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# EFFECT OF GENOTYPE AND DEVELOPMENTAL STAGE OF POLLEN ON THE SUCCESS ANTHER CULTURE OF LOCAL UPLAND RICE VARIETIES FROM EAST KALIMANTAN

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***Key words :*** *Anther culture, Doubled haploid, Leaf flag distance, Spikelet position and East Kalimantan upland rice cultivars*

**Abstract** - Genetic diversity is a main useful tool for plant genetic improvement. East Kalimantan has many local rice cultivars supplying many interesting genes which have not been explorated for rice breeding programs yet. This study was aimed to evaluate the potency of local upland rice varieties in hybrid rice breeding through anther culture. The effect of genotype, four local upland rice varieties, namely Mayas Kuning, Buyung, Geragai and Serai Gunung known as superior taste cultivars, and morphological marker for polen developmental stage were evaluated in this research. The results showed that the response of all varieties was different to the anther culture, in which Geragai showed the best response with the highest anther culture efficiency, followed by Serai Gunung, Mayas and Buyung. The distance between the auricles of the flag leaf and the penultimate leaf, and spikelets position on the panicles as the morphological markers of polen developmental stage affected anther culturability. The flag leaf distance was genotype-dependent in affecting callus induction and plantlet regeneration. The flag leaf distance of 12 cm on Geragai and Serai Gunung as well as 9cm on Buyung and Mayas Kuning resulted in the highest number of callus and plantlet regeneration. The effect of spikelet position was specific for each cultivar. Spikelet at the basal part of the panicle gave the highest anther culture efficiency on Serai Gunung and Buyung. Contrary to that, anthers derived from spikelet at the top position yielded the highest anther culture efficiency on Geragai and Mayas Kuning.

## INTRODUCTION

The assembly of new rice varieties involves the process of collecting superior traits or genes from the parental germplasm. This process is done by using and combining either conventional or inconventional plant breeding techniques. An effective alternative of plant breeding procedure is through plant tissue culture techniques with the application of anther culture technique.

The application of anther culture on rice breeding program has been reported to be able to accelerate the assembly of superior rice varieties through the acquisition of pure or homozygous lines production within short time (Chen *et al.*, 2001). Through this technique, the chromosomes of haploid plants obtained can be doubled by spontaneous diploidization or chromosomal doubling agents like colchicines for homozygous lines production (Nurhasanah *et al.*, 2008).

Doubled haploid lines produced from microspore culture and/or anther culture have been proved to be high potential in genetic improvement by broadening the genetic diversity through production of homozygous lines in one generation compared to conventional breeding methods that require six to eight generations of inbreeding (Foroughi-Wehr and Wenzal, 1989; Xa and Lang, 2011). Doubled haploid character will remain stable from generation to generation. In addition, the traits controlled by either dominant or recessive genes can be expressed in this homozygous plants, increasing efficiency of the selection process over conventional procedures due to better discrimination between genotypes, especially for the traits that are controlled by recessive genes.

The development of homozygous lines through

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anther culture techniques can speed up and simplify the process of hybrid rice breeding program. The hybrid technology can increase rice production to 15-20% compared to inbrida rice (Virmani *et al.*,1997). In addition to potentially higher yields, hybrids rice having other eminent characters such as resistant to biotic and abiotic stress have also been successfully assembled and released (Satoto *et al.*, 2004; Satoto and Suprihatno, 2008; Suprihatno *et al.*, 2009).

Anther culture is not a new method in rice breeding programs (Genovesi and Magill, 1979). In Indonesia, rice anther cultures have been conducted and established using several genotypes (Masyhudi, 1997; Herawati *et al.*, 2008; Munarso *et al.*, 2008) media (Dewi *et al.*, 2004) and methods (Sasmita, 2007) to generate homozygous lines for rice genetics improvement (Abdullah *et al.*, 2006; Bakhtiar *et al.*, 2007). Unfortunately, it has never been applied to East Kalimantan local rice varieties having many potentially interesting and valuable characters. Furthermore, regarding to the hybrid breeding which is based on heterosis phenomenon, the superior performance of F1 hybrids generated from a cross between genetically distant homozygous parents to their midparent value or to the value of the better parent, the development of homozygous lines using East Kalimantan local rice varieties having distantly related individuals to the elite or commercial rice varieties, is very important for rice hybrids production. A number of studies on maize (Benchimol *et al.*, 2000; Stupar *et al.*, 2008), rapeseed(Lefort-Buson *et al.*, 1987), wheat (Martin *et al.*, 1995) and rice (Kwon *et al.*, 2002), showed that the more distant genetic relatonship between the parents in hybrid breeding, the large genetic variability and heterosis effect in its F1 hybrids (Melchinger, 1999).

