Doi: https://doi.org/10.17584/rcch.2023v17i3.16358

Assessment of *Burkholderia glumae* control in rice (*Oryza sativa*) FEDEARROZ 67, using silver nanoparticles (AgNPs) under greenhouse conditions

Evaluación del control de *Burkholderia glumae* en el cultivo de arroz (*Oryza sativa*), FEDEARROZ 67, utilizando nanopartículas de plata (AgNPs) bajo condiciones de invernadero

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Experimental unit of developing rice (*Oryza sativa* L.) under greenhouse conditions.

Photo: G. Chaves-Bedoya

ABSTRACT

Bacterial panicle blight, caused by *Burkholderia glumae*, represents a significant threat to global rice production, jeopardizing future food security. The severity of this seed-borne disease has been amplified by shifting environmental conditions, yet effective control strategies and fully-resistant rice varieties remain elusive. This research was conducted with the FEDEARROZ 67 rice variety sourced from Agua Clara, Cucuta municipality, and tests were undertaken under controlled greenhouse conditions at Universidad Francisco de Paula Santander. Our objective was to assess the potential of electrochemically synthesized silver nanoparticles (AgNPs) for controlling *B. glumae*. We employed a completely randomized design with five treatments: preventive, curative, positive control, negative control, and absolute control. Variables analyzed included the number of chlorotic leaves, plant height, and weight. Statistical analyses encompassed variance analysis and Tukey's mean comparison tests using XLSTATS version 2018. Our findings revealed that the preventive treatment with AgNPs at a 5 ppm concentration exhibited significant phytoprotective effects against *B. glumae*. Plants under this treatment showcased fewer chlorotic symptoms and greater heights and weights compared to controls. These findings underline the potential of nanotechnology as a groundbreaking tool for combating bacterial diseases in essential crops such as rice, thereby contributing to a sustainable future for global agriculture.

Additional key words: bacterial panicle blight; rice disease; bacterial control; plant transformation.

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RESUMEN

El tizón bacteriano de la panícula, causado por *Burkholderia glumae*, representa una amenaza significativa para la producción global de arroz, poniendo en peligro la seguridad alimentaria futura. La gravedad de esta enfermedad transmitida por semillas ha sido amplificada por las cambiantes condiciones ambientales, y aún no se han encontrado estrategias de control efectivas ni variedades de arroz completamente resistentes. Esta investigación se llevó a cabo con la variedad de arroz FEDEARROZ 67, originaria de Agua Clara, municipio de Cúcuta, y las pruebas se realizaron bajo condiciones controladas en invernadero en la Universidad Francisco de Paula Santander. Nuestro objetivo era evaluar el potencial de las nanopartículas de plata (AgNPs) sintetizadas electroquímicamente para controlar *B. glumae*. Empleamos un diseño completamente aleatorio con cinco tratamientos: preventivo, curativo, control positivo, control negativo y control absoluto. Las variables analizadas incluyeron el número de hojas cloróticas, la altura de la planta y el peso. Los análisis estadísticos comprendieron análisis de varianza y pruebas de comparación de medias de Tukey utilizando la versión 2018 de XLSTATS. Nuestros hallazgos revelaron que el tratamiento preventivo con AgNPs a una concentración de 5 ppm mostró efectos fitoprotectores significativos contra *B. glumae*. Las plantas bajo este tratamiento mostraron menos síntomas cloróticos y mayores alturas y pesos en comparación con los controles. Estos hallazgos resaltan el potencial de la nanotecnología como una herramienta innovadora para combatir enfermedades bacterianas en cultivos esenciales como el arroz, contribuyendo así a un futuro sostenible para la agricultura global.

Palabras clave adicionales: tizón bacteriano de la panícula; enfermedades en arroz; control bacteriano; transformación de plantas.

Received: 11-08-2023 Accepted: 17-09-2023 Published: 29-09-2023

INTRODUCTION

Colombia, ranking as the 25th highest rice-producing nation worldwide, considers rice as one of its primary agricultural products (https://www.atlasbig.com/en-au/countries-by-rice-production). Regionally, in the department of Norte de Santander, rice cultivation spans across ten municipalities, with growers primarily affiliating themselves to FEDEARROZ, a sectorial association that provides crucial support to farmers across the nation. Like other rice-growing regions in Colombia and around the world, Norte de Santander's production suffers significantly due to the bacterial panicle blight caused by the *Burkholderia glumae* bacteria.

