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*Full Length Research Paper*

# Callus induction and plant regeneration of Sarawak rice (*Oryza sativa* L.) variety Biris

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**Sarawak Biris rice is a strong aromatic rice variety which can thrive well in rice fields prone to flood, drought and other soil constraints. Tissue culture of Biris rice is important in producing planting materials and conducting genetic improvement work. The present study was conducted to induce callus from Biris rice seed and assess its regeneration ability. Dehusked mature seeds were used as starting materials for callus induction. Sterilized seeds were cultured onto Murashige and Skoog medium containing various concentrations of 2, 4-dichlorophenoxyacetic acid (2, 4-D). The optimum 2, 4-D concentration for callus induction was 2.0 mg/L with a frequency as high as 97%. The calli produced were of desired features (creamy in colour, globular) and relatively bigger in size as compared to other treatments. The calli produced were further tested with plant growth regulator, naphthaleneacetic acid (NAA) in combination with kinetin (Kn) for plant regeneration ability. All uncontaminated calli were found to regenerate. Up to nine shoots were induced by a callus treated with 0.5 mg/L NAA in combination with 1.0 mg/L Kn. The present study should be noted as the first attempt to induce callus and regenerate plants from seeds of Biris rice.**

**Key words:** Biris rice, Sarawak, callus induction, plant regeneration.

## INTRODUCTION

The geographical setup of Sarawak has characterized majority of the rice fields to be located at hilly and undulating terrains. These physical settings as well as, the socio economic backdrop have lead Sarawak rice growers to scrupulously cultivate certain rice varieties which have been proven by generations to have good taste, high in quality and able to thrive well in uphill terrain. These varieties often require low farm inputs as many traditional rice growers do not apply fertilizers and their rice fields are rain-fed (Teo, 2010). These high quality rice varieties are gaining premium market value and the famous fragrant rice varieties such as Biris and Bajong are receiving brand protection from the government of Malaysia through the Geographical Indications (GI) Certificate of Registration Award from the Intellectual Property Corporation of Malaysia (Lim, 2009).

Biris rice originated from rice farms in Simunjan, Kota Samarahan, Sarawak. It made its debut in the early eighties due to its strong aroma and high demand from the affluent society. In the 1980s, the research branch of the Department of Agriculture (DOA) Sarawak rated Biris variety as one of the top three traditional high quality rice varieties in Sarawak with the highest commercial potential as compared to other varieties such as Bajong and Bario (Keng, 2009). Biris is classified as a long slender grain rice according to International Grain Classification System with average grain length of about (6.88 mm), width (2.47 mm) and with a length/width of (3.10 mm). The plant is a tall variety measuring about 160 cm in height. Farmers usually apply a low dosage of fertilizer before transplanting because heavy nitrogen application will result in increment of plant height and little effect on yield. Biris has thick and strong stems and its panicle is long, measuring 30 to 31 cm. Each panicle contains about 230 grains of paddy. Biris can be harvested in 163 to 167 days from the date of sowing (Teo, 2012).

Mass propagation of high quality rice is a matter of concern. In order to mass propagate Biris rice, cell

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suspension culture through tissue culture technique and optimization of the callus induction process are important steps in ensuring its success. Successful calli induction depends on a number of factors such as plant genotype, explant, culture medium, plant growth regulator and the culture environment (Khanna and Raina, 1998). Plant growth regulators are synthetic compounds that act like natural plant hormones which are often manipulated in tissue culture works. They display biological activity which equal or exceed that of the equivalent endogenous hormones (Jiménez, 2001). There are five main classes of naturally occurring plant hormones namely, auxins, abscisic acid, cytokinins, ethylene and gibberellins. Auxin, cytokinins and auxin-cytokinin interactions are usually considered to be the most important and generally required to regulate growth and organize development in plant tissue and organ cultures (Evans et al., 1981; Vasil and Thorpe, 1994). However, effects of natural and synthetic plant growth regulators are rarely specific in their ultimate influence on growth and development. The responses of cells, tissues, and organs *in vitro* can vary with the cultural conditions, explants and genotypes. The present study was conducted to determine the optimum concentration of a synthetic auxin 2, 4-dichlorophenoxyacetic acid (2, 4-D) for callus induction and concentrations of naphthaleneacetic acid (NAA) and kinetin (Kn) on plant regeneration of Biris variety.

