COMPLETE PLANTLET GENERATION AND ACCLIMATIZATION OF THE MEDICINAL HERB *ZINGIBER OFFICINALE VAR. RUBRUM*

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ABSTRACT

Zingiber officinale var. rubrum, also known as red ginger, is a perennial herb that is widely used in traditional medicine. The rhizome extract of Z. officinale var. rubrum has been reported to have antioxidant activity, anti-inflammatory activity, anti-nausea/antiemetic activity, anti-bacterial activity, cytotoxic activity, and antidiabetic activity. Using tissue culture techniques, the mass propagation of red ginger is studied. In a previous experiment, the red ginger shoot multiplication medium was determined, which is Murashige and Skoog (MS) media with 1.0 mg/L BAP supplementation. Even though the plantlets were able to establish a root system during the shoot multiplication stage, an experiment to study the effect of rooting hormone on the red ginger was conducted. The effects of two types of auxin, IBA and NAA, at different concentrations on the root induction of red ginger have been studied. Plantlets, measuring 3–4 cm in length, were cultured in MS media supplemented with IBA or NAA at different concentrations. Media without hormones was used as a control. After 1 week, all plantlets generated a root system in all medium experiments, with the highest number of roots per explant in the medium without hormone. The complete plantlets were acclimatized in four different media in a weaning chamber. The mixture of top soil, baked soil, and peat moss (2:1:1) had the highest survival rate, with 100% survival, and the mixture of peat moss and sand (1:1) had the lowest, with 50% survival. The plantlets that survived were placed in a polybag filled with mixed soil, top soil: baked soil and peat moss (2:1:1), placed under 50% shade, and watered three times daily. After 2 months, the average growth is 5 cm. The protocol developed can be applied for mass production of Z. officinale var. rubrum.

Keywords: *tissue culture, halia bara, root induction, hardening*

INTRODUCTION

Zingiber officinale Roscoe, also known as ginger, belongs to the Zingiberaceae family. It is a popular spice crop in Asia, where it is used in a variety of culinary and medicinal preparations. *Z. officinale* is classified into three varieties based on its size, rhizome colour, and chemical constituents: big white or giant ginger (*Z. officinale var. officinale*), small white ginger (*Z. officinale var. amarum*), and small red ginger (*Z. officinale var. rubrum*) (Supu et al., 2018). Red ginger is grown for its medicinal properties. Its rhizome is used in folk medicine (*jamu*) to treat stomach discomfort, tumours, rheumatic pains, and as a postpartum medicine (Ibrahim et al., 2008). The red ginger resembles common ginger, but its rhizomes are smaller and more pungent, red on the outside with a yellow to pinkish cross-section, and the base of its leaf shoot is red. Unlike common ginger, red ginger's petiole is reddish when young, and the lip is scarlet red mottled with cream (Ibrahim et al., 2008).

Apart from morphology, the chemical constituent of red ginger has a distinct value that aids in *Z. officinale* classification. The chemical constituent content of *Z. officinale* varieties influences their uses. Essential oils (shogaols and gingerol) that produce the gingery odour and taste were found in higher concentrations in red ginger than in the other two varieties. When compared to common ginger, these two compounds gave red ginger a pungent or stronger odour. Previous research found that red ginger has cytotoxic, antibacterial, antihypertensive, antihyperlipidemic, and immunomodulatory properties, and that these biological activities are the underlying causes of red ginger's therapeutic benefits (Zhang et al., 2022).

The red ginger, like other *Zingiber officinale* species, is vegetative propagated by underground rhizome buds. However, this method is limited by the number of rhizome buds that emerge, which is relatively low in a year and ginger plants are prone to fungal, bacterial, viral and mycoplasma diseases (Zuraida et al., 2016). To produce a large quantity of high-quality planting materials for commercial use, an alternative propagation process, tissue culture, is required. However, several factors influence tissue culture success; including plant species, explant selection, culture medium, growth regulator, and culture conditions (Sathyagowri and Seran, 2013). Surface sterilization, shoot multiplication, rooting induction, and acclimatization are all steps in developing a successful tissue culture protocol.

Clean shoot cultures of red ginger have been established and are used in this study for root induction experiments and later acclimatization in the nursery. The completed tissue culture protocol can be used to produce red ginger planting material in the future.

MATERIALS AND METHOD

Root induction

A study on the rooting of ginger using different rooting media was carried out. MS media supplemented with rooting hormone NAA or IBA at different hormone concentrations (0, 1, 2, 3, 4 mg/L) were tested. In this experiment, clean shoot cultures at 3 - 4 cm height were used as explants. Shoots are cultured in bottle jars containing MS medium supplemented with different types of auxins (NAA and IBA at 1.0, 2.0, 3.0, and 4.0 mg/L, respectively), and MS auxin-free medium was used as a control treatment. Each treatment was replicated five times, with four explants per replication. Cultures were kept in a growth room with the temperature set at 24±2 ^{o}C and 16/8 h light. Data related to the percentage of shoots rooted, number of roots per explant, and root length were collected after 1 week.