This bacterium is prevalent in all rice-growing regions globally, including Asia, Africa, Central, and South America. Depending on the cultivar, the production loss ranges from 10 to 75%. *B. glumae* can instigate disease at any stage of development, from seed to maturity, with its most devastating impacts occurring in reproductive tissues. In severe cases, the entire panicle turns straw-colored, and most grains remain unfilled, resulting in erect panicles (Ortega and Rojas, 2021). Despite the agronomic significance of the disease caused by *B. glumae*, including its impact on crops in Norte de Santander, control alternatives are limited. Chemical control methods against bacterial panicle blight are restricted to oxolinic acid. However, populations of *B. glumae* resistant to this compound have been reported since 1998 (Zhou, 2019).

Nanotechnology-based tools have emerged as a promising research area with diverse applications in agriculture, particularly for crop disease control (Ahmed *et al*., 2021). In this context, silver nanoparticles (AgNPs) have shown efficacy in controlling phytopathogens. They have been extensively researched and tested in agricultural research with the aim of improving crop production, efficiency, and sustainability (Gupta *et al*., 2018). AgNPs offer a low-toxicity, broad-spectrum biocidal effect, with a low probability of developing microbial resistance. They are effective against both Gram-positive and Gram-negative bacteria, inhibiting their growth (Wang *et al*., 2017).

Silver nanoparticles (AgNPs) exhibit significant antimicrobial potential, attributed to multiple modes of action. First, it is believed that AgNPs can directly interact with bacterial cell walls. This interaction can cause structural damage and increase the permeability of the cell wall. The consequence of this interaction is potentially the loss of essential cellular components and eventual bacterial cell death. In addition to interacting with the cell wall, AgNPs can also generate reactive oxygen species (ROS) (Ferdous and Nemmar, 2020). These ROS, including peroxides, superoxides, hydroxyl radicals, and singlet oxygen, can cause oxidative damage to cellular proteins, lipids, and DNA, leading to cell death (Dakal *et al*., 2016; Abeer *et al*., 2022). Another theory postulates that AgNPs may interfere with bacterial DNA, inhibiting replication, and thus the growth and spread of bacteria. Binding of AgNPs to DNA can alter the helical structure, preventing transcription and replication machinery from accessing the DNA (More *et al*., 2023).

While these mechanisms of action have been proposed, it is possible that AgNPs operate through a combination of these, and the exact mechanism may vary depending on the type and concentration of AgNPs, as well as the bacterial species in question. To fully understand how AgNPs exert their antibacterial effect against *B. glumae,* further research is needed to explore these mechanisms at a more detailed level. Recently, we reported that at a concentration of 5 ppm, AgNPs inhibit the *in vitro* growth of *B. glumae* (Chaves-Bedoya *et al*., 2022).

In this study, we explore the phytoprotective effects of silver nanoparticles (AgNPs) on the FEDEARROZ 67 rice variety under greenhouse conditions. Our preliminary findings suggest that AgNPs, when applied as a preventive treatment, can effectively control bacterial infection, offering a significant improvement in plant health compared to the untreated control. Building upon these initial results, this research further investigates the potential benefits of AgNPs as a protective measure against bacterial threats in this specific rice variety. Our end goal is to provide valuable insights that could potentially enhance the development of effective strategies for dealing with the widespread issue of *B. glumae*.

MATERIAL AND METHODS

Plant material

Rice seedlings of the FEDEARROZ 67 variety with a germination age of 20 d were obtained from Agua Clara in the municipality of Cucuta (8°14’02.6” N and 72°25’17.6” W). The plant material was transported to the facilities at Los Patios Campus, Universidad Francisco de Paula Santander (7°50’17” N and 72°50’47” W) at an elevation of 410 m a.s.l. and an average temperature of 30°C. The seedlings were planted using soil from the nursery where they were acquired. The planting distances used were 11×6 cm in plastic containers measuring 38.0×26.5×12.5 cm.

