Journal of Life Sciences & Biomedicine, vol. 7(80), No 1, p. 30-36 (2025)

Root plasticity of the Shirvan-Shahi grapevine under phosphorus deficiency: A perspective from microclonal propagation

Aygun Sadigova*, Mahira Mammadova

Plant Biotechnology Laboratory, Institute of Molecular Biology & Biotechnologies, Ministry of Science and Education of the Republic of Azerbaijan, 11 Izzat Nabiyev, AZ 1073, Baku, Azerbaijan;

***For correspondence:** aygunsadigova06@gmail.com*

Received: February 23, 2025; Reviewed: May 19, 2025; Accepted: June 21, 2025

Phosphorus (P) is an essential macronutrient that plays a central role in plant development and metabolism. However, its limited bioavailability in soil often hampers plant growth and reduces agricultural productivity. This study investigated the impact of varying phosphorus concentrations on the *in vitro* microclonal propagation of the locally significant and technically valuable *Shirvan-Shah* grape variety. Murashige and Skoog (MS) basal medium was supplemented with different levels of KH_2PO_4. The most pronounced root development including increased primary root length, a higher number of lateral roots, and greater root biomass was observed in the MS III medium containing ⅔ of the standard KH_2PO_4 concentration. In contrast, shoot growth was more prominent in the standard phosphorus medium (MS I), highlighting an inverse relationship between root and shoot development. The MS II medium, with reduced phosphorus and supplemented with exogenous auxin, demonstrated a synergistic effect on root morphogenesis, resulting in the most vigorous root system formation. Adaptive responses to phosphorus deficiency were primarily mediated by auxin accumulation and a reduction in cytokinin levels. Phosphorus limitation induced notable changes in root system architecture, underscoring the importance of further exploring the hormonal and molecular mechanisms underlying plant adaptation to nutrient stress.

Keywords: *Phosphorus deficiency, in vitro, Shirvan-Shah grape, root morphogenesis*

INTRODUCTION

Phosphate (Pi) is a fundamental macronutrient in plant nutrition, playing a vital role in both structural integrity through components such as phospholipids and nucleic acids (e.g., DNA) - and energy metabolism, including ATP/ADP cycling and phosphorylation processes (Lambers, 2022). Phosphorus is widely recognized as a primary limiting factor for plant productivity across both natural ecosystems and agricultural systems. Its deficiency is particularly detrimental in low-input agricultural regions, where it significantly constrains crop yields (Cong et al., 2020).

At the cellular level, phosphorus regulates a multitude of critical biological functions. Beyond its role in maintaining membrane structures and facilitating the synthesis of biomolecules and high-energy compounds like ATP, it is also indispensable for cell division, enzyme activation, and carbohydrate metabolism. On a whole-plant scale, phosphorus promotes seed germination, enhances root and stem strength, and contributes directly to improvements in crop yield and quality. In legumes, phosphorus further augments soil fertility by enhancing biological nitrogen fixation (Kebede, 2021).

Despite the application of phosphorus fertilizers in well-structured fertilization regimes, plant roots typically absorb no more than 30% of the applied inorganic phosphate (Pi). The remainder is often rendered unavailable due to fixation in soil matrices or microbial competition.

https://doi.org/10.59849/2710-4915.2025.1.30
Available online June 30, 2025

This inefficiency has led to the widespread overuse of phosphate fertilizers, contributing to the nutrient enrichment of water bodies a process that accelerates eutrophication and triggers harmful algal blooms (Razaq et al., 2017).

As a result, there is an urgent demand for strategies that enhance both phosphate acquisition and phosphorus use efficiency (PUE) to mitigate environmental impacts and conserve soil and water resources. In recent years, significant progress has been made in elucidating plant adaptive mechanisms to low-Pi stress and uncovering the regulatory networks that govern phosphate uptake, transport, and utilization (López-Arredondo et al., 2014).

