







Effect of Glycine Betaine and Thiamine Treatments on Germination and Seedling Growth in Eggplant under Salt Stress

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ABSTRACT: This study was carried out to determine the effect of some applications on seed germination and seedling growth of eggplant under salt stress. Egg plant seeds treated with different concentrations of glycine betaine (GB) (5, 10 and 25 μ M) and thiamine (Th) (0.50, 0.75 and 1.00 μ M) were tested at 0, 75 and 150 mM of NaCl doses. In the study, it was found that Th and GB applications increased germination percentage and decreased mean germination time compared to control and decreased the negative effect of salt stress. The best treatment for germination percentage and germination time was 25 μ M GB. Similarly, salt stress had a negative effect on emergence percentage and plant growth parameters such as seedling leaf area, root and leaf fresh and dry weight of eggplant seedlings. GB and Th seed treatments improved these parameters in eggplant seedlings grown under salt stress compared to untreated control and it was determined that salt stress reduced negative effects. According to the results obtained from the study, it is thought that Th and GB treatments can be used as an alternative method in reducing the negative effects of salt stress in eggplant.

Keywords: Salt stress, Eggplant, Hormone, Germination, Seedling

Glisin Betain ve Tiamin Uygulamalarının Tuz Stresi Altında Patlıcanda Çimlenme ve Fide Gelişimi Üzerine Etkisi

ÖZ: Bu araştırma tuz stresi altında patlıcanda bazı uygulamalarının tohum çimlenmesi ve fide gelişimi üzerine etkisini belirlemek amacıyla yürütülmüştür. Farklı konsantrasyonlardaki glisin betain (GB) (5, 10 ve 25 μ M) ve tiamin (Th) (0.50, 0.75 ve 1.00 μ M) ile muamele edilen patlıcan tohumları, 0, 75 ve 150 mM NaCl seviyelerinde denenmiştir. Çalışmada, tuz stresinin patlıcanda çimlenme oranı ve ortalama çimlenme zamanını önemli seviyede olumsuz etkilediği tespit edilmiştir. Bununla birlikte, Th ve GB uygulamalarının kontrole göre çimlenme oranını artırdığı, çimlenme zamanını ise azalttığı ve tuz stresinin olumsuz etkisini azalttığı saptanmıştır. Çimlenme oranı ve çimlenme zamanı için en iyi uygulamanın 25 μ M GB olduğu gözlemlenmiştir. Benzer şekilde, tuz stresi patlıcanda, çıkış oranı fide yaprak alanı, kök ve yaprak yaş ve kuru ağırlık gibi bitki gelişim parametreleri üzerine olumsuz etki yapmıştır. GB ve Th tohum uygulamaları tuz stresi altında yetiştirilen patlıcan fidelerinde incelenen bu parametreleri uygulama yapılmayan kontrole göre iyileştirmiş ve tuz stresinin olumsuz etkilerini azaltmıştır. Çalışmadan elde edilen sonuçlara göre tohumdan uygulanan Th ve GB'nin patlıcanda tuz stresinin olumsuz etkilerini azaltmada alternatif bir yöntem olarak kullanılabilirliği düşünülmektedir.

Anahtar Kelimeler: Tuz stresi, Patlıcan, Hormon, Çimlenme, Fide

INTRODUCTION

One of the most important factors limiting agricultural production in the world is reported to be salinity. Salinity is a factor affecting all metabolism including plant morphology and anatomy (Levitt, 1980). The severity of these effects of salinity varies depending on both the growth period of the plant and the genotype (Shannon and Grieve, 1999). For example, it has been reported that the stages where plants are most susceptible to salt are germination and seedling growth, and salt tolerance increases as

growth and development progresses (Ashraf, 1994). On the other hand, Demir et al. (2003) found that the seedling period was more sensitive to salt stress compared to the germination stage in a Pala variety of eggplant. In addition, there may be differences in salinity tolerance and susceptibility within the same species (Yaşar, 2003) and between species (Maas, 1986). Eggplant is classified as moderately sensitive to salinity and its salinity threshold is 1.5 dSm⁻¹ (Ünlükara et al., 2010). Salinity has been reported to

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delay and reduce germination of vegetables such as melon (Botia et al., 1998), tomato (Cuartero and Fernández-Muñoz, 1998) and eggplant (Akinci et al., 2004). It has been reported that reduced seed germination due to salt stress may result from induction of dormancy, osmotic stress or specific ion toxicity (Shannon and Grieve, 1999).

