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Tailoring Indole-3-Butyric Acid Concentrations for Effective Grape Cultivar Propagation: Insights from Cutting Studies

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Abstract

Grapes (*Vitis vinifera*) are woody perennials propagated mainly via cuttings, with berries that can be green, pink, golden, black, red, or purple. In this study, we investigated the effect of Indole-3-Butyric Acid (IBA) concentrations on the propagation of grape cultivars using cuttings in the monsoon season under greenhouse conditions. The experiment was designed as a Completely Randomized Design (CRD) with each treatment replicated three times. The medium used was solarized sand, free from pathogens and weed seeds. Cuttings, 6–8 inches long and from three grape cultivars ("King's Ruby", "NARC Black", and "Flame Seedless"), were treated with 0 (control), 2000, 3000, and 4000 ppm IBA concentrations. Various parameters were measured, such as the number of leaves per cutting, shoot length, rooting percentage, and survival percentage. The data showed a significant effect of IBA concentrations ($p<0.05$). "King's Ruby" showed the highest number of leaves and roots per cutting at 4000 ppm IBA, while **"**Flame Seedless" responded with the most extended shoot and root length at 2000 ppm IBA. Interestingly, **"**Flame Seedless" without IBA application had the best shoot diameter, rooting percentage, and survival rate. These results suggest that grape cultivars respond differently to IBA concentrations.

Keywords: Berries**,** Cultivar, Plant growth regulator, Stress, Micro-propagation
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Introduction

Grape (*Vitis vinifera*) belongs to the family Vitaceae and is a species of the genus *Vitis*. It is native to central Europe, southwestern Asia, and the Mediterranean region, stretching from northern Portugal and Morocco to southern Germany and east to northern Iran (Galavi et al., 2013). Grapes are woody perennials, and their fruits are considered berries (Sajid et al., 2006). The genus *Vitis* comprises approximately 60 species, which are divided into two sections: *Muscadinia* (2n = 40) and *Euvitis* (2n = 38), based on chromosome number and external morphological features (Wan et al., 2013). Grapes are frequently mentioned in the *Holy Quran* and are among the first fruits cultivated by humans. Since the dawn of civilization, the fermented product of grapes, *wine*, has possibly been a significant way of consuming grapes (McGovern et al., 1996).

In Pakistan, mostly European grape varieties are cultivated. They are non-climacteric fruits that grow in bunches. They are among the most remunerative summer fruits, native to hot, temperate regions between 34°N and 49°S latitude. Sub-mountainous and mountainous zones up to 2000 meters above sea level are suitable for farming (Khan et al., 2009; Nache Gowda et al., 2006). In Pakistan, grapes are primarily grown in the Balochistan region, certain districts of Khyber Pakhtunkhwa, and Gilgit-Baltistan, with an annual production of 122,000 tons and an average yield of 19 tons per hectare, compared to a potential yield of 25 tons per hectare (GOP, 2010). However, due to monsoon rains in central Punjab, grape production declines, which suggests that cultivating early-maturing grape varieties could be more fruitful (Khan et al., 2011). The demand for grapes continues to rise due to their nutritional value and importance in the fruit industry. Commercial grape varieties are primarily propagated through vegetative means, mainly by cuttings.

Auxin, a plant growth regulator (PGR) also known as a phytohormone, plays a key role in promoting adventitious rooting in plant cuttings (Da Costa et al., 2013). Phytohormones play a crucial role in plant cell growth and division, directly influencing rooting and interacting with other plant hormones and growth promoters (Shiozaki et al., 2013). PGRs significantly enhance the propagation potential of grape cuttings (Kelen & Ozkan, 2003) and facilitate root formation in cuttings (Somkuwar et al., 2011). Previous studies have also indicated that phytohormones significantly affect the growth performance of grape cuttings (Yadav et al., 2012). Adventitious root formation and callusing, both critical processes in propagation, are enhanced by auxin (Satish et al., 2007; Galavi et al., 2013). Grapevine cuttings treated with plant growth regulators exhibit significant effects on plant regeneration. Rootstock rooting ability varies with the species, indole-3-butyric acid (IBA) levels, and mother vines (Satish et al., 2007). Generally, IBA is the best root promoter for many plant species (Davies Jr et al., 2017). Therefore, this study investigates the impact of varying IBA concentrations on the propagation of grape cultivars through cuttings.