Plant architecture is widely acknowledged as a critical determinant of stress tolerance and productivity in crops (Guo et al., 2020). The aboveground organs particularly the stem, leaves, and branching pattern play essential roles in key physiological processes such as light interception, photosynthesis, and transpiration, collectively shaping the plant's growth dynamics (Sharma et al., 2021; Berry and Argueso, 2022). Complementing these functions, the root system, as the principal subterranean organ, is integral to the plant's structural stability and physiological viability by mediating water and nutrient uptake from the soil, anchoring the plant body, and providing mechanical support (Li et al., 2018).

The optimization of root architecture (RA) is largely dependent on plant species, environmental factors, and the metabolic demands of the aboveground organs. Alterations in RA such as reduced primary root (PR) length, structural and numerical changes in lateral roots (LR), or disruptions in root hair (RH) development can impair the plant's ability to efficiently acquire water and essential nutrients from the rhizosphere, thereby negatively affecting overall growth and development (Hodge et al., 2009).

Recent advancements in plant biotechnology have opened new avenues for mitigating such developmental limitations through the application of *in vitro* research findings to in vivo systems. In this context, the present study aims to optimize a biotechnological protocol to enhance the efficiency of *in vitro* microclonal propagation of Shirvan-Shahi, a regionally significant and technically valuable grapevine cultivar. The aim of the investigation was to evaluate the impact of varying concentrations of potassium dihydrogen phosphate (KH_2PO_4), a key phosphorus source in the Murashige and Skoog (MS) basal nutrient medium, on morphogenesis, vegetative growth, and the adaptive potential of the root system in micropropagated plantlets

MATERIALS AND METHODS

Plant materials. To optimize components of the technological process and enhance adaptation strategies during *in vitro* biotechnological propagation of valuable aboriginal and technically important grapevine cultivars, Shirvan-Shahi was selected as the model variety. Explants were obtained from the village of Chohranli, located in the Kurdamir district of the Republic of Azerbaijan, and cultured under sterile *in vitro* conditions at the Plant Biotechnology Laboratory of the Institute of Molecular Biology and Biotechnologies, Azerbaijan National Academy of Sciences. Simultaneously, a complementary in vivo collection site was established in the laboratory experimental plot to support phenotypic stability and provide a platform for future comparative phenological and morphological assessments.

Growth conditions and experimental design. Microclonal cultivation was performed on Murashige and Skoog (MS) basal medium (Murashige & Skoog, 1962), supplemented with 0.2 mg/L 6-benzylaminopurine (BAP), 3% sucrose as the carbon source, and 0.8% agar as the gelling agent. Cultures were maintained under a 16-hour photoperiod with a light intensity ranging from 1500 to 2000 lux, at day/night temperatures of 25±2°C and 22±2°C, respectively, conditions shown to be conducive to shoot induction and morphogenic development (Garagozov et al., 2004).

KH_2PO_4 concentrations in the MS medium were experimentally adjusted across three treatment groups:

- MS I (Control): Standard MS macro-and micronutrients + 0.2 mg/L BAP
- MS II: Reduced KH_2PO_4 (½ concentration) in MS macroelements + standard micronutrients + 0.2 mg/L BAP + 0.2 mg/L IAA
- MS III: Reduced KH_2PO_4 (⅔

concentration) in MS macroelements + standard micronutrients + 0.2 mg/L BAP

All other components vitamins, sucrose, and agar were maintained at conventional levels to ensure experimental consistency (Table 1).

Each treatment group was replicated three times, with 25 explants per replicate, to ensure statistical robustness. At the conclusion of the cultivation period, both qualitative and quantitative analyses were performed, assessing parameters such as primary root length, number of lateral roots, total root biomass, root-to-shoot ratio, and overall plant height.

Statistical analysis. The statistical analysis was performed by the mathematical average and standard deviation method. During the analysis of the results, the average mathematical errors and deviations (M±m) were taken into account (Babayev et al., 1999).