Attempts to increase salinity tolerance by conventional plant breeding methods are time consuming, laborious, and depend on existing genetic variability. In addition, many attempts have been made to overcome this stress, including the external use of plant growth regulators, in increasing plant tolerance to saline conditions (Javid et al., 2011). Studies have shown that indole acetic acid (IAA), cytokinins (CK), gibberellic acid (GA), brassinosteroids (BR), jasmonates (JA), salicylic acid (SA) and triazoles (TR) may have effects on salt stress in plants (Javid et al., 2011). Thiamin, also known as vitamin B1, is the first type of vitamin B identified (Funk, 1975). In plants, Th plays a role as a response molecule to abiotic and biotic stresses, and data from the literature suggest that increasing Th content can increase stress resistance (Tunc-Ozdemir et al., 2009). Glycine betaine (GB) is an organic osmolyte that accumulates in various plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation and heavy metals (Ashraf and Foolad, 2007). Studies investigating the effect of GB and Th on seed germination and seedling development in eggplant in salt stress are limited.

The aim of this study was to determine the effects of plant growth regulators such as GB and Th applied on seed germination and seedling growth in eggplant (*Solanum melongena* L.) applied at different concentrations during seed germination and seedling growth periods under salt stress.

MATERIAL AND METHOD

This research was carried out in the laboratory and climate room in Atatürk University, Faculty of Agriculture, Department of Horticulture. Eggplant (*S. melongena* L.) Topan 374 cultivar was used as plant material.

Germination Studies

After the seeds were kept in 1% sodium hypochlorite for 10 minutes, the seeds were washed extensively with tap water and then washing with pure water was repeated 2-3 times. The dried seeds were weighed to 5 g and placed into plastic containers with 20x10x10 cm dimensions and two layers of blotting paper underneath. 25 ml of GB (5, 10 and 25 μ M) and Th (0.50, 0.75 and 1.00 μ M) were then added. In addition, dry seeds without any application were used as control 1 and 25 ml of pure

water was used as control 2. The seeds of eggplant which were kept in solutions in the laboratory for 24 hours and at the end of this period the seeds were washed and spread on blotting paper to dry. The 50 dried seeds for each repetition were placed in a 15 cm petri dish with double layered blotter. Petri dishes were soaked with 10 ml of 0 mM, 75 mM and 150 mM NaCl solutions. Dishes were sealed using parafilm to prevent evaporation. Petri dishes were randomly placed in a germination cabinet at 25 ± 2 °C. Germinated seeds were counted and recorded daily. Germination trials continued for 14 days (ISTA, 1996).

Germination percentage and mean germination time were determined at the end of the germination test. Germination percentage and mean germination time were calculated using the formulas given below (Maguire, 1962).

Mean germination time (day) = $N1 / T1 + N2 / T2 + \dots + Nn / Tn$ (N: Number of germinated seeds, T: Number of days on which germination occurs)

Germination percentage (%) = $\text{number of germinated seeds} / 50 \times 100$

Seedling Studies

The seedling experiment was carried out in the climate room of Horticulture Department. After the above mentioned applications (except dry seed application), the seeds were sown in peat: perlite (2: 1, v:v) filled multi celled trays and watered with NaCl solutions prepared at the above mentioned concentrations. For each replicate 40 seeds were sown. The trays were randomly placed on the shelves in the climate room. Irrigations were repeated every two days. Seedlings were maintained at 26-18 °C (day/night), 70% relative humidity and 16/8 h photoperiod (day/night). 3 replicates were used for each application.

The seedling studies were completed 30 days after the seedling emergence, and the parameters of leaf area, seedling fresh weight, seedling dry weight, root fresh weight and root dry weight parameters of 10 seedlings randomly selected from each replicate were examined. The roots were carefully harvested from the trays, and gently washed to remove the media. Maximum attention was paid to avoid root loss. The plant material for dry weight was dried at 70°C for 48h. The leaf area was determined by leaf area meter (CI-202 Portable Laser Leaf Area Meter by CID Bio-Science, USA).

