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Use of Biological Stress as a Novel Strategy to Control the Rice Blast Disease

Dayaso N. A.[1], Kogi M. P.[1], Adamu R.[1], Aliyu M.[1] and Abubakar Y. S.[1,2,*]

[1] Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria, 810281, Nigeria
[2] State Key Laboratory of Plant-Pathogen Interaction, College of Life Science, Fujian Agriculture and Forestry University, Fuzhou 350002, China

Abstract
***Magnaporthe oryzae,* a hemibiotropic fungus, causes the devastating blast disease of rice, which results in an annual loss of about 15% of the rice produced globally, enough to feed more than 60 million people for a year. Different approached have been adopted to manage the infection, including the use of chemical fungicides. In this study, we analyzed the potentials of using biological stress to hinder the development and pathogenicity of the fungus, thereby controlling its spread and host infection. We induced cell wall/plasma membrane, oxidative and osmotic stresses in the fungus by growing it on media supplemented with 20% w/v SDS, 10 mM H_2O_2 and 1M NaCl, respectively. Under these stresses, we evaluated the vegetative growth, pathogenicity, conidiation and conidia morphology of the blast fungus. Our results revealed that the fungal vegetative growth was seriously hindered by the various biological stresses, with SDS exerting the most pronounced growth inhibition. Conidiation was completely abolished under osmotic stress while oxidative stress significantly reduced conidia production, compared to the unstressed group. However, no significant difference was observed in conidiation following treatment with SDS. We also found that the various stresses have no effect on the physical morphology of the fungal conidia, with the exception of NaCl-induced stress that completely blocked conidiation. In the presence of the biological stress, the pathogenicity of *M. oryzae* has been dramatically affected. No development of disease lesion was observed on rice leaves infected with SDS- and H_2O_2-stressed fungi. A weak lesion was however observed on NaCl-treated fungus. These findings have revealed the crucial potential of biological stress in the control and management of the rice blast disease, which hold a great promise to sustaining the global food security.**

Keyword: Biological stress, Magnaporthe oryzae, Pathogenicity, conidiation, vegetative growth
Corresponding authors Email: ay.saddeeq@yahoo.com
[#]**These authors contributed equally**

Highlights

- The vegetative growth of *M. oryzae* is adversely affected by osmotic, oxidative and cell wall/plasma membrane stresses
- Conidiation of *M. oryzae* is completely abolished under 1M NaCl-induced osmotic stress and significantly reduced under oxidative stress
- The pathogenicity of the fungus is compromised under oxidative and cell wall/plasma membrane stresses, but not under osmotic stress
- All the biological stresses do not alter the normal morphology of the fungal conidia

Introduction

Plants are exposed to a number of factors (biotic and abiotic) that hamper their growth and development (Abubakar et al., 2022). Rice is a

major cereal crop belonging to the family Pocaeae, and it is consumed by more than 50 % of the global population, especially in Asian countries (Yan & Bao, 2014; Thapa & Bhusal, 2020). Rice contributes to about 23 % of the calories consumed by the global human population. It is regarded as a major food for more than 2.5 billion people particularly in developing countries (Muthayya et al., 2014). Rice production is however affected by several factors that can be categorized as biotic and abiotic factors (Acharya et al., 2019). The abiotic factors include drought, pH, temperature and salinity whereas biotic factors include weeds, diseases and pests (Onyango, 2014). Fungal diseases are the major biotic factors that hamper rice production annually (about 14 % yield loss on a global scale), thereby sabotaging global food security (Agrios, 2005).

Magnaporthe oryzae is a filamentous fungal pathogen that causes the most common and destructive disease of rice known as the rice blast. This disease leads to significant reduction in rice yield by about 70-80 % of the total annual production (Nasruddin & Amin, 2012; Miah et al., 2013). In just about 15 to 20 days of infection, *M. oryzae* can damage an entire rice plant. To infect the rice, *M. oryzae* produces a special dome-shaped infection structure called appressorium which is known to generate high turgor, translating into a physical force that aids penetration pegs to pierce through rice leaf cuticles to gain entrance into the host cells (Talbot, 2003). Globally, different strategies have been applied to mitigate the disease transmission including traditional breeding and chemical approaches. In spite of the global effort, all the approaches suffer on limitation or the other, rendering them less effective. This necessitates the need for more alternative approaches for effective control and management of the devastating disease. Therefore, in this study, we investigated the possibility of applying biological (cell wall/membrane, osmotic and oxidative) stresses to suppress the growth and development of the rice blast fungus, thereby abolishing or ameliorating the menace of the rice blast disease.

