

Response of Anther Donor Genotypes (F_1) from *Indica* × *Indica* Crosses to Rice Anther Culture (Respons Beberapa Populasi F_1 Hasil Persilangan *Indica* × *Indica* pada Kultur Antera Padi)

Iswari Saraswati Dewi^{1*}, Nuha Hera Putri², and Bambang Sapta Purwoko²

¹Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Jl. Tentara Pelajar 3A, Bogor 16111, West Java, Indonesia

Tel. (62-251) 8622833; Fax. (62-251) 8622833; *E-mail: iswari.dewi01@gmail.com

²Department of Agronomy and Horticulture, Bogor Agricultural University, Jl. Meranti, Dramaga Campus, Bogor 16680, West Java, Indonesia

Submitted: 23 September 2018; Revised: 19 June 2019; Accepted: 28 June 2019

ABSTRAK

Kultur antera merupakan salah satu teknik kultur *in vitro* yang dapat mempercepat perolehan galur-galur murni dalam bentuk tanaman *doubled haploid* atau dihaploid (DH) hasil androgenesis. Tujuan penelitian ialah mempelajari respons donor antera pada kultur antera padi F_1 *indica* × *indica* dan memperoleh galur-galur DH untuk digunakan dalam program perakitan varietas padi. Tanaman donor antera yang digunakan adalah empat jenis populasi F_1 , yaitu IR85/I-5, BioR-81/I-5, Bio-R81/O18, dan BioR-82/O-18. Hasil penelitian menunjukkan bahwa semua jenis tanaman F_1 memberikan respons yang serupa terhadap induksi kalus, tetapi memberikan respons yang berbeda nyata terhadap regenerasi tanaman. Genotipe tanaman donor antera (F_1) nyata mempengaruhi persentase kalus yang menghasilkan tanaman hijau, tetapi tidak nyata pengaruhnya terhadap persentase kalus yang menghasilkan tanaman albino. Daya kultur antera tanaman donor (F_1) dalam menghasilkan tanaman hijau relatif tinggi, berkisar antara 2,0% dan 7,0%. Dari penelitian ini diperoleh 73 generasi pertama galur DH spontan atau 38,8% dari jumlah tanaman hijau yang ditanam. Galur DH yang diperoleh siap untuk diseleksi lebih lanjut dan digunakan dalam program perakitan varietas unggul baru padi.

Kata kunci: Androgenesis, dihaploid, *indica* × *indica*, padi.

ABSTRACT

Anther culture is one of the *in vitro* techniques that can be used to accelerate the obtainment of purelines in the form of dihaploid (DH) plants resulted from androgenesis. The objectives of this research were to study response of anther donor genotypes in rice anther culture of F_1 populations derived from *indica* × *indica* crosses and obtain spontaneous DH lines to be used further for variety development program. Anther donor plant used for the experiment consisted of four kinds of F_1 populations derived from single crosses, i.e. IR85/I-5, BioR-81/I-5, Bio-R81/O18, and BioR-82/O-18. The result indicated that kindy of all F_1 plants showed similar response to callus induction, but significantly different responses to plant regeneration. Genotypes of anther donor plants (F_1) significantly affected the percentage of calli producing green plantlet, but not significantly affected the percentage of calli producing albino plantlet. Anther culturability of anther donor plants (F_1) was relatively high, ranged from 2.0 to 7.0%. A total of 73 first generation of spontaneous DH lines (38.8%) from the grew out green plantlets was obtained from this study. The resulted DH lines are readily available to be selected and be used in rice cultivar development program.

Keywords: Androgenesis, dihaploid, *indica* × *indica*, rice.

INTRODUCTION

Cross hybridization followed by selection in several generations has been utilized to obtain advanced homozygous or pure lines potential to be released as new varieties in conventional rice breeding (Jeon et al. 2011). The process to obtain pure lines with ~99% homozygosity takes at least 6–8 generations of selection in conventional rice breeding (Foster and Thomas 2005).

