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Response of Superior Maize Verieties to Different Combination of Inorganic and Organic Fertilization

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Abstract

Productivity of superior genotypes often did not satisfyingly achieve its genetic yield potential. Interaction between genetics and environmental factors more likely to driven the final yield of the varieties. The concept of balanced fertilization is carried out by referring to the creation of a balance of macro-nutrients in the soil so that plants can produce optimally. The research was conducted in a form of experimental study at Tarowang village, Takalar Regency during growing season of 2021 from May to September 2021. A split plot design was used with fertilization package as the main plot and superior varieties as subplots. Three fertilization packages combined inorganic and organic fertilizer were used, namely fertilization package consisted of N: P: K with ratio of 225:100:75, fertilizer package that consisted of N: P: K ratio of 200:100:50 + KNO_3 25 kg + Biotani 5cc L^{-1}, and fertilizer package that consisted of N: P: K with a ratio of 200:100:50 + KNO_3 25 kg + Ecofarming 5cc L^{-1}. Six superior varieties used were Nasa 29, JH-37, Bisi 2, Bisi 18, SINHAS 1 and NK7328. Based on the level of both factors, 18 treatment combinations were obtained and repeated three times. Responses of maize varieties to fertilization packages, consisted of different combination and dosage of NPK fertilizers, KNO_3 and organic fertilizers, were varied between varieties. The treatment of fertilization package of N: P: K = 225:100:75 with Bisi 2 variety gave the highest maize productivity, namely 12.39ton ha^{-1}

Keywords: maize, balanced fertilization, superior varieties, inorganic fertilizers, organic fertilizers

A. Introduction

One of the efforts implemented by the Indonesian government to increase the productivity of Maize is plant breeding. This strategy has been implemented and has taken part in the maize self-sufficiency program in order to meet the national demand for the commodity. Despite many high yielding varieties have been released by the Ministry of Agriculture with different superior characters to adapt with diverse growing conditions, in fact the productivity of these superior genotypes often did not satisfyingly achieve its genetic yield potential. Interaction between genetics and environmental factors more likely to driven the final yield of the varieties. If the management of the growing environment is not carried out properly, the high yield potential of these superior varieties cannot be achieved. High genetic potential can only be obtained if environmental conditions, lights, water, and soil nutrient status, support growth and production. In order to optimize this growing conditions, technology on the management farmer used is need to be taken into account. Fertilization package is one of main factors that can provide a good nutrient absorption.

Under conditions of available soil nitrogen, it can improve plant appearance and cob formation. Therefore, additional KNO_3 fertilization and Ecofarming organic fertilizer were carried out to overcome the availability of nutrients in corn plants. The addition of KNO_3 is preferred over KCl because KCl contains chloride which can poison plants if the concentration is more than 0.1%. KNO_3 is very effective to use because the K_2O content is between 45-46% which can improve fruit quality during the generative period of plants (Marschner, 2012). In sweet corn, it can increase the filling of seeds, the number of seeds and the length of the rows of seeds. This is because KNO_3 reacts neutrally so it is more effective to use than urea as a nitrogen source in acidic soils (Widiastoety, 2007). In order to balance the ecosystem, Eco Farming organic fertilizer is added which can improve soil texture and is able to meet the 13 nutrients needed by various plants, so as to increase production by 50 to 100% (Anonymous, 2019).

Balanced fertilization is a combination of inorganic and organic fertilizers. The combination of inorganic and organic fertilizers aims to meet the nutritional needs of sweet corn plants through high and balanced nutrient content in inorganic fertilizers and maintain soil fertility and provide macro and micro nutrients for plants in organic fertilizers. In addition, organic fertilizers applied to the soil will affect the physical, chemical and biological properties of the soil, play a role in soil mineral decomposition, plant nutrient sources, form a stable soil structure and have a direct influence on plant growth and development (Soepardi, 1982).

Cow urine liquid organic fertilizer contains high nitrogen and potassium, as well as growth stimulants that can be used as growth regulators including IAA and can accelerate seed filling on corn cobs. It was further explained that cow urine also had a positive effect on the vegetative growth of corn plants. Normal urine contains a very complex chemical composition, namely: water, urea, creatinine, allanthion, hippuric acid, ammonia, amino acids, sulfates, sulfur, organic salts, urochrome pigments, urobulins (Maspary, 2011). According to Sutedjo (2010) that cow urine has fast-acting properties and can stimulate plant development so as to increase plant production.

The nutrient content of cow urine, especially the amount of nitrogen, phosphorus, potassium, and water is higher than that of solid cow dung which has been used more as organic fertilizer. Cow urine contains growth-stimulating substances that can be used as growth regulators, including IAA. Because of the distinctive smell of livestock urine, it can also prevent the arrival of various plant pests so that cow urine can also function as plant pest control from attacks (Sudiro, 2011).

In the study of Tandisau & Thamrin (2005) regarding the application of complete fertilizers N, P, and K (200:35:100) showed the highest average yield (5.5 t/ha). Purwanto, J. K., K. Agustina & Yursida (2014) showed that sweet corn plants given 50% recommended dose of inorganic fertilizer and cow urine produced 10 -12 tons/ha higher than the national average corn productivity (4.5 tons/ha). The results of Pangaribuan, D.H., Sarno, Kurniawan, M.C. (2017) showed that the use of liquid fertilizer cow urine 7 ml/l or a concentration of 7,000 ppm with application times of 2, 4, 6 and 8 weeks after planting showed that the dose gave production results (11.02 tons/ha) which is better than control (7.3 tons/ha), so this treatment is recommended to sweet corn farmers. Cow urine liquid fertilizer should be applied at intervals of 2 weeks from the beginning of the vegetative growth phase (2 WAP) to the beginning of the generative phase (8 WAP). Yenni & Yayuk's (2015) research on optimizing sweet corn production using organic and inorganic fertilizers resulted in the highest average production of 12.57 t/ha at 200 kg/ha NPK+10 t/ha manure treatment.

The concept of balanced fertilization is carried out by referring to the creation of a balance of macro-nutrients in the soil so that plants can produce optimally. The comparison between N:P:K elements will determine the ability of maize plants in genetic expression to optimally produce prolific cobs, so that the percentage of prolific produced is higher. In order for the potential yield to be more optimal, it is necessary to add organic fertilizer as additional nutrients in the form of biological fertilizers such as the use of eco farming. A research is necessary to be undertaken to study fertilizer package model that provides high productivity (≥12.5 t/ha) for each maize variety.

B. Methology

1. Research Methods

The research was conducted in a form of experimental study at Tarowang village, Takalar Regency during growing season of 2021 from May to September 2021. A split plot design was used with fertilization package as the main plot and superior varieties as subplots. Three fertilization packages combined inorganic and organic fertilizer were used, namely fertilization package P1 consisted of N: P: K with ratio of 225:100:75, fertilizer package P2 that consisted of N: P: K ratio of 200:100:50 + KNO_3 25 kg + Biotani 5cc L^{-1}, and fertilizer package P3 that consisted of N: P: K with a ratio of 200:100:50 + KNO_3 25 kg + Ecofarming 5cc L^{-1}. Six superior varieties used were Nasa 29, JH-37, Bisi 2, Bisi 18, SINHAS 1 dan NK7328. Based on the level of both factors, 18 treatment combinations were obtained and repeated three times resulted in 54 experimental units.

Maize were planted using Legowo planting system of (50 + 100) x 20 cm. Soil tillage were conducted using a maximum tillage prior to setting the experimental plots with a size of 3,5 m x 5 m per plot. Maize seeds were planted in five rows on each plot according to the Legowo planting system. Two seeds were planted in the planting hole using a planting stick and covered with compost subsequently with a total of 1.7 kg/plot or 3 ton.ha^{-1}.

Fertilization were applied with dosage according to treatment. Eco farming and Biotani organic fertilizers were sprayed five times prior to planting at 2 days before planting, and following planting at 10 days after planting (DAP), 30, 50, and 70 DAP. Urea, NPK Ponska and KNO_3 were at the 7, 35, and 50 DAP, except for the SP36 fertilizers that was given once at 7 DAP. Weeding was done by clearing weeds around the plants, while mulching was carried out by elevating the mounds and loosening the soil to create better soil aeration. The first weeding was done before the second fertilization, while the hoarding was done after the second fertilization. Harvesting was conducted when the plant is physiologically ripe which is indicated by the appearance of a black layer on the back side of the seeds. Harvesting was done manually on two middle rows of plants per number and then processed for observation of yield and other yield components.

2. Data Analysis

Data analysis was conducted using Analysis of variance (ANOVA) on growth and production parameters. A further test was conducted on parameters with significant effect of the treatments using Least Significance Difference (LSD) test at a level of confidence of 95%.

C. Result

1. *Effect* of Fertilization packages on Growth of Maize Superior Varieties.

Use of different packages of fertilization that combined inorganic (N,P,K and KNO_3) and organic (Ecofarming/Biotani) significantly affected the growth of various type of superior maize varieties (Tables 1, 2, and 3). Nevertheless, plant height of the maize varieties were determined by the effect of genetic variability of the varieties rather than the fertilization applied (Table 1). Analysis of variance show that plant height varied significantly between varieties. Tallest plant was shown by JH-37 that was not significantly different from other varieties except SINHAS 1 that slightly shorter compared to other varieties. On the other hand, no significant difference between the varieties was found in the parameter of number of leaves. In this parameter, the fertilization package significantly affected the values (Table 2). Application of single N, P, and K resulted in the highest number of leaves (12.48 leaves) compared to other fertilization package that combined the NPK fertilizer with KNO_3 and organic liquid fertilizers. The lowest leaves number was shown by the application of NPK (200:100:50), added by KNO_3 25 kg and Biotani 5cc L^{-1}.

When Ecofarming fertilizer used as the substitute of the Biotani fertilizer at the same concentration, it resulted in a value that did not differen with the fertilization package that gave the highest number of leaves.

Table 1. Average plant height (cm) of superior maize varieties on various fertilization packages.

	P1	P2	P3	Average
V1 (Nasa 29)	230.33	239.33	243.33	237.66 ab
V2 (JH-37)	259.34	248.67	254.22	**254.08 a**
V3 (Bisi 2)	254.33	254.44	250.78	253.18 a
V4 (Bisi 18)	243.89	244.11	251	246.33 a
V5 (SINHAS 1)	228.78	223.33	221.89	224.67 b
V6 (NK7328)	249.67	238.11	245.89	244.56 a
Average	244.39	241.33	244.52	
$LSD_{0.05}$(V)	19.73			

Numbers followed by the same letter in a column (a, b) are not significantly different based on LSD test at α = 0.05. P1=N : P : K (225:100:75), P2=N : P : K (200:100:50) + KNO_3 25 kg + Biotani 5cc L^{-1}; P3=N : P : K (200:100:50) + KNO_3 25 kg + Ecofarming 5cc L^{-1}

Table 2. Average leaves numbers (leaves) of superior maize varieties on various fertilization packages.

	P1	P2	P3	Average	$LSD_{0.05}$(P)
V1 (Nasa 29)	11.89	11.56	11.89	11.78	
V2 (JH-37)	12.44	11.44	11.44	11.78	
V3 (Bisi 2)	13.00	11.67	12.33	12.33	0.50
V4 (Bisi 18)	12.89	11.89	12.56	12.45	
V5 (SINHAS 1)	12.22	11.33	11.33	11.63	
V6 (NK7328)	12.44	12.44	12.78	12.56	
Average	**12.48 p**	11.72 q	12.06 pq		

Numbers followed by the same letter in a column (a, b) are not significantly different based on LSD test at α = 0.05. P1=N : P : K (225:100:75), P2=N : P : K (200:100:50) + KNO_3 25 kg + Biotani 5cc/L; P3=N : P : K (200:100:50) + KNO_3 25 kg + Ecofarming 5cc/L.

Table 3. Average stem diameter (mm) of superior maize varieties on various fertilization packages.

	P1	P2	P3	Average	$LSD_{0.05}$(P)
V1 (Nasa 29)	23.90 $_{ab}{}^{p}$	20.8 $_{bc}{}^{q}$	22.33 $_{ab}{}^{pq}$	22.34	
V2 (JH-37)	23.26 $_{b}{}^{p}$	21.88 $_{abc}{}^{p}$	24.30 $_{a}{}^{p}$	23.15	
V3 (Bisi 2)	**26.99** $_{a}{}^{p}$	22.03 $_{abc}{}^{q}$	23.15 $_{ab}{}^{q}$	24.06	2.72
V4 (Bisi 18)	24.23 $_{ab}{}^{p}$	23.99 $_{ab}{}^{p}$	22.49 $_{ab}{}^{p}$	23.57	
V5 (SINHAS 1)	25.11 $_{ab}{}^{p}$	20.49 $_{c}{}^{q}$	20.76 $_{b}{}^{q}$	22.12	
V6 (NK7328)	26.40 $_{ab}{}^{p}$	24.78 $_{a}{}^{p}$	25.18 $_{a}{}^{p}$	25.45	
Average	24.98	22.33	23.04		
$LSD_{0.05}$(V)	3.34				

Numbers followed by the same letter in a column (a, b), and in row (p, q) are not significantly different based on LSD test at α = 0.05. P1=N : P : K (225:100:75), P2=N : P : K (200:100:50) + KNO_3 25 kg + Biotani 5cc L^{-1}; P3=N: P : K (200:100:50) + KNO_3 25 kg + Ecofarming 5cc L^{-1}.

