bioindicator of genotoxicity. The *Allium cepa* test. *J Environ Contam* 138–156
- Tütünoğlu B, Aksoy Ö, Özbek R, Uçkan F (2019) The effects of gibberellic acid on *Allium cepa* root tip meristematic cells. *Biol Plant* 63:365–370. <https://doi.org/10.32615/bp.2019.042>
- Ünal M, Palavan Ünsal N, Tüfekci MA (2002) Role of putrescine and its biosynthetic inhibitor on seed germination root elongation and mitosis in *Hordeum vulgare* L. *Bull Pure Appl Sci Bot* 21:33–38
- Ünyayar S, Çelik A, Çekic FO, Güzel A (2006) Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Vicia faba*. *Mutagenesis* 21:77–81. <https://doi.org/10.1093/mutage/ge1001>
- Ünal Z (2019) Morphological, physiological and biochemical effects of some abiotic stresses on proline-supported citrus rootstocks. MSc Thesis (Akdeniz Univ Inst Sci) 1–127 p
- Vranova E, Inzé D, Van Breusegen F (2002) Signal transduction during oxidative stress. *J Exp Bot* 53:1227–1236
- Wang XS, Han JG (2009) Changes in proline content, activity, and active isoforms of antioxidative enzymes

- in two alfalfa cultivars under salt stress. *Agric Sci China* 8:431–440. [https://doi.org/10.1016/S1671-2927\(08\)60229-1](https://doi.org/10.1016/S1671-2927(08)60229-1)
- Xia XJ et al (2015) Interplay between reactive oxygen species and hormones in the control of plant development and stress tolerance. *J Exp Bot* 66 (10):2839–2856. <https://doi.org/10.1093/jxb/erv089>
- Yao X et al (2020) Exogenous abscisic acid modulates reactive oxygen metabolism and related gene expression in *Platycladus orientalis* under H₂O₂-induced stress. *Russ J Plant Physiol* 67:85–93. <https://doi.org/10.1134/S1021443720010264>
- Yoshikawa H, Ichik Y, Sakakibara KD, Tamura H, Suiko M (2002) The biological and structural similarity between lunularic acid and abscisic acid. *Biosci Biotechnol Biochem* 66:840–846. <https://doi.org/10.1271/bbb.66.840>
- Zou J, Yue J, Jiang W, Liu D (2012) Effects of cadmium stress on root tip cells and some physiological indexes in *Allium cepa* var. *agrogarum* L. *Acta Biol Cracov Ser Bot* 54:129–141. <https://doi.org/10.2478/v10182-012-0015-x>

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