Compared to that long process in conventional breeding, androgenic spontaneous doubled haploid or dihaploid (DH) plants resulted from rice anther culture is readily available to be used as pure lines at the first generation of culture (DH₀). These pure lines are highly homozygous and can be readily selected as materials suitable for variety development in rice breeding programs (Dewi and Purwoko 2012; Datta 2014; Mishra and Rao 2016; Akhmadi et al. 2017; Anshori et al. 2018). Thus, incorporation of anther culture into rice breeding program would be an advantage because it reduced the time, costs, and labours (Germana, 2017; Hidayatullah et al. 2018). However, the technique should allow production of large number of genetically stable spontaneous DH plants from a wide range of genotypes for effective utilization in rice breeding programs (Dewi and Purwoko 2008).

Most of the breeding programmes in the tropics, including Indonesia, utilized rice subspecies *indica* for rice variety development (Dewi et al. 2009; Dewi and Purwoko 2012; Khush 2013). Unfortunately, rice subspecies *indica* was known as a recalcitrant genotype that exhibits poorer androgenic response than that of *japonica* due to early anther necrosis, poor callus proliferation, and high albino plant regeneration (Chen et al. 1991). By adding 10⁻³ M putrescine into callus induction and regeneration media, Dewi and Purwoko (2008) were able to improve green plantlet regeneration in rice anther culture of subspecies *indica*. However, genotype strongly affected the ability to regenerate plantlets in anther culture of *indica* rice (Dewi et al. 2009).

Rice anther culture can be used to produce DHs with multiple stress tolerances. Dewi et al. (2009) reported the anther culturability of *indica* genotypes used for development of new rice varieties tolerant to aluminum toxicity. Similarly, Purwoko et al. (2010) reported the use of anther culture in upland rice breeding program by producing DH lines tolerant to aluminum stress, shade tolerance, and blast resistance. From the previous research program aimed to develop high yielding rice variety tolerant to

biotic and abiotic stresses, four kinds of F₁ populations were obtained from single crosses between *indica* rice parents, i.e. high yield and resistant to bacterial leaf blight lowland rice lines (Purwoko et al. 2010) and high yield, tolerant to shade and drought, moderately tolerant to Al toxicity, and resistant to blast upland rice lines (Herawati et al. 2010; Safitri et al. 2016). The objectives of this research were to study response of anther donor genotypes in rice anther culture of F₁ populations derived from *indica* × *indica* crosses and obtain spontaneous DH lines to be used further in variety development program.

MATERIALS AND METHODS

Materials

Anther donor plants used in this research were four F₁ populations, each derived from a single cross between lowland rice and upland rice (high yield, tolerant to shade and drought, moderately tolerant to Al toxicity, and resistant to blast), i.e. IR85/I-5, BioR-81/I-5, BioR-81/O-18, and BioR-82/O-18. These donor plants were planted in the greenhouse until booting stage when young panicles were collected.

The media used for callus induction and plant regeneration in anther culture was the same media used by Dewi and Purwoko (2008). Callus induction medium was N6 + 2.0 mg/l NAA + 0.5 mg/l kinetin + 10⁻³ M putrescine + 6.0% sucrose, and regeneration medium was MS + 0.5 mg/l NAA + 2.0 mg/l Kinetin + 10⁻³ M putrescine + 3.0% sucrose (Dewi and Purwoko 2008). Phytigel® (3 g/l) was used as a solidifying agent in both media.

Methods

The experiment was performed using a Completely Randomized Design consisting of ten replications. A single treatment used was an anther donor plant, from single crosses (F₁) i.e. IR85/I-5, BioR-81/I-5, Bio-R81/O18, and BioR-82/O-18. Each experimental unit was one petridish containing anthers from 25 spikelets. Rice anther culture method followed the protocol of Dewi et al. (2004) as follows:

Explant preparation

Young panicles were collected at booting stage when the auricle distance between the flag leaf and subtending leaf of the primary and secondary tillers reached 7–10 cm depending on genotypes. Prior to anther culture, the panicles were incubated at 5°C for 8 days. After cold treatment, panicles were surface

sterilized with 20% commercial bleach containing 5.25% NaClO for 20 minutes under aseptic condition. Spikelets, in which the anther and filament length was no more than half the length of the spikelet, were selected from the middle portion of the panicles. Then, individual spikelets were cut to expose the anthers.

Callus induction and plant regeneration

Anthers containing microspore at the mid-uninucleate to early binucleate stage of development were plated onto petridish containing callus induction medium. The cultures were then incubated in the dark at $25 \pm 2^\circ\text{C}$ to induce callus formation from microspores. Induced calli (± 2 mm in diameter) were subcultured onto the plant regeneration medium and incubated under the light from 80 watts fluorescent bulb (approximately 1,500–1,600 lux) at $25 \pm 2^\circ\text{C}$.