Fertilization packages and varieties significantly affected the stem diameter of maize. The biggest stem diameter was found in the variety of Bisi 2 applied with N: P: K (225:100:75) fertilizers. The use of dosage also seems to resulted in bigger stem diameter in all varieties compared to other fertilization packages except in JH-37 variety that showed the biggest stem diameter obtained by plants applied with fertilization package of N: P: K (200:100:50) + KNO_3 25 kg + Ecofarming 5cc L^{-1} (Table 3).

2. *Effect of Fertilization Packages on the Production Component of Maize Superior Varieties*

Analysis of variance on fertilization packages and superior varieties treatments show that couple of production components i.e. weight of peeled cob and the ratio between kernels net weight and weight of kernels with the corn cob, expressed as percentage of grain yield were affected only by the varieties treatment. No significant effect of the combination of inorganic and organic fertilizers on these parameters (Table 4). On the other hand, yield per hectare or the productivity of the maize were varied between varieties and fertilization applied.

Highest productivity that exceed the 12 $ton.ha^{-1}$ target was shown by variety of Bisi 2 applied with N : P : K (225:100:75), followed by Bisi 18 applied with slightly lower NPK dosage (200:100:50), combined with KNO_3 (25 kg), and Biotani organic fertilizers (Biotani 5cc L^{-1})

Table 4. Average peeled cob weight (kg) and grain yield* (%) of superior maize varieties on various fertilization packages.

Varieties	Peeled Cob Weight (kg)	Grain Yield* (%)
V1 (Nasa 29)	18.28 ab	74.41 ab
V2 (JH-37)	16.50 b	74.05 ab
V3 (Bisi 2)	19.06 ab	75.63 ab
V4 (Bisi 18)	18.50 ab	**76.74** a
V5 (SINHAS 1)	12.94 c	71.85 b
V6 (NK7328)	**20.44** a	72.68 ab
$LSD_{0.05}$(V)	3.32	4.52

Numbers followed by the same letter in a column (a, b) are not significantly different based on LSD test at α = 0.05. *The ratio between the net weight of the corn kernels and the weight of the corn kernels that are still with the cob.

Table 5. Average productivity ($ton.ha^{-1}$) of superior maize varieties on various fertilization packages.

	P1	P2	P3	Average	$LSD_{0.05}$(P)
V1 (Nasa 29)	11.03 $_{ab}^{p}$	10.40 $_{abc}^{p}$	10.39 $_{ab}^{p}$	10.61	
V2 (JH-37)	10.71 $_{ab}^{p}$	8.99 $_{bc}^{q}$	11.24 $_{a}^{p}$	10.31	
V3 (Bisi 2)	**12.39** $_{a}^{p}$	11.01 $_{ab}^{q}$	11.18 $_{a}^{pq}$	11.53	1.36
V4 (Bisi 18)	10.55 $_{ab}^{q}$	12.12 $_{a}^{p}$	10.84 $_{a}^{pq}$	11.17	
V5 (SINHAS 1)	9.88 $_{b}^{p}$	8.61 $_{c}^{pq}$	8.23 $_{b}^{q}$	8.91	
V6 (NK7328)	10.68 $_{ab}^{p}$	9.30 $_{bc}^{q}$	10.23 $_{ab}^{pq}$	10.07	
Average	10.87	10.07	10.35		
$LSD_{0.05}$ (V)	2.21				

Numbers followed by the same letter in a column (a, b), and in a row (p,q) are not significantly different based on LSD test at α = 0.05. P1=N : P : K (225:100:75), P2=N : P : K (200:100:50) + KNO_3 25 kg + Biotani 5cc L^{-1}; P3=N : P : K (200:100:50) + KNO_3 25 kg + Ecofarming 5cc L^{-1}.

D. Discussion

High yielding superior varieties are the results from the breeding program where the pants were designed to have superior characters including yield, resistance to certain pest and disease attacks and can adapt well under limiting growing conditions. Nevertheless, the final yield or productivity of these superior genotypes can be determined by a complex interaction between the genotypes and the environment. In this recent study, it was shown that some superior varieties of Maize had different responses to the soil nutrient management in the form of combination of inorganic and organic fertilizers. According to Adisarwanto (2006), management of the growing environment determine the achievement of the high yield potential of these superior varieties. High genetic potential can only be obtained if environmental conditions, lights, water, and soil nutrient status, support growth and production.

The interaction between genetic potential and environment was shown in the growth parameter such as in stem diameter. Variety Bisi 2 applied with the highest dosage of NPK fertilizers (225:100:75) resulted in the biggest stem compared to other variety. N, P, K fertilizers are needed for plant growth, especially in stimulating the formation of plant height and enlargement of stem diameter. In addition to nutrients N, P, K, organic fertilizers also have a role in supporting plant vegetative growth. Soil with the help of high organic matter content can be ensured to have better soil physical, chemical and biological properties.

Organic fertilizers contain a mix of macro and micro nutrients. Some organic fertilizers also added with different kind of microbes to enrich and improve the benefit of using the fertilizers. Therefore, each type of the organic fertilizer can have different effect on the plant. Recent study shows that in parameter of leaves numbers, use of lower dosage of NPK fertilizers but combined with additional K fertilizers and Ecofarming organic fertilizer resulted in plants with leaves number that similar to plants applied with only inorganic NPK fertilizers. The availability of sufficient nitrogen causes a balance ratio between leaves and roots, hence the vegetative growth is normal.

In the production component, Varieties V4 (Bisi 18) and V1 (Nasa 29) produced the highest average percentage of seed yield, namely 76.74% and 74.41%, and significantly different from the treatment of V5 variety (SINHAS 1), which was 71.85%. The percentage of high corn yield is influenced by the phosphorus content contained an increase in P uptake is needed by plants because it is used as a material for the formation of ATP in the respiration process to increase metabolic processes, including photosynthesis, especially during the seed filling phase.

The highest average productivity was obtained from the interaction of the P1 (N: P: K = 225: 100: 75) fertilization package with the V3 (Bisi 2) variety, which was 12.39 ton.ha-1. Production is an important benchmark that determines the success of a research carried out, both production per plot and production per hectare. Appropriate fertilization and the response of varieties to the given treatment will result in high production.

E. Conclusion

Responses of maize varieties to fertilization packages, consisted of different combination and dosage of NPK fertilizers, KNO_3 and organic fertilizers, varied between varieties. The treatment of fertilization package of N: P: K = 225:100:75 with Bisi 2 variety gave the highest maize productivity, namely 12.39ton ha^{-1}.

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Hydroponic Salinity Screening by Deep Flow Technique on All Paddy Growing Phases

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Abstract

Salinity screening under hydroponic Deep Flow Technique (DFT) has not been widely studied, especially on the nature of rice tolerance to salinity stress. According to previous screening studies, this method was effective in distinguishing the nature of rice tolerance to salinity stress. However, they were tested only at generative phase. Therefore, evaluation on screening method with hydroponic DFT at all phases of paddy growth is essential. The objective of this study is to evaluate the filtering under hydroponic DFT at all paddy phase and to determine secondary character that support productivity which can be utilized as selection character in this screening process. The experiment was arranged with a complete nested group design with nested replication is the NaCl stress. There were 5 (Five) tested rice varieties and the stress environment consisted of three levels: 0, 60, and 120 mM of NaCl, all with 3 (three) replications. The nutrient culture screening was adapted to the modified Egdane method. The results showed that screening under hydroponic DFT was effective at the concentration of 60 nM of NaCl. The best selection character was yields per clump, number of productive tillers and total chlorophyll. The variety of Jeliteng, Ciherang and Inpari 34 of Salin Agritan, were classified as tolerant group. This hydroponic DFT Filtering method could be recommended as one salinity screening method for all paddy growing phases

Keywords: DFT, rice screening, *Oryza sativa,* path analysis, salinity tolerance rice

A. Introduction

Rice has been main food consumed by most Indonesian people. Indonesia's population growth rate in 2025-2030 is estimated to reach 1.49% or 286.02 million people with rice consumption can be as much as 139.5 kg per capita, resulting in rice demand for the nation reaching 39.8 million tons (Directorate General of Agricultural Facilities and Infrastructure, 2013). Efforts for increasing production such as expansion of planting fields, especially in sub-optimal areas. However, such areas have several limiting factors including salinity stress. Salinity has been a main abiotic stresses on coastal and tidal lands. Indonesia being a nation with thousand islands, suffers salt water intrusion to many of its mainland which increase salt concentration in rice farmland, resulting in the decline of productivity. Rad HE, Aref F, Rezaei M. (2012) report that increased salinity has an impact on decreased productivity and also responsible for plants total failure at high concentration of about 12 ds/m. According Ghosh B, Ali Md N, Saikat G. (2016) and Ismail AM, Platten JD, Miro B. (2013) salinity gave negative impact through some stresses such as osmotic, ion toxicity, ion homeostasis and oxidative stress (Ghosh et al., 2016; Ismail et al., 2013). Therefore, the development of tolerant varieties is essential in overcoming the impact of salinity on coastal land.

Tolerant varieties are those which are able to maintain metabolic processes even in stressed conditions. According to De Leon TB, Linscombe S, Gregorio G, Subudhi PK. (2015) salinity tolerant varieties are able to minimize the damage during the stressed period. Anshori MF, Purwoko BS, Dewi IS, Ardie SW, and Suwarno WB. (2018), Anshori MF, Purwoko BS, Dewi IS, Suwarno WB, Ardie SW (2020) reported the nature of tolerance correlates with the rate of plant damage. Hence the lower the damage, the more tolerant the plants. Assembly of tolerant varieties closely relates to the selection method, selection environment and selection parameters.

Selection methods is possible artificially. It has been reported by Ali MdN, Yeasmin L, Gantait S, Goswami R, Chakraborty. (2014), Mondal and Borromeo (2016), Arifuddin M, Musa Y, Farid M, Anshori MF, Nasaruddin N, Nur A, Sakinah AI. (2021) who performed the selection hydroponically and Hariadi YC, Nurhayati AY, Soeparjono S, Arif I. (2014), Safitri H, Purwoko BS, Dewi IS, Ardie SW. (2016). and Anshori et al. (2018) who did it on soil media. Hydroponics method is the most widely performed due to possibility for more controllable selection and the absence of cofactors that affect salinity levels in the testing media (Faiyue, 2012; Safitri, 2016; Anshori et al. 2020; Farid M, Nasaruddin, Musa Y, Ridwan I, Anshori MF. (2021). However, the general hydroponic method focused only on the vegetative phase. According to Ismail et al. (2013) and Kranto S, Chankaew S, Monkham T, Theerakulpisut P, Sanitchon J. (2016) there was no significant correlations between the properties of salinity density tolerance in the vegetative and reproductive phases.

It is an indication that the screening for salinity tolerance on rice by hydroponic method is possible until its reproductive phase. One hydroponic method that is practicable for this screening is the Deep Flow Technique (DFT). According to Arifuddin et al. (2021), selection with DFT methods was effective in distinguishing the tolerant and sensitive properties. However, the research only focused on the generative phase. Therefore, testing and identifying secondary characters for salinity screening at all growing phases of rice through DFT method becomes important. The purpose of this study was to evaluate the DFT hydroponic screening at all growing phases and to determine the secondary production supporting character that can be used as a selection character in the screening process.

B. Methology

Salinity stress experiment was performed on nutrient cultures adapted to the modified method of Egdane JA, Vispo NA, Mohammadi R, Amas J, Katimbang ML, Platten JD, Ismail A, Gregorio GB. (2003). The parallel experiments were conducted greenhouses at the altitude of 22.4 m above sea level from August to November 2020.

1. Research Design and Procedures

The experiment was arranged in a nested and randomized complete group design, where the nested replication was on the NaCl environment. The tested rice varieties were Inpari 34 Salin Agritan, Ciherang, IR 29, Inpari 29, and Jeliteng. The salinity environments in the experiment were 0 mM of NaCl (normal), 60 mM of NaCl and 120 mM of NaCl.

