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Micropropagation of *Rubus chrysophyllus* Reinw. ex Miq. and *Rubus fraxinifolius* Poir.

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ABSTRACT

Rubus chrysophyllus and *Rubus fraxinifolius* are a native species in Indonesia, which has highly potential to be developed as fruits crops. Propagation is an important factor in developing a fruit cultivation. *In vitro* micropropagation is an important instrument to improve the quality of seedling. Our experiment was aimed to study the micropropagation of *R. chrysophyllus* and *R. fraxinifolius*. The shoot tips were cultured on MS medium supplemented with 10 mg/L indole-3-butyric acid (IBA) for enhancing roots of *R. chrysophyllus* and *R. fraxinifolius* in the *in vitro* condition. Then, the rooted plantlets were transplanted into cocopeat media for acclimatization. The results showed that *R. chrysophyllus* and *R. fraxinifolius* gave a different response on the medium contain 10 mg/L of IBA. The *R. fraxinifolius* shows the best results compared to *R. chrysophyllus* on 14 days after subculture. The roots length and roots numbers of *R. fraxinifolius* and *R. chrysophyllus* were 9.13 cm and 11.25; 2.20 cm and 2.00, respectively. Although *R. fraxinifolius* was growing better than *R. chrysophyllus,* but after acclimatization *R. chrysophyllus* were able to grow faster than *R. fraxinifolius*. Moreover, on the parameters of plant height shows that *R. chrysophyllus* (9.20 cm) were growth higher than *R. fraxinifolius* (4.05 cm) during acclimatization.

Keywords: *Rubus chrysophyllus, Rubus fraxinifolius, micropropagation, indole-3-butyric acid (IBA)*

INTRODUCTION

Rubus chrysophyllus and *Rubus fraxinifolius* (Figure 1) are collections of Cibodas Botanical Garden (CBG). *R. chrysophyllus* was collected from Mount Singgalang – West Sumatera on 2525 m above sea level. Van Steenis [1] reported that *R. chrysophyllus* has a sweet taste comparing with others species. Moreover, *R. fraxinifolius* is a garden origin collection in CBG. *R. chrysophyllus* is distributed in Sumatra, Java and Lombok [2], but has not cultivated yet. In Indonesia, *R. fraxinifolius* is distributed in Borneo, Java, Lesser Sunda Islands, Celebes and Moluccas [2]. Moreover, *R. fraxinifolius* also called as *'Arben',* recently it was cultivated in CBG-West Java.

In vitro propagation is an important technique for production of *Rubus* seedling. It is due to this technique guarantees quality and safety compared to traditional production. However, successful *in vitro* propagation have been reported for many members of the genus *Rubus* involving callus culture, shoot tips growth, roots development, and proliferation [3-7], but the study of *in vitro* propagation of *Rubus* spp. from Indonesian Mountain's Forests was very limited. Currently, all of *Rubus* spp. in Indonesia is propagated by seed and cutting techniques. *R. chrysophyllus* and *R. fraxinifolius* are the potential species for breeding material in order to domesticate and develop a new cultivar of wild raspberry.

The *in vitro* propagation of *R. chrysophyllus* and *R. fraxinifolius* have not been clearly studied. The aim of this experiments was to examine the micropropagation methods of *R. chrysophyllus* and *R. fraxinifolius.*

MATERIALS AND METHODS

Plant material

The experiment was conducted in the Laboratory of Cibodas Botanical Gardens. The seeds of *R. chrysophyllus* and *R. fraxinifolius* which were used for the experiments was collected from Cibodas Botanical Gardens.

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(a) (b)

Figure 1. The plants and fruits of *R. chrysophyllus* (a) and *R. fraxinifolius* (b)

Seed germination

Rubus's seeds from mature fruits were first washed under running tap water, continued with a detergent solution for 5 min to remove pulp, then soaked for 15 min in tween 80. In order to minimize fungal and bacteria contamination, seeds were treated with a solution of fungicide (Benlox) and bactericide (Agrept) for 20 min by gentle shaking. Final steps of seeds surface sterilization were transferred to 70% ethanol for 1 min and followed by NaOCl solution (Sunklin® 20%) for 15 min. Each treatment was followed by repeated washing for a minimum of 3 times in sterile distilled water. The seeds were cultured in a bottle containing 20 mL of MS medium [8]. The medium was also enriched with 30 g/L sucrose and solidified with 8 g/L agar. The pH was adjusted to 5.8 before autoclaving at 121°C and 1 atm for 20 min.

