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# Proteins as nitrogen source for plants

## A short story about exudation of proteases by plant roots

Bartosz Adamczyk,[1,2,*] Aino Smolander,[1] Veikko Kitunen[1] and Mirosław Godlewski[2]

[1]Finnish Forest Research Institute; Vantaa Research Centre; Vantaa, Finland; [2]Laboratory of Plant Morphogenesis; Department of Plant Cytology and Cytochemistry; Institute of Plant Physiology, Cytology and Cytogenetic; University of Łódź; Łódź, Poland

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Interest in the problem of plant nitrogen nutrition is increasing. Certain plants can use not only inorganic nitrogen, but also intact amino acids and short peptides. According to our studies, the roots of several agricultural and wild-living plants are able to exude proteases and use them to create a pool of accessible N. This mini-review offers an overview of the problem of protease exudation by plant roots and its potential role in plant nitrogen nutrition.

### Introduction

For a long time it was assumed that inorganic nitrogen (in the form of $NH_4^+$ and $NO_3^-$) was the only source of N for plants. Some authors, during the 20th century, tried to broaden this view to include use of organic nitrogen,[1-3] but it was usually ignored. However, during the last two decades that point of view has changed; amino acids are now treated as another source of N for some plants. The ability of plants to acquire amino acids has been shown in both laboratory and field conditions for plants that are important in agriculture as well as for some forest trees.[4] It has been shown that all of the plant species studied can take up intact amino acids, but with significant differences in effectiveness.[5] Many factors, such as competition with microorganisms[6] or amino acid concentration in soil,[7] influence the level of uptake of amino acids from the soil. The newest results have showed that dipeptides, tripeptides and even oligopeptides can also be used by plants without prior digestion, as was shown by *Arabidopsis thaliana* and *Hakea actities*.[8,9]

However, amino acids in the soil are primarily in the form of proteins.[6] To access this source of N, the action of proteases is needed. Digestion of proteins is assured by enzymes secreted by microbes, which makes plants dependent on the activity of microorganisms. Here our story begins: in a study concerning a different problem, we observed digestion of photographic film emulsion (containing gelatin) that was immersed for a few days in the sterile medium of hydroponically cultivated seedlings. This accidental observation in our laboratory (University of Łódz, Poland) made us suspect that plants are able to exude proteases, which could be potentially important for plant N nutrition.[10]

This review focuses on proteases exuded by plant roots, with special emphasis on the potential role of these enzymes.

*Correspondence to: Bartosz Adamczyk; Email: bartek_adamczyk_79@o2.pl
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### Proteases Exuded by Plant Roots—Prevalence in the Plant Kingdom

To be of great importance for the plant kingdom, many plant species should have the same behavior in common. Exudation of proteases by plant roots was shown for seedlings of 15 plant species[10] (**Fig. 1**) as well as wheat.[11] Secretion of proteases by plant roots was also shown in studies conducted on *Arabidopsis thaliana* and *Hakea actities*.[12] The species studied belong to different plant families, they live in different ecosystems, some being agricultural (i.e., *Triticum aestivum*) others wild-living (i.e., *Ornithogallum umbellatum*); they are mainly mycorrhizal, but non-mycorrhizal plants are also included (*Arabidopsis thaliana, Hakea actities*). However, no mycorrhizal symbionts were included in these studies, nor were tree species studied.

These results point to the fact that exudation of proteases by intact roots could be common in the plant kingdom, but the level of proteolytic activity of root-secreted proteases seems to be species-specific (as shown in **Fig. 1**).[10]

### Biochemical Properties of Proteases Exuded by Roots

Plants contain a wide array of endogenic proteases, which play crucial functions in each metabolic pathway.[13] Their mechanism of action, optimal pH and molecular mass are very diverse and are matched to specific functions. However, our knowledge of the proteases secreted by roots is limited. Today, we know that proteases secreted by the roots of the studied plant species belong to the cysteine protease family; their activity is highest at neutral pH[10] and they operate mainly as endopeptidases, digesting proteins to low-molecular-mass products (**Fig. 2**). Studies with liquid chromatography coupled with mass spectrometry proved, that proteases secreted by *Allium porrum* roots showed some similarities in amino acid sequence to cysteine protease from *Arabidopsis thaliana*, having 51 kDa.[14]

There could be crucial differences between the proteases exuded from the roots of one plant species and those exuded by other species. Moreover, in future our knowledge of the properties