Inoculum preparation of *Burkholderia glumae*

The bacterial strain *B. glumae* ATCC 33617 was incubated in King B medium with 15% glycerol. The bacteria were streaked for isolation on King B agar and incubated for 48 h at 28°C with constant agitation at 150 rpm for 24 h. Subsequently, the microbial biomass was concentrated by centrifugation at 7500 rpm for 5 min. The resulting pellet was resuspended in 20 mL of sterile distilled water, yielding an aqueous suspension of the bacteria with a concentration equivalent to an absorbance of 0.2±0.05 at 600 nm, approximately corresponding to 1×10^{8} colony-forming units (CFU) per mL (Flórez and Uribe, 2011). From this bacterial suspension, 60 mL was used to inoculate each experimental unit via spray.

Synthesis of silver nanoparticles

The silver nanoparticles were synthesized according to the methodology described previously by Chaves-Bedoya *et al*. (2022), resulting in an initial concentration of 25 ppm. To determine the *in vivo* effect on rice plants inoculated with *B. glumae*, dilutions were prepared at 5 ppm. This concentration was determined in preliminary studies as the minimum inhibitory concentration against *B. glumae in vitro.*

Experimental design

Five treatments were analyzed in a completely randomized block design: The treatments are detailed as follows: preventive, spraying silver nanoparticles (AgNPs) at 5 ppm before inoculation; curative, spraying AgNPs after inoculation; positive control, use of oxolinic acid post-inoculation; negative control, only bacterial inoculation; and absolute control, no treatment or infection. Each treatment was performed in triplicate and the experimental unit contains nine plants (38.0×26.5 cm).

The variables analyzed were new chlorotic leaves, plant height and plant weight. Additionally, a follow-up was conducted from the 4th d after establishing each treatment, noting infection symptoms on the leaves such as yellowing and necrosis (Sayler *et al*., 2006). The analysis of variance and Tukey's mean comparison test were conducted using the XLSTATS software, version 2018.

RESULTS

The impact of silver nanoparticles (AgNPs) on the infection of rice plants (*Oryza sativa* L.) by *B. glumae*, both in preventive and curative applications, showed statistical differences with the positive control (application of oxolinic acid) and negative control (untreated).

In table 1, it can be observed that treatments with AgNPs, both in preventive applications and curative applications, showed a lower number of chlorotic leaves after one week compared to the negative control. Additionally, these plants exhibited higher weight and height at both 8 and 12 weeks. Specifically, the preventive treatment resulted in the tallest plants after 8 and 12 weeks, with heights of 35.3 cm and 46 cm, respectively, only surpassed by the absolute control.

To assess the effectiveness of different treatments in mitigating the symptoms of *B. glumae* infection, a statistical analysis of the data was conducted. Figure 1 presents the mean comparison of the different treatments performed using Tukey's test. The results of this test confirmed that there are significant differences between the treatments in terms of the number of chlorotic leaves, plant weight, and height at both 8 and 12 weeks.

In figure 2, the status of rice seedlings at 4 weeks after applying the treatments is shown. The effectiveness of the treatments with AgNPs in counteracting the effects of *B. glumae* after treatment application is visually evident. Plants in treatments preventive, curative and positive control exhibit greener leaves and a lower degree of necrosis compared to treatment control negative control, where the highest level of yellowing and necrosis, characteristic of *B. glumae* attack, was observed.

Finally, figure 3 displays rice plants at 12 weeks under each of the treatments. Here, it is evident that plants in the absolute control have a greater root volume, and the plants are more abundant, thick, and tall. In contrast, plants in negative control have fewer leaves and considerably diminished roots, with clear signs of necrosis and yellowing in the leaves, indicative of the effect of untreated *B. glumae.* Treatments with AgNPs (preventive and curative) as well as oxolinic acid treatment showed better development than positive control, although they exhibited mild signs of necrosis and a less abundant root system compared to absolute control. This long-term visual analysis provides a more comprehensive picture of the effectiveness and residual effects of the different treatments.

Table 1. Impact of treatments on *B. glumae* infection in rice (*Oryza sativa* L.). Parameters include chlorotic leaves per week, 8-week plant weight, and plant heights at 8 and 12 weeks for treatments: AgNPs preventive, AgNPs curative, positive control (oxolinic acid), negative control (no treatment) and absolute control. Values are means from 15 replicates.

