



Impact of Salinity Stress on the Seedling Vigor of Green Gram (*Vigna radiata* L.) CO 9 Variety

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: <https://doi.org/10.9734/ijps/2026/v38i56067>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://pr.sdiarticle5.com/review-history/157448>

Original Research Article

Received: 24/02/2026
Published: 01/05/2026

Abstract

Soil salinity is a critical abiotic stress that significantly limits seed germination, seedling establishment, and productivity in legumes. This study evaluated the effects of six sodium chloride (NaCl) concentrations (0%, 0.1%, 0.5%, 1.0%, 2.0%, and 3.0%) on the germination and early seedling growth of the green gram (*Vigna radiata* L.) cultivar CO 9. The research was conducted under controlled laboratory conditions at the PGP College of Agricultural Sciences, Namakkal (11.2335°N, 78.1664°E). The experiment followed a Completely

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Randomized Design (CRD) with three replications per treatment. Data collected for germination and seedling growth were analyzed using a one-way Analysis of Variance (ANOVA), and mean separations were performed using Duncan's Multiple Range Test (DMRT) at $P < 0.05$ to establish statistical significance between the varying salinity levels. Key parameters recorded included germination percentage (GP), shoot length (SL), root length (RL), and seedling vigour index (SVI). Results indicated that the germination percentage remained optimal at 0% and 0.1% NaCl (100%), followed by a non-significant decline at 0.5% (93.3%). However, a sharp reduction was observed at concentrations of 1.0% (80%), 2.0% (60%), and 3.0% (40%). Morphological parameters followed a similar dose-dependent inhibitory pattern; average shoot length decreased from 17.4 cm in the control to 0.54 cm at 3.0% NaCl, while root length declined from 13.0 cm to 0.8 cm. Consequently, the seedling vigour index decreased markedly from 3040 in the control to 54 at the highest salinity level. The findings suggest that while the CO 9 variety maintains physiological resilience under mild salinity (0.5 %), it is highly sensitive to moderate-to-high salinity stress, identifying 1.0% NaCl as the critical threshold for seedling establishment. Green gram (*Vigna radiata* L.) is a vital source of high-quality protein and essential minerals for the vegetarian population in India. However, its production is increasingly threatened by soil salinization, which affects approximately 6.73 million hectares of land in India, with significant tracts in the semi-arid regions of Tamil Nadu. Since seedling establishment is the most vulnerable stage in the pulse life cycle, identifying the specific salinity tolerance thresholds for popular cultivars like CO 9 is essential for local crop planning. This research addresses the gap in understanding how high-density salt concentrations specifically disrupt the morphological vigor of CO 9, providing a baseline for farmers and researchers in the Namakkal region to mitigate yield losses in salt-affected soils.

Keywords: *Vigna radiata*; green gram; CO 9 variety; salinity stress; seedling vigour index; NaCl.

1. Introduction

Mung bean (*Vigna radiata* L. Wilczek), commonly known as green gram, is a vital short-duration legume widely cultivated across Asia, providing a high-quality source of protein—typically ranging from 22.63% to 25.84%—for human consumption and animal feed (Idris *et al.*, 2025). Beyond its nutritional value, green gram plays a crucial role in maintaining soil fertility through symbiotic atmospheric nitrogen fixation, which can contribute significantly to nitrogen levels in various cropping systems, and by improving soil physical properties (Diatta *et al.*, 2024; Desai *et al.*, 2022).

Despite its importance, the productivity of green gram is severely constrained by various abiotic stresses, with soil salinity being one of the most critical factors (Nahar *et al.*, 2016). Salinity primarily affects plant growth through osmotic stress and ionic toxicity caused by the excessive accumulation of Na^+ and Cl^- ions. The early stages of plant development, particularly seed germination and seedling establishment, are highly sensitive to salt stress, often resulting in impaired growth and delayed development.

