



RESEARCH ARTICLE

Seed priming with sodium nitroprusside enhances the growth of peanuts (*Arachis hypogaea* L.) under drought stress

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Abstract

Peanuts are a nutrient-dense legume with high lipid, protein, vitamin and mineral content. Peanut development is harmed by drought stress, particularly during the germination and seedling stages. Finding ways to mitigate the impacts of drought stress will have positive effects on peanut production. Seed priming, a short-gun strategy for modulating the impact of abiotic stressors on agricultural plants, has lately piqued the attention of researchers to instill drought tolerance in important crops. In this study, peanut seeds (VD01-2 cultivar) were used as material to investigate the role of priming with sodium nitroprusside at different concentrations (10, 15, 20 and 25 mg L-1) in preventing the damage of peanuts triggered by drought stress. Morphological, physiological and biochemical changes during the development of peanuts in the drought stress condition were analyzed. The results show that moderate drought stress (60% of field capacity) reduced germination and seedling growth. Drought stress reduced relative water content, photosynthesis, and the content of chlorophyll and starch significantly over the control. Seed priming with 20 mg L⁻¹ sodium nitroprusside was effective in increasing these above mentioned growth parameters. Further, the priming of 20 mg L-1 sodium nitroprusside enhanced respiration rate and carotenoid, soluble sugar and proline content compared to the control.

Keywords

Arachis hypogaea, drought, peanuts, seed priming, sodium nitroprusside

Introduction

Peanut (*Arachis hypogaea* L.) is one of the world's most important oil and commercial crops. It is a key source of vegetable oil, protein and antioxidants (1, 2). Peanuts are farmed in over 120 countries on a total area of 24.6 million ha, with a global yield of 38.2 million tonnes during 2000 and 2008 (3). The low peanut yields observed in many countries in Africa and Asia are related to water shortages. Droughts are common in these agricultural lands and peanut production suffers as a result. It has become a major stumbling block to increasing peanut output and quality. Season, duration and severity of drought are key variables affecting peanut growth (4). Although peanuts are drought-resistant, water scarcity has a significant impact on growth at some stages. As a result, increasing the drought tolerance of cultivars has become a priority.

Seed priming is a hydration strategy for osmotic up-regulation that accelerates the essential metabolites (5). Seed priming has been proposed as a viable

technique for enhancing plant tolerance to extended stress exposure by increasing nutrient absorption, increasing water usage efficiency, breaking seed dormancy and enhancing crop physiology (6, 7). Water-based, plant growth regulator-based, osmotic solution-based, chemical-based and other priming treatments are often used to improve drought tolerance in various agricultural plants. Although these approaches have a similar feature (partial pre-hydration and early activation of germination processes in seeds), their effectiveness is significantly dependent on the plants being treated and the priming agent used (8). Sodium nitroprusside (SNP) is among the most widely used nitric oxide donors, an essential signaling molecule that has been shown to modulate growth and developmental processes (9). Concentration dependent effects have been seen in reports detailing its favorable influence on the control of growth and stress tolerance in plants (10). The optimal level of NO has been shown to minimize the negative impacts of drought stress by upregulating stress resistance mechanisms such as antioxidant systems and osmolyte accumulation. NO's positive effects assist greatly to growth maintenance by preventing harm to essential cellular functions such as photosynthesis (11, 12). In peanuts, seed priming with 150 μM SNP increased seed germination under the -0.8 MPa drought stress. Moreover, seeds primed with SNP had enhanced soluble sugars, protein content and antioxidant enzyme activities (13). However, the changes in photosynthetic pigments and gas exchange capacity (photosynthesis and respiration rate), which are important indicators to assess drought tolerance, have not been analyzed. The significance of sodium nitroprusside in reducing drought stress damage in peanuts has not been clarified and needs further investigation. Thus, the objective of the study was to evaluate the impact of seed priming techniques with sodium nitroprusside on growth parameters on the physiological and biochemical basis in peanuts under drought stress conditions.

Materials and Methods

Plant materials

Seeds of VD01-2 peanut were collected from the Research Institute of Oil and Oil plant, Ho Chi Minh City, Vietnam.