RESULTS AND DISCUSSION

The growth dynamics of the explants were assessed two weeks after their placement in the artificial climate chamber. By the fourth week, observable and measurable differences between treatment groups allowed for meaningful comparative analysis. The results demonstrated that phosphate (Pi) deficiency-induced notable alterations in the relative growth patterns of shoots, roots, and nodal structures in Shirvan-Shahi grapevine explants. These changes were most pronounced in the root system.

The comparative analysis of MS III and MS I variants clearly illustrates the impact of phosphorus availability on plant development. Although a slight reduction in primary root length was observed under the MS III medium with moderately reduced phosphorus levels there was a notable increase in the number of lateral roots and overall root biomass. These changes reflect an adaptive response in which plants prioritize the development of their root systems under phosphorus-deficient conditions (Table 2).

On the other hand, while shoot length was greater in the MS I medium, indicating the positive effect of higher phosphorus availability on shoot elongation, the root-to-shoot ratio was higher in MS III (65.00%>61.67%). This suggests a physiological shift in resource allocation toward root growth under nutrient-limited conditions.

Table 1. Composition of MS Culture Medium and Its Modifications in Variants

COMPONENTS	MS I (STANDARD MS)	MS II (½ KH_2PO_4)	MS III (⅔ KH_2PO_4)
Macroelements			
NH_4NO_3	1650 mg/L	1650 mg/L	1650 mg/L
KNO_3	1900 mg/L	1900 mg/L	1900 mg/L
KH_2PO_4	170 mg/L	85 mg/L	113 mg/L
$CACL_2 \cdot 2H_2O$	440 mg/L	440 mg/L	440 mg/L
$MgSO_4 \cdot 7H_2O$	370 mg/L	370 mg/L	370 mg/L
Microelements (Combined)	Present	Present	Present
Vitamins And Organics (Combined)	Present	Present	Present
Plant Growth Regulator	0.2 mg/L BAP	0.2 mg/L BAP+0.2 mq/l İAA	0.2 mg/L BAP
Sucrose	30 g/L	30 g/L	30 g/L
Agar	8 g/L	8 g/L	8 g/L

Table 2. Root development parameters of Shirvan-Shahi grapevine microclones under different MS media treatments

INDICATORS	MS I	MS II	MS III
Main Root Length (cm)	5.20±0.10	5.60±0.10	5.10±0.10
Number of Lateral Roots (count)	3.10±0.10	6.40±0.20	4.20±0.10
Root Weight (g)	0.42±0.01	0.48±0.01	0.45±0.01
Root System Development Score (1–5)	3.00±0.00	5.00±0.00	4.00±0.00
Plant Height (cm)	6.80±0.10	5.20±0.10	6.10±0.10
Root/Shoot Ratio (%)	61.67±0.65	73.10±0.60	65.00±0.70