Materials and Methods

The experiment was conducted at the experimental nursery of the Fruit Crops Research Program, Horticultural Research Institute (HRI), National Agriculture Research Centre (NARC), during the 2018 monsoon season.

Experiment design, growth condition, crop husbandry

The experiment was conducted from July to September 2018 at the Horticulture Research Institute, NARC, Islamabad, in a greenhouse located at 33.42°N latitude and 73.08°E longitude, at an elevation of 683 meters. The area receives approximately 1000 mm of rainfall annually. A completely randomized design (CRD) with three replicates per treatment was used. Raised beds filled with solarized sand, free of weed seeds and pathogens, were prepared inside the greenhouse. Cuttings of three grape cultivars (King's Ruby, NARC Black, and Flame Seedless), measuring 6–8 inches and with 3–4 nodes, were taken from mature vines. Each cutting was treated with fungicide (Ridomil Gold, 3 g/liter) for 5 minutes before planting. The cuttings were also treated with different IBA concentrations (0, 2000, 3000, and 4000 ppm). They were planted in raised beds with two buds inside the sand and two outside. Each treatment consisted of 10 cuttings, and 120 cuttings per cultivar. The greenhouse had a sprinkler system, and the cuttings were irrigated twice daily. The data were recorded using the following parameters on grape cultivar cuttings in response to different IBA concentrations.

The number of leaves was counted for six cuttings in each treatment, 40 days after planting, and the average value for each variety was calculated. Shoot length and diameter were measured for six cuttings per treatment using a meter rod and digital vernier caliper, respectively, 40 days after planting, and the average value was calculated for each variety. The sprouting percentage was recorded by counting the rooted cuttings 40 days after planting, with the percentage calculated using Owais' (2010) formula:

Rooting percentage (%) = (Number of rooted cuttings / Total number of cuttings planted) × 100.

Rooting length was measured by carefully separating six sample plants, removing the soil, washing them with tap water, and measuring the root length as described by Satisha et al. (2008). The average root length was then determined using a meter rod. The number of roots per cutting was counted 40 days after planting, and the average was calculated. The survival percentage was recorded 40 days after planting by counting the sprouted cuttings, with the percentage calculated using the formula:

Survival percentage (%) = (Number of survived plants / Total number of sprouted cuttings) × 100.

Statistical analysis

The data were analyzed using Microsoft Office Excel and Statistix 8.1 software. The Least Significant Difference (LSD) test was performed to check the variation in different traits among different varieties in response to different IBA levels.

Results and Discussion

The maximum number of leaves (10.3 leaves per cutting) was produced by the grape cultivar King's Ruby treated with 4000 ppm IBA, followed by King's Ruby (9.33 leaves per cutting) treated with 2000 ppm and 3000 ppm IBA, respectively. The minimum value was recorded in cultivar NARC Black (1.33 leaves per cutting) treated with 3000 ppm IBA (Figure 1).

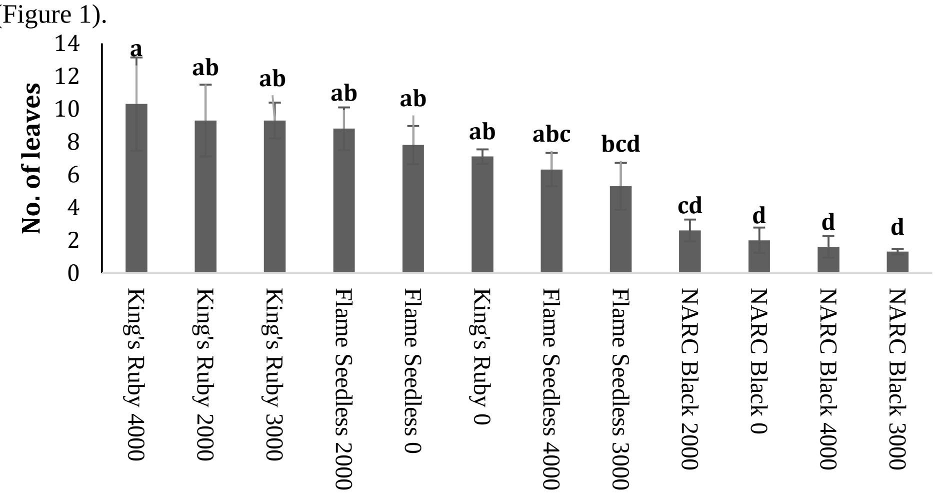

Figure 1. Effect of IBA concentrations on the number of leaves cutting^{-1} in grape cultivars Note: Values followed by different letters are significantly different from one another at P<0.05. The bars represent the average number of leaves per cutting of three grape cultivars in response to IBA concentrations, with ± Standard error.