## MATERIALS AND METHODS

Seeds of Biris rice were obtained from a rice farm in Simunjan, Sarawak, Malaysia. The seeds were freshly harvested, washed, dehusked and surface sterilized in 80% (v/v) ethanol for one minute and rinsed with double distilled water to remove ethanol traces. Subsequently, the dehusked seeds were dipped in 15% (v/v) commercial Clorox (0.78% sodium hypochlorite) solution with an addition of three drops of Tween 20 as a wetting agent for 10 min followed by 30% (v/v) commercial Clorox (1.57% sodium hypochlorite) solution and three drops of Tween 20 for 10 min. Finally, the dehusked seeds were rinsed again thrice with sterilized water. Seeds were kept on a layer of sterilized filter paper in a Petri dish to remove excess water prior to transfer onto culture media.

### Callus induction

Semi-solid Murashige and Skoog (MS) medium was selected as a basal medium for callus induction and plant regeneration (Murashige and Skoog, 1962). Sterilized dried seeds were inoculated onto semi-solid MS media supplemented with 2, 4-D for callus induction. The pH of all culture media was adjusted to 5.8. Cooled autoclave-sterilized MS media were supplemented with different concentrations of 2, 4-D (0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/L). Each treatment consisted of 40 replications. Frequencies (%) of callus induction were counted after six weeks of inoculation and calculated as follow:

Callus induction frequency (%) = number of seeds producing callus/number of seeds inoculated × 100

### Plant regeneration

Calli were cultured onto semi-solid MS media supplemented with different concentrations of NAA and Kn for plant regeneration. All cultures were incubated in the dark for four weeks prior to transfer to light at 25 ± 1°C for two weeks. Frequencies (%) of plant regeneration were recorded after two months of transferring. Observation was done regularly and contaminated cultures were removed from shelves and discarded. The plant regeneration was calculated as follows:

Regeneration frequency (%) = number of plants recovered/number of callus plated × 100

### Data analysis

The effect of treatment in inducing shoot and root growth from callus was analyzed using the Statistical Analysis System (SAS) Window Programme version 9.1. Mean number of shoot and root produced were compared using the Duncan's Multiple Range Test (DMRT) at a significant level of $P \leq 0.05$.

## RESULTS

### Callus induction

Based on results obtained, the percentage of callus induction varied from 80.0 to 97.5% across different concentrations of 2, 4-D (Table 1). The control without any 2, 4-D application failed to induct any callus growth. The greatest (97.5%) and lowest (80.0%) callus induction percentages were observed in T2 (2.0 mg/L 2, 4-D) and T5 (5.0 mg/L 2, 4-D) respectively. It was observed that the frequency of callus produced decreased when the concentration of 2, 4-D exceeded 2.0 mg/L. Different concentrations of 2, 4-D was found to produce various morphology of callus. High concentration of 2, 4-D (3.0, 4.0 and 5.0 mg/L) resulted in yellowish, small or short, compact and fragile calli. Calli generated on MS medium supplemented with 1.0 and 2 .0 mg/L 2, 4-D were creamy to whitish in colour and globular in shape. Calli generated on medium supplemented with 2.0 mg/L 2, 4-D were healthy and bigger (Figure 1).

