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Original Article

Postharvest crown chemical treatments and application timing on internal browning in four pineapple clones

David Chandra[1], Soesiladi Esti Widodo[1*], Muhammad Kamal[1], Sri Waluyo[2] and Kukuh Setiawan[1]

ABSTRACT

Pineapple clones differ in their sensitivity to internal browning (IB), with the Queen group being more sensitive than the Cayenne group. However, there is not much information available on the differences in clone sensitivity between the Cayenne groups. The aim of this research was to study the responses of Smooth Cayenne and MD2 (hybrid) after chemical spraying applied to the pineapple crown on the IB and other fruit qualities. The experimental design used was a 3 factorial completely randomized design (4 x 5 x 2), clone (GP3; HC; GP4; MD2), chemical material (50mg L^{-1} ABA; 200mg L^{-1} AsA [ascorbic acid]; 1 mM jasmonic acid; 2% calcium chloride; control [H_2O]), and application time (0 and 16 days after harvest). The results showed that the MD2 pineapple clone had greater resistance to IB damage than the Smooth Cayenne type. The Smooth Cayenne type itself had different resistance to IB, with the GP3 clone showing the lowest resistance, followed by HC and GP4. Pineapple resistance to IB was followed by endogenous AsA content; MD2 had the highest AsA content. The susceptibility of GP3 pineapple to IB was also characterized by high ion leakage values. The pineapple postharvest crown-coating applications with chemical materials and/or application time effects on Smooth Cayenne and MD2 types were not effective in suppressing IB severity. Therefore, the need for long pineapple storage of up to 46 days with good quality is recommended only for the MD2 clone.

Keywords: Cold storage, Ion leakage, Phenol, Physiological disorders, Shelf life.

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[1]Department of Agronomy and Horticulture, Faculty of Agriculture, University of Lampung, Bandar Lampung, Lampung, 35141, Indonesia
[2]Department of Agricultural Engineering, Faculty of Agriculture, University of Lampung, Bandar Lampung, Lampung, 35141, Indonesia

*Corresponding Author. Address: Department of Agronomy and Horticulture, Faculty of Agriculture, University of Lampung, Bandar Lampung, Lampung, 35141, Indonesia; Email: sestiwidodo@gmail.com

INTRODUCTION

The largest pineapple production in Indonesia was contributed by Great Giant Food (GGF) Co. Ltd. with the Smooth Cayenne type and MD2 (hybrid) pineapple clones. The Smooth Cayenne type has a cylindrical fruit shape, shallow eyes, yellow flesh, sweet taste with light sourness, low fiber, high yield, and smooth leaves (Chan et al., 2003). The MD2 clone pineapple was produced to meet the needs of the fresh fruit market. The MD2 clone pineapple had 50% the same characteristics as the Smooth Cayenne pineapple type. Some of the advantages of the MD2 clone pineapple compared to other types of pineapple are its attractive ripe shell and flesh colour (golden yellow), its higher vitamin C content and total soluble solids than other types, and its sufficient cold storage resistance (Thalip et al., 2015).

Internal browning (IB) was one of the important post-harvest problems of pineapple fruit because it reduced the quality of the fruit. IB is a major constraint in the global pineapple industry, particularly during canning and shipping in refrigerated containers. According to Ko et al. (2006), IB-related losses are estimated at US$1.3 million per year out of a total production value of US$30 million in Australia. Storing fruit at cold temperatures for a relatively long time induced IB. Based on research by Chandra et al. (2023b) regarding the incidence of IB in pineapple clones, GP3 and MD2 stored at 7°C did not appear on the 16th day and were visible on the 23rd day with a relatively low severity of IB (≤ 5%). This was supported by research by Luengwilai et al. (2016), which showed that the occurrence of IB was not observed in MD2 and Pattavia (Cayenne group) pineapples stored at 10°C until a shelf life of 14 days, but it appeared at a shelf life of 21 days. Therefore, delaying post-harvest application was felt to be more effective in prolonging the delay in the incidence and severity of IB.

