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In vitro Apical Shoot Culture and Callus Formation of Durian (*Durio zibethinus Murray*)

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Authors' contributions

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

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ABSTRACT

Backgroud: Durian (*Durio zibethinus* Murray), A tropical fruit with high nutritional and commercial value, is widely cultivated in Southeast Asia. Unfortunately, growing durian is fraught with difficulties. Propagation is the traditional method for conserving plants. This method does have some drawbacks, one of which is that the original tree may suffer as a result. Therefore, a contemporary method for growing durian plants is plant tissue culture.
Aim: The objective of this research is creating a premise for the development of durian high-value varieties by biotechnology, specifically setting up the first step closer to develop durian somatic cell formation protocol *in vitro*.
Methodology: Effects of media MS on shoot culture and Kinetin and 2.4D on callus formation and growth. Experiments were designed by randomized complete block (RCB).

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Results: There are not significant difference between two treatments of 0.1 mg/L BAP (6-benzylaminopurine) added to 1/3xMS and free hormone medium, in an effort to preserve high-value durian varieties. There is research on formating callus from young leaves of durian affected by Kinetin and 2.4D. The outcomes demonstrated that MS medium supplemented with 3 mg/L 2.4D had an impact on the formation of callus on leaves. The combination of 1.0 mg/L Kinetin + 4.0 mg/L 2.4D make a significant to induce callus growth and callus area. Callus was white color, fresh and soft.
Conclusion: the combination of Kinetin + 2.4D was finding the best combination to maintain the survival of callus induction and growth; coconut water treatment was highly effective in sustaining fresh callus.

Keywords: Durian; Durio zibethinus Murr; apical meristem; shoot; callus; induction.

1. INTRODUCTION

The Durian (*Durio zibethinus* Murray) is a popular and valuable fruit for consume and export. Durian is The King of Fruits, originated from Southeast Asia [1]. In Vietnam, durian is an easy-to-consume fruit with a considerably higher price than many other fruits, the producing area is expanding day by day; and of the main fruit with high valuable in export.

Durian is a perennial woody fruit tree and a dicotyledonous plant. The fruit is a loculicidal capsule with five segments that might be spherical, ovoid, cylindroidal, or ellipsoidal (locules). The weight ranges from 1 to 5 kg, and the stem is big and cylindrical. The stem is divided into two segments [2,3]. The bottom section develops from a flower pedicel and extends from the fruit to the abscission zone. The upper section develops from the flower peduncle, from the abscission zone to the attachment point on the branch. When the fruit ripens, it falls off the tree at the abscission zone [4].

The durian tree belongs to the Malvaceae family and is native to Malaysia. Following that, it spread to other tropics countries such as Indonesia, Thailand, the Philippines, Vietnam, Laos, Cambodia, and so on. Furthermore, durian is grown in South and central America, Africa, and Australia. Durian was initially planted in Dong Nai province after being introduced to our country from Thailand. Durian is currently grown in southern provinces such as Mekong River Delta, Tien Giang, Ben Tre, Dong Nai, Binh Duong, and Central Highland of Vietnam [5].

Since durian has a very appealing taste and a high quantity of nutrients such as lipids, proteins, amino acids, and calcium, it is popular among several consumers. Furthermore, tree sections such as bark, seeds, and fruits are utilized as raw materials in the pharmaceutical industry, and the trunk is used as wood material [5].

Although micropropagation of durian is widespread in the world, the species in Vietnam has not been carefully of studied [6]. With the increasing demand for durian from domestic and foreign culinary circles, the output of durian obtained by traditional methods cannot meet the needs of consumers [7-9]. Besides, there are almost no statistical studies on the development of durian in Vietnam, especially in vitro propagation. Currently, durian can be propagated both sexually (via seeds) and asexually (via grafting) in Vietnam.

Each method has its own advantages and disadvantages. Sexual propagation (by seed) is easy, cheap, and has a low propagation coefficient. The tree has strong roots, but it takes a long time to provide fruit, seedlings struggle to maintain the characteristics of the mother tree, and the trees in the garden are not uniform. Asexual propagation (marcotting, grafting) has the advantage of maintaining the mother plant's good characteristics and producing fruit quickly, but it requires experienced farmers and poses a risk of infection through grafting and branch cutting. The propagation coefficient of the two manual methods is limited, and the seedlings are not uniform, so a solution to create a large and uniform source of seedlings to provide for production is considered necessary. Until now, researchers have mainly focused on selecting a few top durian plants having valuable export, except for durian diseases and postharvest storage procedures, rather than in vitro tissue culture from durian [4,10-12].

In this study, an attempt was made to determine the appropriate concentration of BAP for conservation of durian varieties by means of apical culture and to determine the effects of

different concentrations of Kinetin and 2.4D on the formation of durian callus in vitro; leading closer to the development of durian somatic cell generation protocol in vitro. The somatic cell-regenerating aspect of native durian of Vietnam has not been carefully determined. Therefore, this study will contribute to the study of valuable plant species in Vietnam, especially for durian and other members of the Malvaceae family. This study contributes a first step in the conservation and development of durian varieties in Vietnam, offering the potential for higher yields of this crop in the future.

The cytokinin is known by the names BAP and Kinetin. As the name suggests, cytokinin causes cell division; this cell division can result in shoot regeneration in vitro by encouraging the growth of shoot apical meristems and shoot buds. Cytokinin-induced cell division can result in the formation of an undifferentiated callus [13]. A high cytokinin concentration will prevent root development. By causing the release of shoot apical dominance. Cytokinin can encourage the growth of lateral buds and the formation of multiple shoots [14,15].

In addition, the auxin is known by the name 2.4D. Auxin promotes the growth of roots and calluses. The stem extends as a result of auxin. In cambium tissue, auxin stimulates cell division and cell elongation. Phloem and xylem differentiation is stimulated when auxin and cytokinin are combined. A high auxin concentration can trigger somatic embryogenesis [13]. Auxins and cytokinin are crucial for reprogramming somatic cells into a specific state of differentiation. Dedifferentiation and subsequent redifferentiation into a new pathway are brought about by reprogramming. As a result, a cell that was originally intended to grow into a leaf may develop into an embryogenic somatic embryo. Unknown is the mechanism by which auxin induces dedifferentiation. Because they promote the production of concentrations of ethylene, which can inhibit growth, high concentrations of exogenous auxin can be toxic [16,14,17-19].

