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Sodium Nitroprusside Treatment Affects the Expression of Some Defense Genes and Physiological Parameters in *Cucurbita pepo* Infected with Cucumber Mosaic Virus

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Introduction

Considering the importance of vegetables and their constant use in human nutrition, studying the damage caused by plant viruses in vegetables as well as preventing viral diseases is extremely important. Several vegetables are sensitive to viruses and are attacked by various plant viruses. The control of pests and diseases in agriculture often relies on pesticides and chemical fertilizers, which negatively affect the rhizosphere's natural microflora and the ecosystem's balance. Today, researchers are looking to replace these chemicals and pesticides with other environmentally friendly agents to improve agricultural production and control plant pathogens. In recent years, the use of defense inducers has received much attention. Nitric oxide (NO) is a degradable biological activator derived from phytometabolites, which is used today as a substance to increase the defense responses of plants against plant pathogens and abiotic stresses. This molecule is produced in different parts and organelles of plant cells such as cytosol, chloroplast, peroxisome, mitochondria, cytoplasmic membrane and nucleus. Therefore, in the present study, we aimed to investigate the role of sodium nitroprusside (SNP) as a NO donor and biological defensive stimulus, on C. pepo as a sensitive plant to CMV. Furthermore, we tried to study the effects of SNP treatment on the expression of some defense genes and physiological traits of *Cucurbita pepo*.

Material and Methods

The experiment was carried out in a completely randomized design with 3 replications. In two leaves stage, *C. pepo* plants were mechanically inoculated with purified CMV at a concentration of 50 μg/ml. After 48 hours, treatment with SNP at the concentrations of 100, 200 and 300 μg/ml was done in three replications in a completely randomized design as well. The severity of the symptoms was measured 15 days after the treatments. In order to investigate the expression of defense genes and biochemical and physiological indicators, sampling was done from the plant's upper leaves at 7, 14, and 21 days after inoculation (dpi). In this research, the amount of chlorophyll I and II, proline content, total leaf soluble carbohydrates accumulation rate, guaiacol peroxidase (GPX) and catalase (CAT) activity were evaluated. Statistical evaluation of the data obtained from different experiments using SAS 9.1 software and based on ANOVA analysis (Analysis of variance) and Tukey's comparative test at the level of $P \leq 0.05$ done.

Result and Discussion

The results of this research showed that chlorophyll I and II content, soluble carbohydrate accumulation rate, proline content and the activity of defense genes including Pr1, Pr3, LOX1 and GPX were enhanced by the external application of SNP most likely by inducing SAR (Systemic Acquired Resistance) mechanism. The results showed that the increase in the expression level of transcripts of defense genes Pr1, Pr3, LOX1 and GPX in the treatment with different concentrations of SNP was significantly higher compared to the control plants. In other words, the expression of defense genes in the treated plants often increased with increasing the concentration of SNP from 100 μg/ml to 300 μg/ml, which seems that one of the reasons for these results, is the less toxic effects of SNP in relatively high concentrations in *C. pepo*. Also, after contamination, the expression of defense gene transcripts often showed a significant difference compared to the control plants until the second and third week after treatment, and these results indicated the effective role of SNP in the induction of pathway genes. On the other hand, the expression of defense genes in the treated plants often increased with adding the concentration of SNP from 100 μg/ml to 300 μg/ml. The effects of SNP being less toxic in the concentration be relatively high in *C. pepo*.

The application of SNP externally is likely to activate the expression of defense genes in *C. pepo* plants by inducing the SAR resistance mechanism. The study results indicate a significant increase in the expression level of defense gene transcripts such as Pr1, Pr3, LOX1, and GPX when treated with different concentrations of SNP compared to control plants. Additionally, after contamination, the treated plants showed a significant difference in the expression of defense gene transcripts compared to the control plants, especially in the second and third weeks after treatment. This suggests the effective role of SNP in inducing resistance pathway genes. Moreover, the expression of defense genes in treated plants often increased with higher concentrations of SNP, from 100 μg/ml to 300 μg/ml, possibly contributing to these results. In pumpkin, relatively high concentrations of SNP showed less toxicity effects. Other studies have shown that nitric oxide treatment in wheat increases basal resistance and induced resistance against certain pathogens. It also affects various activities such as hydrogen peroxide, guaiacol-peroxidase and catalase activity, callose deposition, relative leaf water content, and membrane stability index. Reports have indicated that the transcript levels of certain biosynthetic and signaling genes were induced with jasmonic acid (JA) and salicylic acid treatment in tomatoes infected with specific nematodes. It was observed that JA and SNP treatment reduced root compound leakage and peroxidation due to root-knot nematodes and increased the expression of Protease inhibitor 2 (PI2) after infection. The production of NO by nitric oxide synthase (NOS) and its interaction with other antioxidants strongly affected the interactions between tomatoes and root-knot nematodes. The involvement of NO in the plant's immune response was first identified in potatoes when a NO donor led to the accumulation of the potato phytoalexin ricin, an endogenous antibiotic compound.