The seedling was grown on rockwool media for 15 days then transferred to a DFT hydroponics system running on PVC pipes. Each pipe has holes with a diameter of 5 cm each and the distance between the holes was 20 cm. The seedlings on the rockwool were placed in a netpot container by ensuring the seedling's root are in contact with the nutrient culture. The nutrient culture media was AB mix with a concentration of 5 ml per liter of water. The volume of nutrients in each DFT installation was 120 L. Induction of salinity stress was applied at the age of 7 days in the DFT. Stress induction was applied gradually to avoid osmotic shock. The first application of NaCl was 50% of the prescribed concentration, it is then increased to 60 mM and 120 mM (as the treatments) in the following three days. The stress condition was sustained for 14 days and then transferred to normal environmental conditions. The nutrient solution was replaced after 7 days under normal conditions. Administration of HCL or NaOH was to maintain the pH of the solution in the range of 5.5-6.5.

The observed characters of this experiment were plant height, root length, number of leaves, number of tillers, number of productive tillers, flowering age, chlorophyll A, chlorophyll B, total chlorophyll, stomata density, wet weight of shoots, dry weight of shoots, dry weight of roots, length of panicles, length of flag leaves, percentage of filled seeds, percentage of hollow seeds, weight of 100 seeds, and production per clump.

2. Data Analysis

The significant different characters in the interactions of each character under the variance analysis were further analyzed with pearson correlation tests, cross-prints, and key component analysis based on the value of each character's stress tolerance index. Heatmap cluster analysis was further performed to visualize kinship patterns of complex variable whish is more simple through color gradation.

The Index of Stress Tolerance was calculated by equation (Fernandez, 1992): ITC = $\frac{Yp \text{ x } Ys}{\bar{Y}p^2}$

Yp = The character value of each variety under normal circumstances/ not stressed.
Ys = The character value of each variety in a stressed state.
Ȳs = Average character values of all varieties under normal/not stressed circumstances

C. Result and Discussion

Results of analysis of the variance for the entire growth phase of rice is shown in Table 1. It shows that the stressed environment affected the entire observed character, whereas the different rice varieties affected almost all of the observed characters except the plant height, percentage of filled seeds, and the percentage of hollow seeds. The interaction of varieties and salinity stress had a significant to very significant effect on the character of root length, number of leaves, number of tillers, number of productive tillers, flowering age, chlorophyll A, chlorophyll B, total chlorophyll, stomata density, shoots wet weight, shoots dry weight, roots wet weight, roots dry weight, length of flag leaves, weight of 100 seeds and production per clump.

Significant interactions shown by the variance analysis are early indicators in stress screening, this has been reported by Anshori (2019); Farid BDR M, Nasaruddin, Anshori MF, Chaerunnisa ANJ. (2020) that characters which have significant interactions have different response patterns among genotypes in normal and stressed environments. Based on the analysis, the characters of root length, number of leaves, number of tillers, number of productive tillers, flowering age, chlorophyll A, chlorophyll B, total chlorophyll, stomata density, shoots wet weight, shoots dry weight, roots wet weight, roots dry weight, length of flag leaves, weight of 100 seeds and production per clump, all can be used as candidates of rice selection criteria for salinity stress on DFT hydroponic screening. However, it is necessary to do more in-depth analysis with several other multivariate analysis series.

The assessment of the variety's response to the stress condition should operate a tolerance index. It has been reported by Mau YS, Ndiwa ASS, and Arsa AGBA. (2014), Aboughadareh AP, Yousefian M, Moradkhani H, Vahed MM, Poczai P, and Siddique KHM. (2019), Aboughadareh AP, Mohammadi R, Etminan A, Shooshtari L, Tabrizi NM, Poczai P. (2020), and Farid et al. (2021) that the Index of Stress Tolerance (ITC) is the appropriate index for characterizing the most stress tolerant ones. The use of ITC values had previously been reported by El-Hashash & Agwa (2018) in drought-stressed barley, Anshori et al. (2018) in salinity stressed rice, Farid and Ridwan (2019) in drought stressed rice, and Fadhli N, Farid M, Rafiuddin, Effendi R, Azrai M, Anshori MF (2020) in drought-stressed maize. The Value of the Tolerance Index for characters with significant interaction values is shown in Table 2. ITC1 is the value of the tolerance index at 60 mM of NaCl, and ITC2 is the value of the tolerance index at 120 mM of NaCl. The value of this index will be used as the basis for the next multivariate analysis in determining the character of selection. Correlation analysis is the most common analysis in identifying the best selection characters. Correlation analysis in this experiment focused on production per clump as its main character. The relationship between other characters to production per clump can be used as the best selection character against salinity stress. The use of this analysis has been reported by Afa L O, Purwoko B S, Junaedi A, Haridjaja O and Dewi I S (2018); Aman, J., Bantte, K., Alamerew, S. & Sbhatu, D.B. (2020); Saleh MM, Salem KFM, Elabd AB. (2020). The results of the correlation analysis based on the values of the Index of Stress Tolerance (ITC) (Table 3) shows that the root length character (0.78), the number of leaves (0.91), chlorophyll A (0.93), chlorophyll B (0.95), total chlorophyll (0.94), stomata density (0.83), shoots wet weight (0.88), shoots dry weight (0.88), roots wet weight (0.93), roots dry weight (0.78), length of flag leaves (0.89) flowering age (0.87), The number of tillers (0.81), the number of productive tillers (0.97), and the weight of 100 seeds (0.93), all have a significant correlation to production per clump. Based on this correlation analysis results, it is not possible to distinguish the direct and indirect effects of each character on the production character. Therefore, it is necessary to continue with cross-print analysis to understand the magnitude of both direct and indirect effects on production (Singh and Chaudhary 2007; Rohaeni and Permadi 2012; Anshori *et al.*2018).

Table 1. Middle square analysis of variance of the observed characters

Character	Varieties	Stress	Var:Stress	Error	CV	Vg	Vp	Repeatability (%)
TT	102.56ns	9588.57**	27.21ns	90.56	15.89	-21.12	69.45	0.00
PA	114.93**	1821.02**	70.02**	11.49	12.60	19.51	31.00	62.95
JD	5.97**	155.78**	2.17**	0.44	10.56tr	0.58	1.01	56.77
JA	1.91**	15.47**	0.86**	0.09	12.62tr	0.25	0.35	73.14
JAP	0.23**	28.12**	0.08*	0.03	12.26tr	0.02	0.05	37.14
UB	123.86**	29767.20**	141.92**	7.20	5.28	44.91	52.11	86.18
Chl A	7270.30**	201173.78**	5888.35**	221.99	11.61	1888.79	2110.78	89.48
Chl B	305.20**	35287.22**	219.04**	25.01	9.08	64.68	89.69	72.11
Chl Tot	11776.69**	421967.17**	9399.63**	411.65	10.84	2996.00	3407.64	87.92
KS	145.92*	13532.79**	123.74*	43.88	19.12	26.62	70.50	37.76
BBT	1043.28**	29669.96**	760.09**	46.43	20.14	237.89	284.32	83.67
BKT	1.55**	50.97**	1.89**	0.30	20.25tr	0.53	0.83	63.81
BBA	0.97**	30.95**	1.34**	0.06	10.91tr	0.43	0.48	88.08
BKA	0.15**	4.90**	0.27**	0.03	17.16tr	0.08	0.11	70.59
PM	7.23*	2654.33**	2.82tn	2.05	10.08	0.26	2.31	11.19
GDP	11.46**	3691.62**	16.94**	2.57	8.95	4.79	7.36	65.10
%JGB	0.00ns	2.60**	0.00tn	0.00	12.45	0.00	0.00	0.00
%JGH	0.00ns	1.54**	0.00tn	0.00	19.44	0.00	0.00	0.00
100BJ	0.08**	15.58**	0.08**	0.01	8.96	0.02	0.04	69.71
Prod	0.04*	48.12**	0.07**	0.01	7.72tr	0.02	0.03	62.33

Notes: *: significant effect, **: very significant effect, tr: results of $\sqrt{x}$ transformation, TT: plant height, PA: root length, JD: number of leaves, JA: number of tillers, JAP: number of productive tillers, UB: flowering age, Chl A: chlorophyll A, Chl B: chlorophyll B, Chl Tot: total chlorophyll, KS : stomata density, BBT: shoots wet weight, BKT : shoots dry weight, BBA : roots wet weight, BKA : roots dry weight, PM : length of panicles, PDB : length of flag leaf, %JGB : percentage of filled seeds, %JGH : percentage of hollow seeds, 100BJ : weight of 100 seeds, Prod : production per clump.

Correlation analysis is an overview of the level of kinship between one character to the others, but the value of correlation cannot explain the causal relationship of the kinship level among characters. Therefore, in order to elaborate the correlation coefficient to be more useful, come the role of cross-print analysis. The results of cross-print analysis could describe how significant the direct and indirect effects of a character to the main character (Rohaeni and Permadi, 2012). The use of correlation analysis and cross-print analysis in determining the character of selection has also been performed by many researchers including Milligan SB, Gravois KA, Bischoff KP, Martin FA. (1990), Akhmadi (2016), Anshori et *al.,* (2019), Fadhli et *al.,*(2020), Farid et *al.,*(2020) and Farid et *al.,* (2021). The cross-print of IST of the production per clump character shows a representative result on the determinant coefficient value of 0.80 (Table 4). Based on the cross-print analysis, the character of total chlorophyll (5.77) had the highest and significant direct effect on the character of production per clump, followed by the character of the number of productive tillers (1.08). Consequently, based on these results the total chlorophyll character and number of productive tillers can be recommended as the selection characters in screening for salinity tolerance throughout the rice growing phase on the DFT hydroponic system. However, these findings need to be supported by other multivariate analyses such as major component analysis and heat map cluster analysis.

Table 2. Average tolerance index of stress on the combination of varieties and concentrations of NaCl

Varieties	PA	JD	Chl_A	Chl_B	Chl_Tot	KS	BBT	BKT	BBA	BKA	GDP	UB	JA	JAP	100BJ	Prod
V1_ITC1	0.76	0.75	0.77	0.8	0.78	0.89	0.26	0.75	0.73	0.72	1.01	1.32	0.89	0.61	0.99	0.25
V2_ITC1	0.87	0.8	0.89	0.9	0.89	0.87	0.13	1.04	0.69	0.6	0.8	0.91	0.88	0.57	0.85	0.24
V3_ITC1	0.68	0.49	0.08	0.5	0.18	1.19	0.06	0.33	0.37	0.39	0.97	1.26	0.42	0.34	0.64	0.1
V4_ITC1	0.97	0.61	0.88	0.89	0.88	0.91	0.2	0.5	0.46	0.53	0.94	1.3	0.79	0.47	0.71	0.17
V5_ITC1	1.47	1.06	0.98	0.97	0.98	1.45	0.34	0.76	0.92	0.97	1.12	1.21	1.56	0.73	0.69	0.23
V1_ITC2	0.37	0.29	0	0	0	0	0.01	0.12	0.15	0.21	0	0	0.49	0	0	0
V2_ITC2	0.46	0.29	0	0	0	0	0.01	0.12	0.25	0.2	0	0	0.4	0	0	0
V3_ITC2	0.33	0.26	0	0	0	0	0.01	0.13	0.16	0.26	0	0	0.29	0	0	0
V4_ITC2	0.47	0.28	0	0	0	0	0.01	0.3	0.18	0.53	0	0	0.37	0	0	0
V5_ITC2	0.45	0.42	0	0	0	0	0.03	0.15	0.23	0.24	0	0	0.45	0	0	0

Notes: ITC1 : index value at 60 mM of NaCl, ITC2 : index value at 120 mM of NaCl, V1 : Inpari 34, V2 : Ciherang, V3 : IR29, V4 : Inpari 29, V5 : Jeliteng, PA: root length, JD: number of leaves, JA: number of tillers, JAP: number of productive tillers, UB: flowering age, Chl A: chlorophyll A, Chl B: chlorophyll B, Chl Tot: total chlorophyll, KS : stomata density, BBT: shoots wet weight, BKT : shoots dry weight, BBA : roots wet weight, BKA : roots dry weight, PM : length of panicles, PDB : length of flag leaf, %JGB : percentage of filled seeds, %JGH : percentage of hollow seeds, 100BJ : weight of 100 seeds, Prod : production per clump.

