Extracellular DNA

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Extracellular DNA (eDNA) refers to any molecule of DNA found outside a cell. eDNA has an important ecological role as a signal molecule in several organisms. In plants, it has shown beneficial effects on important production traits such as defense mechanisms, plant growth and development, and secondary metabolites production that results in yield increment and better-quality food.

eDNA elicitors hormesis sustainable agriculture DAMPs

1. Introduction

Agricultural yields decline largely due to climatic changes, leading to the loss of optimal conditions for crop production, but also due to an increase in the incidence of pest-related losses [1][2][3]. By decreasing crop yields and decreasing global income derived from crop production [4], climate change thus increases food insecurity. Additionally, the use of chemical pesticides, fertilizers, and herbicides in agriculture has also generally decreased its acceptance due to its high environmental, health, and ecological costs, plus the loss of aggregated value of the non-organic crops [5][6].

In addition, current intensive agricultural practices, including land clearing, excessive and inefficient use of fertilizers and pesticides, irrigation, and the use of fossil fuels for agricultural machines, make agriculture a major contributor to greenhouse gas emissions [7], and maintaining this never-ending cycle contributes to environmental problems.

In this context, the use of plant metabolism modifiers (PMFs) has been suggested as a potential treatment that suits most modern agricultural needs. PMFs have been labeled and classified in different ways, principally as biostimulants and elicitors and their use has shown to obtain higher fruit production, enhancement of plant growth, reduction in plant diseases occurrence, an increase in metabolites production as chlorophyll, carotenoids and, protein contents, as well as the production of some important enzyme activities as phenylalanine ammonia-lyase (PAL) and defense enzymes as catalase (CAT), superoxide dismutase (SOD), among others [8][9][10][11][12].

One of the most promising emerging elicitors is extracellular DNA (eDNA), which has multiple roles in plant metabolism.

2. Self-eDNA as a DAMP

The ability to distinguish “self” from “non-self” has been described as the most fundamental aspect of an immune system [50]. Recently, researchers have focused on identifying molecular mechanisms to explain self-inhibition. One of the suggested molecules is DNA, as a highly species-specific, constantly present molecule. Supporting this hypothesis, several works have suggested the role of self eDNA as a danger signal molecule. The first report of it was made by Mazzoleni et al. [13] in a study where multiple species across different taxonomic groups, including plants, were exposed to respective eDNA. The experiments produced highly significant differences in responses to either self or non-self eDNA, treatments with self eDNA always resulted in a concentration-dependent growth reduction. The root growth of all targeted species was significantly inhibited by fragmented self eDNA treatments in a concentration-dependent manner.

As with any other signal molecules, eDNA activates a hormetic dose-response in plants [14]. This means that different doses of the same molecule can cause toxicity or stimulation. In the case of self eDNA, the stimulation of several plant defense responses has been reported. Lima bean (Phaseolus lunatus) and maize (Zea mays) leaves responded to self eDNA with a plasma membrane potential depolarization and calcium signaling, both early response events preceding the build-up of chemical defense in plants [15].

Lettuce seedlings responses were also evaluated in the presence of self and non-self eDNA. All self eDNA treatments, except for the highest concentration (200 ug/mL), showed statistically significant changes in the hypomethylation levels of genomic DNA compared to deionized water in control [16]. Additionally, the self eDNA treatment triggered a concentration-dependent effect in the expression of superoxide dismutase (sod), catalase (cat), and phenylalanine ammonia-lyase (pal) (2–200 ug/mL). Interestingly, non-self eDNA from C. chinense (phylogenetically close to lettuce) treatment activated the expression of sod and cat in the same levels as self eDNA (200 ug/mL) but A. angustissima (phylogenetically more distant from lettuce) did not affect the expression of these genes.

Durán-Flores and Heil [17] reported the effect of eDNA application in the leaves of common bean. Self eDNA caused a significant (almost three-fold) increase in H2O2 compared to plants treated with non-self eDNA that had no detectable effect. Similarly, activation of MAPKs was detectable at 5 min and strongest at 30 min after self eDNA application. Non-self eDNA also showed MAPKs activation but at a lower level. Surprisingly, plants treated with self or non-self eDNA exhibited a decrease in infection rates after the inoculation of the phytopathogen Pseudomonas syringae.

Similar to these studies, Rassizadeh et al. [18], treated Arabidopsis thaliana seedlings with self eDNA in different concentrations and observed an up-regulation of transcription of genes involved in ROS signaling (OXIDATIVE SIGNAL-INDUCIBLE, OXI1 and calcium signaling (CALMODULIN LIKE 37, CML37) but interestingly, the study showed no differential expression in some marker genes regulated by defense-related phytohormones, such as PATHOGENESISRELATED GENE 1 (PR-1) for salicylic acid, PLANT DEFENSIN 1.2 (PDF 1.2), VEGETATIVE STORAGE PROTEIN 2 (VSP2) and JASMONATE RESISTANT 1/JAR1) for jasmonic acid, ETHYLENE RESPONSE FACTOR 2 and 5 (ERF2 and ERF5) for ethylene. Also, induction of resistance against pathogens.