### Plant regeneration

Calli produced on MS media supplemented with 2.0 mg/L 2, 4-D were assessed for their plant regeneration ability. Based on the results (Table 2), T1 (0.5 mg/L NAA+1.0 mg/L Kn) and T2 (1.0 mg/L NAA+1.0 mg/L Kn) produced high number of both shoots and roots; however, these values were not significant when compared with T3 and T4. Whilst a significant higher (55.6%) number of calli in T4 (3.0 mg/L NAA+1.0 mg/L Kn) failed to produce either roots or shoots. T3 (2.0 mg/L NAA +1.0 mg/L Kn) produced significantly ($P \leq 0.05$) high rooted calli as compared with T1 and T4.

**Table 1.** Effect of different concentration of 2,4-D on callus induction.

| Treatment | Concentration of 2, 4-D (mg/L) | No. of seeds inoculated | Frequency (%) |
|---|---|---|---|
| Control | 0 | 40 | 0 |
| T1 | 1.0 | 40 | 92.5$^{ab}$ |
| T2 | 2.0 | 40 | 97.5$^{a}$ |
| T3 | 3.0 | 40 | 90.0$^{abc}$ |
| T4 | 4.0 | 40 | 82.5$^{bc}$ |
| T5 | 5.0 | 40 | 80.0$^{c}$ |

Means within columns with the same superscript are not significantly different at $P \leq 0.05$ by using DMRT.