Table 3. Correlation of the Stress Tolerance Index (ITC)

	PA	JD	Chl_A	Chl_B	Chl_Tot	KS	BBT	BKT	BBA	BKAr	GDP	UB	JA	JAP	100BJ	Prod
PA	1.00															
JD	0.91	1.00														
Chl_A	0.85	0.89	1.00													
Chl_B	0.86	0.90	0.96	1.00												
Chl_Tot	0.86	0.90	1.00	0.97	1.00											
KS	0.84	0.85	0.78	0.90	0.81	1.00										
BBT	0.88	0.92	0.90	0.88	0.90	0.80	1.00									
BKT	0.70	0.82	0.84	0.83	0.85	0.67	0.71	1.00								
BBA	0.87	0.97	0.90	0.91	0.91	0.82	0.93	0.85	1.00							
BKA	0.84	0.84	0.79	0.78	0.79	0.73	0.86	0.77	0.87	1.00						
GDP	0.81	0.84	0.82	0.94	0.86	0.95	0.83	0.74	0.85	0.75	1.00					
UB	0.75	0.78	0.80	0.93	0.84	0.93	0.80	0.69	0.80	0.68	0.98	1.00				
JA	0.90	0.93	0.84	0.80	0.84	0.73	0.92	0.74	0.91	0.84	0.72	0.65	1.00			
JAP	0.86	0.93	0.91	0.96	0.93	0.89	0.91	0.86	0.95	0.84	0.94	0.91	0.86	1.00		
100BJ	0.71	0.81	0.86	0.94	0.88	0.86	0.79	0.81	0.85	0.71	0.95	0.95	0.66	0.92	1.00	
Prod	0.78	0.91	0.93	0.95	0.94	0.83	0.88	0.88	0.93	0.78	0.89	0.87	0.81	0.97	0.93	1.00

Notes: PA: root length, JD: number of leaves, JA: number of tillers, JAP: number of productive tillers, UB: flowering age, Chl A: chlorophyll A, Chl B: chlorophyll B, Chl Tot: total chlorophyll, KS : stomata density, BBT: shoots wet weight, BKT : shoots dry weight, BBA : roots wet weight, BKA : roots dry weight, PM : length of panicles, PDB : length of flag leaf, %JGB : percentage of filled seeds, %JGH : percentage of hollow seeds, 100BJ : weight of 100 seeds, Prod : production per clump.

Table 4. ITC cross-print of rice to the main character of production per clump.

Character	Direct Influence	Indirect Influence														Total Influence	Residual	
		PA	JD	Chl_A	Chl_B	Chl_Tot	KS	BBT	BKT	BBA	BKA	GDP	UB	JA	JAP	100BJ		
PA	**-0.02**		0.38	-3.27	-1.88	4.93	0.07	0.01	0.11	-0.12	-0.09	-0.55	0.46	-0.28	0.92	0.10	0.78	-0.01
JD	**0.42**	-0.02		-3.46	-1.97	5.22	0.07	0.01	0.13	-0.14	-0.09	-0.57	0.48	-0.29	1.01	0.11	0.91	0.38
Chl_A	**-3.86**	-0.01	0.37		-2.10	5.75	0.07	0.01	0.14	-0.13	-0.08	-0.56	0.49	-0.26	0.99	0.12	0.93	-3.57
Chl_B	**-2.19**	-0.01	0.37	-3.69		5.62	0.08	0.01	0.14	-0.13	-0.08	-0.64	0.57	-0.25	1.04	0.13	0.95	-2.08
Chl_Tot	**5.77****	-0.01	0.38	-3.85	-2.14		0.07	0.01	0.14	-0.13	-0.08	-0.59	0.51	-0.26	1.01	0.12	0.94	5.41
KS	**0.09**	-0.01	0.35	-3.01	-1.97	4.68		0.01	0.11	-0.12	-0.07	-0.65	0.57	-0.23	0.97	0.12	0.83	0.07
BBT	**0.01**	-0.02	0.38	-3.46	-1.92	5.19	0.07		0.12	-0.13	-0.09	-0.57	0.49	-0.28	0.99	0.11	0.88	0.01
BKT	**0.16**	-0.01	0.34	-3.25	-1.83	4.88	0.06	0.01		-0.12	-0.08	-0.51	0.42	-0.23	0.92	0.11	0.88	0.14
BBA	**-0.14**	-0.01	0.40	-3.47	-1.99	5.24	0.07	0.01	0.14		-0.09	-0.58	0.49	-0.28	1.03	0.12	0.93	-0.13
BKA	**-0.10**	-0.01	0.35	-3.05	-1.71	4.58	0.06	0.01	0.13	-0.12		-0.51	0.42	-0.26	0.91	0.10	0.78	-0.08
GDP	**-0.68**	-0.01	0.35	-3.18	-2.06	4.95	0.08	0.01	0.12	-0.12	-0.08		0.60	-0.22	1.02	0.13	0.89	-0.61
UB	**0.61**	-0.01	0.32	-3.10	-2.04	4.84	0.08	0.01	0.11	-0.11	-0.07	-0.67		-0.20	0.98	0.13	0.87	0.53
JA	**-0.31**	-0.02	0.39	-3.26	-1.75	4.85	0.06	0.01	0.12	-0.13	-0.09	-0.49	0.40		0.93	0.09	0.81	-0.25
JAP	**1.08****	-0.01	0.39	-3.53	-2.11	5.38	0.08	0.01	0.14	-0.13	-0.09	-0.64	0.55	-0.27		0.13	0.97	1.05
100BJ	**0.14**	-0.01	0.34	-3.31	-2.07	5.10	0.07	0.01	0.13	-0.12	-0.07	-0.65	0.58	-0.20	0.99		0.93	0.13

Notes: R square: 80%, PA: root length, JD: number of leaves, JA: number of tillers, JAP: number of productive tillers, UB: flowering age, Chl A: chlorophyll A, Chl B: chlorophyll B, Chl Tot: total chlorophyll, KS : stomata density, BBT: shoots wet weight, BKT : shoots dry weight, BBA : roots wet weight, BKA : roots dry weight, PM : length of panicles, PDB : length of flag leaf, %JGB : percentage of filled seeds, %JGH : percentage of hollow seeds, 100BJ : weight of 100 seeds, Prod : production per clump.

Major Component Analysis is one of the multivariate analyses that analyzes the data of several interrelated variables. The purpose of this analysis is to extract important information from the data and describe it as a new set of orthogonal variables called the main components, and to display patterns of observational similarity in other words to summarize the data with a smaller number of variables (Ilmaniati and Putro, 2018). The results of the main component analysis (AKU) produced one main component that could describe the character of productivity (Table 5) i.e. the first major component (KU1) with a total proportion of 98% and eigenvalue of 2.93. Based on main component 1 (KU1), the production character (0.58) in stressed condition is within same direction with total chlorophyll character (0.57) and the number of productive tillers (0.58).

Table 5. Main component Analysis of ITC value.

Character	KU1	KU2	KU3
Chl_Tot	0.57	-0.82	-0.07
JAP	0.58	0.46	-0.67
Prod	0.58	0.35	0.74
EV	2.93	0.05	0.02
P	0.98	0.02	0.01
CP	0.98	1.00	1.00

Notes: CP: Cumulative Proportion, EV: EigenValues, KU: Main Component, Chl_tot: total chlorophyll, JAP: number of productive tillers, Prod: production per clump.

Cluster analysis is a common method in plant breeding. There are two main functions of cluster analysis applications: measurement to identify outliers and classifying sample subtypes (Zhao S, Guo Y, Sheng Q, Shyr Y., 2014). However, in the current development, cluster analysis is often combined with heatmap analysis (Virga G, Licata M, Consentino BB, Tuttolomondo T, Sabatino L, Leto C, Bella SL., 2020; Anshori et al. 2020). Based on the GROUPING OF ITC characters, productivity is separate from other character groups, while the other group consists of the number of productive tillers and chlorophyll A. Based on the grouping of varieties, the first group consists of Jeliteng ITC1, Ciherang ITC1, Inpari 34 Salin Agritan ITC1 and Inpari 29 ITC1 while the second group are IR 29 ITC1 and ITC2 for all varieties (Figure 1).

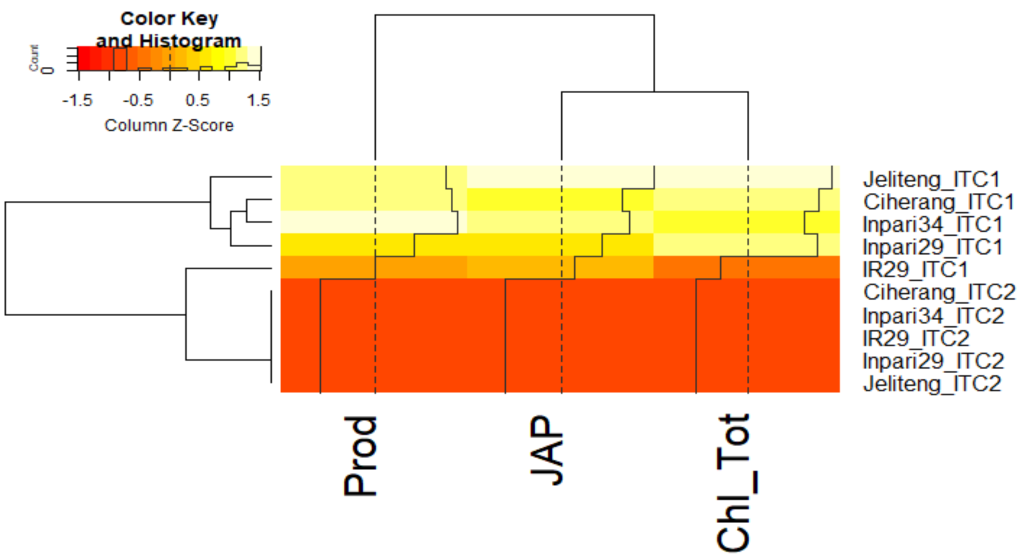

Figure 1. Analysis *of heatmap cluster* on the treatment of 5 varieties on the stress tolerance index (ITC) values and 5 character selection on stress treatment throughout all rice growing phases. Prod: production per clump, JAP: number of productive tillerd, total chlorophyll.

D. Conclusion

The environment at a concentration of 60 mM of NaCl was the best stressed environment in screening rice salinity at all growing phases. A good selection character in this screening procedure was the production per clump, the number of productive tillers and the total chlorophyll. Varieties of Jeliteng, Ciherang, Inpari 34 Salin Agritan were those that considered tolerant to salinity in the screening procedures at all growing phases on DFT hydroponic system. The DFT hydroponic screening method at all growing phases can be recommended as one of the artificial salinity screening methods.

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Genetic Analysis and Distribution of F2 Population Variety of Tomato Crosses

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Abstract

The demand for tomatoes is increasing along with population growth. However, this demand needs to be supported by increasing tomato productivity. One way to increase this is the assembly of high-yielding tomato varieties. In the assembly of varieties, the F2 generation is a generation with high diversity. This becomes important in the process of evaluating and selecting tomato varieties. Genetic variants are the initial capital of plant breeding efforts, which are related to the assembly of varieties to increase the quantitatively and quality of the yield including fruit size, fruit quality and productivity by utilizing the heterotic phenomenon in the assembly of tomato varieties. A research was conducted to evaluate the diversity of generations of biparental crosses using an augmented design. The genotypes used were Mawar (30 plants), Karina (30 plants), F1 genotype of Mawar x Karina (25 plants), Mawar x Karina x Mawar (20 plants), Mawar x Karina x Karina (20 plants), and F2 genotype of Mawar x Karina (200 plants) which were divided into 4 blocks. The results show that the genetic diversity of the F2 population was highest in the characters of plant height, harvest age, fruit length and fruit diameter, with heritability values in the medium to high category

Keywords: F2 population, genetic diversity, tomato cross

A. Introduction

Tomato (*Lycopersicum escelentum* Mill) is a horticultural commodity whose fruit contains lots of vitamins C, A, K and minerals. Tomatoes are favored globally in the world, both consumed as processed foods and as fresh vegetables. This causes tomatoes to continue to develop into an important commodity in international trade. As an agricultural country, Indonesia is ranked at 21st as a tomato producer in the world (Pusat Data dan Sistem Informasi Pertanian, 2014) with a harvested area of 54,780 hectares and total production in 2019 of 1,020,333 tons (KEMENTAN, 2021). Tomato cultivation in Indonesia is carried out by farmers in the lowlands to the highlands with varying areas. The large planting area and growing environment are good opportunities for tomato plant breeders to assemble superior varieties. Continuous increase in demand for tomatoes both in quantity and quality requires the availability of superior tomato varieties. The assembly of high-yielding tomato varieties can be done through plant breeding, including through artificial crosses (Hermanto R, Syukur M, Widodo, 2017).

Artificial crossing is the process of taking pollen from the male parent and placing it on the pistil of the female parent, each of which has its own advantages. The purpose of crosses is to produce offspring that combines superior characters. The success of the tomato plant breeding program is largely determined by the availability of various genetic sources of tomatoes and appropriate breeding methods. Exploration activities are one of the activities that can be carried out to provide genetic resources by utilizing local wisdom that exists in Indonesia (Sutjahjo, S.H., C. Herison, Sulastrini, Marwiyah, 2015).