The success of rice anther culture techniques is influenced by several factors (Li, 1992; Herath *et al.*, 2007), including genotype of donor plants (Yan *et al.*, 1996) and developmental stage of microspore (Chen *et al.*, 2005). This study aimed to determine the effect of those factors to the success of upland rice anther culture originated from East Kalimantan.

## MATERIAL AND METHODS

**Plant material:** Plant material used in this study were four local upland rice cultivars, originated from Kutai Kartanegara district of East Kalimantan, i.e. Buyung (BY), Geragai (GR), Mayas Kuning (MK) and Serai Gunung (SG). The mother plants were grown in green house (Fig. 1).

**Explant preparation:** Panicles were harvested at booting stage when the distance between the auricles of the flag leaf and the penultimate leaf (immediate lower leaf) reach 6, 9 and 12 cm (Figure 1), wrapped with aluminum foil and incubated in cold temperature ±4 °C for 8 days. Prior to inoculation, each panicle was cut into three equally long parts: top, middle and basal to evaluate the effect of spikelet positions in anther culturability. Spikelets were surface sterilized using hipochloride solution 20% for 10 minutes and rinsed with sterillized distilled water two times. The sterilization process of explants was performed in the Laminar Air Flow Cabinet (LAFC).

**Anther culture procedure:** Spikelets were individually cut at the one third base to free the anthers from the filaments. They were plated aseptically onto 0,7% (w/v) agar solidified N6, callus induction medium, supplemented with phytohormones, i.e. 0.5 mg/L Kinetin +2 mg/L NAA and 60 g/L sucrose. Each petri dish is considered as one replication containing ±150 anthers from 25 spikelets. The whole process was done in sterile condition in LAFC. The cultures were sealed with parafilm and incubated in dark room (25+2 °C) for callus induction. Embryogenic calli of at least 2 mm diameter were transferred to regeneration medium (N6+2 mg/L Kinetin + 0.5 mg/L NAA+ 40g/L sucrose) and placed in a light condition (25 + 2 °C). Furthermore, regenerated green plants of 3-5 cm height were transferred on root induction medium (MS+0.5 mg/L IBA). Anther culture procedure followed Sasmita, ((2007) with minor modification.

**Statistical analysis:** Data were analysed using analysis of variance (ANOVA). Prior to variance analysis, data were transformed using vX+1. The differences of mean values were analized using Duncan multiple range test (DMRT).

## RESULTS

**Response of local East Kalimantan upland rice varieties to anther culture:** The succes of anther culture is observed from the ability of microspores in anthers to develop and differentiate to form calli, shoots and green plantlets (Figure 2). The first callus was formed ± 6 weeks after inoculation. The

shoots can be derived either from callogenesis process (callus forming shoots) or regenerate directly without passing through the callus stage, called androgenesis.

Anther culturability, the ability of anther to form callus and the regeneration of callus into plantlet, is an important factor evaluated in the development of haploid/doubled haploid lines through anther culture. Four local upland rice cultivars originated from Kutai Kartanegara districts in East Kalimantan, Buyung, Geragai, Mayas Kuning and Serai gunung showed different response to anther culture observed from different percentage of calli, plantlets and green plantlets regenerated, as well as anther culture efficiency (Tabel 1).

The ability of anthers to develop into callus was high in Serai Gunung cultivar resulting in the highest percentage of callus formation followed by Geragai, Buyung and Mayas Kuning. However callus forming shoots percentage in this cultivar was low, which was only around 60% of calli can develop into plantlets.

It was observed that the percentage of albino plants was high, around 60-95%, producing a low number of green plantlets regenerated. Buyung and Mayas Kuning generated the lowest green plantlet with only 1 among 19 and 18 plantlets, respectively. Among all cultivars, Geragai was observed as the most responsive cultivar followed by Serai Gunung, Buyung and Mayas evaluated from the number of green plantlet regenerated and percentage of anther culture efficiency.

**Effect of flag leaf distance and its interaction with genotype:** Microspore developmental stages, in this case was observed from the distance between the auricles of the flag leaf and the penultimate leaf and its interaction with genotype were evaluated in this study. Variance analysis results showed that genotype and flag leaf distance affected the number of callus, plantlet and green plantlet regenerated (Table 2).