Materials and Methods

Fungal strain, culture condition and stress induction

Magnaporthe oryzae GUY11 strain was used in this study. The fungus was cultured on PDA (potato dextrose agar) at 28 ± 2 °C for 7 days. About 9.75g of PDA was dissolved in 250ml distilled water. The solution was sterilized in an autoclave at 121 ºC for 15 minutes. Four different experimental groups were involved: the control and the three (3) treatment groups. For the control group, no supplementation was made into the media. For the treatment groups, the media were supplemented with 10mM hydrogen peroxide (H_2O_2) to induce oxidative stress, 1M sodium chloride (NaCl) to induce osmotic stress and 0.02% (w/v) Sodium Dodecyl Sulphate (SDS) to induce cell wall/plasma membrane stresses, according to a previous study (Nie et al., 2022).

Conidiation

Conidia were harvested on rice bran media (RBM; 40 g/L powdered rice chaff, 20 g/L agar). Briefly, the fungus was inoculated on RBM (the treatment groups were supplemented with the respective amounts of the stress-inducing agents) and incubated at 28±2°C for 7 days under 12/12 hr. light/dark photoperiod. On the 7th day, the fungal mycelia developed were scraped off from the surface of the culture and the fungal culture was further incubated in the dark for 24 hours. Equal dimensions of the fungal cultures were excised and washed with 2 mL sterile double-distilled water (ddH_2O) and filtered. About 10 µL of the filtrate was pipetted and placed on a hemocytometer, covered with cover slips and counted under a light microscope. Average number of conidia was evaluated and the total number of conidia per mL was computed as previously reported (Wu et al., 2021).

Vegetative growth determination

M. oryzae was cultured on PDA media for seven days and then sub-cultured on a media plate containing the aforementioned amounts of SDS, NaCl, H_2O_2; for the control, DDH_2O was used instead of the stress agents. The cultures were incubated at 28±2°C for 7 days, after which the colony diameters of all the groups were measured and recorded.

Pathogenicity assay

Pathogenicity test was conducted according to a previous study (Chen et al., 2021). Briefly, leaves from 2-week-old rice seedlings were cut using sterile pairs of scissors and placed on damp filter papers in Petri dishes. Mycelial blocks from 5-day-old fungal cultures (plain PDA cultures were used

for the control while PDA cultures containing the stressors were used for the treatment groups) were excised and placed at three different positions on each leaf in the Petri dishes. Little amount of sterile distilled water was added to the filter papers in each Petri dish to keep the environment humid. The Petri dishes were covered and incubated at 28±2°C for 3 days under 12 hours day/night cycles. The plant leaves were carefully observed and clear photos of the infected leaves were taken. The extent of the necrotic lesions formed on the leaves was observed, compared and appropriately recorded.

Conidia morphology assay

The morphologies of the fungal conidia were analyzed using a Nikon scanning confocal microscope (Nikon, Japan). Images were captured using differential interference contrast (DIC) mode.

Statistical analysis

Where appropriate, two-tailed Student's *t*-test was used for paired comparison of the various treatment groups with the control, respectively. Data were analyzed using excel spreadsheet.

Results

Biological stress negatively affects the vegetative growth of M. oryzae

To investigate the impact of biological stress on the vegetative growth of *M. oryzae*, the pathogen was grown on solid PDA media supplemented with 0.01% (36mM) SDS (for plasma membrane/cell wall stress), 10mM H_2O_2 (for oxidative stress), 1M NaCl (for osmotic stress) and double distilled water (DDH_2O) as a control, respectively. The results clearly showed significant ($p<0.05$) reductions in the mycelial growth of the fungus in the plates containing the stress-inducing agents with respect to the control (Figure 1). The plasma membrane/cell wall stress due to SDS presented the most remarkable growth-inhibiting effect, followed by osmotic stress due to NaCl and lastly oxidative stress induced by H_2O_2 (Figure 2). These results demonstrate that biological stress strongly limits the vegetative growth of the rice blast fungus.