Root induction

If somehow plantlet did not have or lack of roots, green plantlet was transferred to a test tube containing MS medium supplemented with 0.5 mg/l IBA to induce root or more developed root. Cultures were kept under 16 hours daily illumination similar to that of the light condition used for plant regeneration.

Acclimatization and greenhouse grew out

Green plantlets, having 3–4 leaves and roots, were acclimatized in sterile water for a week followed by transplanting them into the mud. After 2 weeks in the mud, surviving plants were transferred to 10 litre plastic pot, and placed in the greenhouse. Pests and diseases were controlled as needed.

Observation

Observation on anther response to callus induction was carried out during 60–80 days after anther plating. Observation was conducted on number of anther plated (NAP), number of calli produced (CF), number of calli producing plantlet (CPP), calli forming green plantlets (CGP), number of plantlets (NP), number of calli forming albino plantlets (CAP), number of green (GP) and albino (AP) plantlets, number of survivor green plants (SGP), and number of first generation of DH (DH_0) plants. The above observed primary data were used to obtain:

- Percentage of formed calli = $(\text{CF}/\text{NAP}) \times 100\%$
- Percentage of calli forming green plantlets = $(\text{CGP}/\text{CF}) \times 100\%$
- Percentage of calli forming albino plantlets = $(\text{CAP}/\text{CF}) \times 100\%$

- Percentage of green plantlets = $(\text{GP}/\text{NP}) \times 100\%$
- Percentage of albino plantlets = $(\text{AP}/\text{NP}) \times 100\%$
- Percentage of anther culture ability (ACA) = $(\text{GP}/\text{NAP}) \times 100\%$
- Percentage of DH_0 plants = $(\text{Number of } \text{DH}_0 \text{ plants}/\text{Number of grew out plants}) \times 100\%$

Data analysis

Statistical analysis was performed using variance analysis. The differences between treatments were tested by Duncan Multiple Range Test (DMRT). Data analysis was carried out using the Statistical Tool for Agricultural Research (STAR) version 2.0.1.

RESULTS AND DISCUSSION

Callus Induction

The number of callus produced is not significantly different (Table 1). Thus, the ability of anthers in producing callus was similar in each genotype. Calli were obtained from all genotypes. The onset of callus induction in this experiment started 4–5 weeks after anthers were plated and marked by anther colour changing from yellow to brown followed by the splitting of the anther lobes exposing the microspores. According to Germana (2017), this mechanism is due to the osmotic pressure caused by high levels of carbon source in the media. In this research, we used 6.0% sucrose in the callus induction media. This suggested that callus formation was not derived from somatic tissue of the anther, but was formed from microspores within the anther.

Although it is not significantly different, percentage of callus formation varied between 14.7 to 32.7% (Table 1). Dewi et al. (2009), Bhattacharya et al. (2014), and Dewi et al. (2017) showed that response of rice subspecies *indica* to callus induction in anther culture was usually low (<10%), but the responsive genotype could produce more than one callus from microspores in one anther depending upon the F_1 genotype used.

Plant Regeneration

The ability of callus to regenerate plant in this experiment was not significantly different (Table 2). Two types of calli can be observed in the regeneration medium and could be easily distinguished by their structures and colours. Embryogenic calli, the one that produced plantlets, were creamy, compact, globular in shape, rapidly growing, and with clearly formed green spots prior to the development of somatic embryos, while non-

Table 1. Callus formation in rice anther culture of F₁ plants resulted from *indica* × *indica* crosses.

Genotypes (F ₁)	Number of anther plated	Number of calli formed	Calli formed (%)
IR85/I-5	152.3	41.2	26.5
BioR-81/I-5	145.2	43.1	32.7
BioR-81/O-18	152.2	23.7	14.7
BioR-82/O-18	147.4	36.1	27.0

Table 2. Rate of calli producing plantlet in rice anther culture of F₁ plants resulted from *indica* × *indica* crosses.