A similar effect was reported by OuYang et al. (2015), who found that 2000 ppm IBA increased the cytokinin-auxin ratio, leading to greater leaf production. This was further supported by Al-Zebari and Al-Brifkany (2015), who noted that IBA treatment resulted in higher leaf numbers compared to untreated cuttings. For shoot length, the maximum length (9.91 cm per cutting) was observed in Flame Seedless treated with 2000 ppm IBA, followed by the control (9.75 cm) and King's Ruby (9.25 cm) treated with 4000 ppm IBA. The minimum shoot length was recorded in NARC Black (0.72 cm) with 0 ppm IBA (Figure 2).

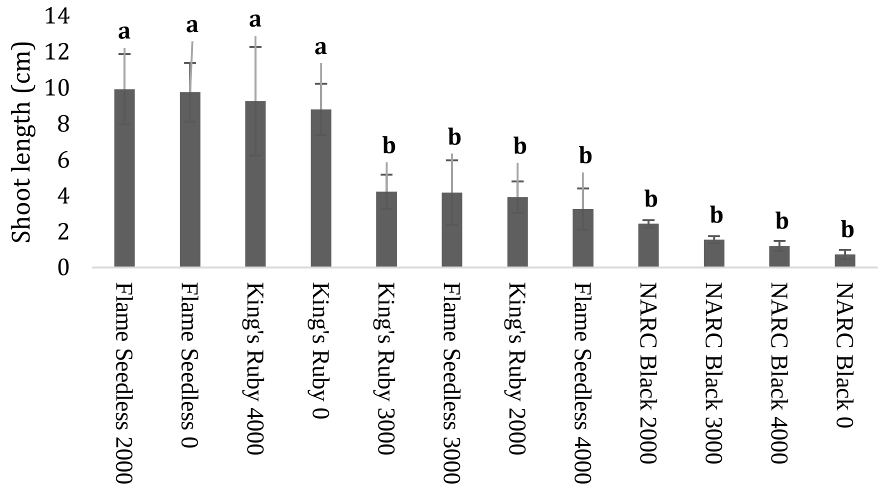

Figure 1. Effect of IBA concentrations on shoot length per cutting in grape cultivars under greenhouse conditions in *the* monsoon season. **Note:** Average bars followed by different letters are significantly different from one another at P≤0.05. The bars represent the shoot length average of three grape cultivars in response to different IBA concentrations with ± Standard error bars.

Patil et al. (2001) found that exogenous plant growth regulators, such as IBA and IAA, increased shoot length by enhancing food material production. This finding was corroborated by Sabir et al. (2004) and Al-Zebari and Al-Brifkany (2015), who observed that shoot length varied with different IBA concentrations. Shoot diameter was greatest (2.88 mm) in Flame Seedless with 0 ppm IBA, followed by Flame Seedless (2.09 mm) and King's Ruby (2.02 mm) with 3000 ppm IBA, while the minimum diameter was recorded in NARC Black (0.94 mm) with 4000 ppm IBA (Figure 3).