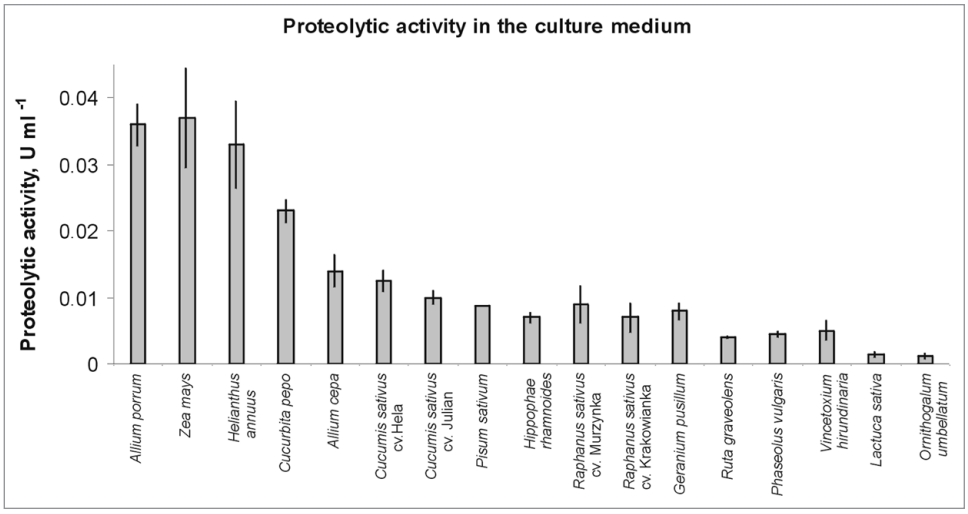


**Figure 1.** Proteolytic activity of the root-secreted proteases of seedlings of different plant species grown in sterile conditions (means ± standard error of the mean, n = 10); azocasein was used as a substrate for proteases. Modified from reference 10.

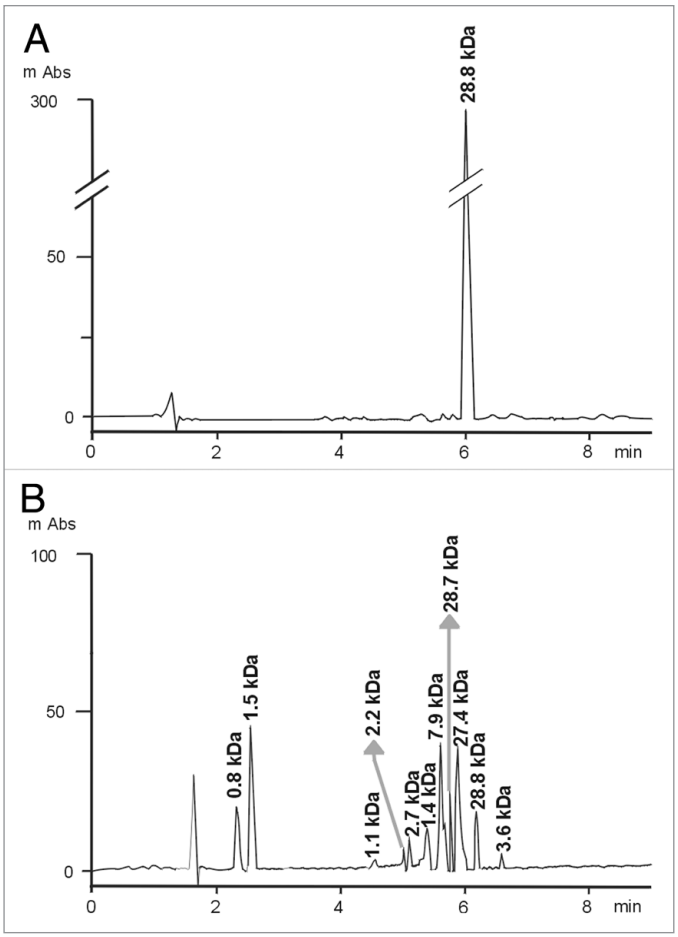


**Figure 2.** Chromatograms of the degradation products of casein by root-secreted proteases, (A) casein with denatured root-secreted proteases (no digestion), (B) casein incubated for 1 hour with root-secreted proteases. Molecular masses are marked above the peaks. Modified from reference 13.

of root-secreted proteases needs to be extended. The better we know these enzymes, the better we can understand their importance for plants, but this problem will be addressed in the next section.