Genetic diversity is the initial capital of plant breeding efforts, which is related to the assembly of varieties to improve yield in terms of fruit size, fruit quality and productivity by utilizing the heterotic phenomenon in the assembly of tomato varieties. This method is quite effective in increasing yields with high fruit quality and good nutritional content. The initial stage in determining the results of crosses between varieties is evaluating the general combining capacity (DGU) and special combining capacity (DGK). This information is needed to obtain a combination of parents that produces offspring that have the potential to have high yields and productivity (Sutjahjo et al., 2015)

According to Saputra, Helfi Eka Saputra, Dwi Wahyuni Ganefianti, Umi Salamah, Yenny Sariasih, Nico Dwi Ardiansyah (2019). the crossover method is a method of uniting each of the superior characters that exist in various genotypes in one genotype so as to produce an F2 population that can segregate maximally. Therefore, the estimation of genetic diversity, heritability, and the number of gene control groups of characters has been done extensively in this population. Analysis of this generation mean has been widely used to determine the type and magnitude of gene action involved in the inheritance of tomato characters. This can induce a superior diversity of tomato species and have a unique shape. However, in the process of observing data, the conventional method has a large bias if it is carried out with logs or lots of plants or fruit.

Therefore, technology in assisting the observation process is indispensable in minimizing observational bias. Genetic diversity and heritability are absolute requirements for the success of a plant breeding program. Genetic diversity can increase the possibility of getting a better genotype through selection. Character diversity and genotypic diversity are useful for knowing the pattern of grouping genotypes in certain populations based on the observed characters and can be used as the basis for selection activities (Agustina and Waluyo, 2017). Analysis of diversity can be done in various ways, namely using markers, one type of marker is morphology in plants. Heritability is a genetic parameter used to measure the ability of a genotype in a plant population to pass on its characters (Rachmadi, M., N. Hermiati, A. Baihaki, dan R. Setiamiharja, 1990). The indicator of the character is genetically controlled based on heritability values. Heritability estimates have functions including determining the success of selection, because they can provide clues that a trait is more influenced by genetic factors or environmental factors (Poehlman, J. M. and D.A. Sleeper, 1995).

Heritability is a genetic parameter used to measure the ability of a genotype in a plant population to pass on its characters. The heritability value has a function to determine the success of the selection, because it can provide an indication of a trait that is more influenced by genetic factors or environmental factors. Heritability values are expressed in fractions or percentages ranging from 0 to 1. The closer the value to 1, the higher the heritability value, on the other hand, the closer to the 0 value, the lower the heritability value (Syukur M, Sujiprihati S, Yuniaanti R., 2012).

A high heritability value indicates that genetic factors play a more important role in controlling a trait than environmental factors. Heritability determines the progress of selection, the greater the value of heritability, the greater the progress of selection, and vice versa. Selection characters must have high diversity and heritability, in order to obtain a target for selection progress (Lubis, K., S.H. Sutjahjo, M. Syukur, dan Trikoesoemaningtyas, 2014). According to Barmawi, M., A.

Yushardi, dan N. Sa'diyah (2013), genetic diversity and heritability are useful for predicting the genetic progress of selection.

Therefore, selection on the F2 population resulting from crossing black rice with white rice can give an indication of a high expected value of genetic progress, if the traits involved in the selection have high genetic diversity and heritability. Thus, selection is expected to result in high genetic advances for some of the desired agronomic characters. The purpose of this study was to determine the hope of genetic progress and the predictability of heritability (inheritance) of agronomic characters of the F2 generation of tomatoes from crosses with superior tomato varieties.

B. Methology

This research was conducted at Experimental Farm, Hasanuddin University on April 2021. The materials used in this study were F2 tomato seeds from crosses (200 genotypes) and 4 comparison varieties, namely Black Cherry, Chung, Mawar, and Karina. The tools used in this research are shovel, hoe, scissors, meter, sieve, caliper, analytical balance, and camera.

1. Research Methods

The The research to evaluate the diversity of generations of biparental crosses was carried out with an augmented design, the genotypes used were 6 biparental generations consisting of P1 vegetable tomatoes (Mawar) 30 plants, P2 fruit tomatoes (Karina) 30 plants, F1 (Mawar x Karina) 25 plants, BCP1 (Mawar x Karina x Mawar) 20 plants, BCP2 (Mawar x Karina x Karina) 20 plants, and F2 (Mawar x Karina) 200 plants which was divided into 4 blocks.

Prior to planting, tomato seeds were sown using Rockwool for two weeks. Subsequently, seedlings were transferred to a 15x 20 cm small polybag for one month. Seedlings that grew well were transferred to the field with a plot size of 2x3 m and a spacing of 50 x50 cm. Fertilization using NPK, urea and SP36 were applied twice, ie 1 week after planting and 3 weeks after planting. Parameters observed were plant height, stem diameter, number of branches, harvest age, fruit fresh weight, fruit diameter, fruit length and fruit height.

2. Data Analysis

All observational data were analyzed by analysis of genetic parameters such as coefficients of genetic diversity, heritability, Skewness, and kurtosis (Singh and Chaudhary 2007; Syukur et al. 2012). Data analysis was performed using Microsoft Excel Software and SPSS v 22.

C. Result and Discussion

Based on the analysis in Table 1, the heritability values were analyzed based on the variance of all characters. There are 4 parameters having high heritability values, 2 parameters having medium heritability values, and 2 parameters having low heritability values. Meanwhile, the characters classified as having high heritability values were plant height (78.08%), stem length (64.37%), fruit height (62.83%), and fruit diameter (58.71%). The results of the analysis showed that the genetic diversity was wide for several characters in this population due to the different genetic backgrounds of the population and the selection would be directed at the characters of plant height, harvest age, stem diameter, stem length, fruit diameter, fruit height, fruit weight and number of branches. Understanding of the genetic background of the population is essential to initiate selection. One of the important components determining the success of a selection program in breeding is genetic diversity, so knowledge related to population genetic diversity is the main thing before starting the selection. According to Pinaria, A, A. Baihaki, R. Setiamihardja dan A.A. Daradjat. (1995), the genetic diversity of a population is influenced by the nature of the population, namely whether the population is a segregated generation from a cross, in what generation and how the genetic background is. In addition, the estimated value of heritability also needs to be known to predict the progress of a selection, whether it is influenced by genetics or the environment (Syukur M, Sujiprihati S, Siregar A., 2010).

Table 1. Results of the distribution of genetic variance values of tomato crosses

Characters	K	M	F1	F2	BCP1	BCP2	Heritabily
TT	42.24	178.12	157.50	574.58	398.11	787.95	78.08
UP	6.09	76.90	252.30	143.00	134.11	611.01	21.84
DB	3.03	4.30	7.74	7.14	5.22	7.22	29.71
PB	82.08	17.67	35.40	126.42	27.35	24.88	64.37

DBU	98.99	26.83	37.97	132.23	15.95	27.43	58.71
TIB	63.21	27.27	40.09	117.10	26.16	17.81	62.83
JC	3.69	2.26	2.30	2.96	0.78	2.37	7.01
BB	153.88	48.01	31.72	64.70	45.67	60.86	0.00

Note: TT: Plant height, UP: Harvest age, DB: stem diameter, PB: fruit length, DBU: fruit diameter, TIB: fruit height, BB: fruit weight, JC: branch numbers

The estimated heritability values between parameters are presented in Table 1. A high heritability value indicates that there is a greater influence of genetic factors than environmental factors and has a great chance of being passed on to their offspring. According to Roy (2000), if the predictive value of heritability is high, the selection is carried out in the early generations because the selection progress will be large. On the other hand, if the heritability is low to moderate, the character needs to be fixed through selection. Genetic diversity and heritability are very useful in the selection process. This is in accordance with the opinion of Syukur (2010) which states that selection will be effective if the population has wide genetic diversity and high heritability. In addition to population variance information, the mean value of each genotype also plays a role in the effectiveness of selection in producing superior tomato varieties.

Table 2. The results of the significance value of the Skewness-Kurtosis Test

Characters	Median	Skewness	Kurtosis
TT	75.51	-0.04	-1.54
UP	120.04	-4.87	1.52
DB	8.57	2.01	4.38
PB	30.32	14.81	40.67
DBU	32.08	13.62	36.90
TIB	27.25	16.07	44.39
JC	4.87	2.67	-1.29
BB	17.63	3.29	0.32

Note: TT: Plant height, UP: Harvest age, DB: stem diameter, PB: fruit length, DBU: fruit diameter, TIB: fruit height, BB: fruit weight, JC: branch numbers.

Table 2 shows the mean population value and the results of the skewness and kurtosis analysis. Analysis of skewness and kurtosis is an approach to predict genetic control descriptively (Roy, 2000). The results of the observed population data distribution based on the data distribution curve on the character of plant height has a skewness of -0.04 and kurtosis -1.54, stem diameter has a skewness of 2.01 and a kurtosis of 4.38, the number of branches has a skewness of 2.67 and a kurtosis of -1.29 based on the value of skewness and kurtosis indicates that these characters are continuous and controlled by many genes. while the length of the fruit has a skewness of 14.81 and kurtosis of 40.67, the diameter of the fruit has a skewness of 13.62 and kurtosis of 36.90, the height of the fruit has a skewness of 16.67 and kurtosis of 44.39 and the weight of the fruit has a skewness of 3.29 and kurtosis of 0.32, indicating that the character is controlled by a few genes. Determination of the nature of the data and the number of genes that control it refers to Roy (2000) and Sihaloho, A.N., Trikoesoemaningtyas, D. Sopandie, D. Wirnas. (2015), which state that the continuous distribution of data is a character controlled by many genes indicated by the kurtosis value of -3 < kurtosis < 3 (mesokurtic form), while characters controlled by a few genes are indicated by kurtosis values > 3 (leptokurtic form) or < -3 (platikurtic form).

The distribution of data that has a skewness value of zero indicates that the character is controlled by additive gene action, a positive skewness value indicates additive gene action has a complementary epistasis effect, while a negative skewness value indicates an additive gene control with a duplicate epistasis effect (Roy, 2000). Based on this, it can be said that the character of plant height is a character controlled by many genes with additive gene action with the effect of duplicate epistasis. Fruit diameter and fruit height are characters that are controlled by a few genes with additive gene action with complementary epistatic effects and harvest age characters are controlled by a few genes with additive gene action having duplicate epistasis effects.

D. Conclusion

High genetic diversity is an important aspect before selection. The genetic diversity of the F2 population was highest in the characters of plant height, harvest age, fruit length and fruit diameter, with heritability values in the medium to high category

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Variations on The Concentration and Purity of Oil Palm Flowers (*Elais guineensis* Jac.) in Some Extraction Methods for Identification of the Flowering Gene

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Abstract

Palm oil production is related to the number of fruit and is thought to be determined since the beginning of the flowering phase as the initial stage of fruit formation. Identification of oil palm flowing genes needs to be done as the first step of knowing the mechanism of flowering in oil palm molecular. Oil male and palm females contain high polysaccharides and polyphenolic compounds so they can inhibit the process of molecular identification. Molecular studies provide accurate and fast information about the potential of oil palm genetics as a commercial commercial commodity. The purity and concentration of DNA and the temperature of annealing are the main requirements for PCR-based molecular studies. The purpose of this study obtained the extraction method of oil palm flowers which produced high DNA concentration and purity and optimized the temperature of annealing to identify the flowering gene. DNA from male flower spike and female flowers is extracted using the A (SDS) method, method B (CTAB), method C (CTAB + PVP), and method D (commercial kit). Primary BMS annealing temperature for amplification of optimized flowering genes using PCR gradients. The extraction of male flowers and female flowers using the C (CTAB + PVP) method produces the best DNA concentration and purity compared to other methods. The best annealing temperature for male flower DNA amplification and female flowers using the BMS primer is 61.1 ^{o}C. Amplification of male flowers and female flowers using Primary BMS produces 1200 PB products. The results of DNA extraction and PCR amplification using the BMS primers in female flower samples are better than male flowers

Keywords: CTAB, DNA Extraction, female flower, flowering gene, male flower, PVP

A. Introduction

Palm oil (Elaeis Guineensis Jacq.) Is one of the national superior commodities because it contributes greatly to the Indonesian economy. Based on Badan Pusat Statistik (2020) the area of oil palm plantations in Indonesia reached 14.33 million hectares with production reaching 42.9 million tons in 2018. The area of oil palm plantations is estimated to increase by 1.88% to 14.60 million hectares with Production increase of 12.92% to 48.42 million tons in 2019. This caused Indonesia to become the largest palm oil exporter in the world during 2020 and contributed 53.3% of the total value of world palm oil exports (workman, 2021).