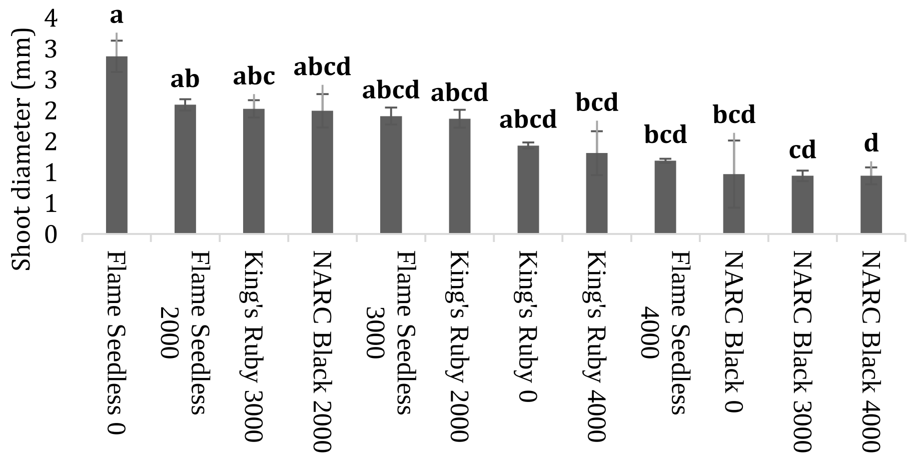

Figure 2. Effect of IBA concentrations on shoot diameter (mm) of grape cultivars under greenhouse conditions in the monsoon season. Note: Average bars followed by different

letters are significantly different at P≤0.05. The bars represent the average diameter (mm) of three grape cultivars in response to IBA concentrations with ± Standard error bars.

Sabir et al. (2004) reported that higher IBA concentrations, above 500 ppm, decreased shoot diameter in one bud Rupestris du Lot (*Vitis rupestris*) American rootstock cuttings. For root length, the maximum length (10.08 cm) was found in Flame Seedless with 2000 ppm IBA, followed by the control (9.66 cm) and King's Ruby (7.33 cm) with 4000 ppm IBA. The minimum root length was observed in NARC Black (2.83 cm) with 3000 ppm IBA (Figure 4).

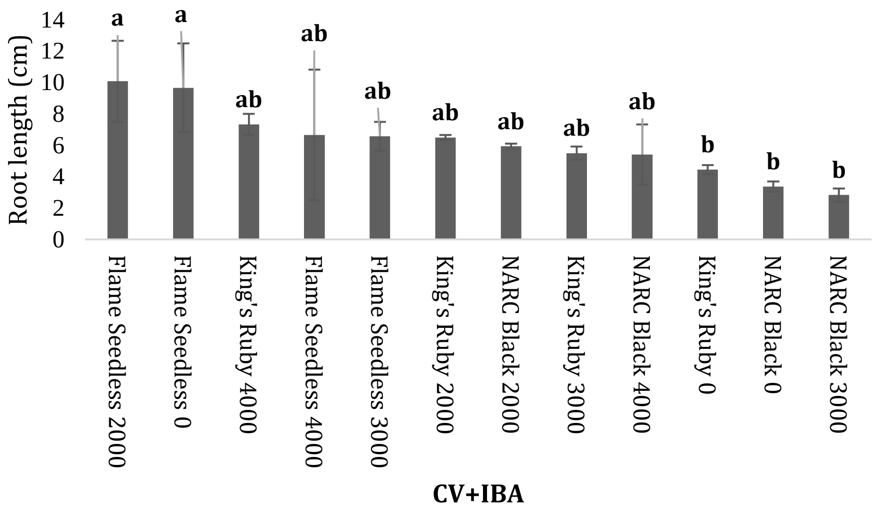

Figure 3. Effect of IBA concentrations on root length (cm) in grape cultivars under greenhouse conditions in the monsoon season. **Note:** Average bars followed by different letters are significantly different from one another at P≤0.05. The bars represent the root length average of three grape cultivars in response to different IBA concentrations, with ± Standard error bars.

Garande et al. (2002) noted that root length was significantly influenced by IBA concentration and variety. The results were also supported by Khajehpour et al. (2014), Tofanelli et al. (2014), and Kareem et al. (2016), who reported positive effects of IBA on root elongation. For rooting percentage, Flame Seedless with 0 ppm IBA produced the highest percentage (86.66%), followed by King's Ruby (73.33%) with 0 ppm IBA and Flame Seedless (56.66%) with 2000 ppm IBA. The minimum rooting percentage was recorded in Flame Seedless (26.66%) with 3000 ppm IBA (Figure 5).

