

Natural PGR trans-zeatin is accessible at reduced cost for plant applications through biotech production platform patented by Acies Bio

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Cytokinins

Cytokinins are a class of plant hormones that promote cell division, differentiation, and plant growth and development. They are involved in the regulation of various processes such as shoot and root development, leaf expansion, and senescence. Cytokinins are produced in various tissues of the plant, including the roots, leaves, and shoot tips, and they are involved in signaling pathways that coordinate growth and development throughout the plant. They are also involved in the regulation of stress responses, such as those caused by drought or salinity.

Cytokinins are currently used commercially in agriculture and horticulture. They are applied to crops and plants to promote growth and development, increase fruit and flower production, and delay senescence. They are also used in tissue culture, to promote cell division and growth, and in regenerating whole plants from small explants. In agriculture, exogenously applied cytokinins are used to increase crop yields and improve the quality of fruits and vegetables as well as seed yield and germination (1, 2). They are also used to regulate the development of root systems, which can help crops to absorb nutrients more effectively and withstand environmental stressors such as drought or high salinity and improve resistance to biotic stress by modulation of plant immunity (3). The most widely used cytokinins in commercial applications are cytokinins 6-benzyladenine (BA) and kinetin, which are currently produced using chemical synthesis. Due to the lack of cost-efficient production methods for natural cytokinins in the past, the predominant active ingredient, BA is a chemical analog of natural cytokinins, which is not present in natural plant physiology. Therefore, the current agricultural practices introduce this unnatural bioactive molecule into the food chain without a complete understanding

of its long-term effects. In contrast, the most potent naturally occurring cytokinin, trans-zeatin, is currently not widely used as an ingredient of registered plant growth regulator products due to the prohibitive costs of its production.

Acies Bio makes trans-zeatin available at a cost competitive with chemical cytokinin analogs

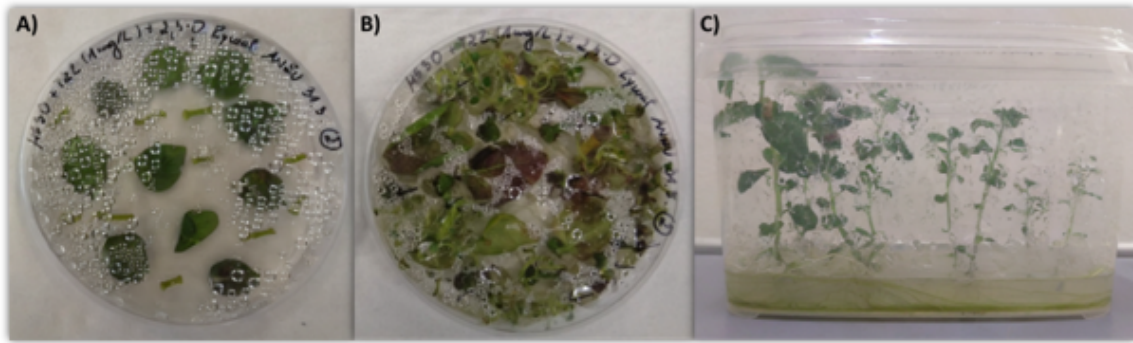
In a quest to provide the next generation of agricultural inputs using natural and sustainable production methods, a leading European microbial biotech company Acies Bio developed a platform for production of natural cytokinins, as well as an efficient bioprocess for production of trans-zeatin, the most efficient natural cytokinin molecule. The process is based on one of Acies Bio's highly efficient microbial platforms with GRAS/QPS status and is expected to lower the cost of trans-zeatin production up to a 100-fold compared to the current production methods. This break-through development opens an array of new possible commercial applications for this superior natural cytokinin. An additional advantage of microbially-produced trans-zeatin is that it is more sustainable than traditional methods of production. The process of producing trans-zeatin through microbial fermentation has several benefits compared to traditional chemical synthesis methods as it diminishes the potential health and safety hazards associated with chemical synthesis. This makes the microbial fermentation process a more environmentally friendly and safer option for trans-zeatin production.

Expected future impact on the cytokinin market

Synthetic cytokinin analogs are the most commonly used and affordable cytokinins in agriculture, but they have several disadvantages, such as lateral root inhibition, growth heterogeneity, problematic acclimatization of plants in the greenhouse, necrosis of shoot tips, and irreversible chloroplast deficiency. Inhibition of root initiation and growth has been attributed to extensive accumulation of unnatural, inactive cytokinin derivatives, resulting from enzymatic inactivation of synthetic cytokinins, such as the commonly used BA (4, 5, 6). The natural cytokinin trans-zeatin, which is now biotechnologically produced at a competitive price, will represent a superior alternative to synthetic cytokinins for use in various agricultural applications. Trans-zeatin is a potent molecule that is part of the natural plant growth cycle and will be a starting point for development of a new generation of products without undesired effects.

In the post-harvest segment, trans-zeatin has been found to extend the shelf life of fruits and vegetables by slowing down the ageing process, this could be a key point for commercial growers or consumers that are looking for long-lasting and fresher products without unnecessary chemical additives. The new plant growth regulator, identical to the molecules naturally present in our food chain, will be easy to use and apply, sustainably produced and possibly a safer alternative to current cytokinin products, which could make it attractive to a wider range of growers, from large commercial operations to small hobbyists.