Emergence percentage was determined according to the formula;

Emergence percentage (%) = $\text{number of emerged seeds} / 40 \times 100$

Statistical Analysis

The experiment conducted with 8 treatments (include control 1 and control 2) and 4 replications for germination study and 7 treatments (include control 2) and 3 replications for seedling study. In the experiment, randomized plot design was used and the obtained data were analyzed using SPSS 20 statistical package program. Percentage data were subjected to arc-sin transformation before analysis of variance. Data were subjected to variance analysis (ANOVA) and differences of means were determined by Duncan multiple comparison test.

RESULTS AND DISCUSSION

In the study, it was determined that salt stress had a negative effect on seed germination percentage in eggplant and the lowest average germination percentage was found to be 50.56% in 150 mM NaCl application. Generally, it was observed that seed applications increased germination percentage compared to control application. The highest

germination percentage at 75 mM NaCl was obtained from 10 µM GB (69.00%) and 0.50 µM Th (74.50%), while in 150 mM was detected in 25 µM GB application with 63.50%. Pure water application (control 2) had a positive effect on germination percentage compared to the control 1 (Table 1).

In the experiment, it was generally observed that seed applications decreased germination time compared to control 1 application. At 75 mM NaCl, the highest germination time was observed in 0.50 µM (6.44 day) Th application, while the highest germination time at 150 mM was detected in the control 1 application with 8.44 day (Table 2).

Eggplant seedlings had lower emergence percentage under salt stress than those under normal conditions. 150 mM NaCl decreased the emergence percentage by 91%. However, all seed treatments increased the emergence percentage. The highest emergence percentage was observed in 10 µM GB (68.89%) and 0.75 µM Th (68.33%) (Table 3).

Table 1. Effect of GB and Th applications on seed germination percentage in eggplant under salt stress (%)

Treatments		NaCl (mM)			Mean
		0	75	150	
Control 1	-	73.50 e***	61.00 e***	41.50 g***	58.67 E***
Control 2	-	86.00 b	65.00 d	45.00 f	65.33 C
GB	5 µM	81.50 c	67.50 c	54.50 b	67.83 B
	10 µM	83.00 c	69.00 bc	52.50 bc	68.17 B
	25 µM	95.50 a	68.50 bc	63.50 a	75.83 A
Th	0.50 µM	79.00 d	74.50 a	50.50 cd	68.00 B
	0.75 µM	72.00 e	73.50 a	49.00 de	64.83 D
	1.00 µM	86.50 b	70.00 b	48.00 e	68.17 B
Mean		82.13 A***	68.63 B	50.56 C	

*** p<0.001; there is no statistical difference between the means indicated by the same lower case letter in same column

*** p<0.001; there is no statistical difference between the means indicated by the same upper case letter in same column and row

The salt stress in the eggplant statistically negatively affected the leaf area and the lowest mean leaf area was determined in 150 mM NaCl application. When each salt level was evaluated separately, it was observed that seed applications generally increased leaf area compared to control 2 application. The highest leaf area under 75 and 150 mM NaCl conditions was determined by application of 0.75 µM Th with 7.51 and 2.45 cm², respectively (Table 4).

Salt stress significantly affected seedling fresh weight in eggplant and the lowest seedling fresh weight was determined with 0.110 g at 150 mM NaCl application. The highest seedling fresh weight was found in 25 µM GB and 0.50 µM Th applications. The maximum seedling fresh weight at 150 mM NaCl was 0.143 g with 10 µM GB application (Table 5).