2 MATERIALS AND METHODS

2.1 Materials

Materials: Shoot apical from durian grafting-plantlet of Khai Hat (small-seed), domestic primary-variety at the durian garden in Tiên Giang province, Vietnam, and were used as starting material for *in vitro* culture. Durian young shoots, after cleaning with tap water, were sown in clean sand and watered twice a day till to germination.

Media: The basic nutrient medium is MS (Murashige and Skoog, 1962) [20], vitamins MW (Morel & Wetmore, 1951) [21], supplemented with 30 g/L sucrose, 10% coconut water, and 8 g/L agar, pH was adjusted to 5.8 by KOH (1N); and additives of plant growth regulators: BAP (6-benzylaminopurine), K inetin (N^6-Furfuryladenine), 2.4D (2,4-dichlorophenoxyacetic acid).

Conditions: Media was sterilized in autoclaving at 121ºC, 1 atm. in 20 minutes. Cultures were maintained at light intensity 37.04 µmol/s/m^2, temperature 26 ± 2 ºC, photoperiod 16-hour

2.2 Method

2.2.1 The sterilization process following steps

(1) Apical shoots (removed the leaves) for experiment 1 and young leaves for experiment 2, 3 from the plant. (2) Use a cotton pad soaked in soap to gently wipe the outside. (3) Rinse with tap-water 3 times; then wash the 4th time with distilled water. (4) Cover the cut-surface with paraffin. (5) Soak the shoot apical meristem in the soapy solution for 15 minutes. (6) Rinse with tap-water 3 times; then the 4th time with distilled water. (7) In the cabinet, wash the sample with 70°C alcohol for 1 minute. (8) Sterilize the sample with sodium hypochlorire (5%) in combination with tween's solution. (9) Wash the sample with 3 times distilled water. (10) Cut the leaves crosswise into pieces, each about 1cm^2 width (for experiment 2, 3).

2.2.2 Experiment 1: *In vitro* apical shoot culture

Shoot apical from durian grafting-plantlet with four leaves was cut for *in vitro* culture by aseptic techniques. The shoot apical meristem was cultured on 1/3xMS medium, 20 g/L sucrose, agar 8g/L; and plant growth regulator: BAP (0.0 - 0.1 mg/L) was tested to increase the formability and quality of shoots.

2.2.3 Experiment 2: Effects of 2.4D on the callus formation

Durian immature leaves were cultured on MS medium treatment with plant growth regulator:

2.4D (0.0 - 0.1 - 0.3 - 0.5 – 1 – 2 – 3 – 4 – 5 - 6 mg/L) are tested for the induction of durian callus. .

2.2.4 Experiment 3: Effects of kinetin and 2.4D on the callus formation

Durian immature leaves from germinated seeds were cultured on MS medium treatment with plant growth regulator: Kinetin (0.0 - 0.1 - 0.3 - 0.5 - 1 mg/L and 2.4D (0.0 - 0.1 - 0.3 - 0.5 – 1 – 2 – 3 – 4 – 5 - 6 mg/L) are tested for the induction of durian callus.

2.2.5 Experiment 4: Effects of kinetin and 2.4D on the secondary callus formation

Durian callus was cultured on MS medium treatment with plant growth regulator: Kinetin (0.0 - 0.1 - 0.3 - 0.5 - 1 mg/L) and 2.4D (0.0 - 0.1 - 0.3 - 0.5 – 1 - 2 mg/L) are tested for the induction of durian callus.

2.3 Data Analysis

Experimental design was followed completely randomized design (CRD). Statistics analysis was used the Statistical Package for the Social Sciences, version 22.0 for Windows (SPSS v.22). All the data from the last two experiments were examined. Data were collected after 4 weeks and analyzed using One-way ANOVA to identify differences between treatment groups, and Duncan's test at p=0.05. Each treatment was prepared 4 replicates, each replicate for 4 bottles, each bottle content 5 samples cultured. Each replicate has 20 samples. Parameters were evaluated: number of shoots, number of leaves, rate of callus formation, rate of callus area (area - mm^2)

Rate of Callus:

- From a jar of culture medium with established treatments containing a young leaf sample cut to create callus.
- Callus is calculated by determining the presence or absence of callus formation on a sample of young leaves.
- Records of these data were made after 4 weeks. This experiment was conducted in a completely randomized design (CRD) with 4 replicates, each replicate having 4 bottles, and each bottle having 5 samples (total 20 samples).
- Rep1: Rate of Callus = Total leaves sample having callus/Total leaves (20 samples) Same as in Rep 2, Rep 3, Rep 4

Mean: Rate of Callus Rep1 + Rep2 + Rep3 + Rep4/Total Rep (4 replicates)

Rate of callus area (mm^2):

- From a jar of culture medium with established treatments containing a young leaf sample cut to create callus.
- Callus area was calculated by determining the total area of callus formed on a young leaf sample. In particular, scar tissue with square or rectangular patches will be calculated by the product of the lengths of the two sides. In addition, scar tissue with a circular shape will be calculated by the product of the coefficient pi and the square of the scar tissue radius.
- Records of these data were made after 4 weeks. This experiment was conducted in a completely randomized design with 4 replicates, each replicate having 4 bottles, and each bottle having 5 samples (total 20 samples).
- Rep1: Rate of Callus area (mm^2) = Total size of leaves Rep1/Total leaves Rep1 (20 samples)
  Same as in Rep 2, Rep 3, Rep 4

Mean: Rate of Callus area Rep1 + Rep2 + Rep3 + Rep4/Total Rep (4)

3. RESULTS

3.1 *In vitro* Apical Shoot Culture

After 8 weeks, cultivation of the shoot apical meristem on the two treatments (hormone free and 0.1 mg/L BAP), there was an increase in the number of shoots in both of medium. However, the growth peak in the BAP medium showed more better compared to the hormone free medium (Fig. 1). In addition, there was an increase in leaf numbers in both media types. Although the number of leaves in the hormone free medium was higher than the number of leaves in the 0.1 mg/L BAP medium after 6 weeks of culture, the number of leaves in the 0.1 mg/L BAP medium had the highest increase after 8 weeks of culture (Fig. 2). In addition, in both treatments, the number of new shoots and new leaves increased until week 6^{th}. However, there was a difference

at week 8: the medium-containing 0.1 mg/L BAP helped explants grow well, while explants in the hormone free treatment stopped development. Overall, there was no significant difference between the two treatments in terms of shoot growth after 8 weeks.