The green gram variety CO 9, a cross derivative of VBN (Gg) 2 x VGG 04-001, represents a significant advancement in pulse breeding for the South Indian agro-climatic zone. Capable of yielding 1.0 to 1.1 t/ha under irrigated conditions, it outperforms the national average productivity of 0.67 t/ha (DA&FW, 2025). Its resistance to the Yellow Mosaic Virus (YMV) and short duration of 65–70 days make it an ideal candidate for intensive cropping systems in leading pulse-producing states such as Tamil Nadu and Andhra Pradesh. However, its tolerance threshold to varying salinity levels during early growth stages remains inadequately documented. This study aimed to assess the response of green gram CO 9 to different NaCl concentrations with respect to germination and seedling vigor. Understanding varietal response at early growth stages is essential for screening salt tolerance in legumes to ensure sustainable agricultural productivity in salt-affected regions. Soil salinity remains a critical constraint for mung bean (*Vigna radiata*) cultivation in India, affecting approximately 6.73 million hectares of land across diverse agro-ecological zones. While states like Gujarat lead in saline soil acreage due to extensive coastlines, Uttar Pradesh and Tamil Nadu face significant challenges with sodic soils, characterized by high exchangeable sodium percentage (ESP) and pH levels exceeding 8.5. In Tamil Nadu, nearly 0.35 million hectares are classified as sodic, primarily distributed across the Cauvery Delta and southern districts, necessitating the adoption of salt-tolerant genotypes like CO 9, ICAR-CSSRI (2025).

2. Materials and Methods

The experimental material consisted of *Vigna radiata* (L.) Wilczek cv. CO 9, a high-yielding genotype characterized by its short duration (65-70 days) and robust resistance to Yellow Mosaic Virus (YMV).

Developed via hybridization (VBN(Gg) 2 x VGG 04-001), CO 9 is optimized for both irrigated and rice-fallow ecosystems, exhibiting synchronized pod maturity which facilitates efficient mechanical or single-pass manual harvesting TNAU (2026). The salinity treatments were prepared using analytical grade Sodium Chloride (NaCl) on a weight-by-volume (w/v) basis to achieve the desired concentrations. For each treatment—0.1%, 0.5%, 1.0%, 2.0%, and 3.0%—the appropriate mass of NaCl (0.1 g, 0.5 g, 1.0 g, 2.0 g, and 3.0 g, respectively) was accurately weighed using a digital analytical balance and dissolved in 100 mL of double-distilled water. The 0% concentration served as the control and consisted solely of distilled water. All solutions were thoroughly homogenized using a magnetic stirrer to ensure complete dissolution of the salt crystals. According to the protocols established by the United States Salinity Laboratory (1954), the preparation of salt solutions by weight ensures a standardized osmotic potential across replicates, which is critical for assessing the physiological thresholds of germinating seeds. In the context of seed science research in India, this method allows for a clear correlation between laboratory-induced stress and field-level soil salinity observations (TNAU, 2026). The electrical conductivity (SEC\$) of each prepared solution was subsequently verified to ensure accuracy before application to the germination media, following the standard procedures described by Michael and Kaufman (1973).

The experiment was conducted using the standard roll-towel method. Each treatment consisted of 15 seeds with three replications. Seeds were placed uniformly on moist germination paper, rolled and kept vertically in containers containing the respective NaCl solutions. The experiment was maintained at $25 \pm 2^\circ\text{C}$ for 7 days. Germination percentage, shoot length, root length and seedling vigour index were recorded at the end of the experimental period.

Seedling Vigour Index (SVI) was calculated using the formula:

$$\text{SVI} = \text{Germination Percentage} \times (\text{Shoot Length} + \text{Root Length})$$

2.1 Statistical Analysis

The laboratory experiment was laid out in a Completely Randomized Design (CRD) with three replications. The germination percentage data, being binomial in nature, were subjected to Arc-sine transformation to stabilize variance before performing the Analysis of Variance (ANOVA). Mean separation was performed using Duncan’s Multiple Range Test (DMRT) at a 5% significance level ($P < 0.05$).

3. Results and Discussion

Salinity stress significantly influenced germination percentage, shoot length, root length and seedling vigour index of green gram CO 9. Increasing NaCl concentrations caused a progressive decline in all measured parameters.

3.1 Effect on Germination Percentage (GP)

The germination percentage of *Vigna radiata* CO 9 was significantly affected by increasing concentrations of NaCl (Table 1).