Table 2. Effect of Gb and Th applications on mean germination time in eggplant under salt stress (days)

Treatments		NaCl (mM)			Mean
		0	75	150	
Control 1	-	5.82 a ^{***}	6.36 ab ^{***}	8.44 a ^{***}	6.87 A ^{***}
Control 2	-	5.42 b	5.94 c	7.14 b	6.17 C
GB	5 µM	4.91 c	5.53 e	6.56 de	5.67 EF
	10 µM	5.00 c	6.29 b	6.48 e	5.92 D
	25 µM	4.98 c	5.61 de	6.61 cde	5.74 E
Th	0.50 µM	5.90 a	6.44 a	6.72 cd	6.35 B
	0.75 µM	5.45 b	5.70 d	6.50 e	5.88 D
	1.00 µM	4.67 d	5.31 f	6.80 c	5.59 F
Mean		5.27 C ^{***}	5.90 B	6.91 A	

*** p<0.001; there is no statistical difference between the means indicated by the same lower case letter in same column

*** p<0.001; there is no statistical difference between the means indicated by the same upper case letter in same column and line

Table 3. Effect of GB and Th applications on seedling emergence in eggplant seedlings under salt stress (%)

Treatments		NaCl (mM)			Mean
		0	75	150	
Control 2	-	83.33 ^{ns}	64.17 e ^{***}	7.50 e ^{***}	51.67 E ^{***}
GB	5 µM	84.17	81.67 a	14.17 d	60.00 C
	10 µM	82.50	76.67 bc	47.50 a	68.89 A
	25 µM	83.33	70.83 d	31.67 c	61.94 B
Th	0.50 µM	83.33	79.17 ab	12.50 d	58.33 D
	0.75 µM	83.33	82.50 a	39.17 b	68.33 A
	1.00 µM	83.33	75.00 c	32.50 c	63.61 B
Mean		83.33 A ^{***}	75.71 B	29.99 C	

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Table 4. Effect of GB and Th applications on leaf area in eggplant seedlings under salt stress (cm²)

Treatments		NaCl (mM)			Mean
		0	75	150	
Control 2	-	16.37 e ^{***}	5.47 d ^{***}	0.00 d ^{***}	7.28 F ^{***}
GB	5 µM	19.03 d	6.69 b	0.00 d	8.57 E
	10 µM	25.55 c	5.23 d	1.27 c	10.68 D
	25 µM	33.26 a	6.23 c	1.41 b	13.64 A
Th	0.50 µM	26.21 c	7.41 a	0.00 d	11.21 C
	0.75 µM	25.91 c	7.51 a	2.45 a	11.96 B
	1.00 µM	28.07 b	6.44 bc	0.00 d	11.50 C
Mean		24.91 A ^{***}	6.43 B	0.73 C	

*** p<0.001; there is no statistical difference between the means indicated by the same lower case letter in same column

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Considering the average values in the study, it was determined that salt stress negatively affected seedling dry weight in eggplant, and lowest seedling dry weight were observed at 150 mM NaCl with 0.08

g. When evaluated in terms of applications, it was found that 25 µM GB application positively affect the dry weight of seedling compared to control 2 (Table 6).

According to the data obtained from the experiment, it was determined that the root fresh weight decreased with the increase in NaCl concentration, and the lowest root fresh weight occurred in 150 mM NaCl with the application of 0.10 g. When the average values were taken into

consideration, the highest root fresh weight was determined in 5 µM GB and 0.50 µM Th application with 0.29 g. It was found that the seed applications used in the experiment reduced the negative effect of salt stress on root fresh weight (Table 7).

Table 5. Effect of GB and Th applications on fresh seedling weight in eggplant seedlings under salt stress (g)

Treatments		NaCl (mM)			Mean
		0	75	150	
Control 2	-	1.35 d ^{***}	0.18 c ^{***}	0.000 b ^{***}	0.51 E ^{***}
GB	5 µM	1.59 a	0.31 a	0.120 a	0.65 AB
	10 µM	1.47 bc	0.26 b	0.143 a	0.61 C
	25 µM	1.57 a	0.30 a	0.120 a	0.67 A
Th	0.50 µM	1.60 a	0.29 ab	0.127 a	0.66 A
	0.75 µM	1.50 b	0.29 ab	0.133 a	0.64 B
	1.00 µM	1.45 c	0.27 ab	0.127 a	0.59 D
Mean		1.50 A ^{***}	0.27 B	0.110 C	

*** p<0.001; there is no statistical difference between the means indicated by the same lower case letter in same column

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Table 6. Effect of GB and Th applications on dry seedling weight in eggplant seedlings under salt stress (g)