3.2 Effects of 2.4D on Callus Formation

Treatments containing different concentrations of 2.4D resulted in a lower callus formation rate and callus area.

Formation of Callus: Table 1 shows the fluctuation in callus formation rate with increasing concentrations of 2.4D. The rate of callus formation in the medium of 3.0 mg/L 2.4D and is the best rate (1.00 ± 0.00). On the other hands, the treatment containing the highest concentration of 6.0 mg/L 2.4D gave the lowest result (0.00 ± 0.00) (Fig. 3).

Callus area: Table 2 shows the callus area at increasing concentrations forming a bell-shaped plot. The callus area obtained in the treatment 4.0 mg/L 2.4D had the highest value (10.11 ± 0.91 mm^2). Next is a treatment 5.0 mg/L 2.4D has a value of 7.21 ± 0.12 mm^2. In contrast, the callus area obtained from the treatment 6.0 mg/L 2.4D had the lowest value (0.00 ± 0.00 mm2) (Fig. 4).

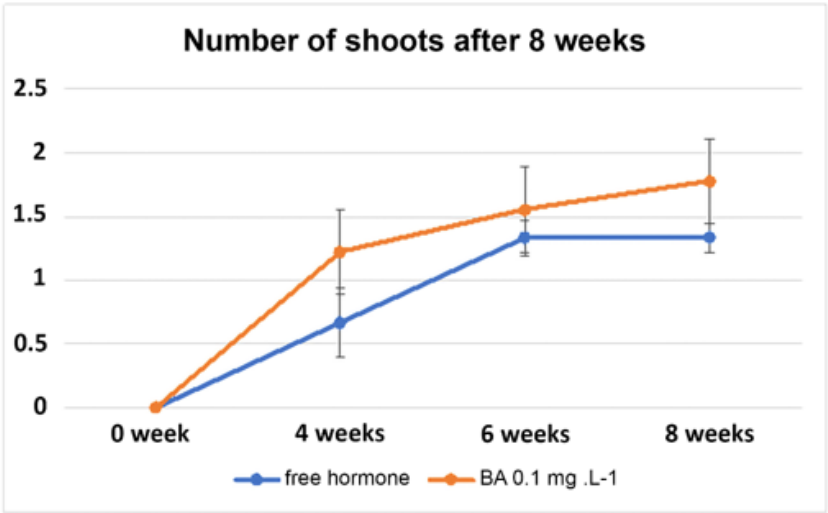

Fig. 1. Number of shoots formed after 8 weeks. Error bars: +/- 1 SE

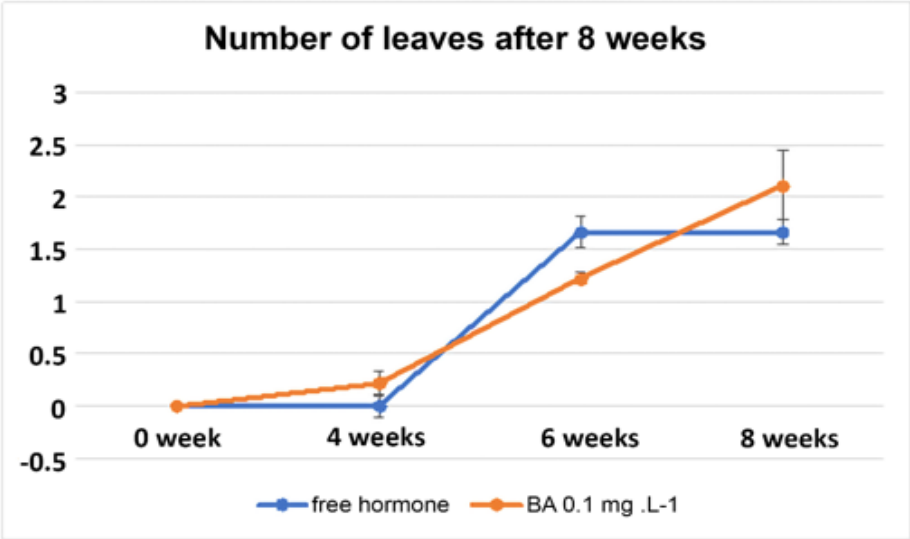

Fig. 2. Number of leaves formed after 8 weeks. Error bars: +/- 1 SE

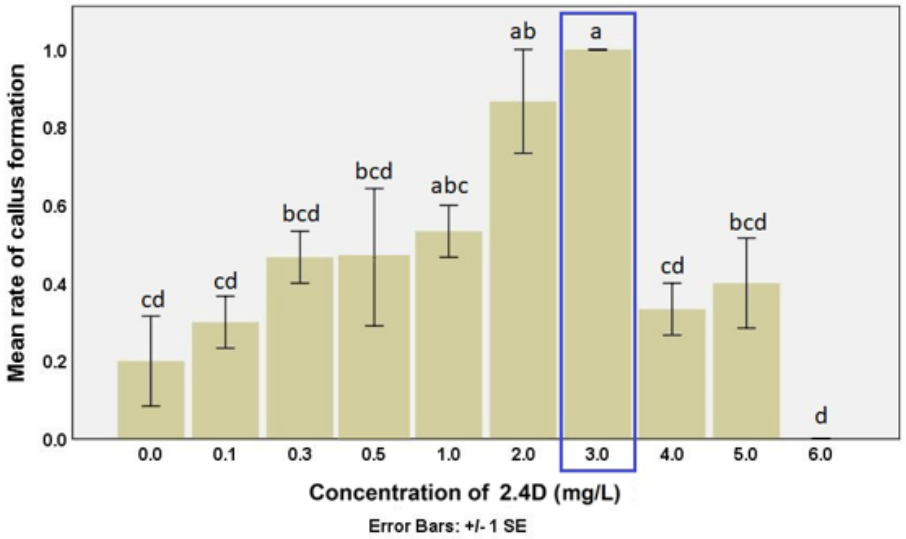

Fig. 3. Effects of different 2.4D concentrations on the rate of callus formation

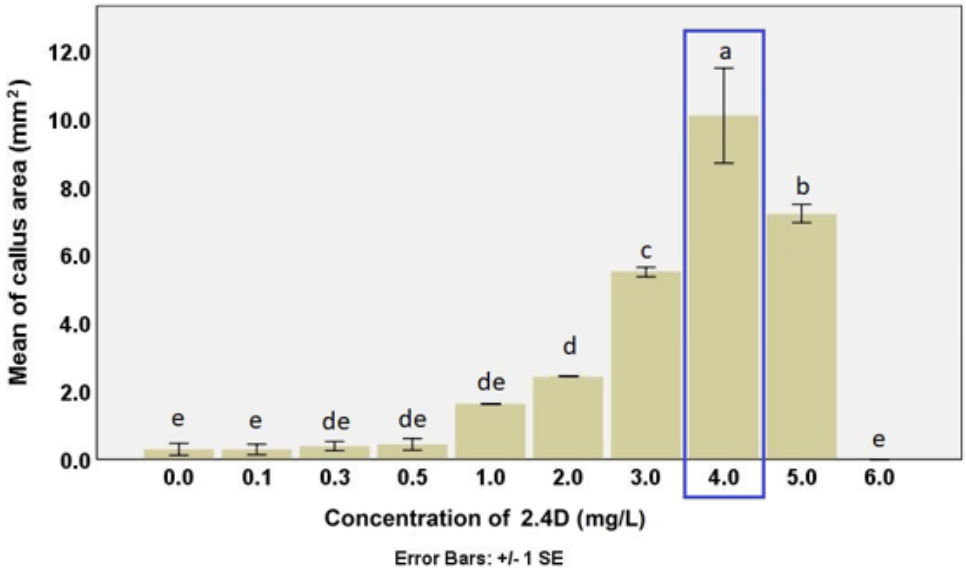

Fig. 4. Effects of different 2.4D concentrations on the rate of callus area

Cultured immature leaf samples demonstrated a response surface manifested by leaf deformation. After 4 weeks, the explants still continued to induce callus on the leaf vein surface. In the treatment with a concentration from 0.0 to 1.0 mg/L 2.4D, the appearance of callus was quite small. But at concentrations from 2.0 to 5.0 mg/L 2.4D, callus showed signs of appearing much more. Callus was white color, fresh and soft. Finally, at a concentration of 6.0 mg/L 2.4D, no callus appeared, and the immature leaves showed signs of death. Moreover, it's 4.0 mg/L 2.4D gave the best result (Fig. 5)

Table 1. The effect of different 2.4D concentrations on the rate of callus formation

Medium	2.4D (mg/L)	Rate
MS	0.0	0.20 ± 0.12^{cd}
	0.1	0.28 ± 0.07^{bcd}