Effect of drought conditions on peanut growth

Seeds were sowed in pots containing normal garden soil at room temperature for growth. Experimental soil had the following physical and chemical properties: an organic matter of 24.91 g kg⁻¹, total N of 0.165%, available phosphorous of 0.062%, and available potassium of 0.93%. The content of Zn, B, Cu, and Mo in soil was 733 mg kg⁻¹, 98 mg kg⁻¹, 26 mg kg⁻¹, and 0.9 mg kg⁻¹ respectively. Drought stress was induced by decreasing the field capacity of soil (FC): 75% (no-drought stress), 70%, 65%, and 60%. During the drought period, the water content in pots was kept by a moisture sensor system and drip irrigation system.

Effect of SNP priming on germination and growth of peanut under the drought stress

Seeds were separately soaked in different sodium nitroprusside solutions: 0 (the control), 10, 15, 20, 25 mg L^{-1} for 18 h at room temperature (30 \pm 2 °C). The ratio of seed

weight (g) to solution volume (ml) was 1:4. Then, seeds were washed 5 times with distilled water to remove sodium nitroprusside (13). Seeds were dried back closer to the original moisture content at 30 °C (4 hr). Seeds were sowed in pots containing soil at 60% of field capacity (moderate drought stress).

Anatomical observations

Anatomical analyses of leaves were conducted by obtaining cross-sections (500 μ m in thickness) through free-hand sectioning. The sections were cleared with 5% NaClO solution for 15 min and rinsed with distilled water (3 times). Then, the sections were flooded with acetic acid 5% (5 min) and washed with distilled water (3 times). Following washing in distilled water, the sections were stained with carmine - iodine dye for 3 mins. Finally, the sections were washed by flooding them with water (repeat until the sections were free of excess dye) and observed under a light microscope (14).

Plant growth parameters

After five days of treatment under drought stress, the germination percentage, mean germination time and root length were measured. Germination parameters were calculated (15). After 14 days, the shoot height, leaf number, total leaf area and root length of seedlings were determined. Fresh weight was measured immediately after picking and dry weight was measured after drying the leaves in an oven at 70 °C until a constant weight was achieved. Turgid weight was measured after floating the leaves in distilled water (4 hr) at room temperature. Relative water contents (RWC) of leaves were calculated according to the formula (16).

Wilt index was measured following the standard method described (17). The peanut wilt grades were visually evaluated. Namely, at grade 0: the peanut leaves were naturally expanded, bright, and glossy. At grade 1: the leaves began to lose water, were dull, and the top leaf was slightly drooping. At grade 2: the drooping of the leaves became more pronounced. Some of the leaves in grade 3 were dry, stiff, and curled. At grade 4: all leaves were drooping, shrinking and turning yellow. At grade 5: the plants had perished and the leaves were entirely dry and hard.

The measure of chlorophyll and carotenoid content

Pigment parameters of the leaves (chlorophyll a, b and carotenoid) were measured following the method (18). 500 mg of leaf samples were ground with 5 mL 95% ethanol solution and then this extract was centrifuged at 3000 g (5 min). Absorbance was measured at 664 nm, 648 nm and 470 nm by using a spectrophotometer.

The measure of respiration and photosynthesis rate

The respiration and photosynthesis rate were determined by using an oxygen electrode with the LD2 electrode chamber of the Leaf Lab 2 system (Hansatech, United Kingdom). The leaf was placed in a chamber with a layer of capillary matting which added 0.1% bicarbonate solution to produce saturating CO2. The chamber was controlled at 28 \pm 2°C and light condition at 0 lx (respiration rate) or

10000 lx (photosynthesis rate). The photosynthesis rate was calculated based on the amount of oxygen released instead of the amount of oxygen absorbed in the respiratory rate (19).

The measure of soluble sugar and starch content

For soluble sugar and starch quantification, 500 mg leaf samples were hydrolyzed with 5 ml of HCl (2,5 N), centrifuged to take the supernatant (supernatant 1). The residue was hydrolyzed by 6.5 ml of HClO₄ (52%) for 24 hr, centrifuged and obtained the supernatant 2. Then, 1 ml of the supernatant 1 was assayed with 1 ml of phenol (5%) and 5 ml of sulfuric acid (96%). On the hand, 1 ml of the supernatant 2 was assayed with 4 ml of the DNS reagent (heated for 5 min). The absorbance of the mixture 1 was read at 490 nm and converted to soluble sugar content using a sucrose standard curve (20). The absorbance of the mixture 2 was read at 540 nm and converted to starch using a glucose standard curve (21).