Genotypes (F ₁)	Calli producing plantlet	Percentage of calli producing plantlet	Number of calli producing green plantlet	Percentage of calli producing green plantlet*	Number of calli producing albino plantlet*	Percentage of calli producing albino plantlet
IR85/I-5	5.7	12.3	1.0	2.4 b	4.7 a	11.4
BioR-81/I-5	4.6	9.4	1.6	3.7 b	3.0 ab	6.9
BioR-81/O-18	3.5	13.2	1.6	6.8 a	1.9 b	8.0
BioR-82/O-18	3.7	8.5	1.7	4.7 ab	2.0 b	5.5

*Numbers in the same column followed by the same letters are not significantly different by DMRT at $P < 0.05$.

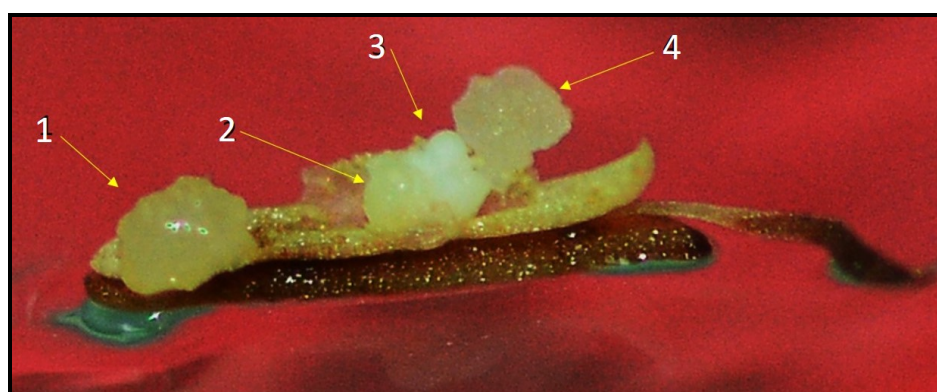


Figure 1. Calli-derived microspores in regeneration medium of rice anther culture. The photograph was taken 6 weeks after callus formation (diameter >2 mm) to show embryogenic calli, creamy colour (arrows no. 1, 2, 4), and non-embryogenic callus (arrow no. 3) developed within gradually senescence anther.

embryogenic calli were white, often friable or watery (Figure 1).

Green plant regeneration started when green spots were observed developing on the embryogenic callus surface approximately 5 days after transferring calli onto regeneration medium. The time taken for calli to regenerate influenced the type of plants formed. Calli that took longer time to regenerate (>10 days) would form albino plants. Similar results were obtained by Gunarsih et al. (2016) and Safitri et al. (2016). Genotype of anther donor plants (F₁) significantly affected the percentage of calli producing green plantlet, but not significantly affected the percentage of calli producing albino plantlet (Table 2). The range was between 2.4–6.8% and 5.5–11.4% for calli producing green plantlet and calli producing albino plantlet, respectively. Interestingly, BioR-81/O-18 produced the highest percentage of calli producing green plantlet (6.8%), although it gave the lower percentage of callus formation (Table 1). Therefore, high callus formation does not always result in high

green plant regeneration. The result was similar to other *indica* rice anther culture studies conducted by Niroula and Bimb (2009), Gunarsih et al. (2016), and Safitri et al. (2016).

In this experiment as in other experiments involving anther culture, calli approximately 2 mm in diameter were transferred into regeneration media. However, not only calli size, but also type of calli transferred into regeneration media determined the outcome of plantlets (Dewi and Purwoko 2008; Tyankova and Zagorska 2008; Germana 2017). Since it was difficult to really distinguished the type of calli when the diameter was so small, thus in this experiment in the regeneration medium there were calli that produced green plantlets or albino plantlets (Table 2, Figure 2).

Previous reports revealed that generally calli with white colour regenerated albino plantlets, while creamy colour calli regenerated green plantlets (Dewi et al. 2004; Safitri et al. 2010). Naik et al. (2017)

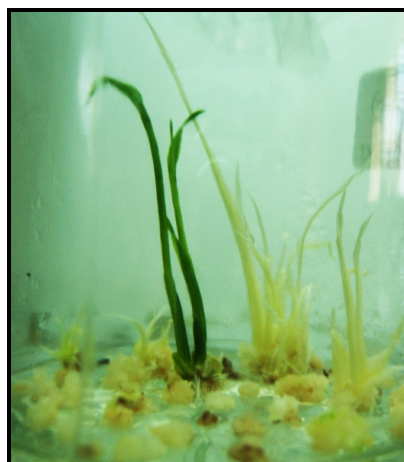


Figure 2. Plant regeneration in rice anther culture showed that green and albino plantlets were obtained from calli.