	0.3	0.47 ± 0.07^{bcd}
	0.5	0.47 ± 0.17^{bcd}
	1.0	0.53 ± 0.07^{abc}
	2.0	0.89 ± 0.11^{ab}
	3.0	1.00 ± 0.00^{a}
	4.0	0.33 ± 0.07^{cd}
	5.0	0.40 ± 0.12^{bcd}
	6.0	0.00 ± 0.00^{d}

Table 2. The effect of different 2.4D concentrations on the rate of callus area

Medium	2.4D (mg/L)	Area (mm^2)
MS	0.0	0.29 ± 0.17^{e}
	0.1	0.24 ± 0.15^{e}
	0.3	0.39 ± 0.03^{de}
	0.5	0.44 ± 0.17^{de}
	1.0	1.67 ± 0.01^{de}
	2.0	2.42 ± 0.02^{d}
	3.0	5.52 ± 0.07^{c}
	4.0	10.11 ± 0.91^{a}
	5.0	7.21 ± 0.12^{b}
	6.0	0.00 ± 0.00^{e}

3.3 Effects of Kinetin and 2.4D on Callus Formation

Formation of Callus: Kinetin combined with 2.4D in Table 3 shows the fluctuation in callus formation rate with increasing concentrations. The highest results went to the treatment containing 1.0 mg/L Kinetin + 4.0 mg/L 2.4D gave formation rate of 0.93 ± 0.07. Callus was white color, fresh and soft. In contrast, the treatment containing combination of 1.0 mg/L Kinetin + 6.0 mg/L 2.4D, gave the lowest formation rate (0.11 ± 0.11) (Fig. 6).

Callus Area: Table 4 shows the callus area when increasing concentrations forming a bell-shaped plot. The callus area obtained in the treatment contained 1.0 mg/L Kinetin + 4.0 mg/L 2.4D had the highest value (17.40 ± 0.17 mm^2). Callus was white color, fresh and soft. In contrast, the callus area obtained from the treatment of 1.0 mg/L Kinetin + 6.0 mg/L 2.4D had the lowest value (0.38 ± 0.38 mm^2) (Fig. 7).

Cultured immature leaf samples demonstrated a response surface manifested by leaf deformation. The explants still continued to induce callus on the leaf vein surface in 4 weeks. In the treatment of only Kinetin of (0.0 – 0.3 mg/L), the appearance of callus was quite small. But, at the combination of (0.5-1.0 mg/L) Kinetin + (0,5-5.0 mg/L) 2.4D, callus showed signs of appearing much more. Callus was white color, fresh and soft. Finally, at the combination of 1.0 mg/L Kinetin + 6.0 mg/L 2.4D, no callus appeared, and the immature leaves showed signs of death. Moreover, concentration of Kinetin combined with (1.0 mg/L Kinetin + 4.0 mg/L) 2.4D gave the best result (Fig. 8).