Acclimatization

Plantlets with an established root system were used for the acclimatization experiment using four different media compositions (M1, peat moss; M2, peat moss: sand (1:1); M3, peat moss: sand: perlite (1:1:1); and M4, top soil: baked soil: peat moss (2:1:1). The tested media were prepared a day before transplanting the plantlets. The complete plantlets were gently pulled out of the medium agar to avoid root damage and washed under tap water to remove any residual agar. Later, the plantlets were soaked in a fungicide solution for 10 seconds and then planted into the respective medium. Each medium was planted with 12 plantlets. The plantlets were acclimatized in a closed weaning chamber for 1 month and placed under shade. The weaning chamber cover was gradually removed beginning in week 3 and was completely removed by the fourth week. The survival percentage and plantlet growth data were collected after the fourth week. The surviving plantlets were transferred into a polybag filled with M4 medium, placed under 50% shade, and watered twice daily.

Statistical analysis

Duncan's Multiple Range test (DMRT) was performed when significant differences among treatments were detected by analysis of variance (ANOVA). The analyses were performed with SAS version 9.1.2 (SAS Institute Inc., Cary, NC, 2000).

RESULTS AND DISCUSSION

Effect of different auxins on complete plantlet generation

Rooting of red ginger was easily induced in all media tested, including in an auxin-free medium. In one-week culture, the explants were started to root, and the percentage of explants rooted was highest in MS medium supplemented with IBA (75%), all other IBA concentrations, and even higher than in control (65%), except for 2 mg/L IBA. In contrast, the percentage of explants rooted was low in NAA in all concentrations when compared to the control. MS media supplemented with 4 mg/L IBA was able to induce the highest percentage of shoot rooted in one week of culture duration. Low concentration of IBA, at 1 mg/L, was also able to induce a high percentage of shoot rooted, 70% (Figure 1).

In one week of culture duration, auxin-free MS media produced the highest number of roots per explant (1.56), followed by 3 mg/L IBA (1.38) and 2 mg/L NAA (1.36) (Figure 2). However, the number of roots produced per explant was not significantly different among the different treatments, including when compared to the control.

On the other hand, root length was increased in IBA when increasing the concentration to 4 mg/L. The longest roots (1.02 cm) were observed in the treatment with 4 mg/L IBA, but they were not significantly different from all other treatments, including NAA. Root length was reduced with treatments of 2 and 3 mg/L NAA compared with control. The shortest roots (0.36 cm) were observed in the treatment with 2 mg/L NAA, but they were not significantly different with other NAA concentrations or even when compared to control and IBA (Figure 2). The percentage of plantlets rooted increased with increasing culture duration. The data collection procedure after 2 weeks and above was quite difficult to perform since the roots would be tangled with each other, hence the reason why data collection was done early, at 1 week after culture.

In previous study, roots were spontaneously induced with the shoot multiplication stage of red ginger (Zuraida et al. 2016). This is in agreement with the previous reports that root induction occurred spontaneously with the shoot multiplication of other ginger species (Sathyagowri and Seran, 2013: Zahid et al. 2021). Although roots were spontaneously produced during the shoot multiplication stage, these roots were insufficient to transfer the plantlets for acclimatization after the numerous shoots were separated. An adequate number of roots per plantlet were required to transplant and survive during acclimatization process. Therefore, a separate experiment must be conducted to discover the suitable media for in vitro rooting of red ginger. The result showed that treatment with auxin-free medium and BAP was sufficient to induce a high percentage of explant rooted and a high root length. The treatment with NAA showed a low effect on root induction after 1 week of culture duration. This is different from the study by Zuraida et al. (2016), whereas NAA showed a good effect on rooting of red ginger compared to control, but the treatment was in combination with BAP. There was a probability that the combination of NAA and BAP played a role during root induction.

Figure 1. Percentage of root induced of *Z. officinale var rubrum* after 1 week in different rooting media