Several post-harvest applications of fruit coatings reduced the incidence of IB. Coating pineapple fruit with ABA 380μM, and 200mg L^{-1} reduced the occurrence of IB in Comte de Paris pineapples (Queen group) (Liu et al., 2017; Zhang et al., 2015; 2016) and ABA 50mg L^{-1} on the severity IB in GP3 pineapple (Smooth Cayenne type) (Chandra et al., 2023b). Postharvest application of exogenous ascorbic acid (AsA) increased the resistance to cold temperatures of strawberry (Saleem et al., 2021) and longan fruit pericarp (Liu et al., 2021). $CaCl_2$ application managed the antioxidant content and quality of pineapple fruit in cold storage (Naradisorn et al., 2022). Dipping apples in 2% $CaCl_2$ for 3mins had higher resistance to electrolyte leakage, acid content and vitamin C than control (Farag & Nagy, 2012). Postharvest application coating of Trad-see-thong pineapple (Queen group) with 1mM methyl jasmonate solution for 5mins then storing the fruit for 20 days at 10±1°C inhibited the occurrence of IB and ion leakage. According to Liu et al. (2017), the application of Comte de Paris pineapple crown coating (Queen group) with ABA 380μM was better than coating on the fruit shell.

Applying a lower concentration of ABA, 50mg L^{-1} to the pineapple crown, would be as effective and more efficient in suppressing the severity of IB as the 380μM concentration. Furthermore, the study aimed to evaluate the effectiveness of other chemicals applied to the pineapple crown in reducing the severity of IB. The results were expected to support the development of post-harvest applications that are easier to implement on a large scale. Delaying post-harvest application was also expected to provide an alternative treatment for pineapple fruit that had been harvested for a long time without treatment, thereby extending shelf life and delaying the development of IB severity.

MATERIALS AND METHODS

The pineapples used in this study were harvested at an early stage of ripeness (ripe fruit with 0% yellow stripes on the shell colour). The fruits were sourced from Great Giant Foods Co. Ltd., Indonesia, in June 2023. The experimental design used a three factorial completely randomized design (4 x 5 x 2), clone (GP3, MD2, HC, and GP4: Smooth Cayenne type), coating (50mg L^{-1} abscisic acid [ABA]; 200mg L^{-1} ascorbic acid [AsA]; 1mM jasmonic acid [JA]; 2% calcium chloride [$CaCl_2$]; and control [H_2O]), and time of application (0 and 12 days after harvest [DAH]). Each treatment combination was repeated 5 times. Coating was carried out by spraying the fruit crown with a treatment solution. The fruit was air-dried for 30mins before being packaged in perforated boxes, with 10 pieces per box. The fruits in the boxes were then stored in a storage room at 7°C for 46 days. The fruits were observed 3 times at 26, 36, and 46 days after storage to determine internal browning and other changes in fruit quality.

Internal Browning

Incidence of IB was calculated based on the percentage of the number of pineapple flesh with IB symptoms to the total sample. Severity of IB was calculated based on the percentage of IB colour score to the highest IB score modified by Chandra et al. (2023a, b) (Table 1). Severity of IB categories were no symptoms; 0%, mild; 1–5%, moderate; 6–10%, moderately severe; 11–20%, and severe; ≥ 21% with Formula 1:

$$\text{IB severity (\%)} = \frac{\text{(IB colour score x IB area)}}{\text{Highest IB colour score x total area}} \text{x100\%} \qquad (1)$$

Table 1. Internal browning color scores (left-right) of pineapple clones

Score	Clone Pineapple			
	GP3	MD2	HC	GP4
1				
2				
3				
4				

*The score for each color in the pineapple clone was the result of preliminary data from the color of IB symptoms

Ion Leakage

Ion leakage was calculated as the percentage of actual ions relative to total ions, using 2 electrolyte leakage (EL) methods based on the modified method of Nukuntornprakit et al. (2015) and changes in fruit pH. Pineapple fruit between the flesh and flesh core was cut into cubes measuring 1 x 1 x 1cm. The fruit pieces were irrigated with deionized water and drained on filter paper. The dried fruit pieces were placed in a glass bottle containing 20mL of 0.4M mannitol solution, shaken, and left for 1h. Electrolyte conductivity 1 (EC_1) of the fruit was measured using an EC meter, and pH 1 (pH_1) was measured using a pH meter. The fruit pieces were autoclaved at 121°C for 20mins, and the Ec_2 and pH_2 of the solution were measured again after the solution reached room temperature. The EL percentage was calculated based on the comparison between EC_1 and EC_2 with Formula 2:

$$\text{Electrolyte Leakage}(\%) = \frac{EC_1}{EC_2} x100\% \quad (2)$$

The percentage change in fruit pH was calculated based on the difference between pH_2 and pH_1 to pH_2 with Formula 3:

$$\text{Changes in fruit pH}(\%) = \frac{pH_2 - pH_1}{pH_2} x100\% \quad (3)$$

Total Phenols Content

Extraction was carried out using the method of Chandra et al. (2023c), namely 1mL of pineapple juice was dissolved in 19mL of 80% methanol using a magnetic hot stirrer at 35°C for 90mins. Calibration curves were made with standard gallic acid concentrations of 100, 150, 200, 250, and 300ppm dissolved in a mixture of methanol: deionized water (1:1). Total phenol content in samples and standards was measured using a UV-vis spectrophotometer at a wavelength of 766nm. The absorbance value of pineapple samples was converted into the form of mg GAE/ 100mL juice.

The thermal image measurement method was based on Chandra et al. (2023a), with modifications, in a storage room at 7°C, and the fruit temperature was analyzed using the MATLAB program with an ROI of 45 x 45 pixels. The hardness of the fruit flesh was measured by the level of hardness of the flesh/core of the fruit using a penetrometer. Analysis of ascorbic acid, soluble solid content, titratable acidity, sweetness, fruit weight loss, shell dehydration, shell colour, and mold methods were performed following the methods by Chandra et al. (2023b) and, the pH of pineapple juice was measured by a digital pH meter.

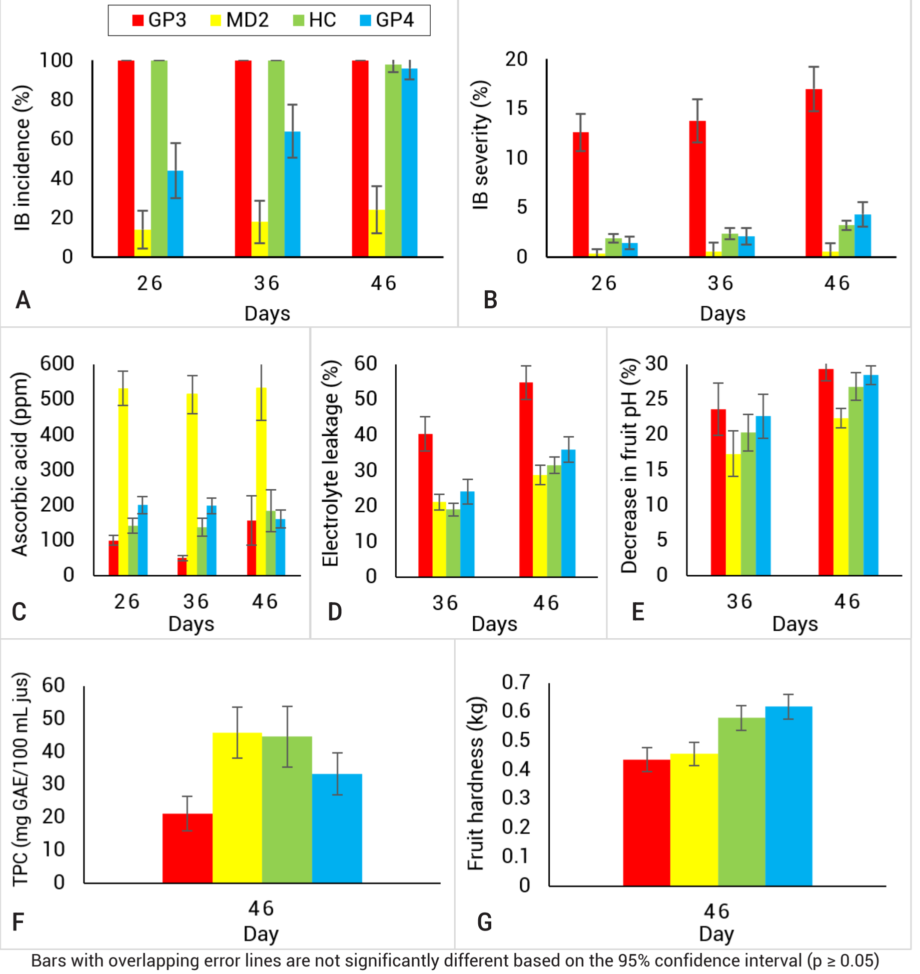
Statistical Analysis

Research data were analyzed using three-way analysis of variance (ANOVA) with a 95% confidence level (p = .05) using the IBM-SPSS version 26 program. Then the significant group was continued by comparing the mean ± 95% confidence interval.