Palm oil production is related to the number of fruit which is thought to be determined since the beginning of the flowering phase as the initial stage of fruit formation. Palm oil is referred to as a temporal dioecious for male and female flower production because the cycle of emergence of male and female flowers alternately on the same plant so as to produce alloogamus reproduction models. Male and female flower production alternately in one tree is influenced by various complex factors such as abiotic factors, metabolic factors and genetic factors (Adam, H., M. Collin, F. Richaud, T. Beule, D. Cros, A. Omore, L. Nodichao, B. Nouy, & J.W.Tregear, 2011). Genetic factors that influence flowering are groups of genes included in the large family of mads-box genes. Mads-Box genes encode the familial of the transcription factor that regulates developments in high-level plants including flower and fruit formation in angiosperme plants (Gramzow, L., L. Weilandt, & G. TheiBen, 2014). Until now the emergence of male and palm male and female palm females cannot be predicted accurately. Identification of oil palm flowing genes needs to be done as the first step of knowing the mechanism of flowering in oil palm molecular.

Molecular studies, especially DNA provide information quite accurately and quickly regarding the potential of oil palm genetics. DNA-based research on the potential of genes that influence a character related to organs that express the character. Research on the flow of flowering is carried out by extracting DNA from male and female flower organs to avoid the sequence of the targeted target gene. Some oil palm DNA extraction protocols generally use leaves as samples (Ihase, L.O., R. Horn, A.G. Anoliefo, C.R. Eke, A.S. Afolabi, & O. Asemota, 2016; Suzana, M., A.R. Rahimah, I. Maizura, & R. Singh, 2015), while the extraction method of oil palm flowers has not been widely published by researchers.

Palm oil plants have a network with high polysaccharide and phenolic content (Sasidharan, S., R. Nilawatyi, R. Xavier, L.Y. Latha, & R. Amala, 2010; Rosalina, T.R.T, S. Mohamed, G.F. Samaneh, M.M. Moordin, Y.M. Goh, & M.Y.A. Manap, 2011). Polysaccharides and proteins are the biggest contaminants during DNA extraction because the two compounds bind DNA and must be eliminated to obtain pure DNA (Samprook and Russell, 2001). The phenolic component in the sample will be quickly oxidized and binding DNA. Samples contaminated with polysaccharides and polyphenolics caused DNA degraded, thus disrupting the analysis of DNA sequencing. Optimization of the DNA extraction of oil palm flower organs needs to be done because every organ organ requires different DNA extraction protocols.

DNA Extraction Methods Are Always Related To Several Extraction Stages To Produce High Quality And Quantity Of DNA So That It Can Be Used For The Next Molecular Identification Process. The Cell Lysis Stage US The Initial Stage of DNA Extraction Generally Uses Chemical Compounds That Can Damage The Barrier Integrity of Cells Walls Such As Cetyl Trimethyl Ammonium Bromide (CTAB) and Sodium Dodecyl Sulfate (SDS) (Cheng, Y.J., W.W. Guo, H.L. Yi, X.M. Pang, & X. Deng, 2003), Especially For The Wall Lysis of Plant Cells With High Polysaccharide and Polyphenol Content. Polyvinyl Pyrrolidone (PVP) Is Often Used To Reduce Phenol Compounds That Can Degrade DNA. PVP Through Hydrogen Bonds, Makes Complex Bonds With Polyphenolic, And Can Effectively Eliminate Polyphenolics From Homogenate (Cheng et al., 2003). Every Plant Has A Secondary Compound Content In Different Plant Cells, Every Plant Requires An Optimum Extraction Procedure. Procedure Optimization can be made to the Composition of the Buffer License Solution or Physical Handling Technique for DNA Separation from other compounds, so that DNA degradation can avoided.

DNA with good concentration and purity is expected to be continued for the amplification process. One factor that affects the amplification process is the temperature of annealing. The appropriate annealing temperature affects the results of amplification. According to Asy'ari & Noer (2005) The temperature of annealing that is too high causes the release of primers that have attached to the DNA template so that the amplicon is not formed, while the annealing temperature is too low, it will cause a primary attachment that is not specific to the DNA template. This study aims to obtain the extraction method of oil palm flowers that produce high DNA concentration and purity and optimize the temperature of annealing to identify the flowering gene.

B. Methology

The study was conducted in biomolecular, PTPP, Laptiab, BPPT, Serpong laboratories. Plant sampling is carried out in Puspitek, Serpong. The material used in this study was the spikelet of male and individual flowers from the spike of the teneral female flower varieties taken from the Puspiptek trial garden, Serpong (Figure 1). Flower samples after being removed from the midrib are immediately stored at -20 oC for DNA extraction.

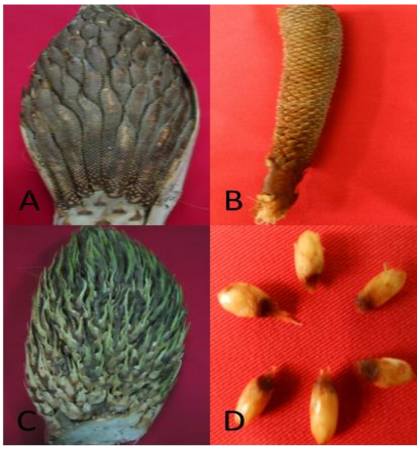

Figure 1. Male flower sign (a), male flower spike (b), female flower bunches (C), individual female flowers (D)

1. DNA Extraction

As many as 0.5 grams of spicles of male flowers and individual flowers from female flower spiketets are crushed using liquid nitrogen until smooth. The method used is as follows:

Method A (SDS). Sample powder is inserted into a centrifuable tube that contains 5 ml extraction buffer (10% SDS; EDTA 0.5 M pH 8; Tris HCl 1 m pH 8; NaCl 5 m; DDH2O; 1% MercaptoenaTOL) that has been incubated earlier at 65 OC.

2. Method B (CTAB)

Sample powder is inserted into a centrifued tube that contains 5 ml extraction buffer (CTAB 10%; EDTA 0.5 M pH 8; Tris HCl 1 m pH 8; NaCl 5 m; DDH2O; 1% MercaptTaHanol) which has been incubated before at 65 OC.

3. Method C

CTAB + PVP). Samples are crushed by adding 0.1 grams of PVP, then the powder is inserted into a centrifuge tube which contains 5 ml extraction buffer (CTAB 10%; EDTA 0.5 M pH 8; Tris HCl 1 m pH 8; NaCl 5 m; DDH2O; 1 % Mercaptoenatol) which had been incubated earlier at a temperature of 65 oC

4. The extraction procedure method A to the C method

The solution is homogenized with vortex then incubated at 65 oC for 30 minutes and turned back every 5 minutes. The solution was incubated at room temperature then added 1 Volume CI (Chloroform: isoamil alcohol = 24: 1) and centrifuged 14000 rpm for 20 seconds at 4 oC. This step is repeated three times. The supernatant was taken then added 1 volume of isopropanol that had been incubated at 4 oC. After being incubated at a temperature of -20 OC for 30 minutes, the solution was centrifuged at 14000 rpm for 10 minutes 4 oC. The supernatant was discarded then the pellet was dried, then added 500 µL, 1/10 volume nach3COO3 3 m pH 7 cold, 2 volume ethanol absolute that has been incubated at 4 oC, then stored at -20 OC overnight. Disentrized solution at 4 oC at a speed of 14000 rpm for 10 minutes. DNA pellets were washed using 400 µL 70% ethanol that had been incubated at 4 °C, then centrifuged at 4 °C at a speed of 14000 rpm for 5 minutes. The dried pellets are then added 100 µL DDH2O and 1/10 volume of RNASE, then incubated at 37 °C for 1 hour to actively in the RNase.

5. ***Method D***

(Commercial Kit). The sample powder was crushed by adding 0.1 grams of PVP, subsequently extracted using a commercial DNA extraction kit in accordance with the procedure recommended by the manufacturer. Each extraction method is repeated three times.

6. ***Quality and quantitative DNA analysis***

The quality of DNA was analyzed using an electrophoresis of gel agarose 0.8% with a tae buffer at a 100 volt voltage for 30 minutes. The electrophoresis results visualized using Gel Doc UV Transilluminator. Analysis of the quantity of DNA extraction was carried out using a 2000 thermo scientific production spectrophotometer. Data of purity and concentration are analyzed using R studio.

7. ***Amplification of flowering genes***

The PCR reaction consists of DNA male flower genomes and female flowers as much as 150-180 ng as templates, 1 µl of Mix 2 mm, 1 µl 10 x Dream Taq Buffer, 0.1 Uream Taq DNA polymerase, 0.5 µl Primary Forward 0 , 2 µm, 0.5 µL primer reverse 0.2 µm, ddh2o nuclease-free to 10 µL. The condition of the PCR used is the initials denaturation of 95 oC 5 minutes, denaturation of 95 oC 30 minutes, the annealing temperature is 65.4 ± 06 oC 30 seconds, the 72 OC extension is 1 minute, this condition is repeated to 35 cycles, and the final extension 72 OC 5 minutes. The primary used for the amplification of the flowering gene is the Primary BMS F AgncacyaCcagnaggcn / r acncactyayggytcccacn for amplification of squamosa flowering genes

C. Result and Discussion

Palm oil flowers are very easy to oxidize, causing brown (browning) that causes degraded DNA, this causes the extraction of DNA to oil palm flowers is quite difficult. The solution to avoid browning on samples of male flowers and palm oil is a room, mortar and pestle must be cold when the sample fleeces. Addition of liquid nitrogen when the grade can prevent degraded oxidation and DNA occurrence. Fresh samples of male and female flowers are soaked in liquid nitrogen and crushed quickly to help break cell walls mechanically, this is done to keep DNA degraded. According to Xin and Chen (2012) the use of fresh tissue, liquid nitrogen and cold mortar on DNA extraction can reduce DNA degradation.

According to Chi, M.H., S.Y. Park, & Y.H. Lee (2009) The DNA extraction process consists of several stages, namely the preparation of the material to be used, the process of destruction of cells (lysis), removal of contaminant compounds, and DNA collection. The phase of the destruction of plant cells greatly affects the purity of DNA so that the appropriate technique and reagent material is needed so that the resulting DNA has a high purity. SDS and CTAB are detergent solutions that function to reduce the surface voltage of the liquid and dissolve lipids at the lysis stage, this causes cell membranes to degradate, so the cell organelets can come out. According to the Bintang (2010), CTAB is a cationic detejen that is destroying cells, unraveling proteins, and separating carbohydrates from nucleic acid. The interaction between SDS and cell membrane proteins affects cell lyzers in DNA extraction, so that cell membrane proteins do not hinder the next DNA process (Perumal, N.V., X. Zhang, M. Yuki, I. Fumio, dan F. Wang, 2016). The addition of SDS and Positively charged CTAB serves to separate the polysaccharide from DNA by binding negatively charged DNA.

Buffer extraction method A, B and C heat is heated at a temperature of 65 oC, because the ability of SDS and CTAB to melisis the cell will be active in conditions of 65 oC. According to Lade, B.D., A.S. Patil, & H.M. Paikrao (2014) Addition of extraction buffers incubated less than 65 oC causes imperfect denaturation in proteins that can produce contamination at the next extraction stage. The addition of heated extraction buffers in frozen samples by liquid nitrogen causes the network to change temperatures quickly so as to avoid damage to DNA. This is in accordance with the opinion of Purchooa (2004) that the addition of a heated CTAB buffer on frozen tissue by liquid nitrogen causes the network to experience a temperature difference from -80 oC to a temperature of 60 oC quickly resulting in high quality DNA.

Analysis of the quality of male and female DNA extraction using the A (SDS) method, method B (CTAB) and method C (CTAB + PVP) on agile gel 0.8% shows the quality of DNA is quite good even though there is still contaminant, while DNA extraction uses a kit Commercial shows degraded DNA ribbons (Figure 2). DNA extraction methods with SDS / CTAB extraction buffers produce good quality DNA from plants containing high concentrations of polysaccharides and polyphenols for Downstream analysis (Niu, C., H. Kebede, D.L. Auld, J.E. Woodward, G. Burrow, & R.J. Wright, 2008).