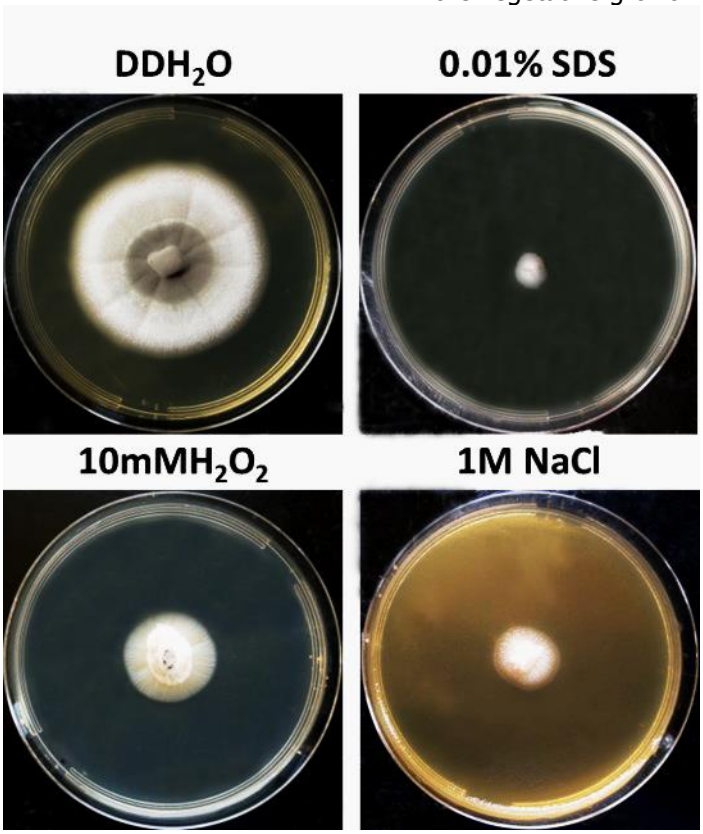

Fig 1: Vegetative growth of *M. oryzae* under plasma membrane/cell wall stress, oxidative stress and osmotic stress.