The measure of proline content

The free proline content was extracted and determined by following the standard method (22). 500 mg of leaf samples were homogenized in 5 ml ethanol (95%), centrifuged at 5000 g for 10 min to take the supernatant. 1 ml of the supernatant was mixed with 2 ml acid ninhydrin in a test tube at 100 °C for 60 min. Then, the absorbance of the mixture was measured at 520 nm. Proline content was quantified spectrophotometrically at 520 nm using L-proline as a standard.

Statistical analysis

The experimental treatments were performed in three independent replicates. The data were subjected to the analysis of variance (ANOVA) valid for a randomized block design. Mean separation was done following Duncan's Multiple Range Test at 5% level of probability using the SPSS 20.0. Values are displayed as mean with standard deviation.

Results

Effects of drought stress on plant growth

After 14 days of treatment under drought conditions, shoot height, leaf number, the total leaf area, and root number significantly decreased in the 60% FC treatment. At 60% of field capacity, these growth parameters decreased by approximately 50% compared to the 75% FC (Table 1). In addition, the wilt index of 60% FC treatment sharply increased to 2.60 \pm 0.24. Thus, the FC of soil at 60% was used as the moderate drought stress condition.

Effects of seed priming with SNP on seed germination and plant growth under drought stress

Under the drought stress condition, seed priming with SNP caused significant decreases in mean germination time and increases in different growth criteria (germination ratio and root length of seedlings) compared with the control (Table 2). Furthermore, SNP treatments of 15, 20, and 25 mg L⁻¹ not only enhance germination metrics under drought stress but also boost the above mentioned criteria as compared to the control.

After 14 days of treatment under drought stress, shoot height, leaf number, total leaf area, and root length of plants reached the highest values at the SNP concentration of 20 mg.L⁻¹ but reduced at lower or higher SNP concentrations (15 and 25 mg L⁻¹) (Table 3). Relative to control, SNP-primed treatments at 10 and 20 mg L⁻¹ exhibited a significant increase in RWC (Fig. 3). Similarly, priming of SNP to drought-stressed seedlings ameliorated the wilt index (Table. 3). Both SNP levels (15 and 20 mg L⁻¹) significantly reduced the wilt of leaves compared to the control. Plants grew from seeds treated with SNP priming developed with fresh, expanded, bright, and glossy leaves, while the leaves of the others non-treated with SNP became wilt (Fig. 1).

The anatomical structure of peanut leaflets under the drought stress showed that the midrib consisted of parenchyma and palisade mesophyll containing many chloroplasts. Palisade mesophyll was located below the adaxial epidermal cells and interrupted with compact collenchyma cells. The midrib has a vascular bundle bordered by fibers that are characteristically smaller and more compact than neighboring parenchyma. Moreover, bundle sheath cells had been noted around the vasculature in peanuts. In the 20 mg L⁻¹ SNP priming treatment, the midrib has 2–3 vascular bundles separated by parenchymal cells compared to the control (Fig. 2).

Changes in photosynthetic pigments

The effect of different SNP levels on photosynthetic pigments of peanuts is shown in Fig. 4. The drought stress treatment (60% FC) caused sharp decreases in chlorophyll a and b and increases in carotenoid content of leaves. Compared with the 75% FC treatment, drought reduced chlorophyll a and b content by 54.74% and 38.16% respectively. On the other hand, SNP priming treatments from 15 to 25 mg L⁻¹ alleviate the harmful effect of drought via increases in chlorophyll a and b components. The highest increases in chlorophyll a and b were achieved by SNP application at 20 mg L⁻¹. Moreover, SNP priming at 20 mg L⁻¹ significantly enhanced carotenoid content with a % increase of 39.89% compared to the control.