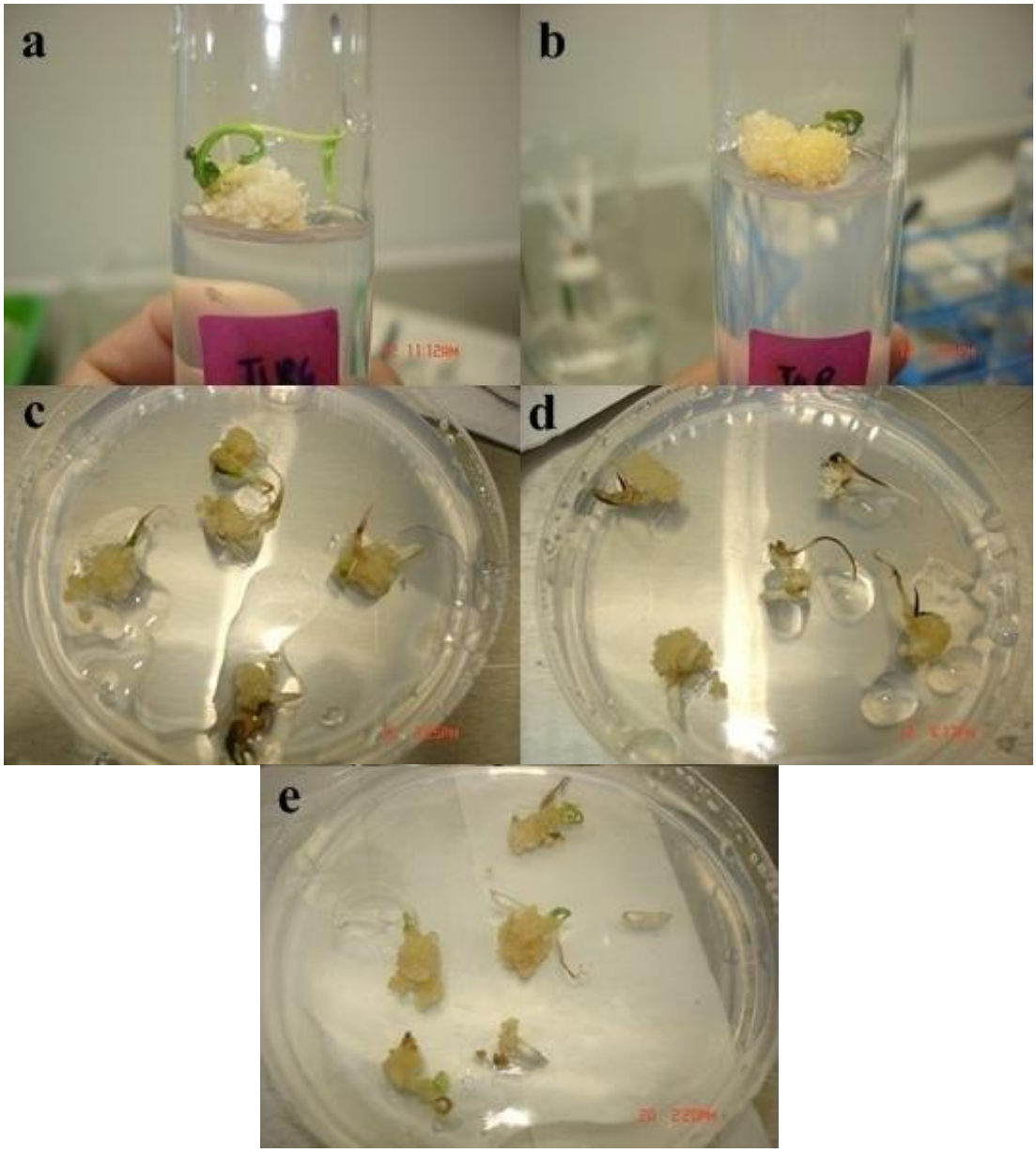


**Figure 1.** Effect of different concentration of 2, 4-dichlorophenoxyacetic acid (2, 4-D) on callus induction of rice seeds. callus induction on Murashige-Skoog medium containing (a) 1.0 mg/L 2,4-D, (b) 2.0 mg/L 2,4-D, (c) 3.0 mg/L 2,4-D, (d) 4.0 mg/L 2,4-D, and (e) 5.0 mg/L 2,4-D. Scale bars = (a) 0.8 cm, (b) 0.9 cm, (c, d) 1.3 cm and (e) 1.2 cm.

**Table 2.** Effects of different NAA concentrations combined with Kn on the production of shoots and or roots from callus.

| Treatment | NAA + Kn concentration (mg/L) | Frequency (%) of shoot and or root formation from callus | | | |
|---|---|---|---|---|---|
| | | Shoot | Root | Shoot and root | No shoot and root |
| T1 | 0.5 NAA + 1.0 Kn | 11.1 | 11.1[b] | 55.6 | 22.2[ab] |
| T2 | 1.0 NAA + 1.0 Kn | 0.0 | 33.3[ab] | 66.7 | 0.0[b] |
| T3 | 2.0 NAA + 1.0 Kn | 11.1 | 55.6[a] | 22.2 | 11.1[b] |
| T4 | 3.0 NAA + 1.0 Kn | 22.2 | 0.0[b] | 22.2 | 55.6[a] |

Means within columns with the same superscript are not significantly different at $P \leq 0.05$ by using DMRT.

**Table 3.** Effects of different NAA concentration in combination with Kn on the mean number of shoots and roots produced per callus.

| Treatment | NAA + Kn concentration (mg/L) | Mean number | |
|---|---|---|---|
| | | Shoot per callus | Root per callus |
| T1 | 0.5 NAA + 1.0 Kn | 9[a] | 12[c] |
| T2 | 1.0 NAA + 1.0 Kn | 6[ab] | 32[b] |
| T3 | 2.0 NAA + 1.0 Kn | 4[ab] | 50[a] |
| T4 | 3.0 NAA + 1.0 Kn | 2[b] | 6[c] |

Means within columns with the same superscript are not significantly different at $P \leq 0.05$ by using DMRT.

### Effect of NAA in combination with Kn on the number of shoots and roots produced per callus

Different levels of growth hormone gave significantly different effects on the number of shoots and roots produced (Table 3). As concentration of NAA increased, the number of shoots per callus decreased. T1 recorded the highest number of shoot per callus with nine. The value was significantly ($P \leq 0.05$) higher than T4. The number of roots produced per callus increased with the increase of NAA concentration until T3 and reduced at the highest concentration. T3 produced significant ($P \leq 0.05$) higher number of roots per callus as compared to three other treatments. The morphology and quality of shoots produced varied with different concentrations of NAA supplemented (Figure 2). Shoots produced in T2 were bigger, healthier and darker, as compared to those produced in other treatments.