The significant interaction between flag leaf distance and genotype exhibited genotype-dependent effect of the flag leaf distance for callus induction and plantlet regeneration. Mean value comparisons showed signifant differences between effect of different flag leaf distances in different genotypes (Table 3-4). Anthers derived from the longest distance of the flag leaf auricle to penultimate leaf auricle, 12 cm, resulted in the highest number of callus for Geragai and Serai Gunung cultivar, followed by the shorter distances 9 and 6 cm. On the other hand, flag leaf distance of 9 cm produced the highest number of callus formation for Mayas Kuning and Buyung, and no anther developed into calus from the panicles harvested at the later developmental stage marked by flag leaf distance of 12 cm. The same pattern was also observed for the number of plantlet regeneration parameter (Figure 3).

High number of green plantlet regenerated from anthers derived from panicles harvested at flag leaf distance of 12 cm from the penultimate leaf for Geragai, 9 cm for Buyung and serai Gunung, and 6 cm for Mayas Kuning, indicating different developmental stages of anther in different genotypes (Figure 3). However, statistically there is no significant interaction between flag leaf distance and genotype for green plantlet regeneration (Table 2 and 5).

**Effect of spikelet position on the panicle:** Statistically, spikelet position on the panicles and its interaction with genotype had no significant effect to the anther culturability (Table 6). However, it was observed that different spikelet position on the panicles resulted in different number of callus, plantlet and green plantlet (Table 7-9). A clear pattern can be viewed in Figure 4. There are two different patterns of spikelet position effect on the callus formation, and callus regeneration ability into plantlets. The first pattern showed that spikelet position on the base of the panicle yielded higher number of callus and plantlets compared with the top part of the panicle in Buyung and Serai Gunung cultivar. While the second pattern showed an opposite indication, the top part of the panicle generated the highest number of callus and plantlet compared with the basal part of the panicle, showed by Mayas Kuning and Geragai. These pattern can not be clearly observed for green plantlet regeneration. The highest number of green plantlet was obtained from anther located on the basal part of the panicles in Serai Gunung, middle part for Mayas, and top part for Geragai and Buyung.

## DISCUSSION

**Effect of genotype in rice anther culture:** Genotypes play an important role to the anther culturability. Each genotype has specific response

**Table 1.** Effect of genotypes to rice anther culturability

| Genotypes | Callus+ | | Plantlet# | | Green plantlet* | | Anther culture efficiency** |
|---|---|---|---|---|---|---|---|
| | Σ | % | Σ | % | Σ | % | % |
| Buyung | 22 | 1.02 | 19 | 86.36 | 1 | 5.26 | 0.05 |
| Geragai | 58 | 2.69 | 43 | 74.14 | 20 | 41.51 | 0.93 |
| Mayas Kuning | 15 | 0.69 | 13 | 86.67 | 1 | 7.69 | 0.05 |
| Serai Gunung | 83 | 3.84 | 52 | 62.65 | 8 | 15.39 | 0.37 |

+Percentage of callus = (Σ of callus formed / Σof anther innoculated (2160)) x 100%
#Percentage of plantlet = (Σ of plantlet regenerated /Σ of callus formed) x 100%
*Pecentage of green plantlet = (Σ of green plantlet regenerated /Σ of total plantlet regenerated) x 100%
**Percentage of anther culture efficiency = (Σ of green plantlet regenerated /Σ of anther innoculated) x 100%

**Table 2.** Variance analysis of the effect of genotype and flag leaf distance to the number of callus, plantlet and green plantlet

| Source of variation | Number of callus | Number of plantlet | Number of green plantlet |
|---|---|---|---|
| Genotypes (G) | ** | * | ** |
| Leaf flag distane (F) | ** | ** | ** |
| G x F | ** | ** | ns |

*Significant at p=0.05; **Significant at p=0.01; ns= not-significant

**Tabel 3.** Effect of genotype and flag leaf distance to the number of callus

| Genotype | Flag leaf distance | | | Mean |
|---|---|---|---|---|
| | 6 cm | 9 cm | 12 cm | |
| Buyung (BY) | 0.00 a<br>A | 3.67 b<br>B | 0.00 a<br>A | 1.22 |
| Geragai (GR) | 0.67 a<br>AB | 3.00 b<br>AB | 6.00 c<br>B | 3.22 |
| Mayas Kuning (MK) | 0.33 a<br>AB | 2.17 b<br>A | 0.00 a<br>A | 0.83 |
| Serai Gunung (SG) | 2.33 a<br>B | 4.33 b<br>AB | 7.17 c<br>B | 4.61 |
| Mean | 0.83 | 3.29 | 3.29 | |

Data presented as mean value from six replications; Different lowercase and capital letters show significant differences in row and column, respectively at the α= 0.05 according to Duncan Multiple Range Test (DMRT)

to the factors influencing the ability of microspore to form callus, and its regeneration to form shoots as well as green plantlets which is the main goal of anther culture. A specific response of genotype was observe in this research. Of the four local upland rice cultivars, Geragai showed the best response to anther culture followed by Serai Gunung (Table 1), producing the highest number of green plantlets thereby resulting in the highest anther culture efficiency. A contrast response was exhibited by the others two genotypes, Buyung and Mayas.