Fig. 1 *In vitro* micropropagation process of potato plants cv. Desiree performed in a culture medium supplemented with Acies Bio trans-zeatin. A) Callus initiation after 1



week of inoculation on callus induction medium: B) Shoots regenerating from calli after 2 months of inoculation C) Plant propagation in a growth chamber using the liquid medium, 2.5 months after the start of the micropropagation process.

The future potential of Acies Bio' s platform for natural cytokinins

The development of new biotechnology tools such as gene editing and synthetic biology, has made it possible to produce a diverse range of agricultural and industrial inputs at lower cost and higher sustainability compared to the chemical synthetic methods developed decades ago. Therefore, making trans-zeatin available at a reduced cost is only the first showcase of the Acies Bio cytokinin platform. The platform has the potential to produce a wide variety of less studied cytokinins which may be shown to have multiple beneficial activities across many cash and row crops. Acies Bio is now working towards producing these new molecules to enable future studies of their complex effects on the physiology of different plants and envision future applications which are expected to play an important role in future sustainable farming practices, such as precision agriculture and regenerative agriculture. Ultimately application of these next-generation plant growth regulators will lead to reduced use of chemical pesticides and fertilizers as well as healthier crops and soil. Addressing the most pressing needs, cytokinins have the potential to enhance the tolerance to abiotic stress such as drought, salinity, and cold in plants, which will be beneficial in the context of global climate change.

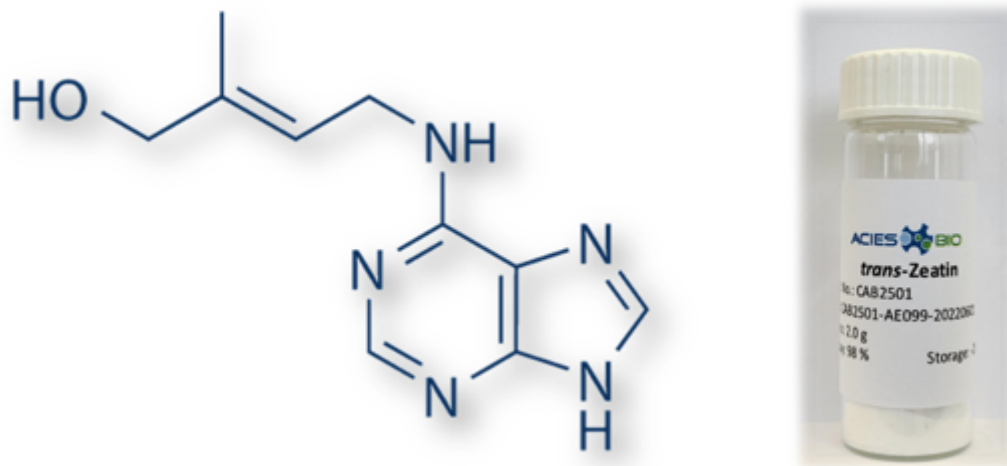
In long term, making trans-zeatin and other cytokinins available to growers at reduced cost is expected to bring about significant changes in the field of agriculture and horticulture, Particularly the increased crop yields, development of more resilient crops, and a reduction in the use of chemicals in farming, will have a big positive impact on the environment and food security.

Partnering with Acies Bio

Based on its unique cytokinin platform technology Acies Bio is looking for partners that are interested in further development of technologies and applications for trans-zeatin and other natural cytokinins with the goal to bring them to the market and make them available to growers. In such partnerships Acies Bio will contribute its proprietary know-how and the technological ability for further improvement of existing technologies and the development of new ones. In the future Acies Bio can also use its European-based fermentation facility to produce the cytokinin active ingredients at a commercial scale. We expect our partners to be experts in the application of plant growth regulators in agriculture and/or horticulture, able to perform functionality testing, regulatory-related activities, and launch the newly

developed products to the market. Samples of trans-zeatin are also available for evaluation purposes and tissue-culture applications.

Fig. 2



Chemical structure of trans-zeatin and purified final product isolated by Acies Bio.

About Acies Bio

Acies Bio is an R&D-specialized biotechnology company using its unique expertise in microbial physiology, strain development and improvement, media optimization, bioprocess development, and isolation of target compounds to develop innovative biologicals for sustainable agriculture. Using its integrated synthetic biology development platform Acies Bio developed in the last 15 years a number of industrially-applicable technologies for a variety of industries, including biocontrol/biopesticides and biostimulants. Acies Bio cooperates with numerous partners from the industry – either as an R&D service provider or as a long-term development partner, leveraging on our technological expertise in biotechnology and partners' in-depth understanding of unmet market needs. Our development goes beyond the strain itself; Acies Bio covers the whole development pathway all the way to industrial-scale pilot production which provides all the parameters for techno-economical analysis and sufficient quantities of the target molecule for advanced formulation and application testing as well as for regulatory purposes.

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