Table 3. Plant regeneration in rice anther culture of F₁ plants resulted from *indica* × *indica* crosses.

Genotypes (F ₁)	Number of plantlets*	Number of green plantlets*	Percentage of green plantlet*	Number of albino plantlets*	Percentage of albino plantlet*	Percentage of anther culturability*
IR85/I-5	31.7 a	3.4 b	10.7 b	28.3 a	89.3 a	2.0 b
BioR-81/I-5	14.0 b	5.9 ab	42.1 a	8.1 b	57.9 b	4.0 ab
BioR-81/O-18	12.4 b	7.7 ab	62.1 a	4.7 b	37.9 b	5.0 a
BioR-82/O-18	18.6 b	9.5 a	51.1 a	9.1 b	48.9 b	7.0 a

*Numbers in the same column followed by the same letters are not significantly different by DMRT at $P < 0.05$.

recently reported that only the creamy colour compact calli were able to pass through the regeneration stage into plants, while the watery callus was unable to regenerate. Generally, calli with size more than 4 mm in diameter was gradually browning and died after transferring them into plant regeneration medium, but when those calli eventually made into plant regeneration only albino plantlets were produced (Sasmita et al. 2001).

Number of plantlet, number of green and albino plantlets regenerated were varied significantly depending upon the genotypes of the anther donor plants (Table 3). IR85/I-5 gave the highest total number of plantlets, while the other three were not significantly different from each other. Genotype was found to be the main limiting factor of *in vitro* androgenesis (El-Hennawy et al. 2011).

In this experiment, all F₁ plants gave more than 10% rates of green plant regeneration from 10.7 to 62.1% (Table 3). This result is higher than that of similar experiment reported by Gioi and Tuan (2004) which showed the results of anther culture of F₁ from several *indica* × *indica* crosses, i.e. between IR64 and new plant types cultivars only reached the highest green plant regeneration rate of 5.72%. According to Dewi and Purwoko (2008), compared to *japonica*, the low androgenic response of rice subspecies *indica* in

anther culture was due to early necrosis or senescence of anthers resulted from a higher rate of ethylene productions by anthers of *indica*. They suggested adding an anti-senescence growth regulator, i.e. 10⁻³ M putrescine, to prolong viability of *indica*'s anther in order to ensure androgenesis. Thus, by adding putrescine into callus induction and plant regeneration media will increase green plant production and lower albinism. In this experiment, the same response was achieved, but percentage of albino plant formation was still high. High rate of albino plant regeneration represented by percentage of albino plants ranged from 37.9 to 89.3% (Table 3).

Like in other cereals, such as maize, wheat (Liu et al. 2002), rye (Immonen and Anttila 2000), oat (Kiviharju et al. 2000), and barley (Makowska and Oleszczuk 2014), albino plants or chlorophyll-deficient plants produced from rice anther culture is a serious problem, especially in *indica* rice (Dewi and Purwoko 2008). In this experiment, genotypes significantly influenced the number and percentage of albino plantlets regeneration (Table 3). Albinism could be due to one or a combination of factors, including genotype (*indica*, *japonica*, or *javanica*), environments (light and temperature during incubation period), meiotic abnormalities, hormonal imbalance, nuclear-plastid genome incompatibility,

Table 4. Dihaploid lines obtained from rice anther culture of F_1 plants from *indica* × *indica* crosses.

Genotypes (F_1)	Number of green plantlet acclimatized	Number of survivor green plantlet	DH ₀	Percentage of DH ₀
IR85/I-5	34	25	15	60.0
BioR-81/I-5	59	36	11	30.6
BioR-81/O-18	77	51	21	41.2
BioR-82/O-18	95	76	26	34.2
Total	265	188	73	38.8

DH₀ = first generation of doubled haploid or dihaploid lines.

deletions in plastid DNA, mutations in genes responsible for chlorophyll biogenesis, a metabolic block in pathways leading to chlorophyll biosynthesis, or an impaired photosynthetic apparatus leading to photo-bleaching (Yao and Cohen 2000; Dewi and Purwoko 2008; Dewi and Purwoko 2012; Makowska and Oleszczuk 2014). Albinism reducing efficiency of green plantlets production and may be the cause of low adoption of anther culture technique for rice breeding (Dewi et al. 2009; Kumari et al. 2009).

