Growth response of diploid and tetraploid taro (*Colocasia esculenta* (L.) Schott) shoot culture to drought stress using polyethylene glycol

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Abstract. Taro genetic improvement through polyploidy induction is expected to be tolerant to abiotic stress. Several studies have shown that polyploidy plants have a higher adaptability to dry environments. *In vitro* selection technique for evaluation of plant tolerance to drought stress can be done by applying polyethylene glycol (PEG) as a selection agent. The aim of the research was to investigate the growth response of diploid and tetraploid taro shoot culture to drought stress using PEG. The experiment was conducted using a completely randomized design with two factors. The first factor was the concentration of PEG at 0, 5, 10, 15 and 20%. The second factor was Bentul taro clones which were 1 diploid clone and 2 tetraploid clones (clones 4.6.3 and 5.4.4). PEG was added to liquid MS medium containing 2 mg/l BAP. Observations of growth variables were carried out every week until the six weeks of culture. Fresh and dry weights, mortality percentage and proline content were determined at six weeks of culture. The results showed that the addition of PEG in liquid medium significantly affected the number of leaves, petiole length, number of roots, fresh and dry weights, as well as shoot mortality percentage. Differences in clones significantly affected the number of leaves, fresh and dry weights. The two factors tested (PEG and clones), gave an interaction on the number of leaves, fresh and dry weights. The proline content in all diploid and tetraploid clones showed an increase with increasing PEG concentration. The LC50 value in diploid clones was 9.82%, in tetraploid clones 4.6.3 and 5.4.4 were 14.14 and 15.45%, respectively. The results showed that PEG at 10% and 15% could be used for *in vitro* selection to drought stress both for diploid and tetraploid taro.

1. Introduction

Taro (*Colocasia esculenta* (L.) Schott) has been known for many years and has great potential to be developed as an alternative food source. In Indonesia, the diversity of taro is very large. The previous selection showed that Bentul is one of the promising taro cultivars to be developed because it has a good taste, soft tuber texture and has few tillers so that it is preferred by farmers [1].

A strategy to develop Bentul into superior taro is through *in vitro* polyploidization. Polyploidy is the number of chromosomes more than two sets in one nucleus. Polyploidization affects the genetic...
and phenotypic constitution of organisms, increasing genetic diversity and adaptation to the environment [2]. Efficiency of in vitro polyploidy induction is influenced by several factors such as plant species, type of antimitotic agent and its duration of application, type of explant, method of application as well the effect before and after treatment. The interaction of some of these parameters is critical, therefore, it is necessary to carry out several experiments to obtain the most efficient method [3,4]. One of the purposes of polyploid induction in food crops is to increase productivity, such as in radish Raphanus sativus L. [5] and rice [6]. In taro, polyploid induction using oryzalin and colchicine resulted in tetraploid cultivars of Bentul and Kaliurang [7,8].

In addition to having a larger appearance and organ size than its wild type diploids, several studies have shown that polyploid plants have a higher adaptability to dry and high-temperature environments such as in Poaceae, i.e. Themeda trianda [9]. The inheritance of polysomic traits in polyploid plants will increase the flexibility of the genome which may result in increased adaptability to environmental changes [10]. Polyploid plants also have a lower osmotic potential and thicker epidermis so they tend to reduce water loss and maintain turgor pressure when water potential decreases [11].

Evaluation of plant tolerance to drought stress can be done by using in vitro selection techniques. The use of this technique can save costs so that it is more efficient. This technique is carried out under controlled laboratory conditions with limited space and time. This technique can also be used to observe the biochemical and physiological responses of plants to environmental stress [12].

In vitro selection can be done by applying a selection agent such as polyethylene glycol (PEG) to the culture medium [13]. High molecular weight PEG is commonly used because it is an inert non-ionic compound that can decrease the water potential of the medium without causing toxicity to plants [14]. The use of PEG molecular weight 6000 in in vitro culture can be adjusted to the actual drought conditions. PEG cannot be absorbed into plant tissues. Cellulose walls on the roots can only be passed by PEG with a maximum molecular weight of 3500 [15]. The purpose of this study was to determine the in vitro growth response of diploid and tetraploid taro to drought stress using PEG 6000.