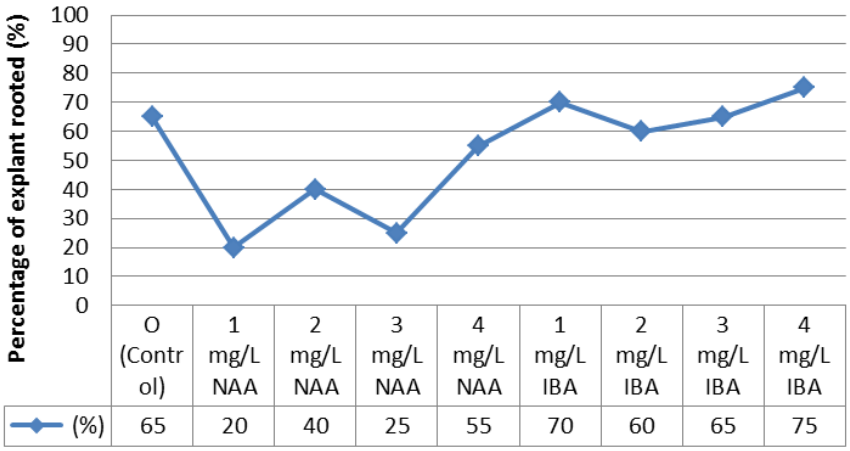
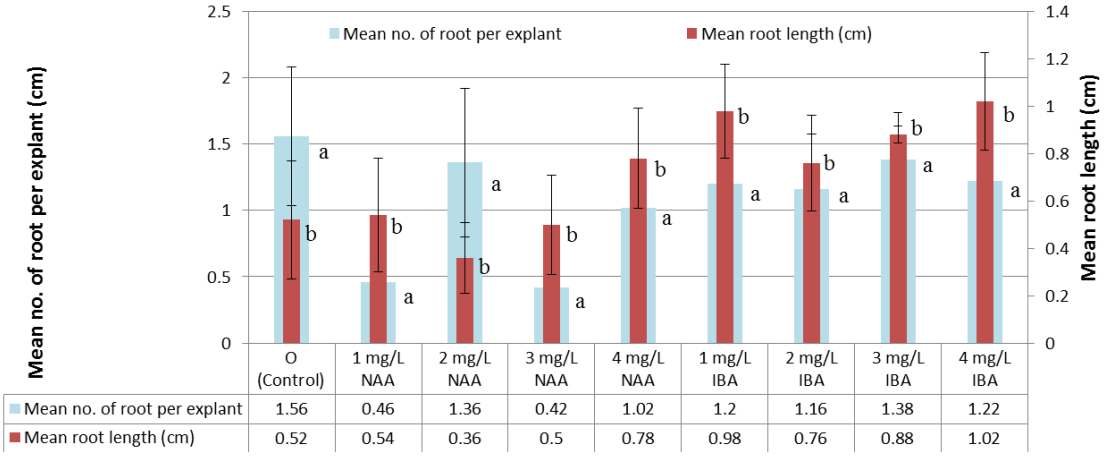


Figure 2. Root induction of *Z. officinale var rubrum* after 1 week in different rooting media



Effect of different medium on plantlets survival during acclimatization

Red ginger in vitro-rooted plantlets (Figure 3) were successfully acclimatized at the highest 100% survival rate in a M4 medium mixed of top soil: baked soil: peat moss (2:1:1 v/v/v). Other tested media also produced a high survival rate—more than 50% after one month. The growth of red ginger was the highest in M4 medium, with a 17.6 cm mean height after 1 month (Figure 4). Figure 5 shows the acclimatization of red ginger (a), and the survived plantlets grow healthy with new leaves after 2 months in a polybag with M4 media. After 2 months of acclimatization, the average growth is 5 cm.

Figure 3 *Z. officinale var rubrum* complete plantlets after 3 months in culture

Figure 4. Survival percentage and growth of *Z. officinale var rubrum* after 4 weeks acclimatized in 4 different media in a weaning chamber

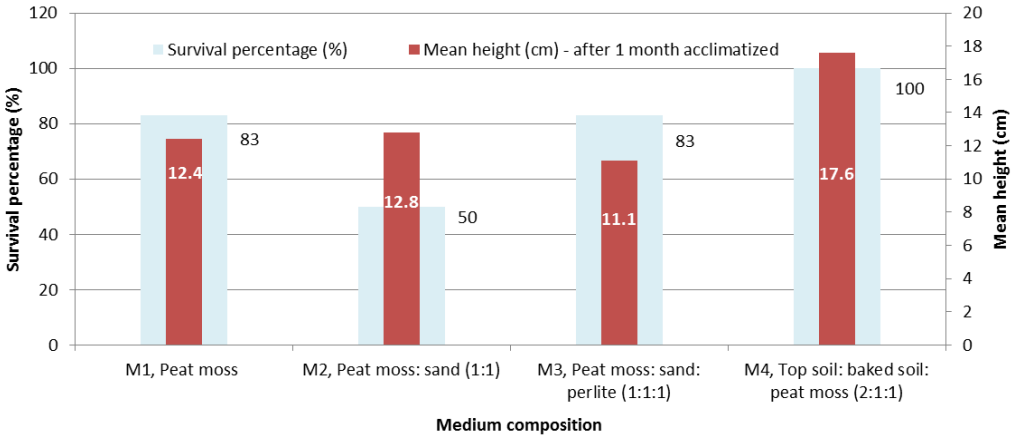


Figure 5. *Z. officinale var rubrum* after acclimatization process a) Acclimatized *Z. officinale var rubrum* in different media after 1 month in weaning chamber b) Tissue culture derived *Z. officinale var rubrum* after 2 months transplanted into M4 media

CONCLUSION

The establishment of red ginger tissue culture complete plantlets has been successfully obtained with suitable medium for rooting, MS without auxin, where the highest number of roots per explant and a second high percentage of explants rooted. The complete plantlets were successfully acclimatized after 1 month. Later, the production of the rhizome will be monitored and tested for secondary metabolite content.

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