For induction of rooting, shoot tips explants of *R. chrysophyllus* and *R. fraxinifolius* seedling were cultured on MS basal medium supplemented with growth regulator indole-3-butyric acid (IBA) 10 mg/L, 30 g/L sucrose and solidified with 8 g/L agar. The observations were made on root length, a number of leaves, buds, and roots.

Acclimatization of plants

Well-rooted shoots were removed from the culture medium, and the roots were washed gently with water to remove agar. The plantlets were then dipped into fungicide (Benlox) for few seconds to disinfect the plants. Plantlets were transferred to small plastic pots (8 cm in diameter) containing a cocopeat media. The plantlets were kept in a sealed chamber to maintain the humidity, after 1 month the plantlets were placed in the greenhouse for acclimatization. The plantlets were observed between 0 to 21 weeks after acclimatization (WAA). The observations were made on plant height, some buds, and the number of leaves.

RESULTS AND DISCUSSION

Micropropagation of *R. chrysophyllus* and *R. fraxinifolius* were studied using a shoot explants. In general, the results show that *R. fraxinifolius* gave a better response on MS medium containing 10 mg/L IBA than *R. chrysophyllus* (Figure 2). The response of *R. chrysophyllus* and *R. fraxinifolius* occurred on 14 days after transfer. On the parameters of roots length and roots numbers, *R. fraxinifolius* shows the best results, with average values, were 9.13 cm and 11.25, while *R. chrysophyllus* shows the average values were 2.20 cm and 2.00. Najaf-Abadi and Hamidoghli [9] reported that the additional of IBA had a significant effect on the number of roots produced and root length. Meng *et al.* [10] reported that auxins from IBA modified by cytokinin were the best plant growth regulator for promoting somatic embryogenesis. Furthermore, in

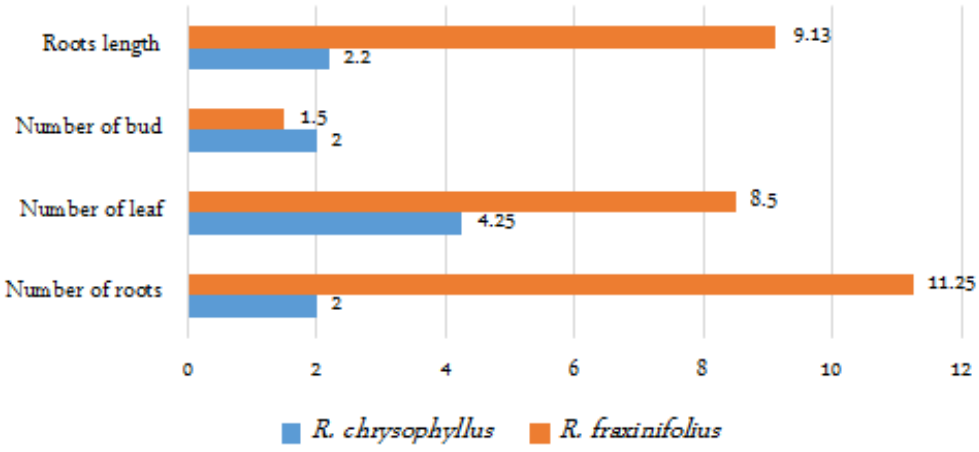

Figure 2. The effect of IBA medium on *R. chrysophyllus* and *R. fraxinifolius* growth by *in vitro* culture

Figure 3. *R. chrysophyllus* (RC) and *R. fraxinifolius* (RF) growths on 50 days after acclimatization

our experiment, the result show that either *R. chrysophyllus* or *R. fraxinifolius* were produced less number of buds (Figure 2). Martinussen *et al.* [5] reported that auxins promoted root formation in vitro but inhibit shoot or bud formations.

On the parameter of leaf number, the results show that leaf number of *R. chrysophyllus* (4.25) was lower than *R. fraxinifolius* (8.50). Reed [3] reported that each species of Rubus has different responses to the *in vitro* medium. It was in line with our experiment which is *R. fraxinifolius* and *R. crysophyllus* gave different responses into the same *in vitro* medium (Figure 2). Moreover, the application of growth regulator application is varied, depending on the genotype and physiological condition of the plant tissue.

Acclimatization from *in vitro* to *ex Vitro* condition is a critical step on Rubus micropropagation. Cocopeat was used as media for acclimatization *R. chrysophyllus* and *R. fraxinifolius* (Figure 3). Three parameters were observed during 21 weeks after acclimatization. The results show that either *R. chrysophyllus* or *R. fraxinifolius* gave good responses. Although during *in vitro* culture *R. fraxinifolius* were able to produce more roots than *R. chrysophyllus,* but after acclimatization, *R. chrysophyllus* could grow faster than *R. fraxinifolius*. It can be seen in figure 4 that the average of plant height of *R. chrysophyllus* was above 8 cm at 21 weeks after acclimatization, and *R. fraxinifolius* were only 4 cm. Martinussen *et al.* [5] reported that two cultivars of cloudberry (*Rubus chamaemorus*), the female 'Fjellgull' and the male 'Apollen', gave different response during *in vitro* propagation and after acclimatization. In the other hand, the opposite results occurred on parameter number of leaves. The results show that the number of leaves which produced by *R. fraxinifolius* (11.20) were higher than *R. chrysophyllus* (6.00) (Figure 4).

