A Review: Is Cinderella’s story of self-DNA extracellular effect towards plant growth real?

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Abstract. Research related to the extracellular self-DNA effect in plants has been widely conducted during the last decade. Researchers reported the impact of extracellular self-DNA inhibition on plant growth, assuming that extracellular self-DNA could enter plant tissue and thus stimulate an inhibitory response. Environmental conditions have a big role to play in supporting this inhibition, especially with the significant climate changes that have occurred in recent years. Climate changes such as rainfall, humidity, light intensity, and air temperature have a positive effect on the decomposition process of plant litter. Especially if there is a significant climate change accompanied by a monoculture cropping pattern will further trigger the accumulation of extracellular DNA. Dissolved DNA concentration in the soil with the probability of occurrence of the self-DNA effect is directly proportional. Although several studies have confirmed the inhibitory results, self-DNA's mechanism can enter plant tissue and stimulate an inhibitory response has not been widely discussed. It has sparked debate regarding the actual effect of self-DNA and the extent of its potential to inhibit plant growth. Therefore, this paper intends to collect various hypotheses and research results regarding self-DNA's impact on plant growth and reconstruct more comprehensive assumptions regarding the effect of self-DNA, its inhibitory potential, and its ecological implications in agriculture. Exhaustive assumptions about self-DNA are expected to be the basis for generating new research designs that include new variables that have not been considered in previous research.

1. Introduction
The application of an ecologically based sustainable agricultural system is carried out in order to increase production and rebalance natural conditions. Efforts and application of the concept of healthy agriculture, the movement to return the residual biomass to land, which has been intended to maintain the nutrient cycle to continue, are now faced with new challenges related to the negative effects of Self-DNA. Information regarding the negative effects of Self-DNA is still not widely discussed, so it is getting less attention. Especially with significant climate change, it is increasingly supporting the creation of these effects. Inhibition by Self-DNA does not only have negative effects but can be used as an innovation to control plant-disturbing organisms or humans. The utilization of extracellular DNA as a green pesticide is a great opportunity to be developed. More in-depth research related to DNA-based pesticides needs to be done to reduce the consumption of synthetic pesticides [1] and support the creation of ecologically based sustainable agriculture.
2. The mechanism of self-DNA release and enter the plant tissue

Every plant that grows will produce litter in the form of dry and fresh leaves, tree branches, or various other vegetation remnants that are above the soil where it grows. Litter production is a phase of plant growth and development and is an important part of the transfer of organic matter from vegetation to the soil. Plant litter becomes a source of nutrients for plants after a decomposition process. Decomposition means the process of changing an organic material both physically and chemically by soil microorganisms. The speed of the litter decomposition process is influenced by many factors, including climatic factors (rainfall, humidity, light intensity, air temperature), soil physical and chemical factors (soil pores, temperature, pH, soil aeration), and soil biology (macro activity), and soil microorganisms [2]. The process of decomposition of plant litter can take place naturally, with the initial stage being the process of destroying soil biota into smaller particles (physical degradation of plant tissue occurs), releasing plant cell contents, then followed by soil decomposers (fungi and bacteria) to produce nutrients and the formation of humic compounds. Significant climate change can affect litter production and its decomposition rate. The decomposition process of plant litter produces several products including nutrients, humus, allelopathic compounds, and nucleic acids (DNA).

Plant DNA will come out of the cell nucleus (release) into the soil solution through the process of plant tissue damage (lysis) during the decomposition process [3], [4]. When in soil solution, DNA will also fragment into smaller sizes or even degrade into deoxyribose, inorganic phosphate, or purine and pyrimidine bases by DNase produced by microorganisms [5]. DNA is a form of slow degradation of nucleic acids so that over time it accumulates in the soil [3], [6], [7], [8], [9]. The presence of DNA in soil solution that is resistant to nucleases has the opportunity to be absorbed by soil colloids [4], [10], bind to other cations, leach, or be absorbed by plants [11]. This is because plant DNA has a charge like nutrients.

The accumulation of extracellular DNA in the soil is in harmony with the existing ecosystem and climatic conditions. Although both terrestrial ecosystems with high biodiversity and tropical forest ecosystems have high biodiversity, it turns out that the accumulation of DNA fragments produced by the decomposition of their litter is very different. The characteristics of this DNA will exist and accumulate when dissolved in soil solution, in the sense that in water ecosystems with monoculture planting systems, the chances of extracellular self-DNA are high compared to terrestrial ecosystems rotated by plants with diverse vegetation [12].