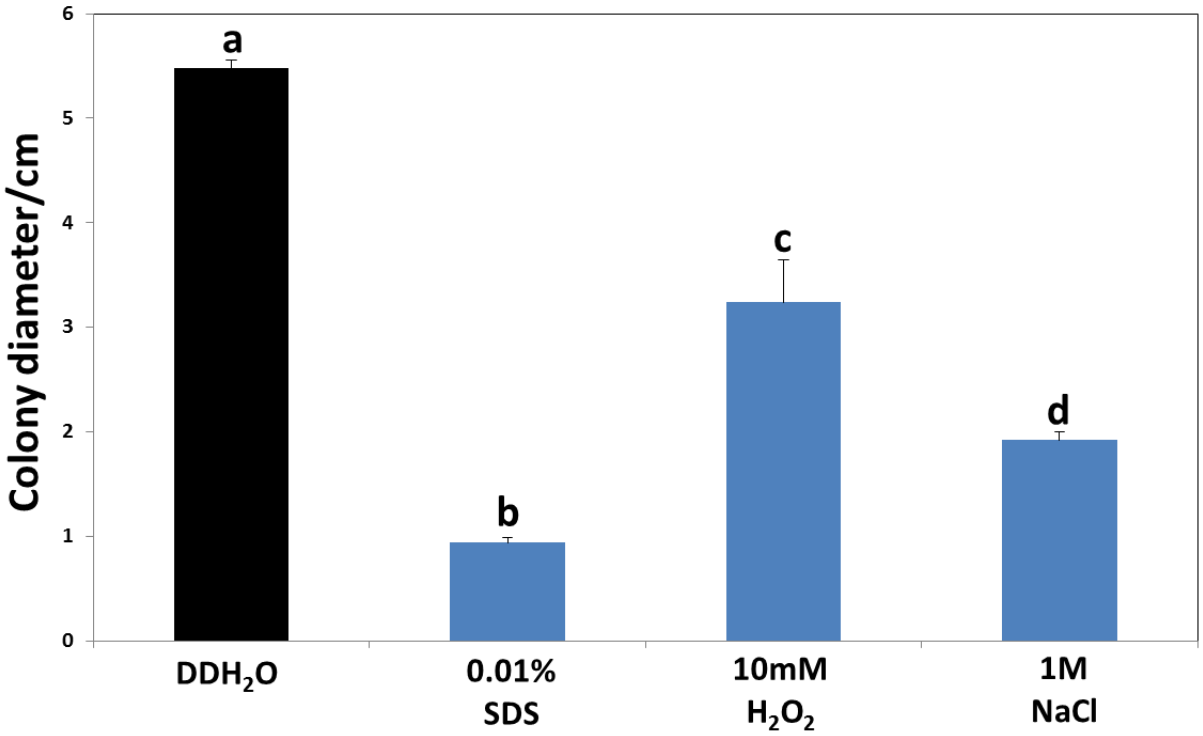

Fig 2: Analysis of the colony diameters of *M. oryzae* grown under plasma membrane/cell wall stress, oxidative stress and osmotic stress. Bars with different letters are significantly different at $p < 0.05$.

M. oryzae conidiogenesis is adversely perturbed by biological stress

M. oryzae produces conidia as primary inoculums for host infection. To investigate the effect of biological stress on the conidiogenesis of M. oryzae, the fungus was cultured on rice bran media (RBM) in the presence of the various stress-inducing agents (0.01% SDS, 10mM H_2O_2, and 1M NaCl) and plain RBM (without any stressor) as a control, respectively. The result clearly showed significant ($p < 0.05$) reductions in the number of conidia produced by the fungus cultured on media containing H_2O_2, compared to the control (Figure 3). Although no significant difference was recorded between the amount of conidia harvested on SDS and RBM, conidiation was completely abolished on the media supplemented with NaCl (Figure 3). These results suggest that osmotic stress critically affects *M. oryzae* conidiation while plasma membrane/cell wall stress has no effect on the spore production.