Anther Culture Efficiency

Anther culture-related green plantlet production efficiency, known as anther culturability, is important in incorporating this technique into the rice breeding program (Zhang 1989). Safitri et al. (2010) stated that anther culture of hybrid from crossing between two desirable parents (F_1) generally showed better anther culturability than anther culture of the parent alone. In this experiment, anther culturability is determined by the percentage of green plants compared to the number of anthers plated which ranged from 2.0 to 7.0% (Table 2). The highest anther culturability was 7% for BioR-82/O-18, followed by BioR-81/O-18 (5%) and BioR-81/I-5 (4%). This result is better than similar research utilizing F_1 plants from crossing of *indica* × *indica* conducted by Safitri et al. (2016) which showed anther culturability between 0.0–3.1%.

Dihaploid Plant Production

The advantage of rice anther culture in producing pure line in one generation of culture due to chromosome doubling that can be spontaneously achieved during culture. Thus, the anther culture technique can be readily incorporated into the rice breeding program (Dewi and Purwoko 2012). From this experiment all green plantlets (265 green plantlets) were acclimatized and the surviving plants (188 plants) were planted in the green house (Table 4).

Pure lines in the form of first generation of homozygous double haploid or dihaploid (DH₀) plants were identified due to their phenotypic performance,

panicle fertility, or chromosome counting. In this research, DH plants were identified through their phenotypic performance. Compared to DH plants, haploid plants was shorter with smaller and narrower leaves, sterile panicle, no ligule and auricle (Sah and Niroula 2007; Herawati et al. 2008; Safitri et al. 2010; Dewi and Purwoko 2012). DH₀ obtained from this experiment were 73 lines or approximately 38.8% from the total survivor plants. BioR-82/O-18 produced the highest number of DH₀ lines, followed by BioR-81/O-18 (Table 4). Those DH₀ lines produced from this research are readily available to be used further to ease selection of phenotypes for quantitative characters in variety development (Herawati et al. 2010; Datta 2014; Akbar et al. 2018). Number of DH₀ lines produced from each F_1 populations is enough to be selected. According to Dewi and Purwoko (2011), population to be selected when using DH lines depends on the number of gene (n) of interest, and assumption of no-linked with other gene. Therefore, if $n = 3$, by using DH lines we only needed minimum of 2^n or 8 lines compared to minimum of 4^n or 64 lines in conventional breeding.

CONCLUSIONS

Callus induction and plant regeneration were found to be independent from each other. All F_1 plants gave similar response to callus induction, but significantly different response to plant regeneration. Anther culturability for F_1 plants used as anther donor plants relatively high, ranged between 2.0–7.0%. Spontaneous first generation of DH obtained from this experiment were 73 lines or approximately 38.8% from the total survivor plants. BioR-82/O-18 produced the highest number (26 lines) of DH₀ lines, followed by BioR-81/O-18 (21 lines). The DH lines resulted from this study can be used in rice cultivar development program.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the Indonesian Center for Agricultural Biotechnology and

Genetic Resources Research and Development (ICABIOGRAD) for providing the facilities for this study.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the manuscript. ISD and BSP planning and supervised the work. NHP performed the experiment. ISD drafted the manuscript and designed the figures. All authors processed the experimental data, performed the analysis, discussed, interpreted the final results and commented on the manuscript.