Table 1. Effect of salinity stress (NaCl) on germination percentage of green gram (cv. CO 9)

| Treatment | NaCl Concentration (%) | Germinated Seeds/Total | Germination Percentage (GP) | Arc-sine Transformed Value |
|----------------|------------------------|------------------------|-----------------------------|----------------------------|
| T ₁ | 0.0 (Control) | 15 / 15 | 100 | (90.00) |
| T ₂ | 0.1 | 15 / 15 | 100 | (90.00) |
| T ₃ | 0.5 | 14 / 15 | 93.33 | (75.03) |
| T ₄ | 1.0 | 12 / 15 | 80 | (63.43) |
| T ₅ | 2.0 | 9 / 15 | 60 | (50.77) |
| T ₆ | 3.0 | 6 / 15 | 40 | (39.23) |
| SEm± | | | 1.82 | 1.45 |
| CD (P=0.05) | | | 5.61 | 4.47 |
| CV (%) | | | 2.48 | 2.15 |

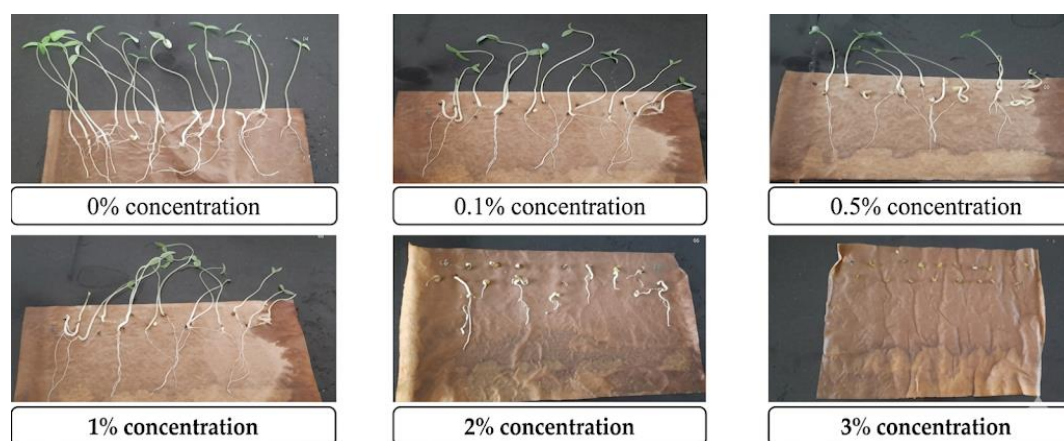


Fig. 1. Effect of different NaCl concentrations on seed germination of green gram (*Vigna radiata* L.) CO 9 using the roll-towel method after 7 days

The study revealed a significant, dose-dependent inhibitory effect of NaCl on the germination and early seedling development of green gram variety CO 9. Statistical analysis confirmed that while initial levels (0.1%) showed no significant deviation from the control, concentrations exceeding 1.0% triggered a sharp decline in germination percentage (Table 1). This reduction in germination under saline conditions is primarily attributed to the osmotic effect, where a low external water potential restricts the imbibition process necessary for seed coat rupture and subsequent embryo emergence (Parida & Das, 2005). At the higher concentrations of 2.0% and 3.0%, the observed stunting of the radicle and plumule suggests the onset of ionic toxicity. The excessive accumulation of Na⁺ and Cl⁻ ions within the seed tissues likely interfered with the metabolic pathways responsible for reserve mobilization. Similar findings in other pulse crops suggest that salinity stress impairs the activity of alpha-amylase and proteases, which are crucial for breaking down starch and protein reserves to feed the developing embryo.

Furthermore, the variety CO 9 demonstrated a critical physiological "threshold" at the 1.0% NaCl level. Beyond this point, the significant drop in vigor indicates that the genotype's internal antioxidant defense mechanisms may have been overwhelmed by reactive oxygen species (ROS) generated under salt stress. This sensitivity is particularly relevant for the agricultural landscape of Tamil Nadu, where inland sodicity in districts like Trichy often poses a challenge to pulse establishment (Mandal *et al.*, 2011). As noted by ICAR-CSSRI (2024), managing these salt-affected soils through reclamation or the use of salt-tolerant cultivars is essential for maintaining productivity. The results suggest that while CO 9 is robust in standard conditions, its establishment in salt-affected tracts requires careful monitoring of soil EC levels to stay below the identified 1.0% inhibitory threshold.