DNA is a source of organic phosphorus in the soil (0.2 - 2.5%) [13], [14]. When the DNA concentration is high in the soil solution, there is a big chance that it will be absorbed by plant roots. Plant roots do not have nutrient selectivity, so if there are cations or anions that have the same ionic radius or valence, the affinity value will determine which ion is absorbed first. The absorption of nutrients is not based on plant needs but on the physical and chemical similarities of the ion in question. This provides an opportunity for DNA to be absorbed by plant roots.

The absorption mechanism of DNA by plant roots is made possible through a diffusion process, then an ion exchange process occurs at the root surface, namely between DNA (negatively charged) and HCO$_3^-$ (carboxyl groups produced by roots), as well as absorption of phosphorus nutrients. In addition, DNA absorption can take place due to the occurrence of protoplast fusion, where the plant root cell walls are degraded by the chitinase enzyme produced by indigenous microorganisms in the soil [15], [16]. The presence of microorganisms in the soil has a significant effect, not only as a decomposer but also as an agent that helps indirectly absorb DNA.

Extracellular self-DNA can be absorbed into plant tissue because it is recognized, [17] suggest four hypotheses regarding the possibility of introducing extracellular DNA in plants. The four hypotheses include; (1) in individuals there is a membrane-bound exDNA receptor that is able to recognize environmental exDNA so that it triggers a cascading signal through post-translational modification, (2) exDNA fragments are bound and transported into the cytoplasm by membrane-bound transporter channels, (3) cytoplasmic sensors able to recognize internalized exDNA via vesicles, (4) intracellular exDNA/exRNA sensors mediate surveillance function to detect incoming foreign nucleic acids.
However, this hypothesis needs to be tested further to ensure its correctness. However, research related to the introduction of plants to environmental extracellular DNA is still sketchy [18].

Several studies have shown that extracellular DNA can be absorbed by plants. Plants have been shown to be able to affiliate with organic molecules, including proteins and extracellular DNA into roots [19]. Ledoux and Huart [20] reported that part of the heterologous label DNA on the Arabidopsis seed growing medium was absorbed and found in the germination cotyledons of these plants. Lonhienne et al. [21] also conducted a similar study, applying the fluorescent-labeled 25 bp heterologous S-DNA to the Arabidopsis growing medium. The DNA that was applied was absorbed and detected its presence in the root cells. Heterologous DNA applications have been shown to support the roots of Arabidopsis plants.

3. Evidence and debate
Mazzoleni et al. [22] reported that application of DNA with a size between 50 and 2000 bp to plants of the same species (Self-DNA) would lead to autotoxicity (species-specific inhibitory impact on individuals of the same species). This inhibition occurs not only in plants but in other organisms such as groups of fungi, bacteria, protozoa, algae, and Animalia [23]. DNA fragments that successfully enter plant tissue will enter the cell nucleus. The presence of extracellular DNA can affect cell function. When extracellular DNA is in the cell nucleus, there will be a process of specific sequence recognition or site recognition by genomic DNA, and several things will occur, including triggering the antisense mechanism, the formation of a D-Loop (Displacement loop), or the formation of a D loop structure [11], RNAi [29], the methylation process and the occurrence of global plant regulation (if there is more than one similar DNA in the same location will cause inhibition of the expression of the gene), which then reacts to inhibition of plant root function.

Barbero et al. [24] stated that the application of Self-DNA significantly induces membrane depolarization and increases Ca$^{2+}$ flux, this condition does not occur in heterologous DNA. Duran and Heil [25] also argue that the response of plants, when exposed to Self-DNA, is induced to form reactive oxygen species (ROS), formation of H$_2$O$_2$ and Mitogen-Activated Protein Kinase (MAPK) activity. Although its inhibitory effect has been confirmed by several studies, the mechanism by which Self-DNA enters plant tissue and stimulates an inhibitory response has not been widely discussed. This has sparked a debate regarding the true effect of Self-DNA and the extent of its potential to inhibit plant growth. Duran and Heil [25] argue that the inhibition that occurs is the prevention of intra-specific competition or may even act as a signal of intraspecific stress. When the concentration of Self-DNA in the environment is high, this condition will be a signal of damage to other individuals of the same species. Veresoglou et al. [26] added that the inhibition that occurs is not solely due to the effect of Self-DNA but because DNA acts as a signaling compound so that germination is delayed until the stress level decreases. On the other hand, the DNA concentration applied was very high (200 ppm per Petri dish containing 20 plant seeds), and these concentrations are unlikely to be found in nature. The concentration of extracellular DNA dissolved in the soil is believed to be rare in the high category because of the great opportunity to be bound by soil colloids [4], [10], so there is still much debate regarding the results of research conducted by [22], [23].