REFERENCES

- Akbar, M.R., Purwoko, B.S., Dewi, I.S., Suwarno, W.B. & Sugiyanta (2018) Agronomic and drought tolerance evaluation of doubled haploid rice breeding lines derived from anther culture. *SABRAO Journal of Breeding and Genetics*, 50 (2), 115–128.
- Akhmadi, G., Purwoko, B.S., Dewi, I.S. & Wirnas. D. (2017) Pemilihan karakter agronomi untuk seleksi pada galur-galur padi dihaploid hasil kultur antera. *Jurnal Agronomi Indonesia*, 45 (1), 1–8.
- Anshori, M.F., Purwoko, B.S., Dewi, I.S., Ardie, S.W., Suwarno, W.B. & Safitri, H. (2018) Determination of selection criteria for screening of rice genotypes for salinity tolerance. *SABRAO Journal of Breeding and Genetics*, 50 (3), 279–294.
- Bhattacharya, A., Mikkilineni, V., Verma, L., Palan, B., Mali, K. & Char, B. (2014) Evaluation of doubled haploid culture conditions and regeneration of an *indica* rice hybrid. *Indian Journal Genetica*, 74 (3), 384–386.
- Chen, C.C., Tsay, H.S. & Huang, C.R. (1991) Factors affecting androgenesis in rice (*Oryza sativa* L.). In: Bajaj, Y.P.S. (ed.) *Biotechnology in agriculture and forestry*. Berlin, Springer-Verlag, pp. 193–215.
- Datta, S.K. 2014. Androgenic haploid: Factors controlling development and its application in crop improvement. *Current Science*, 89 (11), 1870–1878.
- Dewi, I.S. & Purwoko, B.S. (2008) Role of polyamines in inhibition of ethylene biosynthesis and their effects on rice anther culture development. *Indonesian Journal of Agricultural Science*, 9 (2), 60–67.
- Dewi, I.S. & Purwoko, B.S. (2011) Kultur *in vitro* untuk pembentukan tanaman haploid androgenik. Dalam: Wattimena, G.A., Mattjik, N.A., Wiendi, N.M.A., Purwito, A., Efendi, D., Purwoko, B.S. & Khumaida, N. (editor) *Bioteknologi untuk pemuliaan tanaman*. Bogor, IPB Press, hlm. 107–158.
- Dewi, I.S. & Purwoko, B.S. (2012) Kultur antera untuk percepatan perakitan varietas padi di Indonesia. *Jurnal AgroBiogen*, 8 (2), 78–88.
- Dewi, I.S., Purwoko, B.S. & Aswidinnoor, H. (2004) Kultur antera padi pada beberapa formulasi media yang mengandung poliamin. *Jurnal Bioteknologi Pertanian*, 9 (1), 14–19.
- Dewi, I.S., Purwoko, B.S., Aswidinnoor, H., Somantri, I.H. & Chozin, M.A. (2009) Plant regeneration from anther culture of several genotypes of *indica* rice tolerant to aluminum toxicity. *Indonesian Journal of Agriculture*, 2, 1–5.
- Dewi, I.S., Syafii, M., Purwoko, B.S. & Suwarno, W.B. (2017) Efficient *indica* rice anther culture derived from three way-crosses. *SABRAO Journal of Breeding and Genetics*, 49 (4), 336–345.
- El-Hennawy, M.A., Abdalla, A.F., Shafey, S.A. & Al-Ashkar, I.M. (2011) Production of doubled haploid wheat lines (*Triticum aestivum* L.) using anther culture technique. *Annals of Agricultural Science*, 56, 63–72.
- Foster, B.P. & Thomas, W.T.B. (2005) Doubled haploids in genetics and plant breeding. *Plant Breeding Review*, 25, 57–88.
- Germana, M.A. (2017) Anther culture for haploid and doubled haploid production. *Plant Cell, Tissue and Organ Culture*, 104, 283–300.
- Gioi, T.D. & Tuan, V.D. (2004) Anther culture from crosses between IR64 and new plant type cultivars. *Omonrice*, 12, 27–32.
- Gunarsih, C., Purwoko, B.S., Dewi, I.S. & Syukur, M. (2016) Regenerasi dan aklimatisasi kultur antera enam persilangan F₁ padi sawah. *Jurnal Agronomi Indonesia*, 44 (2), 133–140.
- Herawati, R., Purwoko, B.S. & Khumaida, N. (2008) Pembentukan galur haploid ganda padi gogo dengan sifat-sifat tipe baru melalui kultur antera. *Buletin Agronomi*, 36, 181–187.
- Herawati, R., Purwoko, B.S. & Dewi, I.S. (2010) Characterization of doubled haploid derived from anther culture for new plant type upland rice. *Jurnal Agronomi Indonesia*, 38 (3), 170–176.
- Hidayatullah, A., Purwoko, B.S., Dewi, I.S. & Suwarno, W.B. (2018) Agronomic performance and yield of doubled haploid rice lines in advanced yield trial. *SABRAO Journal of Breeding and Genetics*, 50 (3), 242–253.
- Immonen, S. & Anttila, H. (2000) Media composition and anther plating for production of androgenetic green plants from cultivated rye (*Secale cereale* L.). *Journal of Plant Physiology*, 156, 204–210.
- Jeon, J.S., Jung, K.H., Kim, H.B., Suh, J.P. & Khush, G.S. (2011) Genetic and molecular insights into the enhancement of rice yield potential. *Journal of Plant Biology*, 54, 1–9.
- Khush, G.S. (2013) Strategies for increasing the yield potential of cereals: Case of rice as an example. *Plant Breeding*, 132, 433–436.
- Kiviharju, E., Puolimatka, M., Saastamoinen, M. & Pehu, E. (2000) Extension of anther culture to several genotypes of cultivated oats. *Plant Cell Reports*, 19, 674–679.