Table 1. Effect of field capacity of soil (FC) on seedling growth of peanuts after 14 days of treatment

FC%	Shoot height (cm)	Leaf number	Total leaf area (cm²)	Root length (cm)	Wilt index
75	9.90 ± 0.74 ^a	3.20 ± 0.45 ^a	7.83 ± 1.22 ^a	9.76 ± 0.44 ^a	0.00 ± 0.00 ^c
70	9.70 ± 0.44 a	3.10 ± 0.12 ^a	7.71 ± 1.05 ^a	8.92 ± 0.32 b	0.00 ± 0.00 ^c
65	7.10 ± 0.24 b	2.40 ± 0.24 b	4.55 ± 0.16 b	$5.15\pm0.18^{\circ}$	1.20 ± 0.20 b
60	4.14 ± 0.13 ^c	1.40 ± 0.55 °	2.37 ± 0.09 °	3.06 ± 0.09 d	2.60 ± 0.24 a

Values with different letters in a column are significantly different according to Duncan's test (p=0.05)

Table 2. Effect of seed priming with sodium nitroprusside (SNP) on seed germination of peanuts after 5 days of treatment under drought stress (60% FC)

SNP (mg L ⁻¹)	Germination time (h)	Germination (%)	Root length (cm)
0 (Control)	121.50 ± 1.53 ^a	46.00 ± 5.48 °	0.56 ± 0.05 °
10	31.20 ± 2.39 ^b	80.00 ± 7.07 b	0.48 ± 0.05 °
15	27.20 ± 2.28 ^{cd}	98.00 ± 4.47 a	$1.03\pm0.10~^{\rm b}$
20	26.00 ± 0.71 d	98.00 ± 4.47 a	1.49 ± 0.03 ^a
25	29.60 ± 1.14 bc	98.00 ± 4.47 ^a	1.50 ± 0.02 a

Values with different letters in a column are significantly different according to Duncan's test (p=0.05)

Table 3. Effect of seed priming with sodium nitroprusside (SNP) on seedling growth of peanuts after 14 days of treatment under drought stress (60% FC)

SNP (mg L-1)	Shoot height (cm)	Leaf number	Total leaf area (cm²)	Root length (cm)	Wilt index
0 (Control)	4.24 ± 0.05 ^a	2.00 ± 0.00 b	2.77 ± 0.15 ^c	4.84 ± 0.18 a	2.80 ± 0.45 ^a
10	3.04 ± 0.11 $^{\rm b}$	2.00 ± 0.00 b	3.26 ± 0.11 b	2.54 ± 0.11 d	1.00 ± 0.00 b
15	3.16 ± 0.15 b	2.00 ± 0.00 b	4.04 ± 0.11 ^a	3.04 ± 0.15 °	0.00 ± 0.00 ^c
20	4.06 ± 0.11 ^a	2.60 ± 0.55 ^a	4.12 ± 0.08 ^a	3.96 ± 0.11 b	0.00 ± 0.00 ^c
25	3.82 ± 0.48 a	2.00 ± 0.00 b	3.50 ± 0.07 b	3.92 ± 0.19 b	1.00 ± 0.00 b

Values with different letters in a column are significantly different according to Duncan's test (p=0.05)

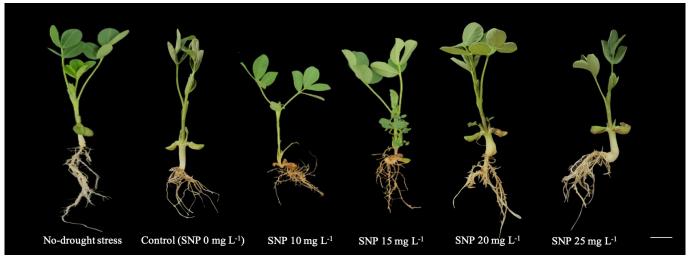


Fig. 1. Effect of seed priming with sodium nitroprusside (SNP) on seedling growth of peanuts after 14 days of treatment under drought stress (60% FC). Scale bar = 1 cm.

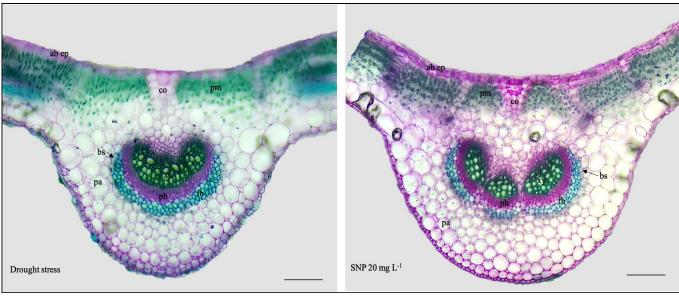


Fig. 2. Effect of seed priming with sodium nitroprusside (SNP) on anatomical changes of peanut leaves after 14 days under drought stress (60% FC). Scale bar = 150 Mm. pm, palisade mesophyll; co, collenchyma; xy, xylem; ph, phloem; pa, parenchyma; fb, fibers; bs, bundle; ab ep abaxial epidermis.