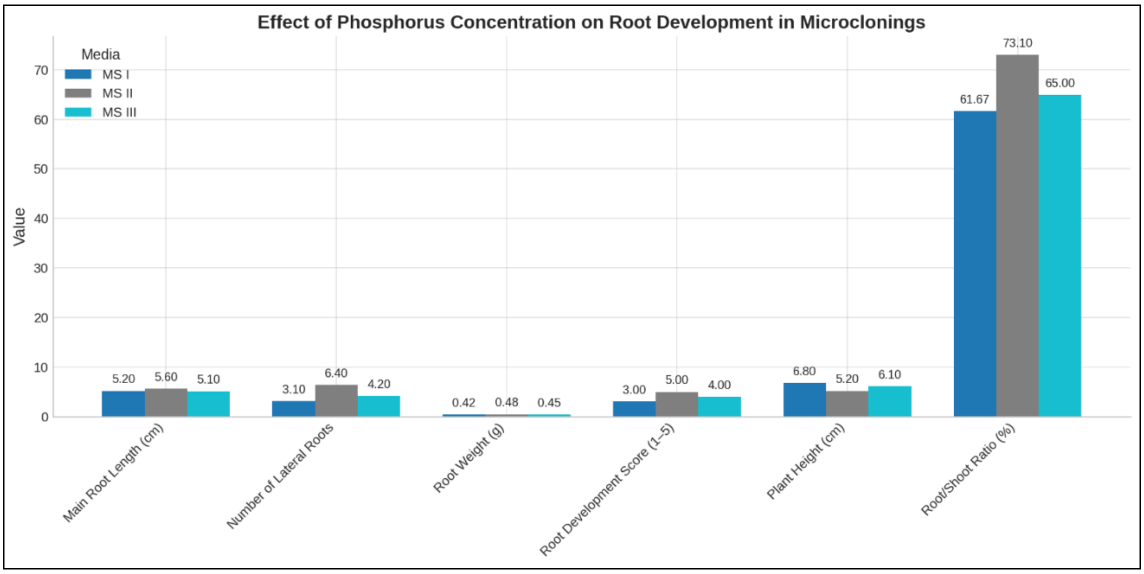

Fig. 1. Root development in microclonings depending on phosphorus concentration.

Collectively, these findings reinforce the idea that enhanced root system development is a core component of plant survival strategies in environments with limited phosphorus availability (Dash et al., 2023; Lu et al., 2024).

These findings suggest the involvement of multiple physiological mechanisms. The duration of phosphorus deprivation and genotypic characteristics of the plant material may significantly influence morphogenetic outcomes. Although the direct and indirect mechanisms through which Pi deficiency regulates plant development are not yet fully elucidated, it is well-established that Pi limitation affects hormonal signaling pathways and nitrogen assimilation, thereby altering developmental processes (Fang et al., 2024; Dandan et al., 2025).

Under phosphorus (P) deficiency stress, a notable decline in meristematic activity within the primary root zone is commonly observed. This suppression is considered a key factor contributing to the preferential formation of lateral roots in regions proximal to the root apex. As a result, cell division and elongation in the primary root are reduced, while meristematic activity in the lateral root zones is stimulated, leading to an increase in lateral root density. Several studies suggest that under low Pi (inorganic phosphate) availability, mitotic activity is redirected toward sites of lateral root initiation. This redistribution of growth potential provides a physiological basis for the altered root system architecture commonly observed under phosphorus-limited conditions.

In particular, explants grown in the MS II medium containing half the standard concentration of KH_2PO_4 and supplemented with exogenous IAA (auxin)-exhibited more vigorous root development. This was evident in the greater length of the primary root, a significant increase in the number of lateral roots, and higher overall root biomass. Root system development scores also reached their maximum in the MS II variant, indicating a healthy and efficient root system formed under moderately reduced phosphorus conditions.

This enhancement is likely the result of a synergistic interaction between phosphorus deficiency and hormonal signaling, especially the role of auxin in promoting the initiation and elongation of lateral roots.

Auxin is primarily synthesized in young aerial organs, particularly in shoot apices, and is directionally transported toward the root system through polar auxin transport mechanisms (Reed et al., 1998). In phosphorus-deficient conditions, auxin accumulates at the root apex, serving as a crucial signal to stimulate lateral root initiation. This spatial redistribution highlights the central role of auxin in the plant’s adaptive response to phosphate (Pi) starvation.

Phosphorus deficiency induces both a redistribution and a localized increase in auxin concentration specifically within the lateral root formation zones. Experimental studies have demonstrated that low Pi availability upregulates the expression of genes encoding auxin transport proteins such as Pın-Formed (PIN) and Auxın Resıstant1 (AUX1) thus enhancing hormone transport toward targeted regions and modulating root system architecture accordingly (Kien et al., 2023).

Interestingly, Pi deficiency not only mimics the physiological effects of auxin but also amplifies its influence in coordination with other hormonal signals, particularly cytokinins. In the second variant of our experiment, where both auxin and cytokinin were supplemented into the nutrient medium, their interaction under low-phosphorus conditions proved to be highly significant. Our data are consistent with the hypothesis that the auxin–cytokinin interplay is critical in orchestrating adaptive root morphogenesis during nutrient stress.