A significant divergence of rice genotypes response to anther culture was also observed in others study (Abe and Futsuhara, 1986; Khanna and Raina, 1998;Khatun *et al.,* 2003). The frequency of callus induction, green plant formation and the amount of homozygous plants were influenced by the genetic of the donor plant (Herath *et al.,* 2007). Therefore, selection of a responsive rice genotypes is very important to obtain a high level of anther culture efficiency.

Genetic studies of anther culture ability in rice

**Tabel 4.** Effect of genotype and flag leaf distance to the number of plantlet

| Genotype | Flag leaf distance | | | Mean |
|---|---|---|---|---|
| | 6 cm | 9 cm | 12 cm | |
| Buyung (BY) | 0.00 a<br>A | 3.17 b<br>B | 0.00 a<br>A | 1.06 |
| Geragai (GR) | 0.50 a<br>AB | 2.17 b<br>A | 4.50 c<br>B | 2.39 |
| Mayas Kuning (MK) | 0.17 a<br>AB | 2.00 b<br>A | 0.00 a<br>A | 0.72 |
| Serai Gunung (SG) | 2.17 a<br>B | 2.17 a<br>A | 4.33 b<br>B | 2.89 |
| Mean | 0.71 | 2.38 | 2.21 | |

Data presented as mean value from six replications; Different lowercase and capital letters show significant differences in row and column, respectively at the $\alpha$= 0.05 according to Duncan Multiple Range Test (DMRT)

**Tabel 5.** Effect of genotype and flag leaf distance to the number of green plantlet

| Genotype | Flag leaf distance | | | Mean |
|---|---|---|---|---|
| | 6 cm | 9 cm | 12 cm | |
| Buyung (BY) | 0.00 | 0.17 | 0.00 | 0.06 A |
| Geragai (GR) | 0.33 | 1.33 | 1.67 | 1.11 B |
| Mayas Kuning (MK) | 0.17 | 0.00 | 0.00 | 0.06 A |
| Serai Gunung (SG) | 0.00 | 0.83 | 0.50 | 0.44 B |
| Mean | 0.13 a | 0.58 b | 0.54 b | |

Data presented as mean value from six replications; Different lowercase and capital letters show significant differences in row and column, respectively at the $\alpha$= 0.05 according to Duncan Multiple Range Test (DMRT)

**Table 6.** Variance analysis of the effect of genotype and spikelet position to the number of callus, plantlet and green plantlet

| Source of variation | Number of callus | Number of plantlet | Number of green plantlet |
|---|---|---|---|
| Genotypes (G) | ** | ns | ** |
| Spikelet position (S) | ns | ns | ns |
| G x S | ns | ns | ns |

*Significant at p=0.05; **Significant at p=0.01; ns = not-significant

**Table 7.** Effect of genotype and spikelet position to the number of callus

| Genotype | Spikelet position | | | Mean |
|---|---|---|---|---|
| | Bottom | Middle | Top | |
| Buyung (BY) | 1.67 | 1.50 | 0.50 | 1.22 A |
| Geragai (GR) | 2.50 | 2.00 | 5.17 | 3.22 B |
| Mayas Kuning (MK) | 0.50 | 0.33 | 1.67 | 0.83 A |
| Serai Gunung (SG) | 7.83 | 4.67 | 1.33 | 4.61 B |
| Mean | 3.13 | 2.13 | 2.17 | |

Data presented as mean value from six replications; Different capital letters show significant differences column at the $\alpha$ = 0.05 according to Duncan Multiple Range Test (DMRT)

**Table 8.** Effect of genotype and spikelet position to the number of plantlet

| Genotype | Spikelet position | | | Mean |
|---|---|---|---|---|
| | Bottom | Middle | Top | |
| Buyung (BY) | 1.33 | 1.33 | 0.50 | 1.06 |
| Geragai (GR) | 2.00 | 1.33 | 3.83 | 2.39 |
| Mayas Kuning (MK) | 0.50 | 0.33 | 1.33 | 0.72 |
| Serai Gunung (SG) | 5.50 | 2.67 | 0.50 | 2.89 |
| Mean | 2.33 | 1.42 | 1.54 | |