Treatments		NaCl (mM)			Mean
		0	75	150	
Control 2	-	0.247 b ^{***}	0.014 b ^{***}	0.00 f ^{***}	0.087 C ^{***}
GB	5 µM	0.277 a	0.150 a	0.06 d	0.182 AB
	10 µM	0.240 b	0.147 a	0.12 b	0.177 AB
	25 µM	0.280 a	0.153 a	0.15 a	0.184 A
Th	0.50 µM	0.270 a	0.153 a	0.08 c	0.183 AB
	0.75 µM	0.243 b	0.150 a	0.12 b	0.176 AB
	1.00 µM	0.233 b	0.150 a	0.04 e	0.170 B
Mean		0.256 A ^{***}	0.131 B	0.08 C	

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Salt stress negatively affected root dry weight in eggplant and lowest root dry weight was determined with 0.037 g in 150 mM NaCl application. At 75 mM NaCl conditions, the highest root dry weight was

observed in 25 µM GB application (0.130 g), whereas at 150 mM the highest root dry weight was determined in 0.75 µM Th application with 0.067 g (Table 8).

Table 7. Effect of GB and Th applications on fresh root weight in eggplant seedlings under salt stress (g)

Treatments		NaCl (mM)			Mean
		0	75	150	
Control 2	-	0.37 c***	0.15 e***	0.00 c***	0.17 D***
GB	5 µM	0.47 b	0.28 a	0.12 ab	0.29 A
	10 µM	0.54 a	0.16 de	0.12 ab	0.27 B
	25 µM	0.53 a	0.18 cd	0.13 a	0.28 AB
Th	0.50 µM	0.55 a	0.21 b	0.11 b	0.29 A
	0.75 µM	0.54 a	0.19 bc	0.12 ab	0.28 AB
	1.00 µM	0.48 b	0.17 cde	0.12 ab	0.25 C
Mean		0.49 A***	0.19 B	0.10 C	

*** p<0.001; there is no statistical difference between the means indicated by the same lower case letter in same column

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Table 8. Effect of GB and Th applications on dry root weight in eggplant seedlings under salt stress (g)

Treatments		NaCl (mM)			Mean
		0	75	150	
Control 2	-	0.090 d***	0.023e***	0.000 d***	0.038 C***
GB	5 µM	0.147 c	0.067 d	0.050 b	0.088 B
	10 µM	0.160 b	0.120 ab	0.047 b	0.109 A
	25 µM	0.157 b	0.130 a	0.023 c	0.103 A
Th	0.50 µM	0.173 a	0.110 abc	0.043 b	0.109 A
	0.75 µM	0.160 b	0.107 bc	0.067 a	0.111 A
	1.00 µM	0.150 bc	0.093 c	0.027 c	0.090 B
Mean		0.148 A***	0.093 B	0.037 C	

*** p<0.001; there is no statistical difference between the means indicated by the same lower case letter in same column

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Abiotic stress conditions are recognized as a major threat to plant growth and crop productivity worldwide. Stress conditions that negatively affect crop production have been reported to cause more than 50% reduction in yield (Maggio et al., 2005). Salinity adversely affects almost every aspect of plant physiology and biochemistry and significantly reduces yield. This is one of the most serious threats to the major environmental factors that limit agricultural production and productivity (Ashraf et al., 2008; Munns and Tester, 2008). The aim of this study was to determine the effect of GB and Th on seed germination and seedling growth under salinity conditions in eggplant.

In the study, it was determined that different seed and salt level applications significantly affected seed germination and seedling growth in eggplant. With the increase in salinity, germination percentage and seedling emergence decreased but germination time increased. Furthermore, salinity stress caused to decrease the leaf area, seedling fresh and dry weight,

root fresh and dry weight of eggplant seedlings. However, it was determined that seed applications reduced this negative effect caused by salt stress compared to the control 1 application (Table 1-8). Eggplant is reported to be moderately sensitive to salt stress (Heuer et al., 1986; Savvas and Lenz, 1996). However, tolerance to salt stress varies between eggplant varieties (Ünlükara et al., 2010). A 50% yield loss of 8.5 dSm⁻¹ soil salinity was observed in the eggplant (Shalhevet et al., 1983). Chartzoulakis and Loupassiki (1997) concluded that the first growth stages, ie germination and seedling stages, are the most sensitive to salinity stress. For example, salt (NaCl) stress has been reported to cause a significant reduction in germination percentage and ratio, root and hypocotyl fresh and dry weights (Akinci et al., 2004). Tıprıdamaz and Ellialtıođlu (1997) reported that salt stress caused a decrease in plant height, dry matter amount and chlorophyll content in eggplant. Yaşar et al. (2006) reported a decrease in growth

parameters such as shoot length, leaf area and leaf dry matter in eggplant at 50 mM NaCl.

