

## **Biomass Enhancement of Stevia (*Stevia rebaudiana* Bertoni) Shoot Culture in Temporary Immersion System (TIS) RITA® Bioreactor Optimized in Two Different Immersion Periods**

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**Abstract :** Stevia plant contains steviol glycosides which is estimated to be 300 times sweeter than sucrose. However in Indonesia, conventional (in vivo) propagation of *Stevia rebaudiana* was not effective due to a poor result. Therefore, alternative methods to propagate *S. rebaudiana* plants is needed, one of it is using in vitro method. Multiplication with a large quantity of stevia biomass in relatively short period can be conducted by using TIS RITA® (Recipient for Automated Temporary Immersion System). The objective of this study was to evaluate the effect of immersion period of the medium on growth and the medium bioconversion into the production of shoot biomass. The study was conducted to determine the effect of different intensity period of medium to enhance biomass of stevia shoots. Shoot culture of *S. rebaudiana* was grown in full strength MS medium supplemented with 1 ppm Kinetin. RITA® bioreactors were set up with two different immersion periods, 15 min (RITA® 15) and 30 min (RITA® 30), scheduled every 6 hours and incubated for 21 days. The result indicated that immersion period affected the biomass and growth rate ( $\mu$ ). Thirty-minutes immersion showed greater percentage of shoot multiplication ( $93.44 \pm 0.83\%$ ), percentage of leaf growth ( $85.24 \pm 5.99\%$ ), growth rate ( $0.042 \pm 0.001$  g/day), and productivity ( $0.066$  g/L medium/day) compared to that immersed in RITA® 15 min ( $76.90 \pm 4.85\%$ ;  $79.73 \pm 7.76$ ;  $0.045 \pm 0.004$  g/day, and  $0.045$  g/L medium/day respectively). Enhancement of biomass in RITA® 30 reached  $1,702 \pm 0,114$  gr, whereas in RITA® 15 only  $0,953 \pm 0,093$  gr. Additionally, the pattern of sucrose, mineral, and inorganic compounds consumption followed the growth of plant biomass for both systems. In conclusion, the bioconversion efficiency from medium to biomass in RITA® 30 is better than RITA® 15.

**Keywords :** intensity period, shoot culture, *Stevia rebaudiana*, TIS RITA®

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