RESULTS

Based on ANOVA analysis of the incidence and severity of pineapple, IB was only influenced by clone group effect; there were no interaction effects of clone with crown coating and/or postharvest application time on the incidence and severity of IB up to a shelf life of 46 days. The interaction was observed only in the GP4 clone and at the application times of 26 and 36 days of storage, where delayed application exacerbated the interaction between IB and the GP4 clone.

The MD2 clone pineapple had a significantly lower incidence and severity of IB up to 46 days after being stored at 7°C followed by clones GP4, HC, and GP3 (Smooth Cayenne type pineapples). Although the GP3 and HC clones showed the same response on IB incidence, the GP3 clone had more severe IB symptoms (rather severe symptoms) than the HC clone (mild symptoms). The incidence of IB in the pineapple clone GP4 showed moderate symptoms at a shelf life of 26 days after storage and increased to severe symptoms at a shelf life of 46 days. An increase in IB severity occurred from 26 to 46 days of shelf life in pineapple clones GP3, HC, and GP4 (Smooth Cayenne type), but did not occur in MD2 (Figure 1.A, B).



Bars with overlapping error lines are not significantly different based on the 95% confidence interval ($p \geq 0.05$)

Figure 1. Response of pineapple clones for 46 days after harvest at a storage temperature of 7°C

Pineapple clones showed varying levels of resistance to IB incidence. The distribution pattern of GP3 IB symptoms appeared from around the flesh core and spread to the surrounding fruit flesh. The distribution pattern of IB symptoms in the GP4 pineapple clone was similar to that of the GP3 clone, and the MD2 clone was in the form of spots on the flesh of the fruit, while the HC pineapple clone started from the edge of the fruit core (Figure 2).

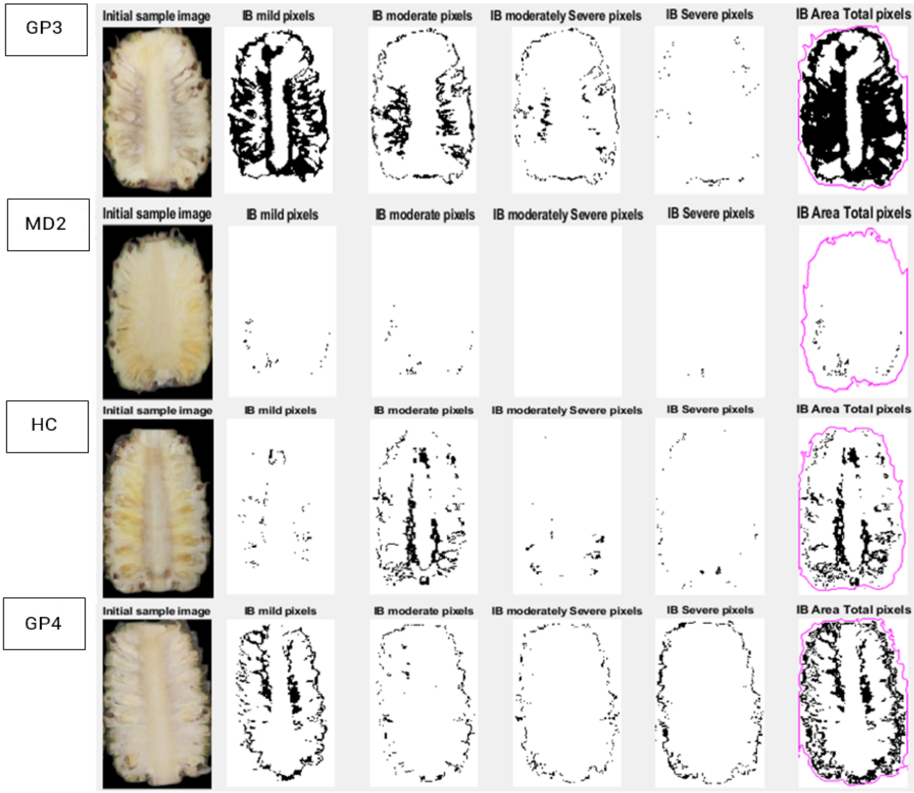
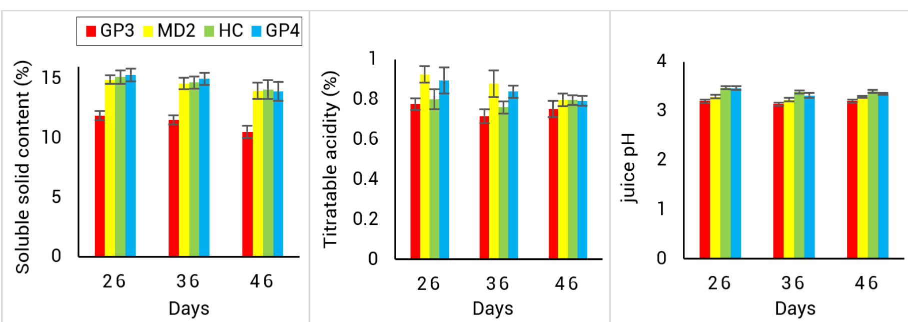