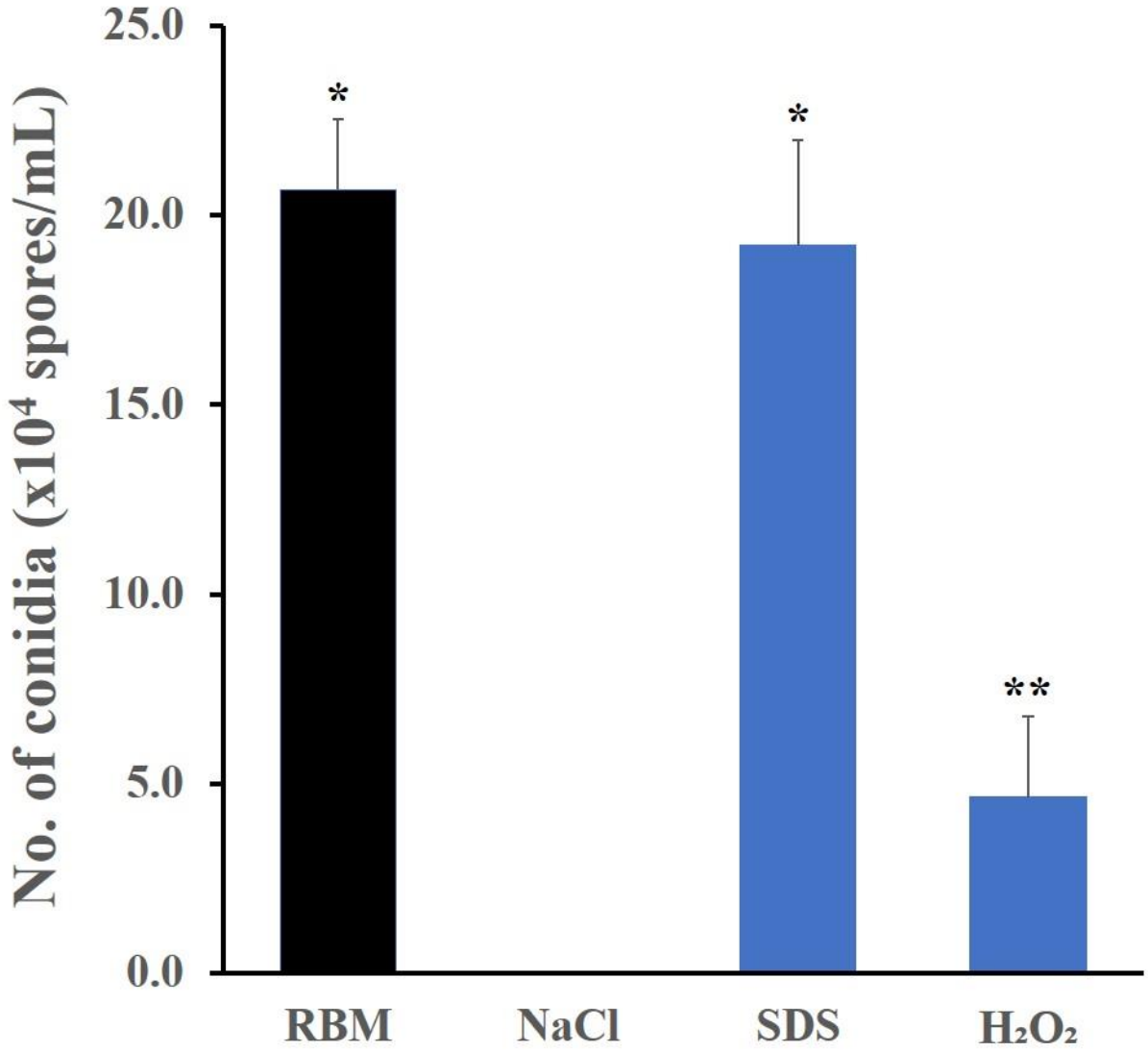

Fig 3: Effects of plasma membrane/cell wall, oxidative and osmotic stresses on *M. oryzae* conidiogenesis. Single and double asterisks show significant difference at $p<0.05$

Biological stress does not influence M. oryzae Conidia morphology

Conidiation is an important part of the life cycle of *M. oryzae*. Therefore, production of normal conidia is an important step that determines the pathogenicity of the fungus. As such, we investigated whether biological stress has any effect on the morphology of the fungal conidia. To achieve this, we harvested conidia RBM in the presence and absence of the stressors. The physical morphology of the spores was observed under a scanning confocal microscope. The results revealed no obvious difference in the conidia harvested on media containing the stress inducers compared to the ones harvested on RBM, although those harvested from H_2O_2 appeared with slightly rough surfaces (Figure 4). Based on this result, we conclude that biological stresses are dispensable for conidia morphology.

Fig 4: The role of biological stress on the physical morphology of *M. oryzae* conidia. The images were captured using the differential interference contrast (DIC) mode of a confocal microscope.

M. oryzae pathogenicity is affected in the presence of biological stress

To investigate the effect of biological stress on the pathogenicity of *M. oryzae*, the fungus was cultured on PDA supplemented with the various stress inducers for 5 days. Mycelial blocks were excised from the culture and used to infect apparently healthy rice leaves. The results revealed the absence of disease lesions on the leaves infected with SDS- and H_2O_2-stressed fungi, contrary to the control plants (Figure 5). However, weak disease lesions were observed on the leaves infected with NaCl-stressed fungus, suggesting the efficacy of biological stress in controlling the blast disease.