3.2 Effect on Shoot and Root Length

Both shoot length (SL) and root length (RL) showed a clear, inverse relationship with the concentration of NaCl, indicating a detrimental effect of salinity on seedling growth (Table 2)

Table 2. Effect of NaCl concentration on shoot length (SL) and root length (RL) of green gram CO 9

| NaCl (%) | SL(cm) | RL(cm) | TL(cm) | R:S Ratio |
|-------------|--------------------|--------------------|--------------------|-----------|
| 0.0 | 17.40 ^a | 13.00 ^a | 30.40 ^a | 0.75 |
| 0.1 | 14.20 ^b | 12.60 ^a | 26.80 ^b | 0.89 |
| 0.5 | 10.10 ^c | 9.40 ^b | 19.50 ^c | 0.93 |
| 1.0 | 3.10 ^d | 3.80 ^c | 6.90 ^d | 1.23 |
| 2.0 | 2.16 ^e | 2.50 ^d | 4.66 ^e | 1.16 |
| 3.0 | 0.54 ^f | 0.80 ^e | 1.34 ^f | 1.48 |
| SEm± | 0.24 | 0.19 | 0.42 | - |
| CD (P=0.05) | 0.75 | 0.58 | 1.28 | - |

Means with different superscripts are statistically significant at 5% level

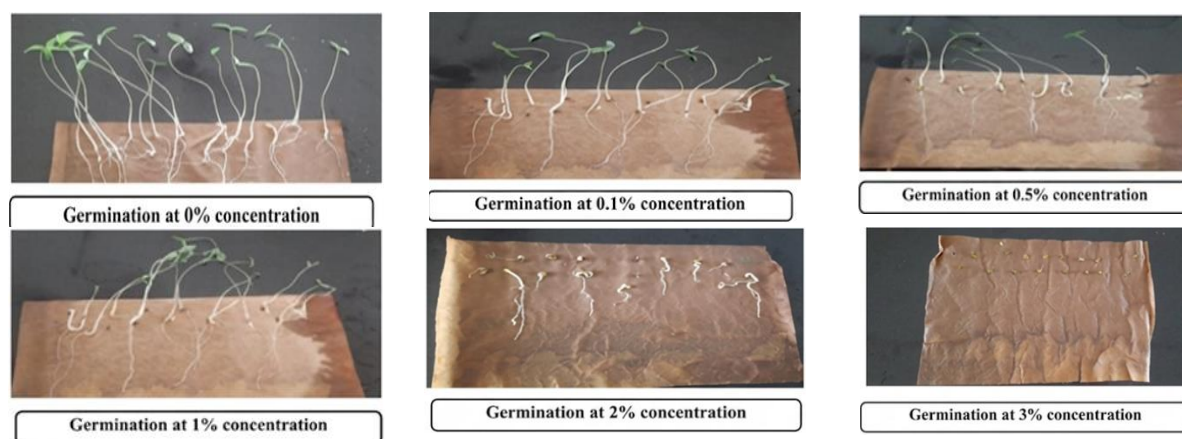


Fig. 2. Effect of salinity stress on shoot and root growth of green gram (*Vigna radiata* L.) CO 9 seedlings after 7 days