Table 3. The effect of different combinations of Kinetin and 2.4D on the rate of callus formation

Medium	Kinetin (mg/L)	2.4D (mg/L)	Rate
MS	0.0	0.0	0.20 ± 0.12^{bc}
	0.1	0.1	0.56 ± 0.11^{abc}
	0.3	0.3	0.56 ± 0.11^{abc}
	0.5	0.5	0.89 ± 0.11^{a}
	1.0	1.0	0.89 ± 0.11^{a}
		2.0	0.67 ± 0.07^{ab}
		3.0	0.78 ± 0.11^{a}
		4.0	0.93 ± 0.07^{a}
		5.0	0.22 ± 0.11^{bc}
		6.0	0.11 ± 0.11^{c}

Table 4. The effect of different combinations of Kinetin and 2.4D on the rate of callus area

Medium	Kinetin (mg/L)	2.4D (mg/L)	Area (mm^2)
MS	0.0	0.0	0.88 ± 0.49bc
	0.1	0.1	0.51 ± 0.13c
	0.3	0.3	0.55 ± 0.17c
	0.5	0.5	2.22 ± 0.17bc
	1.0	1.0	4.73 ± 0.42bc
		2.0	3.18 ± 0.07bc
		3.0	6.35 ± 0.75bc
		4.0	17.40 ± 0.17a
		5.0	10.08 ± 5.73ab
		6.0	0.38 ± 0.38c

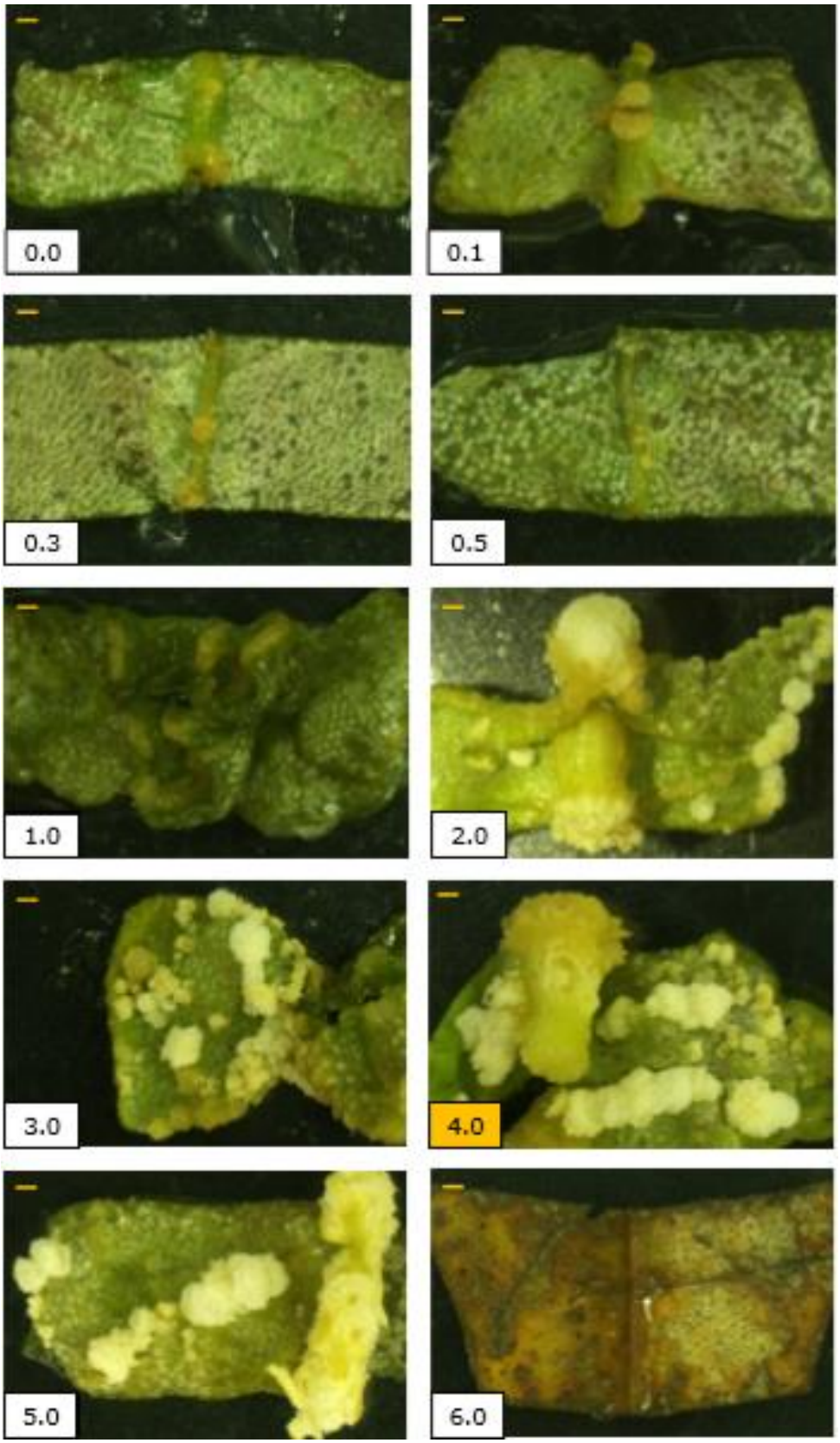

Fig. 5. Effects of different 2.4D concentrations on the formation of callus after 4 weeks *in vitro* culture. Bar = 1 mm