2. Materials and methods

2.1. Materials

Plant materials used in this experiment was in vitro-shoot of taro (Colocasia esculenta (L.) Schott) var. Bentul diploid (control) and two tetraploid clones 4.6.3 and 5.4.4 cultured in MS medium without plant hormone for 1 month. Tetraploid clones were obtained by induction with oryzalin [7].

2.2. Methods

2.2.1 PEG treatment

This study used a two-factor completely randomized design. The first factor was 5 levels of PEG at 0, 5, 10, 15 and 20%, while the second factor was three different Bentul taro clones which were one diploid clone and two tetraploid clones 4.6.3 and 5.4.4. The combination of both factors obtained 15 treatments. Each treatment had 6 replicates so there were 90 experimental units in total.

The basic media used was MS liquid medium [16]. The medium pH was adjusted to 5.8 with the addition of 1N KOH. Twenty five ml of media was poured into a culture bottle with a base filled with synthetic foam sized at 3 x 3 x 0.5 cm. The culture bottles were closed, then sterilized by autoclaving for 20 min at 121°C at 1 atm [17].

The explants used were in vitro shoots with a petiole length of 0.5 cm. The explants were planted on synthetic foam that had been perforated with a diameter of 2 mm. All cultures were incubated in a culture room at 25-26°C with continuous photoperiod.

2.2.2 Growth observation

Leaf number, petiole length and root number were observed every week until six weeks of culture. The number of leaves was determined by counting all the green leaves on each shoot. The length of
the petiole was determined by measuring the longest petiole on each shoot. The number of roots was determined by counting all roots on each shoot. Fresh and dry weights of shoots were determined after six weeks of culture. LC$_{50}$ value was determined after six weeks of culture using probit analysis of mortality percentage.

2.2.3 Proline analysis
Analysis of proline content was carried out according to method [18] using spectrophotometer. Proline content was determined after six weeks of culture. Whole parts of shoots were harvested for proline analysis. Pure L-proline was used as a standard for proline quantification. The proline concentration was determined based on a standard curve and calculated on a fresh weight basis as follows:

\[
\frac{(\mu g\text{ proline/ml} \times \text{ml toluene})}{115.5 \, \mu g/\mu mole} / \frac{(g\text{ sample})/5}{\mu moles\text{ proline / g of fresh weight material.}}
\]

2.3. Statistical analysis
Data were analyzed using analysis of variance by software DSAASTAT version 1.1 and further tests were carried out using Duncan Multiple Range Test (DMRT) at $\alpha= 5\%$ for parameters showing a significant effect in the analysis of variance ($P<0.05$).

3. Results and discussion

3.1. Statistical analysis of growth after 6 weeks of culture
Analysis of variance on number of leaves, petiole length, number of roots, fresh and dry weight of diploid, tetraploid 4.6.3 and tetraploid 5.4.4 taro clones are presented in Table 1. The results showed that clones had a high significant effect on leaf number, fresh weight and dry weight. but it was not significant on petiole length and number of roots. PEG treatment had a high significant effect on all observed growth variables. Clones and PEG concentrations had a high significant interaction on the number of leaves, fresh weight and dry weight. However, no significant interaction was found between clones and PEG on petiole length and number of roots.

| Variable         | Clone | PEG | Clone vs PEG |
|------------------|-------|-----|--------------|
| Leaf number      | **    | **  | **           |
| Petiole length   | ns    | **  | ns           |
| Root number      | ns    | **  | ns           |
| Fresh weight     | **    | **  | **           |
| Dry weight       | **    | **  | **           |

Note: ** very significance at $\alpha 1\%$; ns : not significance

The average number of leaves, petiole length, number of roots, fresh and dry weights on media containing PEG after 6 weeks of culture is presented in Table 2. The results showed that all growth parameters in all clones decreased with increasing PEG concentrations. In all clones, leaves were only formed on media without and with 5% PEG. On medium containing 10, 15 and 20% PEG, leaves were not formed. Similarly, the number of roots in all clones was also not formed on media containing 15 and 20% PEG. The petiole length, fresh weight and dry weight also decreased with increasing PEG concentrations. Decreased growth was also found in in vitro screening of sugarcane cultivar for tolerance to PEG-induced water stress (19).
Table 2. Leaf number, petiole length, root number, fresh and dry weights of diploid and tetraploid taro (Colocasia esculenta (L.) Schott) cultured on MS medium containing PEG six weeks after culture.