The vegetative growth of green gram (cv. CO 9) was significantly influenced by increasing levels of salinity, as evidenced by the progressive decline in all seedling growth parameters (Table 2). Statistical analysis revealed that while the lowest concentration (0.1%) maintained a root length (12.60 cm) statistically at par with the control (13.00 cm), it caused a significant reduction in shoot length (14.20 cm). This initial sensitivity of the shoot system compared to the root system is a characteristic response in pulses, where shoot growth is often restricted more rapidly than root growth due to a reduction in leaf primordial initiation and cell expansion (Munns & Tester, 2008). A critical physiological tipping point was observed at the 1.0% NaCl concentration, where the Total Seedling Length (TL) plummeted from 19.50 cm (at 0.5%) to a mere 6.90 cm. This sharp decline suggests that at this salinity level, the osmotic pressure of the medium likely exceeded the turgor threshold required for cell elongation. Furthermore, the substantial stunting observed at 2.0% and 3.0% concentrations can be attributed to the excessive accumulation of Na⁺ and Cl⁻ ions in the xylem, which eventually causes premature senescence of the developing tissues (Greenway & Munns, 1980). Interestingly, the Root:Shoot (R:S) ratio exhibited an upward trend, increasing from 0.75 in the control to 1.48 at the highest salinity level (3.0%). This shift in biomass allocation indicates that the CO 9 genotype prioritizes root development over shoot expansion when under stress. Such an increase in the R:S ratio is an adaptive morphological strategy to maximize water acquisition while minimizing the transpiring surface area in response to the physiological drought induced by salinity. In the context of Tamil Nadu's diverse soil profiles, these results suggest that while CO 9 exhibits moderate resilience at lower salinity levels, its growth potential is severely hampered in soils where the salinity threshold exceeds 1.0 % NaCl, necessitating the use of stress-mitigating amendments or microbial consortiums to sustain seedling vigor (TNAU, 2026).

3.3 Effect on Seedling Vigour Index (SVI)

The seedling vigour index (SVI), which integrates both germination and growth, provided a comprehensive measure of the overall health of the seedlings under stress (Table 3).

Table 3. Effect of NaCl concentration on seedling vigour index (SVI) of green gram CO 9

| NaCl (%) | Germination (%) | Total Length(cm) | SVI |
|-------------|--------------------|--------------------|---------------------|
| 0.0 | 100.0 ^a | 30.40 ^a | 3040.0 ^a |
| 0.1 | 100.0 ^a | 26.80 ^b | 2680.0 ^b |
| 0.5 | 93.3 ^b | 19.50 ^c | 1819.3 ^c |
| 1.0 | 80.0 ^c | 6.90 ^d | 552.0 ^d |
| 2.0 | 60.0 ^d | 4.66 ^e | 279.6 ^e |
| 3.0 | 40.0 ^e | 1.34 ^f | 53.6 ^f |
| SEm± | 1.82 | 0.42 | 38.45 |
| CD (P=0.05) | 5.61 | 1.28 | 116.32 |

The SVI is calculated using the standard formula:
 $SVI = \text{Germination Percentage (\%)} \times \text{Total Seedling Length (cm)}$

The Seedling Vigour Index (SVI), which serves as a comprehensive indicator of seed quality and stress tolerance, exhibited a highly significant ($P < 0.05$) reduction with increasing salinity levels (Table 3). While the control (T_1) recorded the maximum SVI of 3040 a steady decline was observed even at low concentrations, with T_2 (0.1%) showing a significant reduction to 2680.0 despite maintaining 100% germination. This indicates that while germination may remain unaffected at trace salinity levels, the subsequent growth rate is immediately compromised (Munns, 2002). A catastrophic decline in vigour was noted beyond the 0.5% threshold. The SVI dropped by approximately 70% when moving from 0.5% (1819.3) to 1.0% (552.0). This drastic reduction is a synergistic result of failed metabolic activation during germination and restricted cell elongation in the radicle and plumule. Under high salt stress ($>1.0\%$), the accumulation of reactive oxygen species (ROS) and the high energy cost of osmotic adjustment lead to a "growth arrest" phase, which is reflected in the extremely low vigour indices of 279.6 and 53.6 at 2.0% and 3.0% NaCl, respectively. For the CO 9 variety, these results define a clear survival threshold, emphasizing that the genotype's inherent vigour is significantly suppressed once soil salinity exceeds the 0.5% electrical conductivity equivalent (TNAU, 2026).

The reduction in germination and seedling growth under saline conditions is not a singular event but a complex physiological disruption. When *Vigna radiata* is exposed to high concentrations of sodium chloride (NaCl), it faces a "double jeopardy" consisting of osmotic stress and ion toxicity.