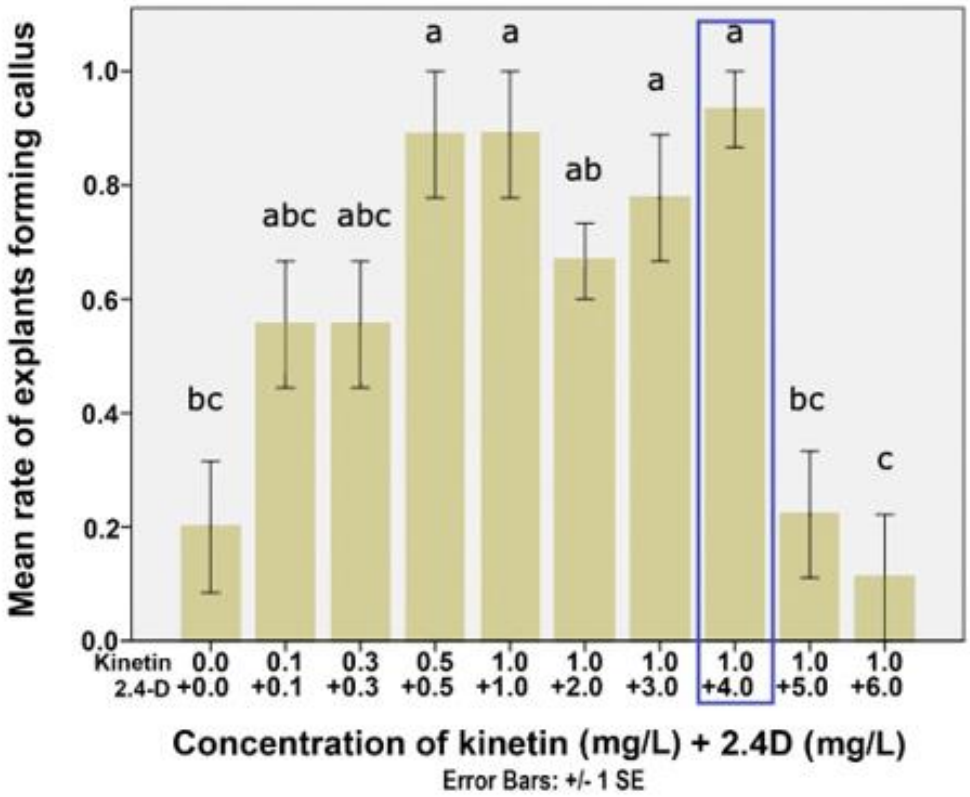

Fig. 6. Effects of different combinations of Kinetin and 2.4D concentrations on the rate of callus formation

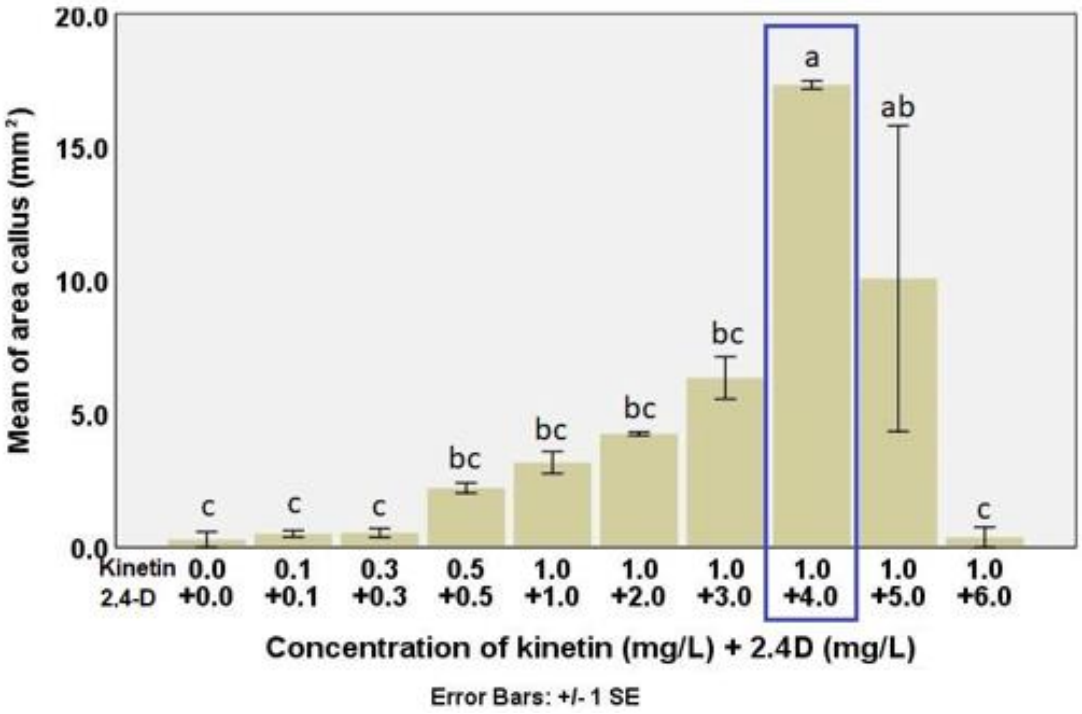

Fig. 7. Effects of different combinations of Kinetin and 2.4D concentrations on the rate of callus area