| Clone | PEG concentration (%) | Leaf number | Petiole length (cm) | Root number | Fresh weight (mg) | Dry weight (mg) |
|-------|-----------------------|-------------|---------------------|-------------|------------------|----------------|
|       |                       | 0           | 1.3 ± 0.11c         | 4.8 ± 0.40a | 2.5 ± 1.38c      | 366.5 ± 6.22c  |
| Diploid | 5                     | 0.2 ± 0.17d | 1.6 ± 0.10c         | 0.1 ± 0.08c | 226.4 ± 1.98f    | 22.4 ± 0.86f   |
|       | 10                    | 0d          | 1.0 ± 0.10d         | 0.1 ± 0.08c | 127.6 ± 1.97f    | 18.4 ± 0.45g   |
|       | 15                    | 0d          | 0.7 ± 0.00ed        | 0c          | 96.4 ± 0.59j     | 15.0 ± 0.44h   |
|       | 20                    | 0d          | 0.7 ± 0.00ed        | 0c          | 38.1 ± 0.66j     | 8.9 ± 0.21i    |
| Tetraploid 4.6.3 | 0             | 3.4 ± 0.24a | 3.7 ± 0.35b         | 1.0 ± 0.26bc | 514.0 ± 3.32a    | 43.1 ± 0.63a   |
|       | 5                     | 0.1 ± 0.08d | 1.4 ± 0.21c         | 0.2 ± 0.11c | 290.9 ± 7.49g    | 40.7 ± 0.96b   |
|       | 10                    | 0d          | 0.8 ± 0.10d         | 0.1 ± 0.08c | 198.4 ± 2.39e    | 26.7 ± 0.72c   |
|       | 15                    | 0d          | 0.8 ± 0.00d         | 0c          | 67.2 ± 0.70k     | 15.1 ± 0.29h   |
|       | 20                    | 0d          | 0.6 ± 0.00d         | 0c          | 40.5 ± 0.61l     | 9.1 ± 0.38l    |
| Tetraploid 5.4.4 | 0             | 2.7 ± 0.21b | 3.8 ± 0.82b         | 1.8 ± 0.66ab| 472.2 ± 4.92a    | 36.3 ± 0.56c   |
|       | 5                     | 0.1 ± 0.08d | 1.5 ± 0.19c         | 0.5 ± 0.26c | 353.1 ± 5.71d    | 34.4 ± 0.85cd  |
|       | 10                    | 0d          | 0.9 ± 0.06d         | 0.1 ± 0.08c | 184.7 ± 4.03h    | 26.1 ± 0.80c   |
|       | 15                    | 0d          | 0.8 ± 0.00d         | 0c          | 69.2 ± 0.61k     | 15.7 ± 0.64d   |
|       | 20                    | 0d          | 0.6 ± 0.00d         | 0c          | 41.9 ± 0.60l     | 10.7 ± 0.65j   |

Note: The numbers followed by the same letter in the same column are not significantly different according to Duncan's multiple range test at α = 5%.

3.2. Leaf numbers
The results showed that the number of leaves on control medium (0% PEG) in all clones increased from 1 to 6 weeks of culture. The number of leaves in diploid and tetraploid 5.4.4 clones treated with 5% PEG increased only at 6 weeks of culture (Figures 1.A and 1.C), while in tetraploids 4.6.3 clone the number of leaves began to increase at 5 weeks of culture (Figure 1.B). The addition of 10, 15 and 20% PEG in all clones resulted in the clones not forming leaves until 6 weeks of culture (Figure 1.A-C).

The decrease in the number of leaves with increasing PEG concentration was due to the shoots having difficulty in absorbing water and nutrients from the media, so that growth was inhibited, as in banana plantlets [20] and potato plantlets [21]. Limited water availability interferes with cell division and expansion [22]. The decrease in plant growth is a physiological response of plants to drought stress by decreasing their metabolic rate [19].

3.3. Petiole length
The results showed that the petiole length of diploid clones on control medium (0% PEG) increased rapidly from the first week to the third week, but at 4 to 6 weeks of cultures, there was only slightly increased. From week 0 to 6, tetraploid taro clones 4.6.3 and 5.4.4 grown on control media showed increased petiole length. The addition of 5% PEG in all clones resulted in clones with a longer petiole than that in the media with addition of PEG at 10, 15 and 20%. More than 5% PEG decreased petiole length lower than the addition of 5% PEG (Figure 2.A – C).
The inhibited petiole growth due to PEG treatment was thought to be a defensive response of plants to reduce transpiration rate, as in tomatoes, which decreased shoot height significantly due to PEG treatment. Reduction of shoot height usually promotes root development, and this is the strategy of plant to find more water and nutrients in the soil. The character of shoot height can be used as a rapid initial selection criterion to determine the level of tolerance of the genotype to drought [23].