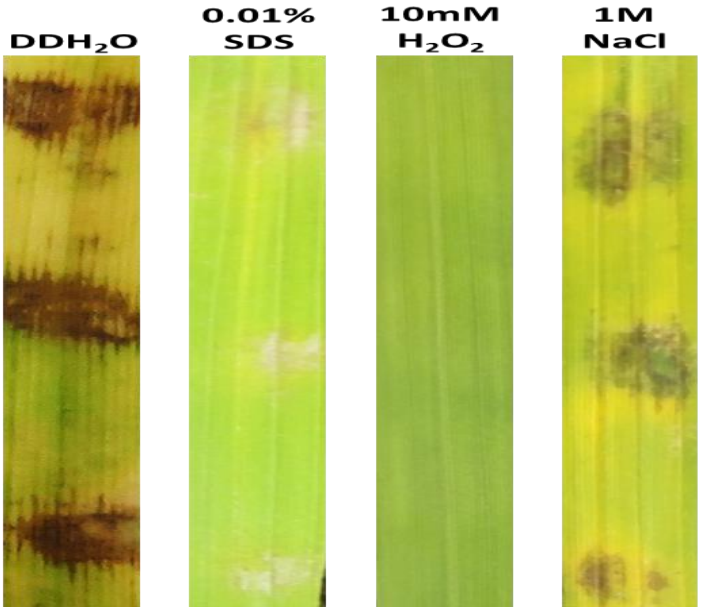

Fig 5: Pathogenicity of *M. oryzae* under plasma membrane/cell wall, oxidative and osmotic stresses. SDS = Sodium dodesylsulfate (cell wall stress induction), DDH_2O = Double distilled water (control), H_2O_2 = Hydrogen peroxide (oxidative stress induction), NaCl = Sodium Chloride (osmotic stress induction)

Discussion

One of the most serious impediments to increased rice production is the presence of the rice blast fungus *Magnaporthe oryzae*, which drastically affects rice yields every year and indirectly increases production costs (Skamnioti et al., 2009). Rice blast is one of the most frequent and costly rice diseases in temperate

rice-growing regions worldwide (Wang et al., 2009).

The disease poses a serious threat to global food security which results on increased malnutrition by decreasing global rice production by about 15 %, which is enough to feed more than 60 million people for a year (Simkhada & Thapa, 2022). Developing an effective and sustainable method to reduce crop loss from the rice blast could have a significant impact on food security (Spence et al., 2015). Conidiation is an important developmental process of *M. oryzae* life cycle and it involves a series of morphological changes starting from hyphal growth to conidiophores formation (Park et al., 2010). Conidia are the fungal asexual spores that initiate the process of infection which eventually leads to colonization of the host tissues. Hence, targeting conidia production of the fungus might serve as an important strategy in controlling the disease. In this research work, the effects of cellular stresses on conidia production were evaluated. The biological stresses induced on the fungus have shown a serious effect on conidia production (Figure 3). Although cell wall stress induced by SDS did not show any significant reduction in conidia production, conidiation was completely abolished in the presence of osmotic stress induced by NaCl. Moreover, a significant ($p < 0.05$) reduction in conidiation was observed following oxidative stress by H_2O_2, compared to the control. Therefore, this study generally revealed the role of biological stress in conidial growth of M. oryzae, similar to the findings of Adam et al., (1998) in *Aspergillus nidulans*. This result unveils a means of reducing the fungal spores, which in turn will reduce the spread of the fungus to new uninfected areas/plants, thereby reducing its effects in the environment.

The rice blast fungus is a hemibiotrophic pathogen which initially invades a few host cells, steals nutrients, but does not kill the host cells. Eventually, the fungus becomes necrotrophic, destroying the colonized tissue and proceed to the neighboring ones (Compos-soriano et al., 2014). Hence, the effect of this biological stress was also investigated in the pathogenicity of the rice blast fungus. Interestingly, it was found that the biological stress significantly reduced the pathogenicity of the fungus especially cell wall/plasma membrane and oxidative stresses. There were no necrotic lesions on the leaves infected with the fungus grown on medium supplemented with hydrogen peroxide and SDS, indicating that the pathogenicity of the fungus seriously compromised when subjected to these stress-inducing agents. However, there was only a weak reduction in the pathogenicity of the fungus when exposed to osmotic stress. This shows that biological stresses especially oxidative stress has significant effect on *M. oryzae* pathogenicity.

In the vegetative growth, a remarkable inhibition of the growth of the fungus was observed in the cell wall induced biological stress using SDS followed by osmotic stress due to NaCl and the growth inhibition is less in oxidative stress induced by H_2O_2. From this we could understand that cellular biological stresses can be used to target the vegetative stage of the fungal life cycle, thereby reducing its growth which reduces the negative effect caused by the fungus after infecting the plants. Hence, this study has demonstrated that induction of biological stress can serve as an alternative technology to control the rice blast disease. However, a great challenge to be resolved by future studies is on how to induce such stresses on the fungus without affecting the host plant. Until this control technology is established, this serves as the major limitation of this work.

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