Figure 2. Measurement of the severity of internal browning symptoms in pineapple cross sections after storage for 28 days after harvest at a temperature of 7°C

The GP3 clone pineapple had a significantly lower titratable acid content than the other clones (Figure 3). However, the soluble solid content was also low which made the GP3 pineapple clone less sweet than the other pineapple clones. The highest fruit weight loss occurred in pineapple clone GP4, followed by HC and MD2, and the lowest in clone GP3. The lowest pineapple shell dehydration occurred in the GP3 pineapple clone, followed by the GP4, HC, and MD2 clones, although in general it was not significant.



Bars with overlapping error lines are not significantly different based on the 95% confidence interval ($p \geq 0.05$)

Figure 3. Response of pineapple clones when stored for 46 days after harvest at a temperature of 7°C on other fruit qualities

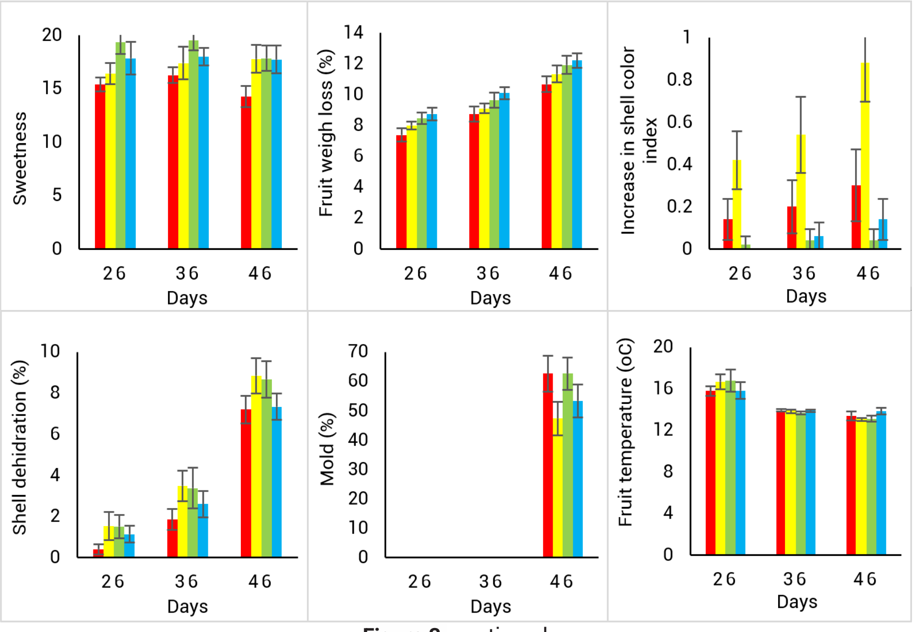


Figure 3. continued

DISCUSSION

The incidence and severity of IB of the MD2 clone could be maintained at mild symptoms from a shelf life of 26 to 46 days. Pineapple resistance to IB was also demonstrated by its high AsA content and low ion leakage value. According to Chandra et al. (2023b), the high AsA (ascorbic acid) content of MD2 was more resistant to IB than the GP3 clone, which had a much lower AsA content. The AsA content in pineapple suppressed the physiological damage caused by IB, which was linked to the antioxidant activity of AsA (acidifying and chelating the PPO enzyme) and its role as a ROS scavenger, thereby protecting cells from damage (Min et al., 2020; Queiroz et al., 2008). These results were also supported by several other studies, which stated that the MD2 cultivar had quite high resistance to IB (Ahmadi et al., 2015; Luengwilai et al., 2016; Souleymane et al., 2019). According to Luengwilai et al. (2016), the sclerenchyma of pineapple cultivar MD2, which was observed using scanning electron microscopy, had a layer structure of sclerenchyma fibres that was thicker and twice as large as that of the susceptible cultivar Trad-see-thong (Queen group) and the tolerant Pattavia (Cayenne group). The sclerenchyma tissue in the MD2 clone pineapple also formed concentric rings around the phloem and xylem.

Ion leakage is an indication of stress-induced damage to agricultural products (Yuan et al., 2024). Increased electrolyte leakage and decreased pineapple pH occurred in all pineapple clones on day 46 compared to day 36 (see Figure 1.D, E). This increase in storage time also increased the IB damage that occurred. The higher the value of electrolyte leakage and the decrease in pineapple pH, the higher the IB damage. According to Dolhaji et al. (2019), MD2 pineapple had a lower IB severity compared to Josephine (Spanish and Smooth Cayenne hybrid) and Morris (Queen group), which positively correlated with lower ion leakage as well. This was supported by research by Luengwilai et al. (2016), that the incidence of IB was positively correlated with ion leakage. The Pattavia cultivar (Cayenne group) had higher resistance compared to the Trad-see-thong cultivar (Queen group) (Luewilai et al., 2018; Pusittigul et al., 2012).