Data presented as mean value from six replications

**Table 9.** Effect of genotype and spikelet position to the number of green plantlet

| Genotype | Spikelet position | | | Mean |
|---|---|---|---|---|
| | Bottom | Middle | Top | |
| Buyung (BY) | 0.00 | 0.17 | 0.17 | 0.11 A |
| Geragai (GR) | 1.17 | 0.50 | 1.67 | 1.11 B |
| Mayas Kuning (MK) | 0.00 | 0.17 | 0.00 | 0.06 A |
| Serai Gunung (SG) | 1.17 | 0.17 | 0.00 | 0.44 B |
| Mean | 0.58 | 0.25 | 0.46 | |

Data presented as mean value from six replications; Different capital letters show significant differences in column at the $\alpha = 0.05$ according to Duncan Multiple Range Test (DMRT)

**Table 10.** Effect of flag leaf distance and spikelet position to the anther culturability

| Morphological markers for polen developmental stage | Callus+ | | Plantlet# | | Green plantlet* | | Anther culture efficiency** |
|---|---|---|---|---|---|---|---|
| | Σ | % | Σ | % | Σ | % | % |
| Flag leaf distance | | | | | | | |
| 6 cm | 20 | 0.69 | 17 | 85.00 | 3 | 17.65 | 0.10 |
| 9 cm | 79 | 2.74 | 57 | 72.15 | 14 | 24.56 | 0.49 |
| 12 cm | 79 | 2.74 | 53 | 67.09 | 13 | 24.53 | 0.45 |
| Spikelet position | | | | | | | |
| Basal | 75 | 2.80 | 56 | 74.67 | 14 | 21.82 | 0.61 |
| Middle | 51 | 1.77 | 34 | 66.67 | 6 | 14.71 | 0.26 |
| Top | 52 | 1.81 | 37 | 71.15 | 11 | 29.73 | 0.48 |

+Percentage of callus = (Σ of callus /Σ of anther innoculated (2880)) x100%
#Percentage of plantlet = (Σ of plantlet/Σ of callus) x100%
*Pecentage of green plantlet = (Σ of green plantlet/Σ of total plantlet) x100%
**Percentage of anther culture efficiency = (Σ of green plantlet /Σ of anther innoculated) x 100%

showed that the efficiency of anther culture is controlled by gametic, additive, maternal and sitoplasmic effect (Yan *et al.*, 1996; Yamagishi *et al.*, 1998). In which, the ability of callus induction from explants is affected by gametic additive effects with less effect of the maternal effects, while the ability of callus regenerating green plants was controlled by gametic-maternal effects. The role of maternal effects on the regeneration of green plants can be evaluated from the response of the female parent in the intersection of anther culture. The plants (F1) produced from a cross between parent or females parent responsive to anther culture will have a good response to this technique (Sasmita, 2007).

**Fig. 1** Explants source taken at different pollen developmental stage marked from the distance between the auricles of the flag leaf and the penultimate leaf (immediate lower leaf). From left to right, distance of the flag leaf 6, 9 and 12 cm, respectively.

A high percentage of albino plants (Table 1) was observed in this study. Serai Gunung cultivar which is actually generated the highest number of calli and plantlets more than Geragai, unfortunately it generated the lower amount of green plantlets less than a half of that in Geragai exhibiting a high frequency of albino plants. The high frequency of albino plant is one of the problems faced in rice anther culture (Herath *et al.,* 2007). The amount of green plant regenerants is controlled by a gene located on chromosome 10 in rice showing that the genotype of the donor plant plays an important role in determining the efficiency of anther culturability (Yamagishi *et al.,* 1998).

**Effect of pollen developmental stage:** The level of development stage of the microspore in the anther can be observed through the morphological markers, such as the distance between the flag leaf auricle and the penultimate leaf auricle and the spikelet position on the panicles. Both of these factors influence the efficiency of rice anther culture (Table 10).

In this study, generally, anthers derived from the panicles harvested when the flag leaf auricle is as far as 9 cm to the penultimate leaf auricle generated the highest efficiency of anther culture, followed by the 12 cm length. A very low anther culture efficiency marked by low number of green plantlets was obtained when the flag leaf distance is 6 cm away from the immediate lower leaf (Table 1). On the other hand, spikelet position on the panicles also influences anther culturability, in which spikelet located on the basal part of the panicles resulted the highest percentage of anther culture efficiency.

The distance between the flag leaf auricle to the penultimate leaf auricle provides a useful means in identifying the reduction of pollen division stage (Yoshid, 1981). The process of panicles differentiation is synchronous with leaf development, and the meiosis stages coincides with the auricle of the flag leaf distance above the auricle of the penultimate leaf. The distance between the flag leaf auricle to the penultimate leaf auricle was closely associated with the developmental stage of pollen (Li, 1992) which will further affect the physiological state of the pollen and determine the regeneration ability in rice anther culture (Sasmita, 2007). From several studies, it was reported that the success rate of the anther culture is higher when pollen is at uninucleate stage characterized by the distance of the auricles of the flag leaf ranges from ± 7-12 cm to the penultimate leaf.