The findings demonstrate that S-nitroso-glutathione (GSNO) serves as an active intracellular NO storage center and is regulated by S-nitrosoglutathione reductase (GSNOR) in plants. According to Yu et al. (2020), the external application of NO through s-nitrosylation of the GSNOR protein and inhibition of its activity enhances the resistance of peach fruits to *Monilinia fructicola*. Other studies have also revealed that the external application of NO on fruits post-harvest exerts inhibitory effects on pathogens such as *Botrytis cinerea*, *Penicillium expansum*, and *Colletotrichum gloeosporioides* (Zheng et al., 2011; Lai et al., 2014; Hu et al., 2019). Additionally, Lazalt et al. (1997) found that the external application of NO preserves chlorophyll levels in potato leaves infected with *Phytophthora infestans*. Furthermore, research has highlighted the importance of plant RNA-dependent RNA polymerase 1 (RDR1) in the RNA silencing pathway against viruses in plants. While the induction of RDR1 expression by viral infection and salicylic acid is well-known, the signaling mechanisms during this process remain incompletely understood. Investigations into the interaction between tubamo-tobacco mosaic virus (TMV) and plants such as *Nicotiana tabacum*, *N. benthamiana*, and *Arabidopsis thaliana* have revealed the involvement of hydrogen peroxide and NO in the induction of RDR1 production. Notably, inoculation of TMV on the lower leaves of N. Tabacum led to rapid accumulation of H_2O_2 and NO, followed by an increase in RDR1 transcripts in non-inoculated upper leaves. Pretreatment of upper leaves with external H_2O_2 and NO resulted in elevated expression of RDR1 and systemic acquired resistance (SAR) genes against TMV (Liao et al., 2013). Moreover, NO treatment of hemp, along with inoculation of the plant with begomo-vein yellow mosaic viruses (MeYVMV) and beta-satellite, as well as the cotton Multan leaf complex virus (CLCuMB), led to an increase in the production of PAL, LOX1, and Pr genes not only in the infected leaves but also in the roots and aerial parts of the plant. In addition, it has been observed that the expression of genes related to brassinosteroids (BRs) increases after corn infection with Maize Chlorotic Spot Virus (MCMV). External NO treatments resulted in the accumulation of 2,4-epibrasinolide (BL) and brassinazole (BRZ). These changes, along with alterations in the content of chlorophyll and soluble sugars, played a positive role in reducing the incidence of MCMV disease (Sarkar et al., 2010). Furthermore, MCMV infection of maize was delayed when plants were treated with NO, suggesting that BR reduces the susceptibility of maize to MCMV infection in an NO-dependent manner. Additionally, research has indicated that sensitive Arabidopsis plants, which exhibited high CMV proliferation, experienced less damage after treatment with NO scavengers or synthetic NO inhibitors (Cao et al., 2019).

After being infected with rice black vein dwarf virus (RBSDV), rice plants show a significant decrease in nitric oxide (NO) production. Studies have demonstrated that using NO-releasing compounds such as sodium nitroprusside (SNP) and glutathione (GSNO) in rice plants can reduce the occurrence of RBSDV disease. Following RBSDV infection, there is a notable increase in the transcript levels of OsICS1, OsPR1b, OsWRKY 45, salicylic acid, and protein S-nitrosylation in rice plants, which leads to decreased sensitivity to RBSDV. Recent studies have also indicated that melatonin and NO work together to reduce rice stripe virus (RSV) contamination in rice (Lu et al., 2020).

Conclusions

The external treatment of nitric oxide played an important role in the response to CMV infection and also in regulating the expression of some resistance-related genes. In general, the results of the gene expression pattern and the improvement of the physiological characteristics showed the effectiveness of SNP in inducing resistance to CMV. On the one hand, taking into account the environment friend nature of SNP, as well as the high costs of chemical, SNP is recommended to reduce the disease damage and even decline the effects of chemicals abused and help us to design novel and effective disease management strategies to protect plants against pathogens.

Keywords: Defense genes inducers, Resistance, Physiological characteristics.

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Declaration of conflict of interest

The authors declare no conflict of interest.