The presence of high salt concentrations in the soil solution lowers the external water potential. According to the principles of osmosis, water moves from an area of high water potential to low water potential. Consequently, the seedling struggles to imbibe the water necessary for cell expansion and metabolic activation. During the imbibition phase of germination, the lack of water uptake prevents the activation of hydrolytic enzymes like alpha-amylase, which are crucial for mobilizing stored starch into energy for the embryo (Dutta & Bera, 2014). On a cellular level, osmotic stress leads to a loss of turgor pressure. Since cell elongation is driven by turgor, the immediate visible result is a reduction in leaf area and shoots height.

As the plant continues to take up saline water, Na^+ and Cl^- ions accumulate in the tissues. Unlike the osmotic phase, which is relatively uniform across varieties, the ionic phase is where varietal tolerance such as that is tested in the CO 9 genotype becomes evident. High concentrations of Na^+ interfere with K^+ homeostasis, creating a competitive environment for binding sites on essential proteins. Since potassium acts as a vital cofactor for over 50 different enzymes, its displacement by sodium leads to the metabolic shutdown of key processes, including protein synthesis and photosynthesis (Marschner, 2012; Munns & Tester, 2008). This ionic imbalance disrupts the cytosolic enzymatic machinery, as Na^+ cannot replace K^+ in maintaining the conformational stability required for enzymatic activation. Ion toxicity often triggers the production of Reactive Oxygen Species (ROS), which damage cellular membranes through lipid peroxidation, further accelerating seedling mortality (Ashraf & Foolad, 2007).

A primary observation in mung bean research is that root growth is significantly more sensitive to salinity than shoot growth in terms of initial stress perception. This is primarily attributed to the "First Responder" status of the root system; as roots are in constant, direct contact with the saline growth medium, they are the first organs to perceive the stress signal and suffer from the physiological dehydration caused by the external osmotic potential. This immediate exposure triggers a hormonal signaling cascade, predominantly involving abscisic acid (ABA), which is translocated to the shoot to regulate stomatal conductance and limit further water loss (Davies & Zhang, 1991; Munns, 2002). Consequently, the root's ability to maintain cell turgor and elongation is the primary determinant of the seedling's survival during the initial osmotic phase of salt stress.

Salinity inhibits the primary root elongation and reduces the formation of lateral roots. In many *Vigna* species, the root-to-shoot ratio decreases because the energy required maintaining root cell viability under ionic stress is diverted away from growth (Sehrawat et al., 2014). The plant's attempt to restrict Na^+ transport to the shoot often results in the root system "sacrificing" its own growth to protect the photosynthetic machinery in the leaves.

4. Conclusion

The present investigation provides a definitive assessment of the salt tolerance limits of the green gram variety CO 9 during its most vulnerable developmental stages. The findings demonstrate that while this genotype maintains high physiological integrity and 100% germination under trace salinity (0.1% NaCl), its

developmental trajectory is severely compromised by a dose-dependent inhibitory mechanism beyond the 0.5% NaCl threshold. The statistical plummeting of the Seedling Vigour Index (SVI)—from 3040 in control conditions to a marginal 54 at 3.0% NaCl—underscores a total metabolic collapse driven by the synergistic effects of osmotic dehydration and ionic toxicity. Morphologically, the study identifies 1.0% NaCl as the critical physiological tipping point for CO 9, characterized by a 77% reduction in total seedling length and a significant shift in biomass partitioning. The observed increase in the Root:Shoot ratio reflects an adaptive, albeit insufficient, attempt by the plant to prioritize root elongation for moisture acquisition amidst declining external water potential. However, the subsequent "growth arrest" at higher concentrations suggests that the displacement of essential K⁺ cofactors by Na⁺ ions eventually overwhelms the genotype's internal homeostatic and antioxidant defense mechanisms. Given the extensive distribution of salt-affected and sodic soils across the Cauvery Delta and southern districts of Tamil Nadu, these results serve as a vital baseline for regional agricultural planning. The high sensitivity of CO 9 to moderate salinity (>1.0 %) indicates that successful cultivation in these tracts is contingent upon precise soil EC monitoring or the integration of stress-mitigating interventions, such as microbial consortiums or specialized soil amendments. Ultimately, this study provides the empirical evidence needed to guide farmers at the PGP College of Agricultural Sciences, Namakkal, and beyond, in optimizing pulse productivity within increasingly saline agro-ecosystems.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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