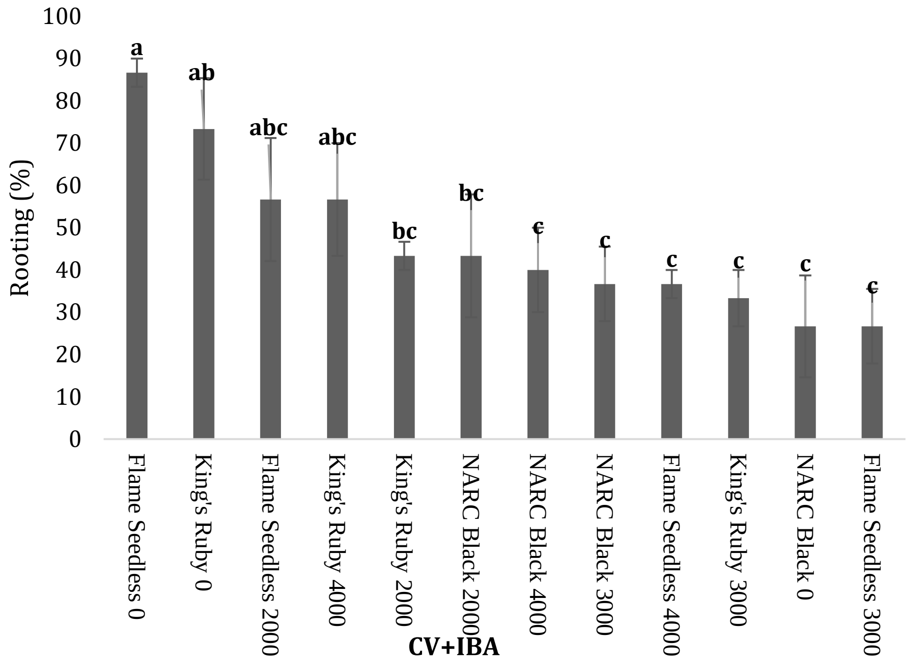

Figure 4. Effect of IBA concentrations on rooting percentage in grape cultivars under greenhouse conditions in the monsoon season. **Note:** Values followed by different letters are significantly different from one another at $P \leq 0.05$. The bars represent the mean of three grape cultivars and IBA concentration, ± Standard error of means.

Patil et al. (2001) found that rooting percentage was more affected by the interaction between IBA concentration and cultivar. King's Ruby produced the maximum number of roots per cutting (23.66) treated with 4000 ppm IBA, followed by King's Ruby (23.16) with 2000 ppm IBA and King's Ruby (18.33) with 3000 ppm IBA. The minimum number of roots was recorded in NARC Black (4.33) with 3000 ppm IBA (Figure 6). Kaplan and Gökbayrak (2012), Satisha et al. (2008), and Abu-Qaoud (1999) observed significant differences in rooting responses among grape rootstocks, which could be attributed to the reserve materials in cuttings. This finding was also confirmed by Somkuwar et al. (2011), who discovered that higher numbers of root primordia were associated with increased levels of reducing sugars and carbohydrates. For survival percentage, Flame Seedless treated with 0 ppm IBA showed the highest survival rate (8.66%), followed by King's Ruby (7%) with 0 ppm IBA and Flame Seedless (5.33%) with 2000 ppm IBA. The minimum survival percentage was recorded in Flame Seedless (2.66%) treated with 3000 ppm IBA (Figure 7). Patil et al. (2001) suggested that survival percentage was influenced by the interaction of cultivar, cutting time, length, and IBA concentration, while Rolaniya et al. (2018) found that IBA treatment at 2000 ppm improved survival percentages in grape cuttings.