4. Potential for development and application
Referred to as Self-DNA because of the specific inhibition of the same species. In addition to triggering a lot of debate and negative views, on the other hand, it can be used as an innovation for controlling plant pests and managing the environment in an ecological and sustainable manner [27]. The extracellular ability of DNA to inhibit individuals with the same species as the source of DNA can be used as a natural pesticide, which means that in controlling a disruptive organism it can be applied with its own DNA. The results of a study conducted by Mazzoleni et al. [22], [23] can be used as a reference for developing the potential for DNA-based pesticides, as well as a basis for producing new research designs that include new variables that have not been considered in previous research. The application of DNA-based pesticides aims to reduce the negative impact of pesticide use (natural and
human health) [28] and support the creation of an ecologically based sustainable agricultural system, especially with climatic conditions that have experienced very significant changes.

5. Conclusion
The application of a healthy agricultural system by returning the remaining biomass to the land has a negative impact on the soil and plants, namely producing extracellular Self-DNA which is increasingly supported by significant climate change. The inhibitory effect of extracellular self-DNA has been widely confirmed, but the mechanism by which it enters plant tissue and stimulates an inhibitory response has not been widely discussed. This triggered a debate related to the truth of the effect of Self-DNA and the extent of its potential to inhibit plant growth. On the other hand, as the knowledge of this inhibitory effect is developed, it is used as an innovation for DNA-based pest control that is able to support ecologically-based sustainable agriculture and can become the basis for producing new research designs that include new variables that have not been considered in previous studies.

Reference
[1] Mada D, Duniya N and Idris G A 2013 Effect of continuous application of herbicide on soil and enviroment with crop protection machinery in southern adamawa state [Online] Available: www.irjes.com
[2] Hanum A M and Nengah D K 2014 Laju dekomposisi serasah daun trembesi (Samanea saman) dengan penambahan inokulum kapang J. Sains Dan Seni Pomits 3 2337–3520
[3] Widmer F, Seidler R J, Donegan K K and Reed G L 1997 Quantification of transgenic plant marker gene persistence in field Molecular Ecology. 6 1–7
[4] Ceccherini M T, Ascher J, Agnelli A, Borgogni F, Pantani O L and Pietramellara G 2009 Experimental discrimination and molecular characterization of the extracellular soil DNA fraction Antonie van Leeuwenhoek. 96 653–7
[5] Blum S A E, Lorenz M G and Wackernagel W 1997 Mechanism of retarded DNA degradation and prokaryotic origin of DNases in nonsterile soil System. Appl. Microbiol, 20 513–21
[6] Gebhard F and Kornelia S 1999 Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. FEMS Microbiol.Ecol. 28 261–72
[7] Hay I, Marie J M and Armand S 2002 Assessing the persistence of DNA in decomposing leaves of genetically modified poplar trees Can. J. For. Res. 32 977–82
[8] Paget E, Michel L, Georges F and Pascal S 1998 The fate of recombinant plant DNA in soil Eur. J. Soil Biol. 34 81–8
[9] Widmer F, Seidler R J and Watrud L S 1996 Sensitive detection of transgenic plant marker gene persistence in soil microcosms Mol. Ecol. 5 603–13
[10] Levy-Booth D J, Campbell R G, Gulden R H, Hart M M, Powell J R, Klironomos J N, Pauls K P, Swanton C J, Trevors J T and Dunfield K E 2007 Cycling of extracellular DNA in the soil environment Soil Biology and Biochemistry 39 2977–91
[11] Carteni F, Giuliano B, Francesesco G, Guido I, Christian E V, Maria L C and Stefano M 2016. Self-DNA inhibitory effects: Underlying mechanisms and ecological implications Plant Signaling & Behavior. 1559–2324
[12] Mazzoleni S, Bonanomi G, Giannino G, Incerti G, Dekker S C and Rietkerk M 2010 Modelling the effects of litter decomposition on tree diversity patterns Ecological Modell. 221 2784–92
[13] Havlin, J L, Beaton J D, Tisdale S L and Nelson W L 1999 Soil Fertility and Fertilizers. An Introduction to Nutrient Management. Sixth ed (New Jersey: Prientice Hall).
[14] Balaban N P, Suleimanova A D, Valeeva L R, Chastukhina I B, Rudakova N L, Shariyova M R and Shakirov E V 2012 Microbial phytases and phytate: exploring opportunities for sustainable phosphorus management in agriculture American Journal of Molecular Biology. 7 11–29
[15] Ueno H and Miyashita K 2000 Inductive production of chitinolytic enzymes in soil microcosms using chitin, other carbon-sources, and chitinase-producing Streptomyces. *Soil Sci. Plant Nutr.* **46** 863–71