## DISCUSSION

### Effect of 2, 4-D on callus induction of Biris seeds

Several studies have reported that plant growth regulator 2, 4-D is the most suitable auxin for callus induction in rice tissue culture (Chen et al., 1974; Maeda, 1980; Bajaj, 1991). However, the optimum concentration of 2, 4-D in callus induction varied, depending on the explant source and genotype of rice (Raina, 1989). In this study, the effectiveness of different concentrations of 2, 4-D alone were evaluated for callus induction from dehusked seed of Biris rice. Different concentrations of 2, 4-D was observed to produce various morphology of callus. Semi-solid MS medium supplemented 2.0 mg/L 2, 4-D gave the greatest frequency of callus induction (97.5%) and has the most desired calli features (Figure 1b). This indicated that 2.0 mg/L 2, 4-D was optimum for callus induction from Biris rice seeds. Results of the present study were in agreement with those of Pandey et al. (1994), Thadavong et al. (2002) and Abeyaratne et al. (2004) which showed that 2.0 mg/L 2, 4-D was the most optimum concentration for callus induction from mature rice seeds. Moreover, based on ten rice genotypes used, calli produced on semi-solid MS media supplemented with 2.0 mg/L 2, 4-D produced the most desired features (Pandey et al., 1994).

### Effect of NAA in combination with Kn on plant regeneration

In this study, NAA was tested at various concentrations in combination with 1.0 mg/L Kn, to assess plant regeneration from calli produced on semi-solid MS media supplemented with 2, 4-D. The most optimum concentration of NAA in combination with 1.0 mg/L Kn for plant regeneration was 1 mg/L which gave the highest plant regeneration frequency (67%), producing both shoots and roots. Results of current study also showed that as the concentration of NAA increased, the frequency of callus producing both shoots and roots decreased. This may due to the high concentration of NAA (2.0 and 3.0 mg/L) which could inhibit cytokinin