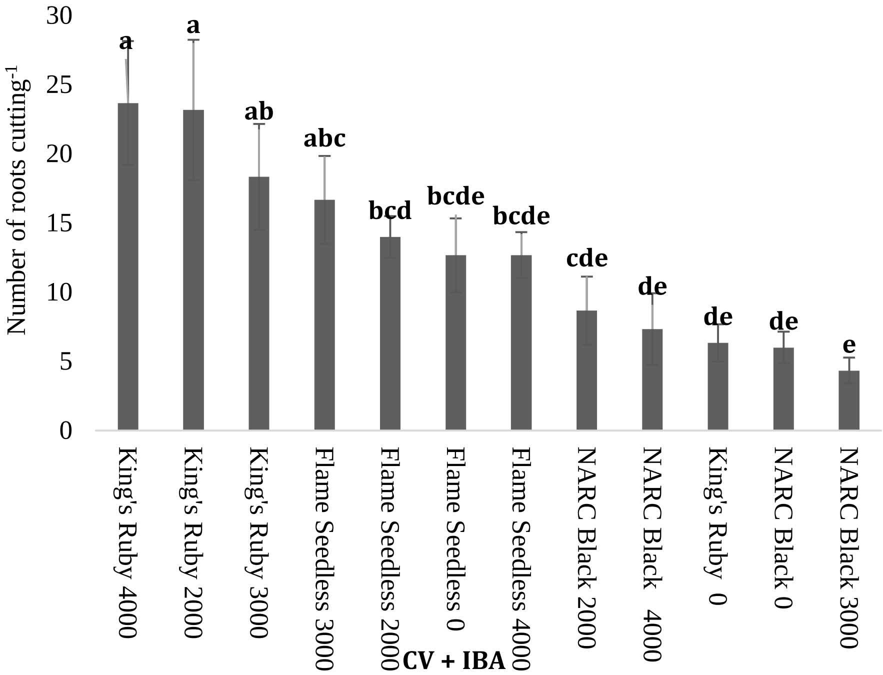

Figure 5. Effect of IBA concentrations on the number of roots of grape cultivars under greenhouse conditions in the monsoon season. Note: Average bars followed by different letters are significantly different from one another at $P \leq 0.05$. The bars represent the number of roots, the average of three grape cultivars in response to different IBA concentrations, with ± Standard error bars.

Conclusions

We observed that application of 4000 (ppm) IBA on cultivar King's Ruby resulted in a higher number of leaves and roots cuttings^{-1}. In contrast, a 2000 ppm IBA application on Flame Seedless resulted in maximum shoot and root length. Furthermore, Flame seedless with no IBA application was found to be superior in terms of shoot diameter, rooting percentage, and survival percentage. The present study concludes that different concentrations of IBA affect propagation differently across various grape cultivars via cuttings during the monsoon season under greenhouse conditions.

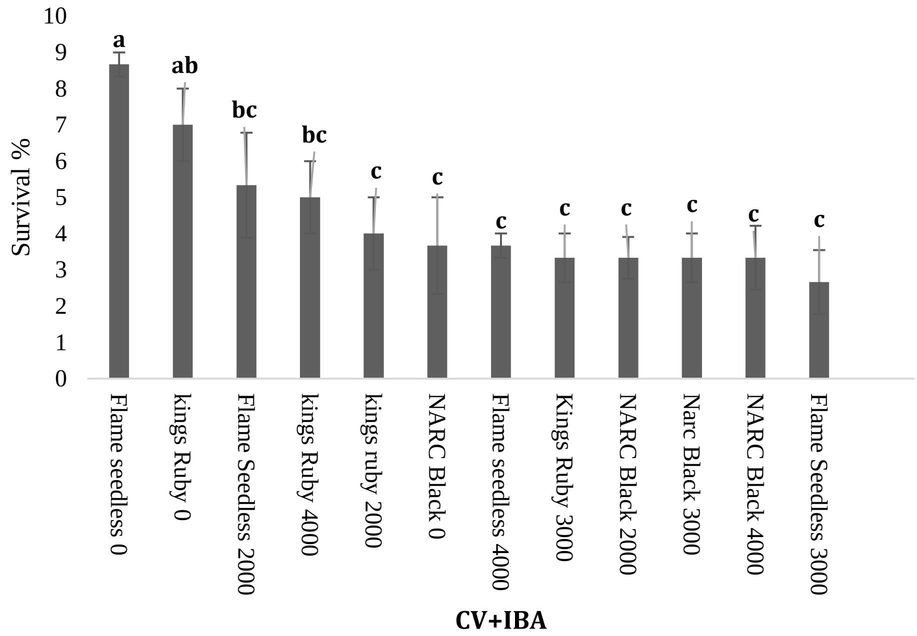

Figure 6. Effect of IBA concentrations on survival percentage of grape cultivars under greenhouse conditions in the monsoon season. Note: Average bars followed by different letters are significantly different from one another at $P \leq 0.05$. The bars represent survival percentage average of three grapes cultivar in response to different IBA concentrations with ± Standard error bars.

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Competing Interests

The authors declare that the research was conducted without any commercial or financial relationships that could be perceived as a potential conflict of interest.

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Ethical statement: This article does not contain any studies involving human or Animal Subjects.

Code availability: Not applicable.

Consent to participate: All authors participated in this research study.

Consent for publication: All authors submitted consent to publish this research.

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