The phenol content of the GP3 clone pineapple was lower compared to that of MD2, HC, and GP4 clones on day 46 after storage at 7°C. The MD2 clone pineapple had the highest phenol content, followed by HC and GP4 clones (see Figure 1.F). According to Queiroz et al. (2008), phenolic compounds were substrates for enzymatic browning reactions. In this study, the high phenol content in the MD2 clone did not cause high IB damage. The high content of AsA caused tissue damage to be inhibited, so that the high level of phenol could not react with the catalytic enzyme to form melanin (Chandra et al., 2023b). The product genotype influenced the antioxidant pathway. If the product was resistant enough, during stress, the reaction of the oxidant pathway will be higher in overcoming the stress. The level of fruit hardness was a result of the fruit's genetics in forming the fruit's eating structure which was not very significant for ion leakage and the incidence of IB.

Based on the research results, the response to pineapple crown application with $50mL^{-1}$ ABA did not affect IB symptoms. However, according to Chandra et al. (2023b), coating with $50mL^{-1}$ ABA on GP3 pineapple shells effectively suppressed IB severity. However, according to Liu et al. (2017), the application of pineapple crowns with 380μM ABA effectively suppressed IB in Trad-see-thong and Pattavia pineapples. This indicates that the response to chemical application can differ depending on the type of pineapple or its concentration.

CONCLUSION

Pineapple clones' responses to internal browning (IB) varied depending on their ascorbic acid (AsA) content and ion leakage. MD2 clones had higher resistance to IB compared to Smooth Cayenne pineapples. GP3 pineapple clone had the lowest IB resistance and AsA content and highest ion leakage compared to HC and GP4, even though all three belonged to the same pineapple group (Smooth Cayenne type). There were no significant differences in other fruit qualities, except that MD2 clone had higher shell dehydration and shell color indices and lower mold levels than GP3 on day 46. Coating compounds and delayed post-harvest coating applications on pineapple crowns were ineffective in suppressing IB.

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Author Contributions

DC, SAW, MK, and SW conceptualized and designed the work; DC and SAW carried out field and laboratory, collected the data, interpreted the data, and wrote the original draft, review and editing; MK, SW, and KS analyzed the data and made visualization.

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Availability of Data and Materials

Data and materials generated in this article and its supplementary files, and/or available from the corresponding author upon request.

Ethical Considerations

Data and materials generated in this article are available from the corresponding author on request.

Competing Interest

The authors declare that they have no conflict of interest.

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