- Kumari, M., Clarke, H.J., Small, I. & Siddique, K.H.M. (2009) Albinism in plants: A major bottleneck in wide hybridization, androgenesis, and doubled haploid culture. *Critical Review of Plant Science*, 28, 393–409.
- Liu, W., Zheng, M.Y. & Konzak, C. (2002.) Improving green plant production via isolated microspore culture in bread wheat (*Triticum aestivum* L.). *Plant Cell Reports*, 20, 821–824.
- Makowska, K. & Oleszczuk, S. (2014) Albinism in barley androgenesis. *Plant Cell Reports*, 33, 385–392.
- Mishra, R. & Rao, G.J.N. (2016) *In vitro* androgenesis in rice: Advantages, constraints, and future prospects. *Rice Science*, 23 (2), 57–68.
- Naik, N., Rout, P., Umakanta, N., Verma, R.L., Katara, J.L., Sahoo, K.K., Singh, O.N. & Samantaray, S. (2017) Development of doubled haploids from an elite *indica* rice hybrid (BS6444G) using anther culture. *Plant Cell, Tissue and Organ Culture*, 128 (3), 679–689.
- Niroula, R.K. & Bimb, H.P. (2009) Effect of genotype and callus induction medium on green plant regeneration from anther culture of Nepalese rice cultivars. *Asian Journal of Plant Science*, 8 (5), 368–374.
- Purwoko, B.S., Dewi, I.S. & Khumaida, N. (2010) Rice anther culture to obtain doubled haploids with multiple tolerances. *Asian Pacific Journal of Molecular Biology and Biotechnology*, 18 (1), 55–57.
- Safitri, H., Purwoko, B.S., Dewi, I.S., Wirnas, D. & Abdullah, B. (2010) Daya kultur antera beberapa persilangan padi gogo dan padi tipe baru. *Jurnal Agronomi Indonesia*, 38 (2), 81–87.
- Safitri, H., Purwoko, B.S., Dewi, I.S. & Ardie, S.W. (2016) Kultur antera untuk mendapatkan galur padi toleran salinitas. *Jurnal Agronomi Indonesia*, 44 (3), 221–227.
- Sah, B.P. & Niroula, R.K. (2007) Successful regeneration and characterization of anther derived rice hybrid plants from *O. sativa* L. × *O. rufipogon* Griff. *Science World*, 5 (5), 14–18.
- Sasmita, P., Dewi, I.S. & Purwoko, B.S. (2001) Pengaruh generasi kalus terhadap regenerasi tanaman pada kultur antera padi (*Oryza sativa* L.) cv. Gajah Mungkur. *Sain Teks (special edition)*, 179–188.
- Tyankova, N. & Zagorska, N. (2008) Factors affecting *in vitro* androgenesis in cereals. *International Journal of Plant Development Biology*, 21, 59–78.
- Yao, J.L. & Cohen, D. (2000) Multiple gene control of plastome-genome incompatibility and plastid DNA inheritance in interspecific hybrids of *Zantedeschia*. *Theoretical and Applied Genetics*, 101 (3), 400–406.
- Zhang, Z.H. (1989) The practicability of anther culture breeding in rice. In: Mujeeb-Kazi, A. & Stich, L.A. (eds.) *Review of advances in plant biotechnology, 1985–88*. Los Baños, Philippines, International Maize and Wheat Improvement Center, International Rice Research Institute, pp. 31–42.
-