Changes in gas exchange parameters

Drought (60% FC) reduced photosynthesis rate by 30.00% but increased 18.55% in respiration rate compared to the

75% FC treatment. SNP priming mitigated the drought-induced decline to a considerable extent, imparting 36.95% and 6.80% amelioration in photosynthesis and

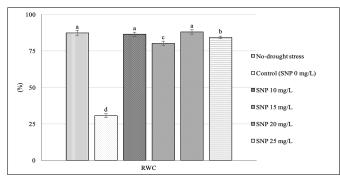


Fig. 3. Effect of seed priming with sodium nitroprusside (SNP) on relative water content (**RWC**) of leaves after 14 days of treatment under drought stress (60% FC). Values with different letters in a column are significantly different according to Duncan's test (p=0.05).

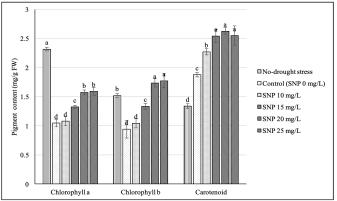


Fig. 4. Effect of seed priming with sodium nitroprusside (**SNP**) on chlorophyll and carotenoid content of peanut leaves after 14 days of treatment under drought stress (60% FC). Values with different letters in a column are significantly different according to Duncan's test (p=0.05).

respiration rate respectively, compared to the control (Table 4).

Changes in soluble sugar, starch and proline content

Results showing the effect of drought and SNP priming on the accumulation of soluble sugars, starch, and proline are shown in Table 4. Drought stress (60% FC) resulted in increased soluble sugar and proline accumulation compared to the 75% FC treatment, with maximum increases of 49.48% and 619.00% respectively. The seed priming with 20 mg L⁻¹ SNP was found to be effective in improving the content of soluble sugars and proline by 1.21-fold, and 1.41-fold respectively, over the control. The

Discussion

Germination rate, germination time, and root length of seedlings are critical for plant growth rate under stress conditions (23). Seed priming, as evidenced by several reports, increases the tolerance of plants when exposed to stress. This study found that seed priming with SNP improved peanut seed germination (Table 2). Under stress conditions, 0.1 mM SNP supplementation enhanced the germination rate in Triticum aestivum (24). SNP supplementation at lower doses significantly improved seed germination in Oryza sativa (25). Seed germination, seedling length and biomass of Brassica chinensis were observed to be escalated with SNP treatments (26). It was opined that the increase in germination rate could be explained by genetic and structural repair as well as the rapid development of immature embryos caused by seed priming (27). Moreover, seed priming was reported to diminish physiological non-uniformity in seeds, which might lead to a greater seed germination rate and more synchronized germination. At a physiological level, priming treatments cause distinct metabolic changes in seeds as the imbibition process begins. Major cellular activities in seeds, including synthesis of proteins and nucleic acids, ATP generation, antioxidant activation, and DNA repair mechanisms, are regulated by rehydration during seed priming (28). Various storage proteins that occur exclusively during seed priming were found during a proteome investigation of Arabidopsis seed germination following priming (29). Other reserve mobilization enzymes, such as isocitrate lyase (for lipid mobilization) and amylases (for carbohydrates), are also induced by seed-priming treatments (30). Upregulation of the tubulin subunit proteins, which are critical in cell signaling, occurs during priming treatments (31).