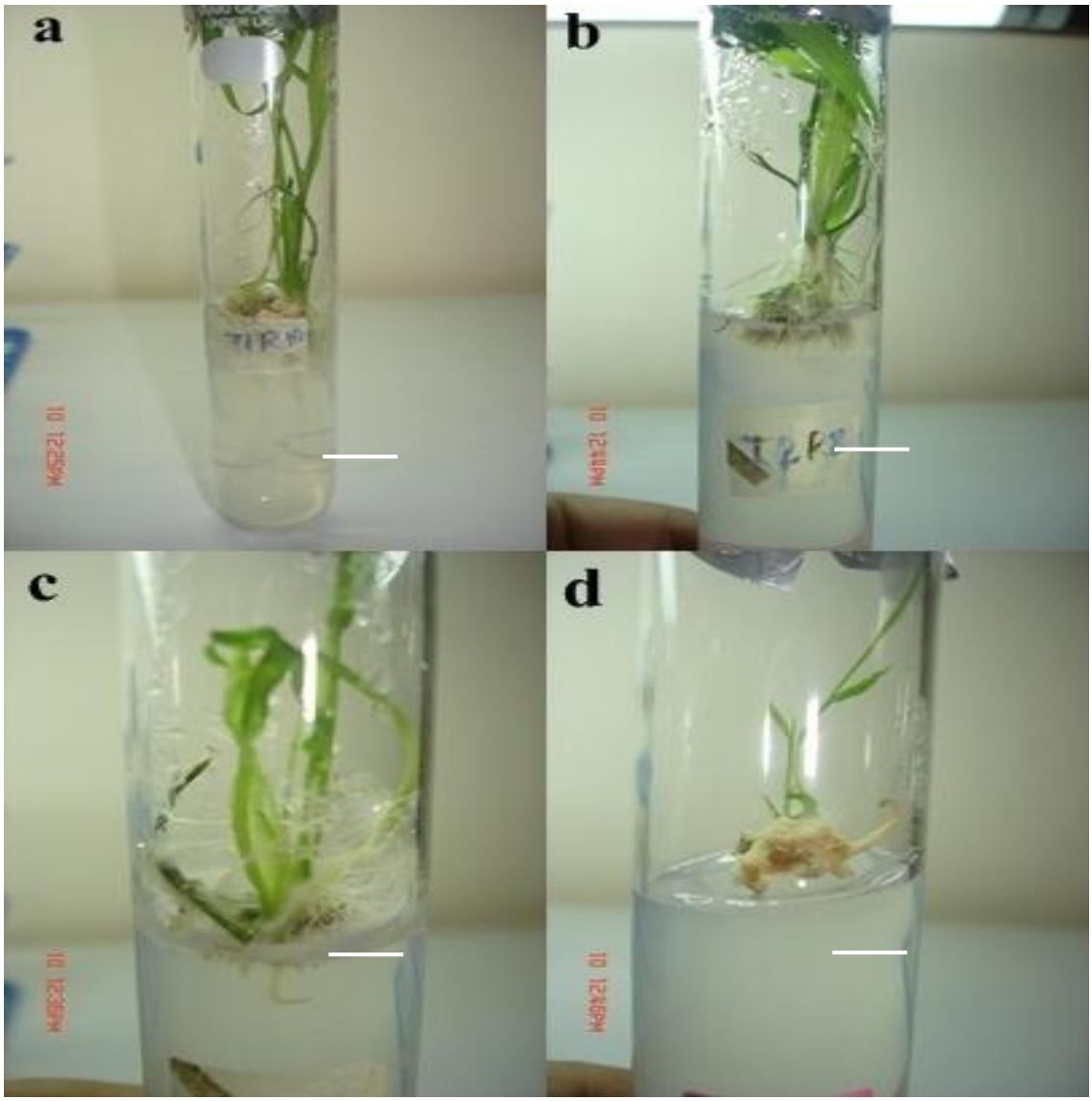


**Figure 2.** Effect of various NAA concentrations in combination with 1.0 mg/L Kn on rice regeneration from callus. Plant regeneration on medium containing 0.5 (a), 1.0 (b), 2.0 (c) and 3.0 (d) mg/L NAA. scale bars = (a) 1.5 cm, (b) 1 cm, (c) 0.8 cm.

accumulation, and suppress growth and morphogenesis (Gasper et al., 1996).

The effect of different NAA concentrations in combination with Kn on root production was contrary to shoot production. The mean number of root produced per callus increased with an increase in NAA concentration. Increase of NAA concentration in the medium increases the ratio of auxin to cytokinin and this consequently, enhanced root regeneration. Such phenomenon was reported by Thadavong et al. (2002). However, when the NAA concentration is equal or exceeded 3.0 mg/L, the number of root produced decreased. At this concentration, NAA significantly suppressed growth and morphogenesis. Results of this study indicated that calli produced in MS media supplemented with 2.0 mg/L of 2, 4-D have the highest regeneration ability (100%) when combined with 1.0 mg/L NAA and 1.0 mg/L Kn.

## Conclusion

The current study indicates that the MS medium supplemented with 2.0 mg/L 2, 4-D gave the highest frequency (97.5%) of callus induction from matured seeds of Biris rice. The qualities of calli produced showed the most desired features which were creamy, globular and big in size. The most effective concentration of NAA in combination with 1.0 mg/L Kn for plant regeneration

from calli of Biris rice was 1.0 mg/L NAA. Calli produced by dehusked Biris seeds in MS media supplemented with 2.0 mg/L of 2,4-D were suitable as starting material for cell suspension in order to mass propagate and genetically improve plant material for commercial planting.

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