Regardless to the explanations above, we observed that the effect of the auricles distance between the flag leaf and the penultimate leaf in rice anther culture is genotype-dependent (Figure 3). It was also confirmed by the statistical anlysis results showing a significant interaction between the flag leaf distance and the genotype of the mother plant (Table 2-5). The highest green plantlets regeneration was resulted by the auricles distance of 12 cm between the flag leaf and the penultimate leaf for Geragai, 9 cm distance for Buyung and Serai Gunung and 6 cm distance for Mayas.

These results showed that the same length of the flag leaf distance to the penultimate leaf could result in different pollen developmental stage on different genotypes. The 12 cm distance between the flag leaf and the penultimate leaf might be too

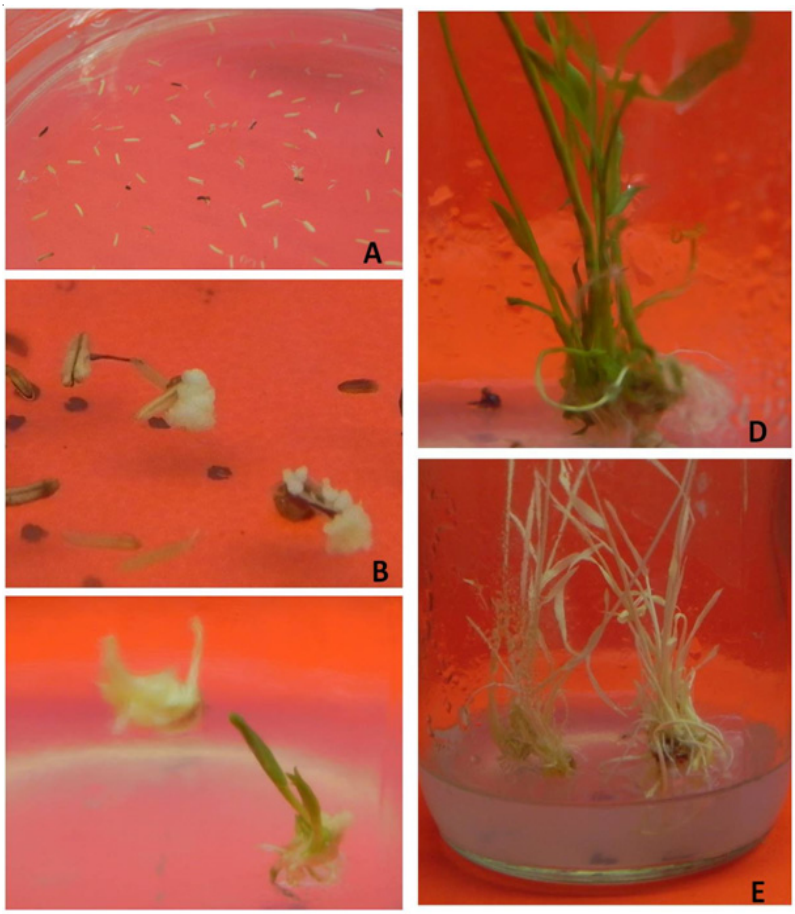


**Fig. 2** Rice anther culture of East Kalimantan local upland rice cultivars; A. Anther inoculated; B. Anther forming callus; C. Callus forming shoot; D. Green plantlet; E. Albino plantlet

late for one genotype like Mayas, but it might be not for another such as Geragai. It was supposed that pollen developmental stage was late for Geragai. Anther derived from the panicles harvested at 12 cm flag leaf distance still containing early to mid-uninucleate stage in this cultivar. On the contrary, pollen developmental stage in Mayas was faster compared to other genotypes. Panicles harvested when the auricle of flag leaf distance of 6 cm to the subtending leaf containing uninucleate stage of polen, while the longer flag leaf distance of 12 cm already contains microspore at bi-nucleate stage. This might explain that no regeneration observed from anthers derived from the flag leaf distance of 12 cm in Mayas. It shows that the reduction division stage of pollen ends earlier before the flag leaf auricle appears 12 cm length from the sheath of the penultimate leaf in this cultivar.

Spikelets position on the top, middle and basal part of the panicles also indicated a different developmental stage of pollen showed by different anther culturability. A previous reserach reported that anthers from the basal part of the panicles harvested at the flag leaf distance of 7 to 13 cm, resulted in the highest percentage of anthers forming calli (Afza *et al.,* 2000) which is confirmed by this research (Table 10 and Fig. 4). The anthers of the basal parts contain pollen at uninucleate stages, including early, middle and late generating higher callus induction frequency (Afza *et al.,* 2000).