Treatment	New chlorotic leaves	Plant weight (g)	Plant height (cm)	
	1 week	8 weeks	8 weeks	12 weeks
Preventive	0.67	4.6	35.3	46
Curative	1.67	3.4	30.3	43
Positive control	7.67	3.5	29.6	35
Negative control	13.3	3.1	26.3	34
Absolute control	0	4.7	36	52

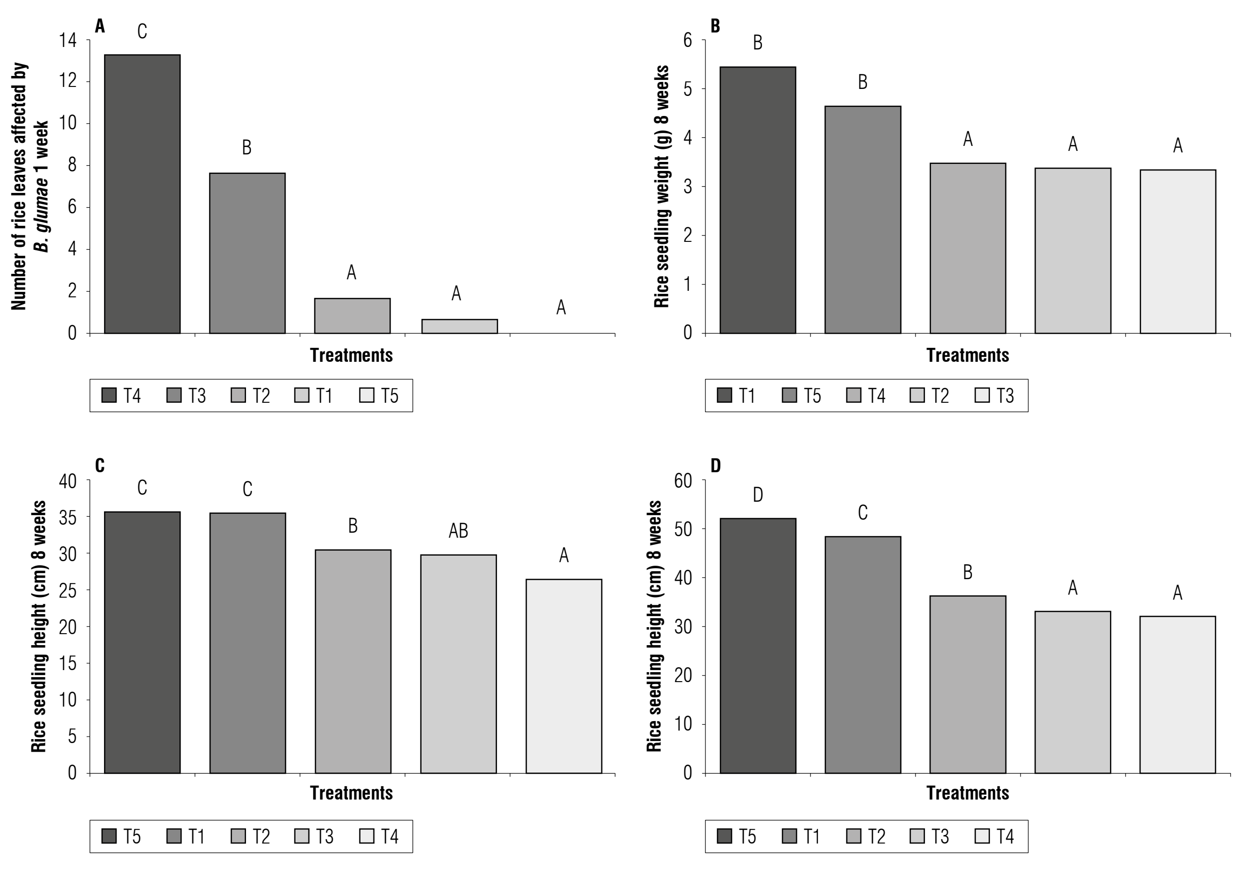

Figure 1. Effect of different treatments on rice plants (*Oryza sativa* L.) infected with *B. glumae*. The treatments are detailed as follows: T1 (preventive, spraying silver nanoparticles (AgNPs) at 5 ppm before inoculation), T2 (curative, spraying AgNPs after inoculation), T3 (positive control, use of oxolinic acid post-inoculation), T4 (negative control, only bacterial inoculation), and T5 (absolute control, no treatment or infection). The evaluated parameters include the number of new chlorotic leaves at week 1 post-inoculation, plant weight at 8 weeks, and plant height at 8 and 12 weeks. The values represent the mean of 15 replicates. Significant differences between treatments were determined using Tukey's test (*P*<0.05).