## The Role of Proteases Exuded by Plant Roots in Nitrogen Nutrition

As discussed in the preceding chapters, we know that many plants are able to exude proteases, and we know some of the biochemical characteristics of these enzymes. But, how can these enzymes be useful for plants? The idea is that they can create a pool of accessible organic nitrogen by digestion of proteins,[10,14] and such low-molecular-mass products of digestion can be taken up by plant roots.[8,9] Moreover, it was shown that wheat seedlings can adjust the level of proteolytic activity of the root-secreted proteases according to the source of nitrogen in the culture medium.[11] In these studies, wheat seedlings obtained from isolated embryos were cultivated on Murashige and Skoog medium[15] with inorganic nitrogen only or without nitrogen or with casein as the only source of N. The highest proteolytic activity and greatest plant fresh weight were obtained for medium with casein (**Fig. 3**); this suggests that seedlings adjusted the proteolytic activity of root-secreted proteases to use added protein effectively as a source of N.[11] The ability of plant roots to secrete proteases and then to use these proteases to access protein from the culture medium could be used to improve the growth of seedlings in sterile cultures.

But what about the role of root-secreted proteases in field conditions? One should bear in mind that soil microorganisms secrete many enzymes, including proteases,[16] and they also take up organic nitrogen. However, the protein distribution in soil varies; there may be patches of organic nitrogen originating from organic fertilizers, but also from dying soil animals and plant debris.[17] In such patches, competition for organic nitrogen could be decreased; and at least part of these materials can be used by plants.

Another problem is the recalcitrance of complexes of proteins with other soil compounds, such as tannins,[18] which can also influence proteolytic activity directly.[19] Moreover, species-dependent differences in the activity of root-secreted proteases, the presence or absence of mycorrhizal symbiont and different soil conditions (pH value, organic matter content etc.,) can strongly influence the use of soil proteins by plants. Our studies were conducted on seedlings only, and we must keep in mind that the age of plants can potentially affect root-secreted proteolytic activity. These problems should be included in future studies of the use of proteins by plants. Furthermore, these studies were not conducted on tree species, so the question about secretion of proteases by tree species remains unanswered.

Secretion of proteases by plant roots in order to degrade soil proteins and obtain organic N can potentially change the actual stage of knowledge about nitrogen cycling and also about plant fertilization strategy. Nowadays, inorganic nitrogen fertilizers are commonly used, but because of their high mobility in the soil, especially that of nitrates, they easily undergo leaching[20] which could cause eutrophication of water reservoirs.[21] On the other hand, organic nitrogen fertilizers are more stable in the soil.[20] Development of environmental-friendly methods of plant cultivation is important for sustainable agriculture. The use of organic fertilizers, including proteins as a N source, could be the answer to this need. To decrease the costs of such a practice some agricultural organic waste material can be used as organic fertilizers.

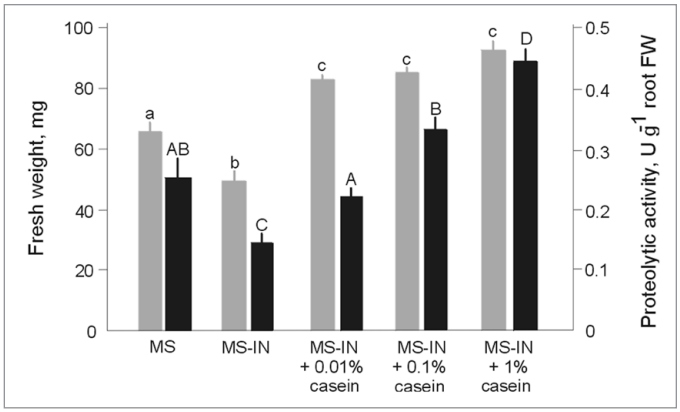


**Figure 3.** Fresh weight of shoot (grey columns) and proteolytic activity (black columns) in the culture medium of seedlings cultivated on different media (means ± standard error of the mean, n = 6). Azocasein was used as a substrate for proteases. Statistically significant differences ($p < 0.05$) between fresh weight are indicated by different letters, and significant differences between proteolytic activity are indicated by capitals. MS—Murashige and Skoog medium, MS-IN—Murashige and Skoog medium without inorganic nitrogen, FW—fresh weight. Modified from reference 14.

## Conclusions and Perspectives

Our knowledge of nitrogen nutrition in plants is still developing. Many plants can use a wide range of nitrogen sources: from inorganic N to amino acids, peptides, and with the help of root-secreted proteases, also proteins. The future challenge is to find agricultural species that have a good ability to use proteins and then to establish a fertilization strategy. This could limit the use of inorganic nitrogen fertilizers, which may be ecologically and economically problematic. Use of proteins by plants could be of great importance in developing sustainable agriculture based on fertilization with organic nitrogen.


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