Micropropagation has been extensively used for the rapid multiplication of many plant species. However, acclimatization of *in vitro* grown plants is very difficult needing an understanding of the growth habit and factors including the root apparatus of the plants to be acclimatized, surrounding temperature, humidity, and soil template responsible for plant growth under *in vitro* and *ex Vitro* conditions. As the *in vitro* cultivated plants are very sensitive to each of these even a very small lag in the look after of the cultured tissue plants can lead to damage and death [11]. The specific *in vitro* environment, with artificial medium usually sup-

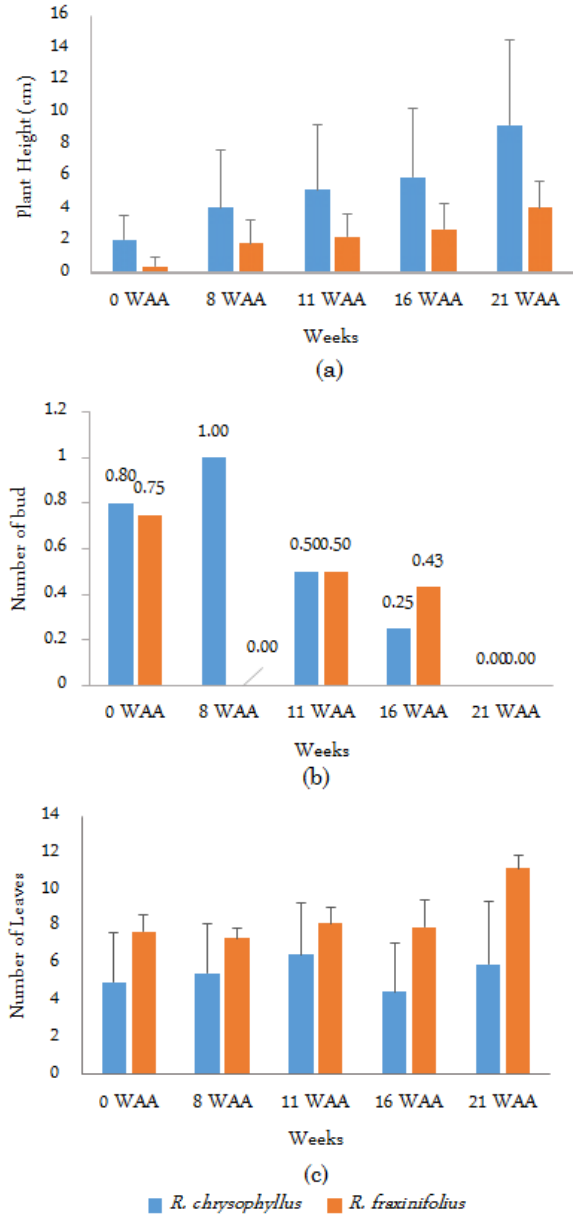

Figure 4. Height (a), number of bud (b) and number of leaves (c) of *R. chrysophyllus* and *R. fraxinifolius* from 0 to 21 weeks after acclimatization (WAA).

plied with sugar(s), the growth of plantlets in small air-tight vessels with high air humidity, low gas exchange and thus a CO2-shortage during almost the whole photoperiod, ethylene production and relatively low photosynthetic photon flux density, induces disturbances in plant development and photosynthetic performance [12, 13, 14]. Furthermore, the *in vitro* propagation is an intensive labor, and the acclimatization step is needed additional treatment to produced plantlet before it is commercialized and transported to the growers.

CONCLUSION

Rubus chrysophyllus and *R. fraxinifolius* gave a different response on the medium contain 10 mg/L of IBA. *R. fraxinifolius* was more responsive during *in vitro* culture compared to *R. chrysophyllus.* Although *R. fraxinifolius* was growing better than *R. chrysophyllus,* but after acclimatization *R. chrysophyllus* were able to grow faster than *R. fraxinifolius.* It was indicated by the value of plant height of *R. chrysophyllus* was higher than *R. fraxinifolius.* A further experiment is necessary to determine the excellent medium for *R. chrysophyllus.*

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