3.4. Root numbers
On media without PEG (control), all diploid and tetraploid clones of taro began to form roots in the first to third week after culture. The fastest root induction occurred in the first week of tetraploid 5.4.4. In the diploid taro, roots started to form in the second week, while in the tetraploid 4.6.3 root formation was slower than that of diploid and tetraploid 5.4.4, which was in the third week of culture. Root formation of diploid clones with 5 and 10% PEG was slower which was at the fifth and sixth week after culture, whereas at 15 and 20% PEG no roots were formed (Figure 3.A).

Root formation of tetraploid 4.6.3 in the media with 5 and 10% PEG treatment was faster than that in diploid clones, which was in the fourth and fifth weeks of culture. Similar to diploid clones, in the tetraploid 4.6.3 root formation was slower which was in the third week of culture. Root formation of diploid clones with 5 and 10% PEG was slower which was at the fifth and sixth week after culture, whereas at 15 and 20% PEG no roots were formed (Figure 3.A).

Root formation of tetraploid 4.6.3 in the media with 5 and 10% PEG treatment was faster than that in diploid clones, which was in the fourth and fifth weeks of culture. Similar to diploid clones, in the media with 15 and 20% PEG, no roots were formed (Figure 3.B). Root formation of tetraploid clones 5.4.4 on media containing 5% PEG was the fastest compared to other clones, starting in the first week, while root formation on media containing 10% PEG started in the third week. The addition of 15 and 20% PEG resulted in all clones not forming roots (Fig. 3.C). In the media containing 5 and 10% PEG, the tetraploid clone 5.4.4 was faster to root and formed more roots than the other clones.

The decrease in the number of roots along with the increasing concentration of PEG is a consequence of the occurrence of drought stress. The same results were also shown in Satureja rechingeri. Roots are the first earliest organ showing a plant's adaptive response to drought and this can be used as an important indicator to identify tolerant genotypes [24]. Research on hybrid taro showed that growth parameters such as number of roots and number of leaves could be used as indicators of tolerance to osmotic stress [25].

![Figure 1](image-url)

**Figure 1.** Number of leaves of taro (Colocasia esculenta (L.) Schott) shoot culture on MS medium containing PEG at 0-6 weeks in culture. A. diploid; B. tetraploid 4.6.3; and C. tetraploid 5.4.4.
Figure 2. Length of petioles of taro (*Colocasia esculenta* (L.) Schott) shoot culture on MS medium containing PEG at 0-6 weeks in culture. A. diploid; B. tetraploid 4.6.3; and C. tetraploid 5.4.4.

Figure 3. Number of roots of taro (*Colocasia esculenta* (L.) Schott) shoot culture on MS medium containing PEG at 0-6 weeks in culture. A. diploid; B. tetraploid 4.6.3; and C. tetraploid 5.4.4.
3.5. Fresh and dry weights
The highest average fresh and dry weights on control media without PEG were found in tetraploid clone 4.6.3 followed by tetraploid clone 5.4.4 then diploid control clone. However, on media containing 5% PEG, the highest fresh weight was found in tetraploid clones 5.4.4. The highest dry weight was found in clone 4.6.3. Similarly, on media containing 10% PEG, tetraploid clones 4.6.3 and 5.4.4 had higher fresh and dry weights than diploid clones. Overall, the increase in PEG concentration resulted in a decrease in the fresh and dry weights of the in vitro shoots. Tetraploid clones had higher fresh and dry weights compared to diploid clones (Figure 4).

The decrease in biomass is a consequence of the decrease in photosynthetic rate and the deviation of energy that was originally used for growth to activate and maintain the metabolism associated with adaptation to drought. This response was found in triploid banana AAB and tetraploid AAAB [19]. The same response was also found in rice [26] and wheat [27].