The adverse effects of drought stress on plant growth may be attributed to the low osmotic potential of the soil solution. Furthermore, drought stress altered physiological and biochemical processes (reduction in carbon fixation and photosynthesis), resulting in decreased plant growth and yield. The detrimental effects of dryness on peanut growth (Table 3) are congruent with those reported earlier on soybean and on mung bean (32, 33). However, SNP priming boosted peanut growth under

Table 4. Effect of seed priming with sodium nitroprusside (SNP) on physiological and biochemical changes of peanut leaves after 14 days of treatment under drought stress (60% FC)

		Drought stress		
Physiological and biochemical changes	No-drought stress —	Control (SNP 0 mg L-1)	SNP 20 mg L ⁻¹	
Respiration rate (μmol O ₂ g ⁻¹ h ⁻¹)	45.13 ± 1.25 °	53.50 ± 0.93 ^b	57.14 ± 0.28 ^a	
Photosynthesis rate (μ mol O_2 cm $^{-2}$ h^{-1})	66.67 ± 1.00 a	46.44 ± 0.99 °	63.60 ± 0.67 ^b	
Total soluble sugar content (mg g¹)	18.31 ± 0.66 °	27.37 ± 0.36 ^b	33.14 ± 0.22 a	
Starch content (mg g ⁻¹)	37.82 ± 1.04 a	23.01 ± 1.46 °	33.44 ± 0.69 b	
Proline content (mg g ⁻¹)	0.21 ± 0.02 ^c	1.51 ± 0.04 b	2.13 ± 0.16 ^a	

Values with different letters in a row are significantly different according to Duncan's test (p=0.05)

content of starch declined under the drought condition with a reduction of 39.16% compared to the 75% FC treatment. However, seed priming with SNP increased starch content by 45.33% compared to the control.

drought stress (Table 3). The effect of SNP on peanut growth criteria could be attributed to an increase in relative water content by the maintenance of cellular osmotic adjustment (34). This can be seen clearly in the increase in RWC (Fig. 3).

The amount of chlorophyll is an important significant indicator of photosynthetic efficiency (35). Drought stress reduced the amount of chlorophyll a and b in peanuts (Fig. 4). The increased formation of reactive oxygen species (ROS) and oxidative stress that damaged chloroplast membranes might explain these declines. Furthermore, these decreases might be viewed as an essential regulatory step in preventing excessive light absorption and hence reducing ROS formation (36). When compared to the non-priming treatment, SNP treatments resulted in significant increases in photosynthetic (chlorophyll and carotenoid) (Fig. 4). This promotive effect of SNP in increasing chlorophyll might be due to the reduction of lipid peroxidation and ROS production (37). Moreover, SNP enhanced chlorophyll level, carbonic anhydrase and nitrate reductase activity by preserving membrane integrity (38). Carotenoids have a key part in plant defense against drought stress because of removing singlet oxygen. Thus, carotenoids synthesis was enhanced during drought stress conditions. Under stress conditions, the application of SNP promoted gaseous exchange (photosynthesis and respiration) in barley seedlings (39) and tomatoes (40). In B. juncea, SNP improved photosynthesis in response to stress by increasing the size of the chloroplast and the shape of thylakoids (41). In addition, SNP-mediated increases in the absorption and transport of several macro-and micronutrients were shown to boost photosynthesis and respiration (42).

Increased buildup of carbohydrates, sugars, and proline due to SNP priming was observed in the current research under drought stress (Table 4). Compatible organic osmolytes safeguard membrane architecture, enzyme activity and water content in cells, preventing stress-induced damage. The study demonstrated that SNP treatments result in increased accumulation of proline under stress conditions (13). Increased accumulation of osmolytes like proline results from improved nitrogen assimilation (43). Moreover, SNP treatments can increase the activity and transcript levels of 1-pyrroline-5carboxylate synthase (P5CS), the primary enzyme in proline production (14). Under stress conditions, exogenous SNP treatments convert starch to sugars in wheat by increasing the function of α - and β -amylase (15). Similarly, by enhancing efficient amylase activity, SNP pre-treatment of wheat under stress conditions considerably minimized unfavorable impacts (44). Furthermore, SNP may also impact soluble sugars through its involvement in photosynthetic regulation (45).

Conclusion

The peanut was drought-stressed at 60% FC. In the drought stress condition, the shoot height, leaf number and area and root length decreased sharply. Similarly, the chlorophyll content, photosynthetic intensity, RWC and starch content dropped, but respiration intensity, carotenoid, soluble sugars and proline content increased. The seed priming with SNP at 20 mg L⁻¹ increased seed germination and seedling growth (improving in shoot height, leaf number and area and root length). The RWC, respiration, photosynthesis, starch, soluble sugar and proline content of leaves were higher than the control.

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Authors contributions

TTT carried out the experiments and drafted the manuscript. HTT and VBT conceived of the study and participated in manuscript editing, its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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