The percentage of haploid embryos resulting from the crossing of two white-seeded genotypes with three dark-seeded genotypes of maize

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Abstract. In maize, there is a special genotype, inducer, that able to induce haploid embryo formation. This study aims to evaluate three dark-seeded genotypes in in vivo induction of haploid embryos in two white-seeded genotypes. To achieve this goal, two white-seeded genotypes (Kumala and Paramita) were pollinated with three dark-coloured genotypes (Blue Jade, IPB J1, and IPB J2). The level of ploidy of the embryos resulting from the cross between two white-seeded genotypes with IPB J2 is difficult to determine due to the hybrid seeds are light yellow. The cross of two white-seeded genotypes with Blue Jade and IPB J1 produced purple hybrid seeds with haploid embryos of 8-12 percent.

1. Introduction
Haploids can occur spontaneously in nature or as a result of in vitro culture [1]. Haploid embryos have a very important role in the development of hybrid maize. The haploid embryo can be developed into a double haploid line through colchicine treatment. This method will greatly shorten the length of time to develop homozygous lines, only two cropping seasons as opposed to 6–8 seasons using the traditional method of recurrent self-pollination. Even though spontaneously haploid plants in maize have been described by Randolph [2], and found in some maize populations by Chase [3], they have not been used intensively in the development of hybrid maize in Indonesia.

The implementation of the doubled haploid method in maize requires the use of haploid inducer lines. Generally, these haploid inducer lines have a temperate origin, which hampers the handling and development of these lines in tropical conditions [4]. In addition, for haploid embryos to be easily observed, the two crossed genotypes must have contrasting seed colour and the dark ones used as pollinator. Faculty of Agriculture, Andalas University, has a collection of five maize genotypes, two white seeds and three dark-coloured seed genotypes. The ability of these three dark-seeded genotypes to induce in vivo haploids has never been tested. This study aims to evaluate three dark-seeded genotypes in inducing in vivo haploid embryos in two white-seeded genotypes.

2. Materials and methods
The field experiment was carried out at Experimental Farm of the Faculty of Agriculture, Andalas University, Padang (150 m altitude) in November 2017 to April 2018. The genotypes expected to become inducers of in vivo haploid are Blue Jade (black seed), IPB J1 (purple seed), and IPB J2 (with red seed) which are used as pollen donors. The genotypes used as female parents are Kumala and...
Paramita, both of which are white seeds. The culture treatments were conducted according to recommendations for maize culture.

2.1. Planting

Results of the preliminary experiments showed that the three genotypes of pollinator (Blue Jade, IPB J1, and IPB J2) were anthesis at around 63 - 68 DAP (days after planting) and both white seed genotypes were silking at around 45-50 DAP. Therefore, to synchronize anthesis-silking period, Blue Jade, IPB J1, and IPB J2 were planted 20 to 4 days earlier than Kumala and Paramita, with 7-days planting intervals as shown in Table 1.

| Genotypes   | Planted on the day |
|-------------|--------------------|
|             | 1  3  7  10  14  17  21  24 |
| Kumala      |                  |
| Paramita    |                  |
| Blue Jade   |                  |
| IPB J1      |                  |
| IPB J2      |                  |

The experiment was a half diallel cross between two white-seeded genotypes and three dark-seeded genotypes. There are six combinations of crosses with 3 replications. Thus, the experiment consisted of 18 experimental units. Each experimental unit is a 150 cm x 250 cm plot that is planted with a spacing of 75 cm x 25 cm (20 plants per plot).

2.2. Identification of haploid embryos

Maize is a plant that has a xenia effect. The xenia effect can be interpreted as a direct effect of pollen on seeds or fruit [5]. The effect is separate from the genetic contribution of pollen towards the next generation. Observation of haploid embryos was carried out on all seeds in each ear formed. They were observed visually on the colour of the endosperm and embryo. A seed was considered to have a haploid embryo if the endosperm was coloured and the embryo was colourless. A coloured embryo indicates that the embryo is a hybrid [6]. Percentage of haploid embryo seeds was determined based on the number of seeds in each ear. After harvesting, the haploid seeds on each ear are calculated in percentage. The data obtained were averaged and analysed statistically by calculating the standard deviation to determine its variation in each pair of crosses.

3. Results and discussion

In this experiment, a cross between white seed and dark seed produced a variety of seed colours as presented in Table 2. Blue Jade and IPB J1 produced purple seeds in Kumala and Paramita. However, IPB J2 produced light yellow on both Kumala and Paramita. This non-contrasting colour of the seed due to pollination of IPB J2 will clearly complicate the determination of haploid embryos, so that, in this experiment, their haploid levels were not counted (Table 3).

Table 3 shows that Blue Jade and IPB J1 are good enough to induce haploid embryos. Paramita produces haploid about 8% and Kumala can produce haploid up to 12%. Thus, Blue Jade and IPB J1 have a high enough haploid induction rate (HIR). An HIR of around 2% can already be utilized in a maize breeding program [7]. The current haploid-inducing line generally has an induction rate of around 7.2 to 12.8% [4].
Table 2. The color of the seeds from the cross between white and dark genotype.

| Female          | Male         |
|-----------------|--------------|
| Kumala          | Blue Jade IPB J1 IPB J2 |
| Kumala x Blue Jade | Kumala x IPB J1 Kumala x IPB J2 |
| Paramita        | Paramita x Blue Jade Paramita x IPB J1 Paramita x IPB J2 |

Table 3. Percentage of haploid resulting from crossing of several maize genotype.

| Male        | Female          | Percentage of haploid | Standard deviation |
|-------------|-----------------|-----------------------|--------------------|
| Blue Jade   | Kumala          | 12.13                 | 2.52               |
|             | Paramita        | 8.17                  | 1.11               |
| IPB J1      | Kumala          | 10.42                 | 2.61               |
|             | Paramita        | 7.97                  | 1.53               |

4. Conclusions
Based on this research it can be concluded that Blue Jade and IPB J1 can induce haploid embryos by 8 to 12%, while IPB J2 cannot be used as haploid embryo inducers because it produces a light yellow seeds.

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