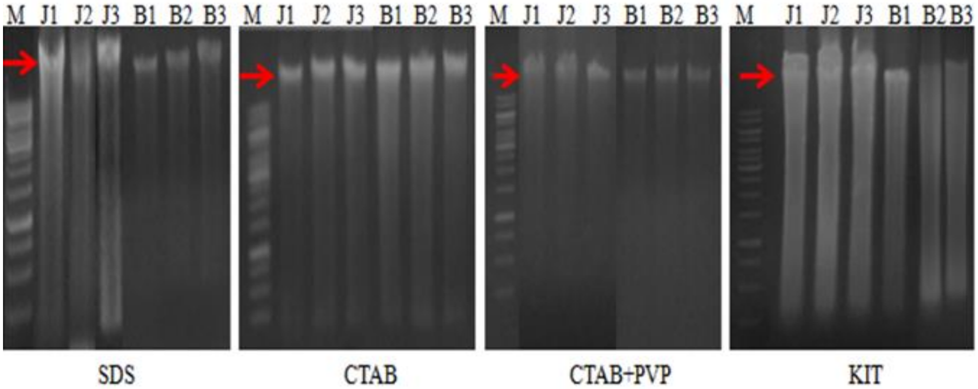

Figure 2. Analysis of the quality of male and female flowers DNA results using the A (SDS) method, method B (CTAB), C method (CTAB + PVP) and D method (kit)

Extraction using the D method (Kit method) produces degraded DNA shown by the existence of smears on the DNA band. The use of kits to extract DNA male and female flower organs is not recommended because this organ contains a fairly high phenolic compound. The reagents used on kits cannot reduce phenolic compounds produced by flower organs so that DNA is degraded. Commercial DNA extraction kit is an ideal procedure for DNA extraction because it does not use dangerous reagents, but in some commercial research kits produce low DNA results and varied quality (buldewo and jaufeerally-fakim 2002; Keb-Llanes, M., G. González, B. Chi-Manzanero, & D. Infante, 2002; Horne, E.C., S.P. Kumpatla, K.A. Patterson, M. Gupta, & S.A. Thompson, 2004).

Quantitative analysis shows that as much as 50% of male flower samples and extracted female flowers use the A (SDS) method, method B (CTAB), and the C (CTAB + PVP) method produces a concentration of DNA more than 500 ng / µL and 50% more DNA purity Of the 1.5 (Figure 3). Aboul-Maaty and Oraby (2019) recommended the ratio of DNA purity greater than 1.5 and better close to 1.8, while according to Healey, A., A. Furtado, A. Cooper, & R.A. Henry (2014) for NGS-based Molecular activities (next generation sequencing) needed DNA with a comparison of A260 / A280 between 1.8 to 2.0. The DNA extraction using the D method (Kit Method) is not quantified due to degradation.

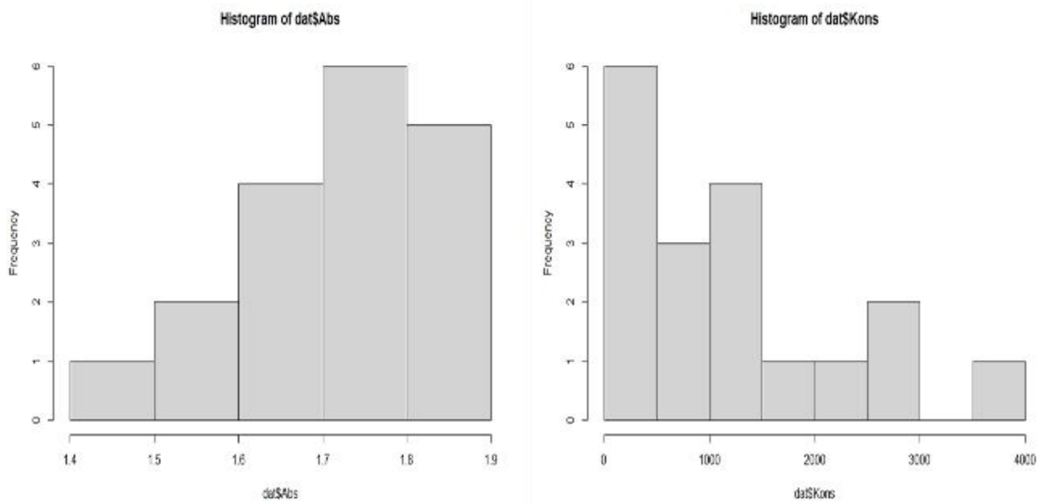

Figure 3. Histogram concentration and purity DNA extraction results using the A (SDS) method, B (CTAB) method, and method C (CTAB + PVP)

The male flower DNA extraction using the B (CTAB) method produces the lowest concentration and purity of DNA compared to samples and other methods (Figure 4). The extraction of female flower DNA uses the B (CTAB) method and the C (CTAB + PVP) method shows good purity, while the male flower extraction using the C (CTAB + PVP) method produces better DNA purity than male interest extracted using other methods. Method C (CTAB + PVP) produces the concentration of DNA from male flowers and the highest female interest compared to the A (SDS) method and B (CTAB) method. This shows the method C (CTAB + PVP) is able to produce the concentration and purity of male flower DNA and oil palm female flowers higher than other methods. This result is in accordance with the results of Murtiyaningsih's research (2017) that the extraction of DNA pineapple leaves using CTAB and PVP buffers show good purity between 1.8 - 1.9.

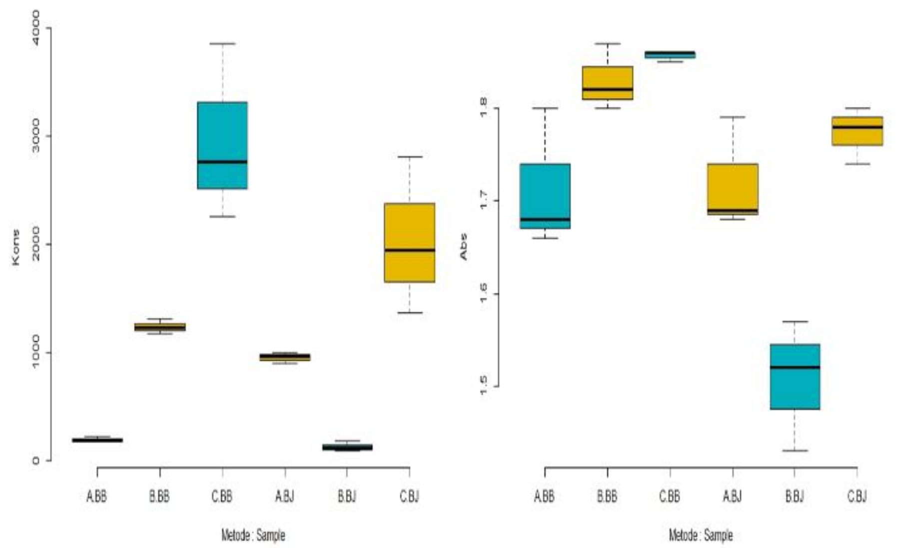

Figure 4. Boxplot concentration and purity DNA male and female flowers extraction using method A (SDS), method B (CTAB), and method C (CTAB + PVP)

The purity of female flowers DNA differs significantly with the purity of male flowers DNA, while DNA purity is no different between method A (SDS), method B (CTAB) and C method (CTAB + PVP) (Figure 5). The concentration of male flower DNA is no different from the concentration of female flower DNA, but the sample extracted using the C (CTAB + PVP) method produces a significant different concentration with other methods. Addition of PVP when the grade is thought to be able to inhibit the oxidase polyphenol enzyme. The polyphenol enzyme can degrade DNA and cause the oxidation of phenol compounds. Oxidation is characterized by the formation of brown color on the plant tissue to be isolated. This is in accordance with the opinion of Utami, A., R. Meryalita, N.A. Prihatin, L. Ambasari, P.A. Kurniatin, & W. Nurcholis (2012) Insulation with CTAB as an extraction buffer requires additional PVP to eliminate phenol contamination.

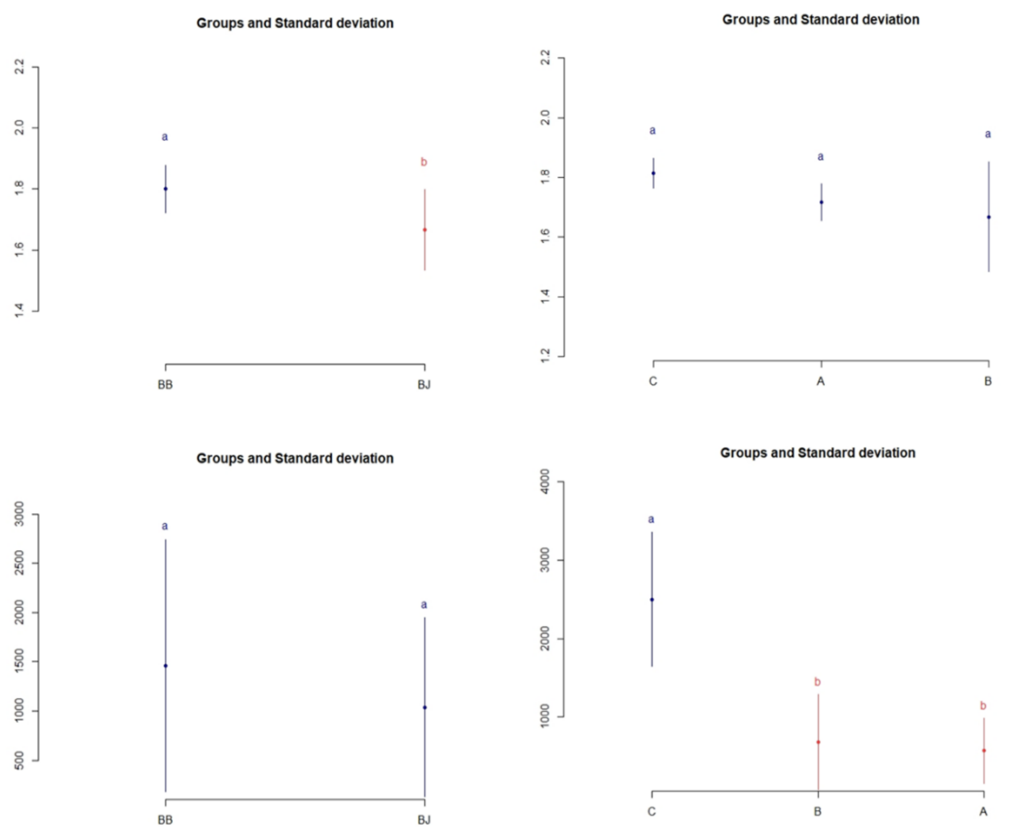

Figure 5. Advanced Test Duncan Purity and Concentration of Male Flower DNA and Female Interest Results Using the A (SDS) method, method B (CTAB), and method C (CTAB + PVP)

Extraction of male flowers and female flowers use method B (CTAB) and C methods (CTAB + PVP) show the same pattern, while the A (SDS) method shows a different direction in the parameters of purity and DNA concentration (Figure 6). The extracted female flowers using the B (CTAB) and C (CTAB + PVP) method shows higher purity and concentration than male flowers, while extracted female flowers using the A (SDS) method shows lower purity and concentrations than male flowers. This shows a CTAB buffer that is applied to a single and modified with the addition of PVP capable of producing the concentration and purity of oil palm female DNA better than the SDS buffer application. The extracted male flowers using SDS buffers produce purity and concentration of DNA better than Buffer CTAB and CTAB + PVP.

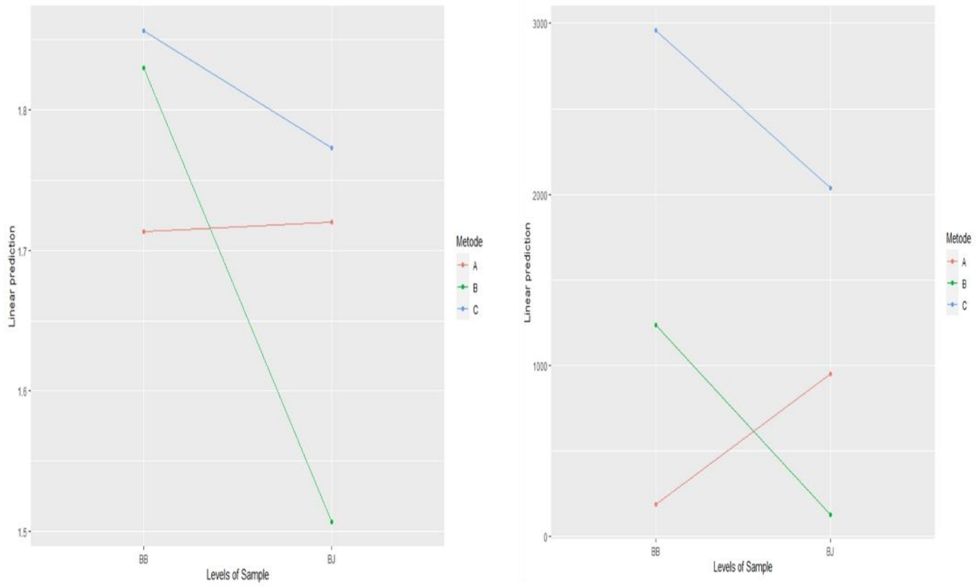

Figure 6. Pattern of interaction methods and types of samples on the extraction of male flowers and female flowers

DNA male flowers and female flowers extraction using the C (CTAB + PVP) method are widelyified using the BMS primer for the amplification of squamosa flowering gene. The PCR reaction uses DNA male flower genomes and female flowers as templates, the PCR reaction of each sample is repeated three times to get consistent results. The results of the amplification of male flowers and female flowers at various temperatures of primary BMS annealing using PCR gradients showing female flower DNA produces better PCR product quality than male flower DNA (Figure 7). PCR products female flower samples look more clear than male flowers.