![Figure 4](image_url)

**Figure 4.** Fresh weight (A) and dry weight (B) of taro (Colocasia esculenta (L.) Schott) shoot culture on MS medium containing PEG after 6 weeks of culture

3.6. In vitro shoot performance
The results showed that in vitro shoot performance of all clones on control media without PEG had optimal growth. On media containing 5% PEG, some clones decreased in growth. On media containing 10% PEG, growth was inhibited even though the shoots were still green for up to 6 weeks of culture. Differences in growth response between clones were found in media containing 15 and 20% PEG. The diploid clones on this medium were stunted, the shoots withered and turned brown starting 4 weeks after culture (Figure 5-I).

A similar response due to 15 and 20% PEG treatment was also shown by tetraploid clone 4.6.3, shoots were stunted, but remained green 4 weeks after culture, then began to brown in the sixth week (Figure 5-II). Tetraploid clone 5.4.4 grew better on the media containing 15 and 20% PEG than diploid and tetraploid clones 4.6.3. In media containing 10% PEG, although there was inhibition of shoot growth, the shoots were still fresh and green for up to 6 weeks after culture. Shoot wilting occurred on media containing 20% PEG 6 weeks after culture (Fig. 5-III).
3.7. Proline content

The results showed that in all clones, there was an increase in proline content with increasing PEG concentrations as presented in Table 3. The highest proline content was found in shoots grown in media containing 20% PEG. Tetraploid 4.6.3 shoots growing on media without PEG (control) and on media added with PEG had the highest proline content, followed by diploid and 5.4.4 tetraploid clones were the lowest.

At the cellular level, drought signals promote production of stress-protectant metabolites such as proline, trigger the antioxidant system to maintain redox homeostasis and deploy peroxide enzymes to prevent acute cellular damage and membrane integrity [28]. A previous study of PEG treatment in Tacca leontopetaloides showed that proline content increased along with the increase of PEG concentrations [29].

### Table 3. Proline content of diploid and tetraploid taro (*Colocasia esculenta* (L.) Schott) cultured on MS medium containing PEG, six weeks after culture

| Clone          | Proline content (µmol/g FW) |
|----------------|-----------------------------|
|                | 0% PEG | 5% PEG | 10% PEG | 15% PEG | 20% PEG |
| Diploid        | 1.53   | 1.76   | 2.02    | 2.06    | 2.32    |
| Tetraploid-4.6.3| 1.70   | 1.75   | 2.97    | 2.97    | 3.20    |
| Tetraploid-5.4.4| 1.08   | 1.28   | 1.43    | 1.58    | 1.68    |
3.8. Lethal concentration \( (LC_{50}) \)

Recording of alive shoots after PEG stress is critical for determining the 50% lethal dose \( (LC_{50}) \). Determination of \( LC_{50} \) value was effective for \textit{in vitro} selection to identify stress tolerant genotype as it showed in peanut [17]. The \( LC_{50} \) value for PEG concentration was used to facilitate ease and time saving of \textit{in vitro} screening for drought tolerance [14]. Alive taro shoots were recorded at 6 weeks after culture. The results showed that in all clones the percentage of mortality increased with increasing PEG concentrations. Diploid clone showed a higher mortality percentage than tetraploid clones 4.6.3 and 5.4.4 (Table 4).

Determination of the \( LC_{50} \) value based on the mortality percentage data using probit analysis showed that the PEG concentration in the \( LC_{50} \) increased with increasing ploidy level (Table 4). Diploid clones had the lowest \( LC_{50} \) value compared to tetraploid clones 4.6.3 and 5.4.4. The tetraploid clone 5.4.4 showed a slightly higher \( LC_{50} \) value than clone 4.6.3.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Clones & Death explant (%) & 0% PEG & 5% PEG & 10% PEG & 15% PEG & 20% PEG & \( LC_{50} \) (\%)
\hline
Diploid & & 0 & 25 & 40 & 60 & 90 & 9.82
\hline
Tetraploid 4.6.3 & & 0 & 15 & 30 & 55 & 65 & 14.14
\hline
Tetraploid 5.4.4 & & 0 & 15 & 25 & 55 & 60 & 15.45
\hline
\end{tabular}
\caption{Percentage of death explants and \( LC_{50} \) value of diploid and tetraploid taro (\textit{Colocasia esculenta} (L.) Schott) cultured on MS medium containing PEG six weeks after culture.}
\end{table}

4. Conclusion

The growth response of diploid and tetraploid taro was inhibited after PEG treatment. The proline content in all clones showed an increase with increasing PEG concentration. Based on the \( LC_{50} \) value, PEG from 10 to 15% can be used for \textit{in vitro} selection for drought in diploid and tetraploid clones of taro.