Under normal nutrient conditions, cytokinins promote shoot development and suppress excessive root proliferation, particularly the formation of lateral roots. However, phosphorus deficiency alters this hormonal equilibrium. A marked reduction in cytokinin synthesis and concentration within the root tissues is observed as an adaptive shift that alleviates the cytokinin-mediated suppression of lateral root initiation. Furthermore, phosphorus scarcity impairs cytokinin translocation from roots to shoots, thereby enhancing auxin sensitivity in root cells and reinforcing root expansion (Mehmood et al., 2024). This hormonal antagonism between auxins and cytokinins under phosphorus-deficient conditions orchestrates a regulatory shift in favor of auxin dominance in the root zone, facilitating enhanced lateral root development and overall root plasticity. The plant's capacity to adapt to low Pi availability is thus largely dependent on this dynamic hormonal rebalancing.

It is also important to note that phosphorus is predominantly taken up by plants in the form of inorganic phosphate (Pi), namely $H_2PO_4^-$ and HPO_4^{2-}. However, the efficiency of Pi acquisition and its influence on root development is highly variable, depending on plant species, genotype, and the complex cross-regulation between hormonal signaling networks (Ristvey et al., 2007; Mellor et al., 2016; Bhosale, 2020).

To elucidate the molecular underpinnings of these adaptive responses collectively referred to as the phosphate starvation response (PSR**)** comprehensive studies have been conducted in model species such as *Arabidopsis* and *Oryza sativa* (rice), providing significant insights into how plants respond to phosphorus limitation at both cellular and whole-organism levels (Høgh-Jensen et al., 2020; Wu et al., 2014; López-Arredondo et al., 2014). For instance, in *Arabidopsis thaliana*, phosphorus (Pi) deficiency results in an immediate cessation of cell division and a sharp reduction in primary root elongation following exposure to low-Pi conditions. Simultaneously, Pi limitation promotes the prolific formation of lateral roots and root hairs, which exhibit elevated expression of phosphate transporters and phosphatases key components of the plant’s compensatory mechanisms (Péret et al., 2011).

It is known that under field conditions, the concentration of bioavailable phosphorus in soil solution can be as low as 10 μM (Nascimento et al., 2015). Although plants have evolved sophisticated signaling networks to sense and respond to fluctuating external and internal Pi levels, these natural mechanisms are often insufficient to prevent severe yield losses, particularly in agriculturally important crop species (Høgh-Jensen et al., 2002; Nascimento et al., 2018).

Moreover, similar trends have been observed in both wild and cultivated species such as maize, rice, and tomato. Pi availability influences a broad spectrum of root developmental parameters, including root primordium initiation, primary and lateral root elongation, lateral root growth angle, and overall spatial configuration of the root system (Guo et al., 2025; Kaur, 2024).

Taken together, these findings align closely with the results of our current investigation. Specifically, under reduced phosphorus conditions, we observed a consistent pattern of suppressed primary root elongation accompanied by increased lateral root density and length. This shift in root system architecture is widely interpreted as an adaptive strategy that enhances nutrient foraging efficiency under conditions of limited Pi availability.

Phosphorus deficiency leads to significant alterations in root system architecture, primarily by stimulating lateral root proliferation as an adaptive mechanism to enhance nutrient uptake under stress conditions. These findings underscore the importance of refining nutrient management strategies and optimizing micropropagation protocols to improve root plasticity and stress resilience in agrobiologically valuable grapevine cultivars.

In the present study, visual assessments consistently revealed changes in overall plant morphology and root system structure across all explants under Pi-modified conditions. These observations highlight the necessity of further investigations into the molecular and hormonal responses of plants to phosphorus starvation, with the goal of advancing our understanding of nutrient stress adaptation.

REFERENCES

Babayev T.A., Bunyatov A.R., Efendiyev G.J. (1999) Application of computational techniques and the mathematical theory of the experiment in scientific research. Baku: Elm, 102 p. (in Azerbaijani).