New studies have focused on understanding the effect of self eDNA in plants in a more global way. Barbero et al. [19] identified as an effect of self eDNA treatment in tomato plants a negative regulation of genes related to gene ontology terms such as metabolic and biosynthetic processes of Myo-inositol (>100-fold enrichment), nitric oxide metabolic and biosynthetic processes (>70-fold enrichment), biosynthetic processes of ROS, cell wall, jasmonic acid, and sucrose transport (>47 fold enrichment). Additionally, several genes were identified as upregulated and related to the following gene ontology terms: oxygen transport, defense against Gram-negative bacteria, and lactate biosynthetic processes (>66-fold enrichment), adenine biosynthetic, and metabolic processes, auxin influx, and cellular ion homeostasis processes (>33-fold enrichment). Contrastingly, an up-regulation of MPK3 and OXI1 genes resulted from treatments with broccoli, citrus, bean, and maize eDNA.

In a more recent study [20], DNA extracted from A. thaliana plants and common herring (Clupea harengus) were applied to A. thaliana plants as self and non-self eDNA treatments. In self DNA responses, genes related to oxidative stress, toxic substances, and ions were up-regulated, involving genes encoding detoxification and anti-oxidation protective enzymes, while downregulating typical stress-responsive genes, as PAD4 gene that has been related to pathogen resistance response mediated by TIR-NB-LRRs. This contrasts with the up-regulation of PAD4 as an effect of non-self-DNA treatment, as well as several genes involved in systemic acquired resistance. These responses evidence differences in self and non-self-DNA activated mechanisms, consistent with DAMP-, P/MAMP-like responses, respectively.

Another important difference between responses is the up-regulation of genes related to ABA and jasmonic acid in the first hour after self eDNA treatment, while in non-self eDNA analysis an up-regulation of genes related to ABA and salicylic acid was revealed [20]. In self DNA response, an up-regulation of most of the genes belonging to the cytokinin oxidase/dehydrogenase family also suggests cytokinin-mediates processes affected, possibly involving cell cycle regulation, cell proliferation, and shoot and root development.

3. eDNA as a MAMP/PAMP

Different from the role of self eDNA, the perception of eDNA not only non-self but specifically coming from a prokaryotic source interpreted as the presence of a possible pathogenic microbe has been suggested to activate defense mechanisms in plants [21] [22] [23].

The treatment with a mixture of fragmented eDNA from different phytopathogenic organisms (Phytophthora capsici, Fusarium oxysporum and Rhizoctonia solani) also showed a protective effect in chili pepper against wilt and root rot disease [22]. In this study, both disease severity and plant mortality were measured with a reduction of 60% and 40% compared to the infected control, respectively. The plants immune system activation was also measured by total phenolics determination as well as by phenylalanine ammonium lyase and chalcone synthase gene expression analysis. The mixture of microbial fragmented eDNA treatment showed an immune system activation effect that can be related to a decrease in disease severity by the inoculated pathogenic complex in capsicum.

In another study, the application of short sequences of non-self eDNA with cytosinephosphate-guanine oligodeoxynucleotide motifs in a concentration of 9.5 10?5 g/liter statistically reduced the lesions in leaves of wheat plants by the pathogenic fungus Z. tritici, showing a similar effect to a commercial fungicide [23].

With all these observations, the ability of plants to distinguish microbial eDNA from other kinds of eDNA is clear. The common hypothesis is that the content of CpG DNA motifs helps plant receptors to respond differently to microbial eDNA, and this can confer an ecological advantage to plants to identify near pathogens or beneficial bacteria. Thus, this natural mechanism can be seen as a potential agricultural application that could replace chemical pesticides at some level or completely. More tests are needed to complete the information about hormetic curves in multiple plant species.

4. Technical Challenges of eDNA Application as an Agricultural Treatment

The use of eDNA as an agriculture treatment could be questionable in terms of the cost-benefit of extraction, fragmentation, and application of eDNA, even knowing the ideal treatment concentration. In this section we propose a redefinition of the protocol needed for DNA extraction suitable for agricultural application by identifying the common steps in lab DNA extraction protocols and determining which steps are needed for this specific purpose, having in mind that the conditions needed for this goal are different from the conditions desired in lab extracted DNA. Usually, the lab DNA extraction aims not only for the significant quantity of nucleic acids, but for the high integrity and purity of the molecule, and some of the most expensive steps have these purposes. Otherwise, eDNA for agricultural treatments has shown a better immune response in plants in a fragmented state, and a high level of purity may be of lower importance [17].

5. Conclusions

The evolution of agricultural practices has significantly increased crop yields by the application of improved crops, mechanical plowing, chemical fertilizers, and pesticides [24], but recently, a negative face of traditional agricultural systems has been identified, this related to a negative environmental, ecological and health impact. Later, organic agriculture has emerged as a group of diverse green techniques with the great challenge of meeting the needs of an everyday growing world population in a matter of quantity, yield, food quality, nutritional benefit, efficient management of plant pests, and diseases and reducing the environmental impact of technological change [25].

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