Acknowledgements

The authors would like to thank Dr Andri F Martin, Yudha Hadi Pratama, Gabriella V. Besari and Yusup Lukman for their assistance during the research and BRIN-By Research Program for scholarship funding.

References

[1] Prana M, Kuswara T. 2002 1st ed. Bogor: Medikom Pustaka Mandiri
[2] de Carvalho Santos T, de Oliveira Amorim V, dos Santos-Serejo J, da Silva Ledo C, Haddad F, Ferreira C, et al. 2019 \textit{Mol Breed}. 39.
[3] Dhooghe E, Van Laere K, Eeckhaut T, Leus L, Van Huylenbroeck J. 2011 \textit{Plant Cell Tissue Organ Cult}. 104:359–73.
[4] Touchell DH, Palmer IE, Ranney TG. 2020 \textit{Front Plant Sci}
[5] Pei Y, Yao N, He L, Deng D, Li W, Zhang W. 2019 \textit{Sci Hortic} (Amsterdam) 257
[6] Chen C, Chen Z, Chen J, Huang J, Li H, Sun S, et al. 2020 \textit{Polymers} (Basel) [Internet] 12(2). Available from: www.mdpi.com/journal/polymers
[7] Wulansari A, Martin A, Ermayanti T. 2016 \textit{J Biol Indones}. 12(2):297–305
[8] Ermayanti T, Wijayanta A, Ratnadewi D. 2018 \textit{J Biol Indones}. 14(1):91–102
[9] Godfree R, Marshall D, Young A, Miller C, Mathews S. 2014 \textit{R Soc open sci}. 4:170934
[10] Parisod C, Holderegger R, Brochmann C. \textit{New Phytol} 186:5–17
[11] Guang-You H, Lucero M, Sanderson S, Zacharias E, Holbrook N. 2013 \textit{New Phytol}. 190:970–8
[12] Jan N, Qazi HA, Ramzan S, John R. 2018 Biotechnologies of Crop Improvement, Volume 1 327–72
[13] Rai MK, Kalia RK, Singh R, Gangola MP, Dhawan A. 2011 \textit{Environ Exp Bot} [Internet].
71(1):89–98. Available from: www.elsevier.com/locate/envexpbot

[14] Pradhan S, Singh SK, Srivastav M, Prakash J, Lal SK, Padaria JC, et al. 2021 Plant Cell Tissue Organ Cult. 145

[15] Patade VY, Bhargava S, Suprasanna P. 2012 Plant Cell Tissue Organ Cult. 108:279–86

[16] Murashige T, Skoog F. 1962 Physiol Plant. 15:473–97

[17] Rahayu ES, Sudarsono S. 2015 Emirates J Food Agric. 27(6):475–87

[18] Bates L, Waldren R, Teare I. 1973 Plant Soil. 39:205–7

[19] Hernandez-Perez CA, Gomez-Merino FC. 2021 Agronomy. 11(598)

[20] Marssaro AL, Morais-Lino LS, Cruz JL, da Silva Ledo CA, dos Santos-Serejo JA. 2017 Pesqui Agropecu Bras. 52(12):1301–4

[21] Labeb RK, Zaki HEM, Abdel-Ati YY, Zaky MH. 2020 SVU-International J Agric Sci. 2(1):53–61

[22] Sahoo MR, DasGupta M, Kole P, Mukherjee A. 2018 Photosynthetica. 56(4):1069–80

[23] Naveed MS, Manzoor A, Javed A, Tariq MA. 2019 World J Biol Biotechnol. 4(3):15–9

[24] Shariat A, Karimzadeh G, Hassan AM, Esfahan EZ. 2016 J Plant Physiol Breed. 6(1):1–12

[25] Sahoo MR, DasGupta M, Mukherjee A. 2006 Ann Trop Res. 28(2):1–11

[26] Miftahudin M, Putri RE, Chikmawati T. 2020 Biodiversitas. 21(8)

[27] Firdausya AF, Khumaida N, Ardie DSW. 2016 J Agron Indones (Indonesian J Agron.) 44(2):154

[28] Gupta A, Rico-Medina A, Cano-Delgado AI. 2020 Science 368(6488):266–9

[29] Martin AF, Hapsari BW, Ermayanti TM. 2018 Int J Agric Technol. 14(5):705–16.