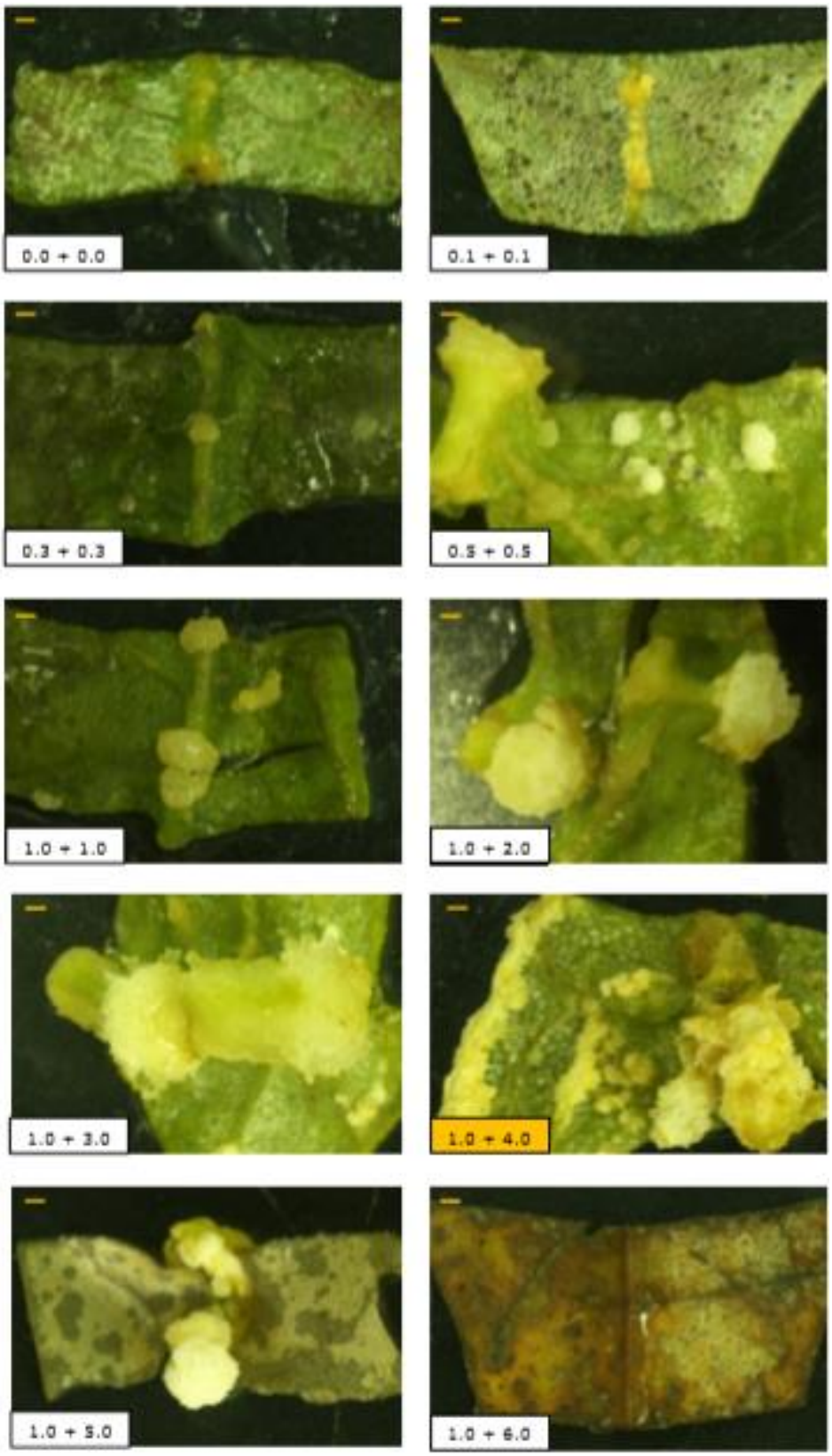

Fig. 8. Effects of different combinations of Kinetin - 2.4D concentrations on the formation of callus after 4 weeks *in vitro* culture. Bar = 1mm

Table 5. The effect of different combinations of Kinetin and 2.4D on the rate of callus survival secodary formation

Medium	Kinetin (mg/L)	2.4D (mg/L)	Rate
MS	0.0	0.0	0.10 ± 0.01[e]
	0.1	0.1	0.10 ± 0.01[e]
	0.3	0.3	0.20 ± 0.03[d]
	0.5	0.5	0.40 ± 0.06[c]
	1.0	1.0	0.53 ± 0.07[b]
	0.1		0.70 ± 0.10[a]
	0.5	2.0	0.67 ± 0.11[a]
	1.0		0.10 ± 0.03[e]

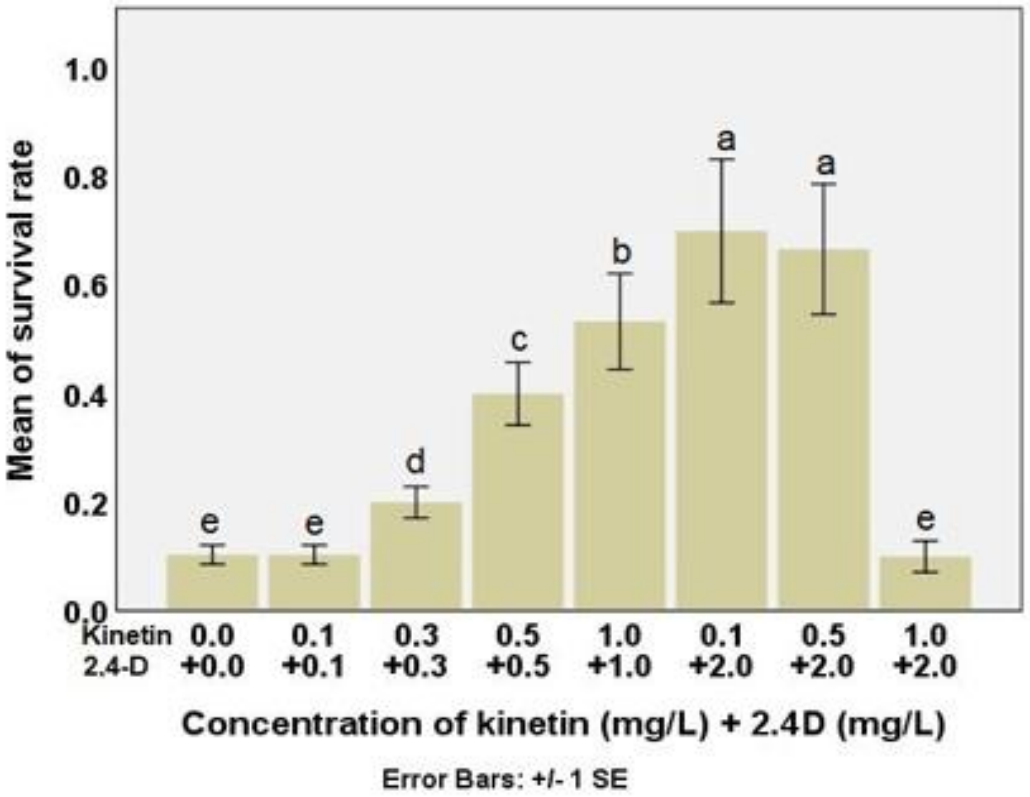

Fig. 9. Effects of different combinations of Kinetin and 2.4D concentrations on the rate of callus survival secodary formation