A heterogenity assimilates distribution including amino acids and soluble carbohydrates on different part and developmental stage of the panicles (Mohapatra and Sahu, 1991) might correlate with the different callusing ability of the anthers derived from different part of the panicles in different genotypes.

## CONCLUSION

Genotype plays an important role to the success of rice anther culture. Different rice genotypes resulted

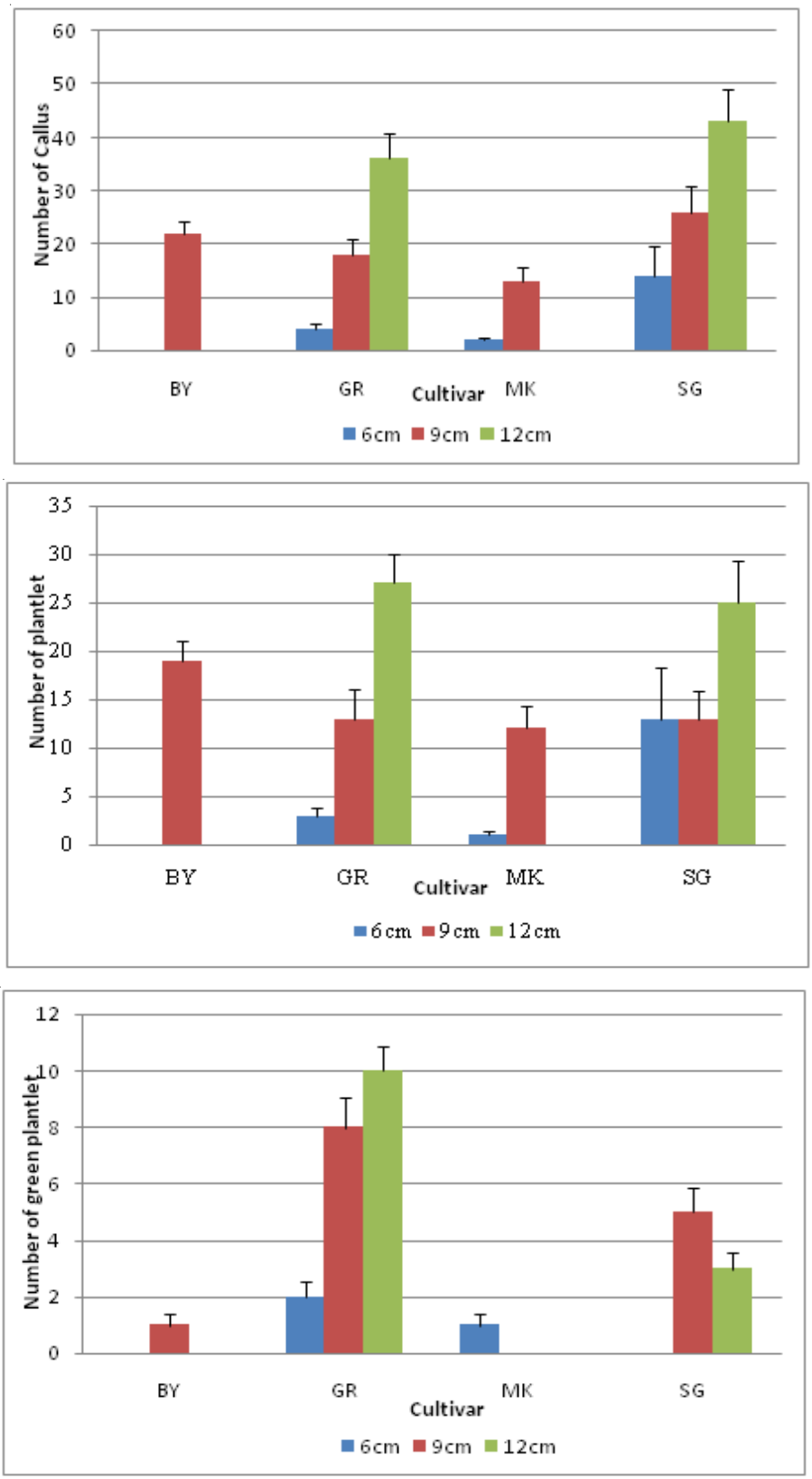


**Fig. 3** Effect of different flag leaf distances, 6 cm, 9 cm and 12 cm, to the number of callus, plantlet and green plantlet in different local upland rice cultivars, BY: Buyung; GR: Geragai; MK: Mayas Kuning; SG: Serai Gunung.