DISCUSSION

The use of nanotechnology in agriculture has emerged as a promising solution to overcome the limitations of traditional disease management strategies. In particular, silver nanoparticles (AgNPs) have garnered significant attention due to their unique antimicrobial properties, rendering them effective against a range of phytopathogens (Anees *et al*., 2020). Among the innovations, AgNPs synthesized by endophytic *Fusarium concolor* have shown efficacy in the in vitro control of fungal phytopathogens, including *Colletotrichum guaranicola* (Almeida *et al*., 2021). Moreover, in phytopathogenic fungi, smaller-sized nanoparticles have demonstrated greater toxicity than their larger counterparts. Specifically, AgNPs have been reported to inhibit the growth of fungi such as *Aspergillus fumigates*, *A. niger*, *A. flavus*, *Trichophyton rubrum*, Cand*ida albicans*, and various Penicillium species (Mansoor *et al*., 2021). Supporting these findings, another study presented evidence that AgNPs exhibited potent antibacterial and antifungal activities against notable pathogens, including *Meloidogyne incognita*, *Ralstonia solanacearum*, and *Fusarium oxysporum* (Khan *et al*., 2021)

The management of bacterial diseases in crops is a global challenge due to increasing antibiotic resistance and the need for more sustainable and environmentally friendly agricultural practices. Successfully

Figure 2. **Rice plants (*Oryza sativa* L.) four weeks after infection with *B. glumae* under five different treatment conditions. T1 shows plants that received a preventive treatment with silver nanoparticles (AgNPs) before infection. T2 represents plants treated with AgNPs post-infection. T3 shows plants that received oxolinic acid post-infection as a positive control. T4, the negative control, represents plants inoculated with *B. glumae* but received no additional treatment, showing evident signs of infection and damage. T5, the absolute control, shows non-infected and untreated rice plants, exhibiting normal growth and no signs of infection. Each photograph is representative of the observed effects in each treatment.**

Figure 3. **Rice plants at 12 weeks after infection with *B. glumae* under five different treatments. The plants in treatment T1 (preventive with AgNPs) and T2 (curative with AgNPs) show signs of healthy growth, but with some differences in root abundance and leaf thickness compared to the non-infected and untreated plants (T5). The plants treated with oxolinic acid (T3) exhibit yellowing and necrosis in the leaves, and a smaller root system compared to T5 but larger than T4. The plants in the negative control (T4), only inoculated with *B. glumae* and without treatment, show the greatest impact with yellowing leaves, necrosis, and a highly reduced root system. The plant heights are as follows: T1, 46 cm; T2, 43 cm; T3, 35 cm; T4, 34 cm; T5, 52 cm. Each photograph is representative of the general response observed in each treatment.**

handling Bacterial Panicle Blight (BPB) caused by *B. glumae* is a significant step in mitigating the harm inflicted by the disease and bolstering production outcomes. However, the methods currently available for dealing with this disease are relatively restricted. Only a small selection of rice varieties with partial resistance are commercially available. Despite some countries having used oxolinic acid as a primary countermeasure against BPB for an extended period, no chemical control strategies are universally accepted. The emergence of *B. glumae* strains resistant to oxolinic acid poses a challenge, limiting the broader application of this antibiotic compound for BPB management (Zhou, 2019). In this context, our study presents significant evidence of the efficacy of silver nanoparticles (AgNPs) in controlling *B. glumae* infection in rice plants.