Berry H.M., Argueso C.T. (2022) More than growth: Phytohormone-regulated transcription factors controlling plant immunity, plant development and plant architecture. Curr. Opin. Plant Biol., **70:** 102309; doi: 10.1016/j.pbi.2022.102309.

Bhosale R., Giri J., Pandey B.K., Giehl R.F.H., Hartmann A., Traini R., Truskina J., Leftley N., Hanlon M., Swarup K. et al. (2018) A mechanistic framework for auxin dependent Arabidopsis root hair elongation to low external phosphate. *Nat. Commun*., **9:** 1409.

Cong W-F., Suriyagoda L.D.B., Lambers H. (2020) Tightening the phosphorus cycle through phosphorus-efficient crop genotypes. *Trends Plant Sci*., **25:** 967–975.

Dandan H., Jinyu Z., Yuming Y. et al. (2025). Molecular mechanisms underlying plant responses to low phosphate stress and potential applications in crop improvement. *New Crops*, **2:** 100064; doi: 10.1016/j.ncrops.2024.100064.

Dash G.K. et al. (2023). Redesigning Root Architecture for Improved Performance of Plants Under Stress. In: T.Aftab (ed.) *New frontiers in plant-environment ınteractions. environmental science and engineering*. Springer, Cham.; doi: 10.1007/978-3-031-43729-8_19

Do Nascimento C., Pagliari P., Schmitt D. et al. (2015) Phosphorus concentrations in sequentially fractionated soil samples as affected by digestion methods. *Sci. Rep*., **5:** 17967; doi: 10.1038/srep17967.

Do Nascimento C.A.C., Pagliari P.H., Faria L.D.A., Vitti G.C. (2018) Phosphorus mobility and behavior in soils treated with calcium, ammonium, and magnesium phosphates. *Soil Sci. Soc. Am. J*., **82(3):** 622–631.

Fang X., Yang D., Deng L., Zhang Y., Lin Z., Zhou J., Chen Z., Ma X., Guo M., Lu Z., Ma L. (2024). Phosphorus uptake, transport, and signaling in woody and model plants. *Forestry Research*, **4:** e017; doi: 10.48130/forres-0024-0014

Garagozov T.H., Mammadova M.H., Asadova S.Sh., Alimammadzade I.M., Hasanov F.E. (2004) Grapevine expedited reproduction. Baku: Elm. 78 p.

Guo H.L., Tian M.Z., Ri X., Chen Y.F. (2025) Phosphorus acquisition, translocation, and redistribution in maize. J. Genet. Genomics, **52(3):** 287–296; doi: 10.1016/j.jgg.2024.09.018.

Guo Y., Wu Q., Xie Z., Yu B., Zeng R., Min Q. et al. (2020) OsFPFL4 is involved in the root and flower development by affecting auxin levels and ROS accumulation in rice (*Oryza sativa*). *Rice*, **13:** 1–15; doi: 10.1186/s12284-019-0364-0.

Hodge A., Berta G., Doussan C., Merchan F., Crespi M. (2009) Plant root growth, architecture and function. *Plant Soil*, **321:** 153–187; doi: 10.1007/s11104-009-9929-9.

Høgh-Jensen H., Schjoerring J., Soussana J.F. (2002) The influence of phosphorus deficiency on growth and nitrogen fixation of white clover plants. *Ann. Bot*., **90(6):** 745–753.

Kaur A., Zhawar V.K., Dhillon B.S. (2024) Phosphorus deficit response between shoots and roots of rice (*Oryza sativa* L.) at stages before anthesis. *Cereal Res. Commun.*, **2024;** doi: 10.1007/s42976-024-00597-1.

Kebede E. (2021) Contribution, utilization, and ımprovement of legumes-driven biological nitrogen fixation in agricultural systems. *Frontiers in Sustainable Food Systems*, **5:** 767998; doi: 10.3389/fsufs.2021.767998

Kien L.U., Lukas S., David L.S., Ulrich Z.H., Bjørn P.P. (2023) Substrate recognition and transport mechanism of the PIN-FORMED auxin exporters. *Trends Biochem. Sci.*, **48(11)**: 937-948; doi: 10.1016/j.tibs.2023.07.006.