In our study, it was found that GB and Th applications reduced the negative effects of salt stress on eggplant during seed and seedling period. In parallel with our findings, plant growth regulators such as tryptophan (Gerekli, 2015), IAA and IBA (Iqbal and Ashraf, 2006), melatonin (Li et al., 2012), GB (Şirikçi, 2010; Abbas et al., 2010), GA, kinetin and ethylene (Çavuşoğlu et al., 2007), 24-epibrassinolide (EBR) (Ding et al., 2012), jasmonic acid (JA) (Manar et al., 2013) and salicylic acid (SA) (Khodary, 2004) have been reported to reduce the negative effect of salt stress on many plant species including eggplant. Abbas et al. (2010) suggested that the use of GB and sugar beet extracts reduced the negative effects of salt stress on two eggplant varieties. Similarly, it has been found that GB applications reduce the negative effects of various stress factors in rice (Cha-um and Kirdmanee, 2010), tomato (Li et al., 2011) and pepper (Korkmaz and Şirikçi, 2011). GB has important functions in the involvement of enzymes and complex proteins, in stabilizing the lipids of photosynthetic structures and maintaining the stability of the membranes under stress conditions (Papageogiou and Murata, 1995; Xing and Rajashekar, 1999).

In the study of Pushpalatha et al. (2011) 5, 10, 15, 20 and 25 mM concentrations of Th were applied to seeds of *Pennisetum glaucum* (L). In the experiment, 20 mM thiamine seed application was found to have a positive effect on plant growth. It has been reported that Th treatment to seed induces rapid LOX gene expression and provides resistance to hairy mold diseases. In two mustard varieties (Rohini and Varuna), the effect of different concentrations of thiamin hydrochloride (0.01, 0.02 and 0.03%) was investigated on plant growth. At the end of the study, it was found that immersion of Varuna mustard seeds in 0.03% thiamine hydrochloride solution provided better growth and nutrient uptake under local conditions (Sajjad et al., 2017).

Many strategies have been proposed, including mechanical, chemical and biological, to cope with the harmful effect of salt stress and improve plant growth and productivity. Recently, exogenous application of GB-containing organic and inorganic chemicals to stressed plants has attracted attention (Habib et al., 2012). The effect of GB on plant growth may be due to the neutralization of salt stress-induced toxicity. Our findings are in accordance with those of Abbas et al. (2010) who suggested that GB application improves the negative effect of salt stress on eggplant growth. Th formation in plants has spread to organs such as leaves, flowers, fruits, seeds, roots, tubers and onions. In plants, Th is known to play a co-factor role for important metabolic activities (Colinas and

Fitzpatrick, 2015). Th has been reported to be a major regulator that plays an important role in the plant's primary regulatory system (Bocobza and Aharoni, 2014). In plants, Th plays a role as a response molecule against abiotic and biotic stresses, and data from the literature suggest that increasing Th content can increase stress resistance (Dong et al., 2016). In addition, Th has been reported to have antioxidant capacity (Asensi-Fabado and Munné-Bosch, 2010).

In our study, it was determined that salt stress negatively affected seed germination and seedling growth in eggplant. However, GB and Th applications improved the parameters examined in eggplant grown under salt stress compared to untreated control. According to the results obtained from the study, GB and Th can be used as an alternative method in reducing the negative effects of salt stress on eggplant during germination and seedling.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

EY, ME, and RK conceived and designed research. FG, EY, ME and RK set up and conducted the experiment. ME analyzed the data. EY ME, and RK wrote the manuscript. All authors read and approved the final manuscript.

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