3.4 Effects of Kinetin and 2.4D on Secondary Callus Formation

In Table 5, treatments of Kinetin and 2.4D shows the fluctuation in the survival rate of callus with increasing concentrations. The survival rate of callus gave high value in the treatment 0.1 mg/L Kinetin + 2 mg/L 2.4D (0.70 $\pm$ 0.10[a]) and 0.5 mg/L Kinetin + 2 mg/L 2.4D (0.67 $\pm$ 0.11[a]) gave. Callus was white color, fresh and soft. In contrast, the callus area obtained from the treatment contained 0.1 mg/L Kinetin + 0.1 mg/L 2.4D had the lowest value (0.10 ± 0.01) (Fig. 9).

4. DISCUSSION

4.1 Experiment 1: *In vitro* Apical Shoot Culture

In comparison to other applications, when the meristems were cultured on MS medium supplemented with 5 and 10 µM BAP, multiple shoot regeneration occurred in coffee (*Coffea arabica* L.) [22]. Besides, in culture of wheat (*Triticum aestivum* L.) apical shoot from seedlings 7 day-old on medium containing BAP and 2.4D produced axillary and adventitious

shoots as well as somatic embryos [23]. The best results were obtained when 2 and 4 mg/L BAP and 0.5 mg/L 2.4D and were combined with various phytohormone concentrations [23].

In experiment 1, there was shoot growth and leaf count in both treatments during the 8 weeks of study. The treatment containing 0.1 mg/L BAP showed better results than the other treatment. During observation, some shoots in both treatments showed secondary callus appeared. Bapat and Rao [24] studies on *Pharbitis Nil* showed that 90% of apical shoots grown on medium of MS + 1 mg/L BAP and MS + 1 mg/L Kinetin produced robust plantlets in 25–30 days. An extensive callus formed from the explant's basal end along with the development of plantlets. Green, nodular, and compact described the callus.

4.2 Experiment 2: Effects of 2.4D on Callus Formation

Eexplants formed callus when treated with 5 mg/L 2.4D at the highest rate (Lizawati et al., 2012). On the majority of cultured explants, the addition of 3.0 - 5.0 mg/L Picloram without BAP was found to be effective in promoting callus proliferation (Zulkarnain, 2013) [8]. According to Yustinus et al. [17] culture of flower bud explant of durian gave callogenesis (69.2% or 0.69 rate) in combination of 45 g/L sucrose and 0.8 ppm TDZ for inducing callus growth; most of the callus showed white colour and friable callus. Following of Rd. Selvy Handayani et al. [14] application of 0.5 ppm of Kinetin or 0.5 ppm 2.4D on durian leaves gave the best result compared to others.

According to experiment 2, the rate of callus formation in the medium with 3.0 mg/L 2.4D gave the highest rate (1.00 ± 0.00a). Callus was white color, fresh and soft. High rate of callus area in treatment of 4.0 mg/L 2.4D was the most dominant (10.11 ± 0.91a).

4.3 Experiment 3: Effects of Kinetin and 2.4D on Callus Formation

The formation of callus gave good results in medium supplemented with 0.5 mg/L Kinetin or 0.5 mg/L 2.4D respectively. The in vitro application of Kinetin and 2.4D had no effect on the growth of the leaf callus at any of the measured parameters (Handayani et al., 2019). Results of Agung et al. [19] show that the treatment of 5 ppm 2.4D, 2 ppm 2.4D + 1 ppm Kinetin, 5 ppm 2.4D + 1 ppm Kinetin produced rolled up explant of young durian leaves and formed the callus. Additionally, cytokinin application is crucial in promoting explant growth and development. The cell division of tissue may be inhibited by small amounts added to the culture media [25]. This finding supports the claim that adding auxin to MS media caused callus growth on durian leaf explants [26]. To promote callus formation, auxin must be present in high concentrations and auxin will stimulate callus formation and inhibit morphogenesis at this high concentration [8,27,28].

Results in experiment 3, treatments with a combination of 1.0 mg/L Kinetin + 4.0 mg/L 2.4D were significant difference in callus formation rate of 0.93 ± 0.07^{a}. Callus was white color, fresh and soft. In callus area, the treatment containing 1.0 mg/L Kinetin + 4.0 mg/L 2.4D gave the best results (17.40 ± 0.17^{a}). Kinetin application in a culture medium promotes callus growth

4.4 Experiment 4: Effects of Kinetin and 2.4D on Secondary Callus Formation

In experiment 4, after 4 weeks sub-culture of calluses from experiment 2, the highest callus survival rate (0.70 ± 0.10) was obtained in the treatment of 0.1-0.5 mg/L Kinetin + 2 mg/L 2.4D. Callus was white color, fresh and soft. The results in Table 5 showed that at different concentrations of Kinetin and 2.4D, there were different callus survival rates. The opportunity to further study of durian callus formation, it can be promised that the combination of Kinetin + 2.4D will help callus grow more steadily in terms of survival rate and callus area.

5. CONCLUSION

Based on the results of this research, the effects of hormone-free and BAP (0.1 mg/L) are not significant for durian apical shoot culture. Additionally, the concentration of Kinetin and 2.4D added to the culture medium had an impact on the stimulation of callus proliferation in immature leaf specimens in *in vitro*. The application of 3 mg/L 2.4D gave the best results compared to others. The combination of Kinetin + 2.4D increased callus area, with the best combination of 1.0 mg/L Kinetin + 4.0 mg/L 2.4D. Callus was white color, fresh and soft. Although the embryogenic properties of all the callus that were formed in this study showed similar features.

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 writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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