Our findings reveal that the rice plants in the negative control group, inoculated with bacteria but devoid of any treatment, exhibited the most pronounced yellowing. This aligns with the understanding that *B. glumae* generates phytoalexins, primarily toxoflavin and fervenulin, known to induce chlorotic symptoms in rice leaves (Kim *et al*., 2012). Conversely, plants subject to preventive treatment and those in the absolute control, which had no bacterial exposure, maintained healthier, greener leaves. This suggests a diminished production or impact of these phytoalexins. These results evidence that AgNPs, when applied preventively, can inhibit the production of toxoflavin and fervenulin by *B. glumae*. However, despite the decrease in yellowing in the preventive treatment, this effect did not translate into an increase in plant weight compared to absolute control, suggesting that AgNPs may have secondary effects on plant metabolism. Drawing upon our study, numerous research primarily emphasizes the detrimental effects of AgNPs on plants, influencing aspects ranging from their morphology and physiological functions to cellular structure and molecular interactions. However, there are instances where silver nanoparticles have shown to enhance plant development and growth (Khan *et al*., 2023). These contrasting outcomes emphasize the complexity of plant reactions to AgNPs. It's important to note that these reactions are influenced not just by the AgNPs' attributes such as their size, concentration, and physical form, but are also strongly connected to the specifics of the plant organism in question (Yan and Chen, 2019).

Comparatively, treatments curative and positive showed a decrease in plant weight compared to absolute control and preventive, indicating that AgNPs applied after infection or the use of oxolinic acid may not be sufficient to fully reverse the negative impact of *B. glumae*. This result highlights the importance of preventive disease management in agriculture (He *et al*., 2019) and underscores the need to optimize AgNPs application protocols.

When assessing plant development in terms of height, leaf color, presence of necrosis, and root volume, significant differences among the treatments become evident. Notably, the preventive application of AgNPs (preventive) curtailed the initial infection and prevented leaf damage. At the 8-week mark, the height of plants in preventive was similar to that of the uninfected plants (absolute control), implying that this treatment not only staved off the infection but also preserved the plants' overall health during this period. However, by the 12-week mark, the plants in absolute control exhibited significantly greater heights than those in preventive, underlining the longer-term impacts of the treatments on plant development. Nevertheless, this treatment prevented the infection and maintained the overall health of the plants.

This result is especially relevant considering that *B. glumae* infection often results in a significant reduction in rice crop yield, a critical factor in global food security (Pedraza *et al*., 2018). Contrarily, the curative use of AgNPs (curative) and oxolinic acid showed less efficacy. While oxolinic acid had a positive effect on treating the infection, it did not outperform the preventive treatment with AgNPs. Given the increasing resistance of bacteria to quinolones (Jacoby, 2005) like oxolinic acid and their potential environmental impact, AgNPs could serve as a more environmentally friendly and potentially more effective alternative. However, it is crucial to recognize that, while AgNPs proved to be effective in controlling *B. glumae* infection, these treatments could also have secondary effects on plant development.

The observation of a less abundant root system and the presence of thinner leaves in certain treatments could have implications on photosynthesis and, therefore, on biomass production and grain yield. The study's results point to a promising future for the use of silver nanoparticles in managing bacterial diseases in rice cultivation. Despite possible side effects that could affect plant growth, like a decrease in root system development, the beneficial effects appear to outweigh these drawbacks, especially when compared with the significant damage caused by an untreated infection.

The study's findings highlight the preventive efficacy of AgNPs in controlling bacterial infection, representing a crucial advance in understanding the potential of nanotechnology in plant disease management. This approach could open new horizons for effective, sustainable, and resistance-resistant strategies against *B. glumae*. The application of AgNPs not only offers a promising solution to the challenges posed by this bacterium but could also revolutionize disease management strategies in the global rice industry. Nevertheless, more research is still needed to optimize the use of AgNPs in agricultural environments and fully explore their potential and limitations.

CONCLUSSION

The present research shows that the use of silver nanoparticles (AgNPs) can be an effective strategy for controlling *B. glumae* infection in rice plants, both preventively and curatively. This control is reflected in greater plant height, fewer chlorotic leaves, and fewer signs of necrosis compared to untreated infected plants. However, although AgNPs treatments demonstrated significant effectiveness in controlling the infection, it was also observed that these could have secondary effects on plant development, like a less abundant root system and the presence of thinner leaves. These side effects could have an impact on the final yield of rice plants and should be considered when evaluating the use of AgNPs in the field.

Declaration of competing

The authors declare that there are no conflicts of interest.

ACKNOWLEDGMENTS

Authors thanks the University Francisco de Paula Santander's University Research Fund (FINU) for the funding of Project 004-2022.

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