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NITRATE REDUCTASE AND ITS ACTIVITY IN PLANTS UNDER THE INFLUENCE OF ENVIRONMENTAL STRESS FACTORS

НІТРАТРЕДУКТАЗА ТА ЇЇ АКТИВНІСТЬ В РОСЛИНАХ ЗА ДІЇ СТРЕСОВИХ ЧИННИКІВ НАКОЛИШЬОГО СЕРЕДОВИЩА

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In the last decade, researchers have been interested in studying the effect of stress factors on plant growth and development. According to modern thinking, stressors include not only extreme temperatures and high salt concentrations, but also other factors that are not traditional for the life of this form of plant. The nitrogen metabolism enzyme nitrate reductase (NR) deserves special attention, as understanding the mechanisms of its functioning under normal and stressful conditions, to which plants are often exposed, is not only of fundamental, theoretical importance, but also of great practical significance. The next stage of research will certainly be the task of

experimentally obtaining plant forms with altered metabolism [3, p. 2; 11, p. 201; 12, p. 2].

Active plant growth under stressful (critical) conditions can only be supported by functional enzymes of various kinds, since in the case of minimised metabolism, only passive survival is possible at best. First of all, this applies to nitrogen metabolism, as this element is necessary for any living system primarily to create its own structural and functional proteins.

Among all the natural forms of nitrogen in the plant organism, the nitrate form (NO^{3-}; N^{5-}) is available. The chain of protein assimilation is as follows: $N^{5+} \rightarrow N^{3+} \rightarrow N^{+} \rightarrow N^{-} \rightarrow N^{3-}$. It is clear that the nitrogen deficit will increase with the reduction (waste) of the links. Therefore, the first link, namely the nitrate→nitrite ($N^{5+} \rightarrow N^{3+}$) conversion, is considered to be the key link. This reaction is catalysed by the enzyme nitrate reductase (KF 1.6.6.1.) [1, p. 205; 5, p. 317; 10, p. 1].

Nitrate reductase (NR), a homodimer encoded by nuclear genes in higher plants, catalyses the reduction of nitrate to nitrite. The NR enzyme complex consists of two parts that are sequentially involved in the transfer of electrons from NAD(P)H to nitrate. This is the diaphorase part, which contains FAD and catalyses the transfer of electrons from NAD(P)H to cytochrome c or other acceptors. And the terminal (reductase) part, which contains molybdenum and transfers electrons to nitrate [4, p.708; 9, p. 2010]. The parts differ significantly. The diaphorase complex is exposed to IVM, sulfhydryl groups, and is thermolabile. The terminal part is sensitive to osmotic stress and redox transformations [2, p. 2; 5, 13, p. 2; 14, p. 3].

To test the proposed assumption, a model selective system containing lethal doses of VO^{3-} or WO_4^{2-} was created. Hexavalent tungsten (in the form of tungstate anion) is an analogue of molybdenum and can replace the latter as a cofactor of the nitrate reductase enzyme. All tungsten-containing enzymes, with the exception of the form of anaerobic dehydrogenase, are inactive [6, p. 3; 7, 1313; 8, p. 1258].

According to our proposed methodology, tobacco cell lines resistant to tungstate ions were obtained. These cellular variants were cultured on medium containing nitrate form of nitrogen and tungstate anion simultaneously. The presence of WO_4^{2-} in such a selective system inactivates conventional NP and thus completely disrupts the overall nitrogen assimilation chain. Cell culture growth under such conditions is in favour of obtaining a new functioning (stress-resistant) modification of HP. To test this assumption, the resistant cell lines were transferred to the condition of another nitrate reductase inhibitor, vanadate (VO^{3-}).

Vanadate inhibits enzyme activity without being incorporated. W-SCLs were also resistant to vanadate, which was observed by an increase in the relative growth of crude biomass. A similar situation was observed in the

cultivation of V-SCL. In this case, the resistance was manifested even under the combined action of two alternative factors. Since the used anions-inhibitors have different mechanisms of harmful effects on conventional HP (affecting different parts of the enzyme complex), it is clear why the activity of relative biomass growth depended on the type of factor. More important is the fact that the resistance of the cell culture did not depend on the ion in the presence of which the primary selection was carried out. It is likely that the selection resulted in the selection of NR variants with changes in the parts of the enzyme molecule that unite parts of the NR or coordinate their individual functions.

In our experiments, mutants with increased HP were regenerated from two resistant cell lines isolated in selective media. Their frequency of occurrence was 10^{-6}, it corresponded to the frequency of spontaneous mutants. The resistance trait was directly correlated with the phenomenon of nitrate reduction.

Thus, for the first time, mutant forms of plants with an increased level of NR activity under normal conditions and functional activity in the presence of inhibitors were obtained.

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