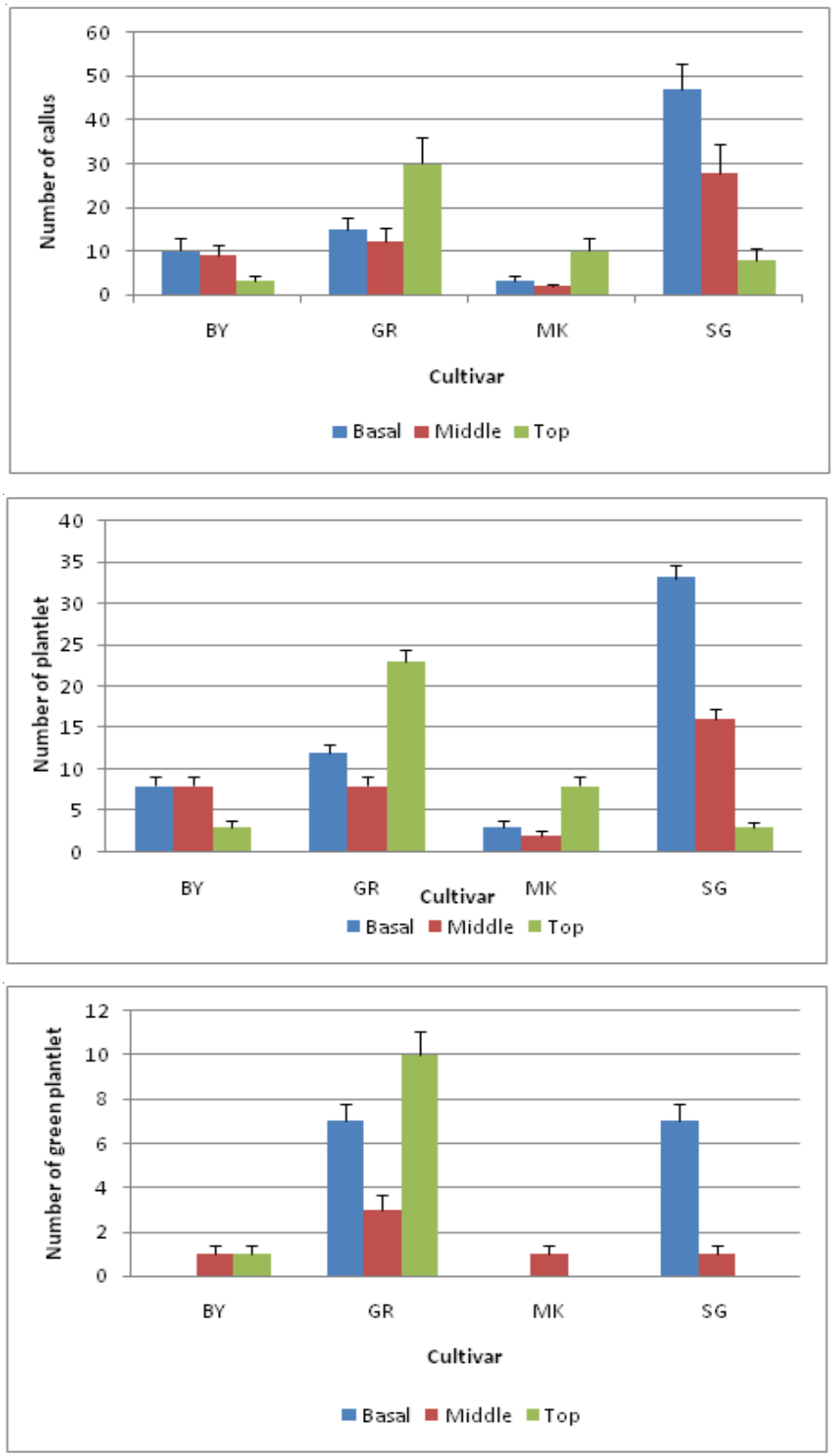


**Fig. 4** Effect of different spikelet position, basal, middle and top part of the panicle, to the number of callus, plantlet and green plantlet in different local upland rice cultivars, BY: Buyung; GR: Geragai; MK: Mayas Kuning; SG: Serai Gunung.

in the different anther culturability. The distance between the auricles of the flag leaf and the penultimate leaf can be used as a morphological marker to evaluate pollen developmental stage affecting the ability of anther to develop into callus and callus regeneration into plantlet and green plantlet. The effect of flag leaf distance was genetic-dependent in rice anther culture. Anthers derived from different position of the spikelet on the panicles yielded different anther culture efficiency indicating different pollen developmental stage.

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