Lambers H. (2022) Phosphorus acquisition and utilization in plants. *Annu. Rev. Plant Biol.*, **73:** 17–42.

Li K., Liang Y., Xing L., Mao J., Liu Z., Dong F., et al. (2018) Transcriptome analysis reveals multiple hormones, wounding and sugar signaling pathways mediate adventitious root formation in apple rootstock. *Int. J. Mol. Sci.*, **19(8):** 2201; doi: 10.3390/ijms19082201.

López-Arredondo D.L., Leyva-González M.A., González-Morales S.I., López-Bucio J., Herrera-Estrella L. (2014) Phosphate nutrition: improving low-phosphate tolerance in crops. *Annu. Rev. Plant Biol.*, **65:** 95–123.

Lu H., Ren M., Lin R., Jin K., Mao C. (2024) Developmental responses of roots to limited phosphate availability: Research progress and application in cereals. *Plant Physiology*, **196(4):** 2162–2174; doi: 10.1093/plphys/kiae495

Mehmood J., Sajid M., Weicai J., Wenhao Z., Shaolong Z., Yanjie L., Yueni Z., Jinlong L., Haifeng L., Raheel M., Qiang Y., Muhammad A., Guoping W. (2024) Modulating root system architecture: Cross-talk between auxin and phytohormones. *Front. Plant Sci*., **15:** 1343928; doi: 10.3389/fpls.2024.1343928

Mellor N., Band L.R., Pencik A., Novak O., Rashed A., Holman T., Wilson M.H., Voss U., Bishopp A., King J.R. et al. (2016) Dynamic regulation of auxin oxidase and conjugating enzymes AtDAO1 and GH3 modulates auxin homeostasis. *Proc. Natl. Acad. Sci. U.S.A.*, **113:** 11022–11027.

Péret B., Clément M., Nussaume L., Desnos T. (2011) Root developmental adaptation to phosphate starvation: better safe than sorry. *Trends Plant Sci.*, **16:** 442–450; doi: 10.1016/j.tplants.2011.05.006.

Razaq M., Zhang P., Shen H.L., Salahuddin. (2017) Influence of nitrogen and phosphorus on the growth and root morphology of *Acer mono*. *PLoS One*, **12:** 1–13.

Reed R.C., Brady S.R., Muday G.K. (1998) Inhibition of auxin movement from the shoot into the root inhibits lateral root development in Arabidopsis. *Plant Physiol.*, **118:** 1369–1378; doi: 10.1104/pp.118.4.1369.

Ristvey A.G., Lea-Cox J.D., Ross D.S. (2007) Nitrogen and phosphorus uptake efficiency and partitioning of container grown azalea during spring growth. *J. Am. Soc. Hortic. Sci*., **132:** 563–571.

Rivas M.Á., Friero I., Alarcón M.V., Salguero J. (2022) Auxin-cytokinin balance shapes maize root architecture by controlling primary root elongation and lateral root development. *Front. Plant Sci.*, **13:** 836592; doi: 10.3389/fpls.2022.836592.

Sharma M., Singh D., Saksena H.B., Sharma M., Tiwari A., Awasthi P. et al. (2021) Understanding the intricate web of phytohormone signalling in modulating root system architecture. Int. J. Mol. Sci., **22(11):** 5508: doi: 10.3390/ijms2211550.

Wu Y., Wen Y., Zhou J. et al. (2014) Phosphorus release from lake sediments:Effects of pH, temperature and dissolved oxygen. KSCE *J. Civ. Eng.*, **18:** 323–329.

ORCIDS:

Aygun Sadiqova: https://orcid.org/0000-0002-8605-5752

Mahirə Mammadova: https://orcid.org/0000-0002-9055-9400