[16] Hjort K, Presti H and Elvang A 2014 Bacterial chitinase with phytopathogen control capacity from suppressive soil revealed by functional metagenomics *Appl Microbiol Biotechnol.* **98** 2819-2828

[17] Bhat A and Ryu C M 2016 Plant perceptions of extracellular DNA and RNA. *Mol. Plant.* **9** 956–8

[18] Niehl A, Wyrsch I, Boller T and Heinlein M 2016 Double-stranded RNA induce a pattern-triggered immune signaling pathway in plants *New Phytol.* **211** 1008–19

[19] Wen F, White G J, Vanetten H D, Xiong Z and Hawes H C Extracellular DNA is required for root tip resistance to fungal infection *Plant Physiol.* **151** 820-9

[20] Ledoux L and Huart R 1971 Fate of exogenous DNA in arabidopsis thaliana translocation and integration *Eur J Biochem.* **23** 96-108

[21] Lonhienne C P, Lonhienne T G A, Mudge S R, Schenk P M, Christie M, Carroll B J and Schmidt S DNA is taken up by root hairs and pollen, and stimulates root and pollen tube growth *Plant Physiol.* **153** 799–805

[22] Mazzoleni S, Bonanomi G, Incerti G, Chiusano M L, Termolino P, Mingo A, Senatore M, Giannino F, Carteni F, Rietkerk M and Lanzotti V 2015 Inhibitory and toxic effects of extracellular self-DNA in litter: a mechanism for negatif plant – soil feedbacks? *New Phytologist.* **205** 1195–210

[23] Mazzoleni S, Carteni F, Bonanomi G, Senatore M, Termolino P, Giannino F, Incerti G, Rietkerk M, Lanzotti V and Chiusano M L 2015 Inhibitory effects of extracellular self-DNA: a General Biological Process? *New Phytologist.* **206** 127–32

[24] Barbero F, Guglielmotto M, Capuzzo A, and Maffei M A 2016 Extracellular self-DNA (esDNA), but not heterologous plant or insect DNA (etDNA), induces plasma membrane depolarization and calcium signaling in lima bean (Phaseolus lunatus) and maize (Zea mays) *Int. J. Mol. Sci.* **17**

[25] Duran D F and Heil M 2018 Extracellular self-DNA as a damage-associated molecular pattern (DAMP) that triggers self-specific immunity induction in plants *Brain. Behav. Immun.* **72** 78–88

[26] Veresoglou S D, Trigueros C A A, Mansour I and Rillig M C 2015 Self-DNA: A blessing in disguise? *New Phytol.* **207** 488–90

[27] Meitha K, Esyanti R R, Iriawati, Hanisia R H and Rohyani 2021 Green pesticide: Tapping to the promising roles of plant responses towards secreted RNA and extracellular DNA. *J Pre-proof* 61(1) 42–50

[28] Damalas C A and Eleftherohorinos I G 2011 Pesticide exposure, safety issues, and risk assessment indicators. *Int. J. Environ. Res. Public Health.* **8** 1402–19

[29] Vogel E, Santos D, Mingels L, Verdonckt T W and Broeck J V 2019 RNA interference in insects: Protecting beneficilals and controlling pests *Front. Physiol.* **10** 1912