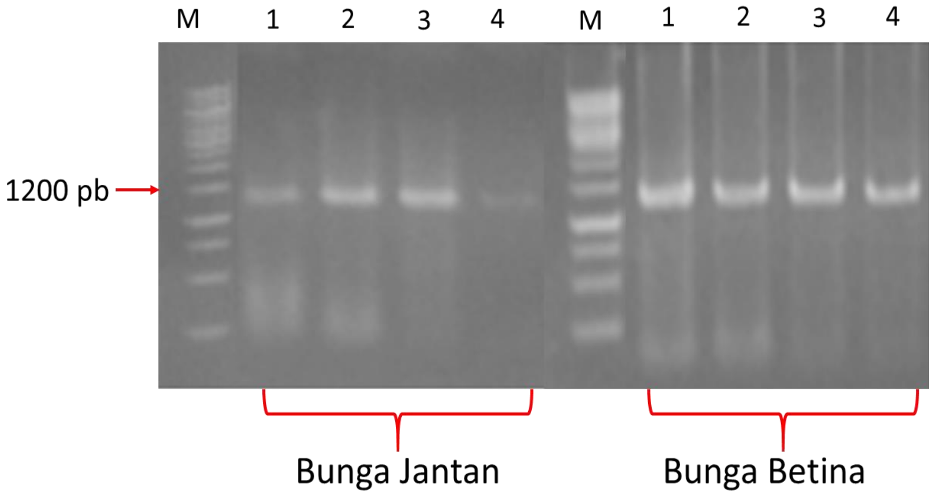

Figure 7. PCR Gradient Primary BMS at 59.4 oC (1); 60.2 oC (2); 61.1 OC (3); 62.1 OC (4)

The results of the Gradient PCR show amplification of male flower DNA using the BMS primary produces PCR products at 59.4 oC annealing temperatures; 60.2 oC: 61.1 OC and not produced PCR products at annealing temperature 62.1 oC, whereas in DNA female flower PCR products are produced at all annealing temperatures. These results show a type of sample and annealing temperature allegedly influencing the quality of PCR products. The best annealing temperature for male and female DNA amplification using the BMS primer is 61.1 oC. Amplification of male flowers and female flowers using Primary BMS produces 1200 PB products.

In general, the results of this study show extraction of DNA and PCR reactions to identify the flowering gene from female flower samples producing purity and concentration and and PCR products are better than male flowers, because they are suspected of male flowers containing more contaminants that can reduce DNA purity and concentration, thus disrupting the PCR reaction. One source of contaminants in male flowers is the presence of pollen composed of polysaccharide compounds. The high polysaccharide male flower causes male flower DNA extraction and the PCR reaction is more difficult than female flowers.

D. Conclusion

The extraction of male and female flowers DNA uses the CTAB + PVP method produces the best DNA concentration and purity compared to the SDS method and the CTAB method. The best annealing temperature for male and female DNA amplification using the BMS primer is 61.1 oC. Amplification of male flowers and female flowers using Primary BMS produces 1200 PB products. DNA extraction and PCR amplification using primary BMS on female flower samples is better than male flowers.

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In Vitro Culture of Dragon Fruit (*Hylocereus polyrhizus*): Callus and Anthocyanin Production

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Abstract

Tissue culture biotechnology has been widely used to produce secondary metabolites. Anthocyanin as the secundary metabolic content in Dragon Fruits was thought to be able to liberate free radicals and have pharmacological activities as antioxidants and anti-aging. Anthocyanin can be produced in vitrothrough callus production by tissue culture. The study aims to produce callus cultures that have the potensial to produce secondary metabolites. This research was conducted in the tissue culture laboratory. The study used a completely randomized design method with a factorial pattern. Factor 1 : Type of explants (M1): Explants from young shoots; (M2) Explants from dragon fruit callus. Factor II: The number of explant each culture tube. There are J1,J2, 3 and J4 with (1;2;3;4 explants in each culture tube. Murashige and Skoog + 15 % sucrose were used as media culture. The results of study showed that: callus began to form at 7 weeks after planting. (1) MS Media with the addition of 15 % sucrose had a significant effect on increasing the anthocyanin content in the callus of Dragon fruit formed. (2) Callus formed from Shoot Dragon fruit contain Anthocyanin 0,74 % ; (3) The Callus formed from Shoot and callus Dragon Fruits contain secondary martabolites of Anthocyanins ranging from 0,68%–0,76%. The highest Anthocyanin content (0,76%) was produced in treatmant J3 (3 Explant each culture tube)

Keywords: anthocyanin, callus, dragon fruit, in vitro

A. Introduction

Dragon fruit is atropical plant with many benefits. The content of anthocyanins in fruit has pharmacological activity as an antioxidant and anti-aging (Indarwati,I. Sri Arijanti Jajuk H., Ristani W. Primawan P Nugrahadi, 2020). Red dragon fruit is a plant that contains substances that can increase endurance and improve metabolism (Dragon fruit has been reported for its high antiradical activities with the presence of phenolic compounds. Some significant characteristics of plant pigment are betacyanin, anthocyanin, and other flavonoids. (Prabowo I., Utomo, E. P., Nurvayzy, A., Widodo, A., Widjajanto, E., & Rahadju, P., 2019). Red dragon fruit peel extract

(*Hylocereus polyrhizus*) containing phytochemical compounds is an effective antioxidant from natural plant sources, with anti-cardiogenic and anti-inflammation properties, and may help with other degenerative disorders.

With the developpment of biotechnology; secondary metabolite can be produced in Vitro with Tissue culture technology. (Rahmawati, 2006) reported thatatissue culture techniques have been widely used in pharmaceutical field to produced secondary metabolites in larger quantities in short time for medical purposes. Mahmudah (2021) added that tissue culture is an alternative technology that can be used to produce secondary metabolites. The tisuue culture method can be used to increase the content of metabolic coumpounds in callus by manipulating the level of compunnent in media or adding precursor compound, and other alternatieves; by increasing the productivity of cells maintened on a variety of aseptic artificial media (Rahmawati, 2006)

Mahmudah (2021) reported that the addition of combination of Plant Growth Regulator (PGR) to the culture of the young leaves of Plectranthus scutellarioides (Iler plant) called callus contained the flavonoid quercetin with a concentration of 33.7 mg/gram callus. Indarwati, I., D, R, Suryaningsih, Sri Arijanti and A. W. Qurotin (2021), reported that in vitro tissue culture technology could be used to produce papain from papaya leaf callus; callus formed from sliced papaya varieties (California, Bangkok and Gantung) contain papain in range (11.06 % - 19.5%)

Indarwati *et al*. (2020) in her research reported that MS Media manipulation with addition of a sucrose elicitor in callus culture succeeded in incresing the Anthocyanin content in Dragon fruit callus formed. The addition sucrose Elisitor on MS media, the callus formed from the development of young shoots contained the hihest Anthocyanins (0.14%) comapared to other treatment while the addition of 5% carbohidrates elicitor callus that is formed only contains anthocyanins ranging (0.06% -0,09%).

This study aims to produce callus culture that have the potential to produce better secondary matabolites (Anthocyanins) by modifying the variety of explant materials and the number of explants planted in the media (MS + 15 % sucrose).

B. Literature Review

1. Overview of Anthocyanin on Dragon Fruit

Dragon fruit is a tropical fruit that many Indonesian people like. The exotic aesthetic characteristics of dragon fruit with attractive pulp in purple-red color make it also very popular in the European and United States markets. Dragon fruit, including tropical fruit that has high economic value. This is due that Dragon fruit not only used as an ornamental plant but also used fruit. Dragon fruit is unique its stems are triangular and have very short spines which differ from the general shape of stems that are round or rectangular. Judging from the flower of this plant has a funnel-shaped crown and stars blooming at dusk and bloom enterily at midnight with a fragrant smell.

Dragon fruit also has health benefits, because of the bioactive components contained in the fruit. Red dragon fruit is a plant that contains substances that can increase endurance and improve metabolism. Some research results on dragon fruit skins have been done. Dragon Fruit Skin is known to have antioxidant content of vitamin C, flavonoids, tannins, alkaloids, steroids, and saponin. Antioxidant compounds play a very important role in helping to overcome carcinogenic. There are natural antioxidants found from bio resources, plants, medicinal plants, and metabolites produced from various microbes that are currently used as natural active pharmaceutical ingredients (NAPIs) and nutraceuticals to improve human health (Kitts, D.D.; Wijewijckreme, A.N.; Hu, C. 2000).

Dragon fruit is good for health has been proven through an analysis conducted by "Taiwan Food Industry Development and Research Authorities". The benefits of consuming dragon fruits are : (1) Albumin which can realease toxins; (2) Anthocyanin can free radicals and slow the Aeing process/ anti-ageing; (3) Vitamin C which can beautify and make skin brigher; (4) Rich in fibre/ soluble fibre, so its useful for dieting; (5) Reducing diabetes; (6) Preventing colon cancer and launching bowel movement. Indarwati (2020) reported that Dragon fruit contain metabolic sekundair (Anthocyanin) in vegetable and fruit. Dragon Fruits exptract containt anthocyanin aboutt 26,46 ppm. Anthocyanin are dyes that give red to blue colors. Anthocyanin belong to pigments called Flavonoids.

Dragon Fruit is also known as natural dyes because it contains anthocyanin, play a role in giving red colour so that are often used for various food Industries. .Anthocyanins are found in all plant organt of Dragon fruits. The strong red colour has been used as natural dye, Dragon Fruit skins have been applied to the food and tested in white rats, the test results showed coloring dragon fruit can be used as a natural dye food (Handayani,E., Samudin,S. & Basri, Z., 2013). Zhang, Y., S.K.Vareed., M.G. Nair. (2005) reported that anthocyanins are one of the flavonoid groups thatb have successfully known thei benefits as bioactives that inhibit the growth of caccer cell in humans. The results of Yamuangmoru and Prom (2021) reported that Anthocyanin have been Shown to lower the risk of severel illnessses including cancer and obesity, as well as to have anti viral, anti-inflammatory, and anti-aging properties

Some of the secondary metabolites contained in the skin and fleshof Dragon Fruit can also be used anti-microbial substances. Alkaloid compound work by interfering with the components that make up peptidoglycan in bacterial cells, so that the bacterial cell wall layer becomes unstable (Suhartati, 2018). The destruction of peptidoglycan can be through the destruction of hydrogen bonds between the peptides that make it up which results in the cellwall layer being incompletely formed and bacteria can die (Ainurrochmah, ,2013). Flavonoid compounds can inhibit bacterial growth naturally, these compounds can couse bacterial cell wall to be damaged and in hihit the movement of bacteria (Zubaidah, N., Juniarti, D. E., & Basalamah, F., 2018).

2. Tissue Culture: Biotechnology to Produce Secondary Metabolites

Tissue culture is the cultivition of plant /cells intoa whole plant. Tissue culture is often called in vitro culture or cell/ tissue culture in glass tubes. In its development, tissue culture is widely used to modify plants and improve plants (Harahap, 2011). Further statement by Arijanti, Ribkahwati dan Retno D. (2009), Tissue culture is a method for isolating parts of plants such as protoplasm, cells, tissues, or organs and growing them in aseptic conditions so that these parts can regenerate into whole plants again. Through tissue culture, it is hoped that the seeds can produce the same plant seedsas their parents, as well as the seeds obtained, are free from pests and disease, and produce uniform seeds

In tissue culture techniques, several media are often used in implementation but Murashige and Skoog (MS) and Vacin and Went media are relatively good media because nutrients, both macro and micro, and vitamins for plant growth and development can be fulfilled. In the method of propagation through in vitro culture, the growth and development of the explants are strongly influenced by the type of basic media and growth regulators. MS medium is the basic medium that is generally used for the propagation of a large number of plant species. The basic media is rich in minerals that stimulate organogenesis.

The use of in vitro culture technology, which was previously used for plant breeding and propagation, has now begun to be directed towards producing large amaount of secondary metabolites in a short time. The use of this technology can at the same time answer the problem of limited land , and maintain the balance of biodiversity by avoiding overexploitation of germplasm as a source of natural medicine

Some of the advantages of using plant tissue culture techniques for the production of secondary metabolites include : (1) not depending on environmental factors such as climate, pests and diseases, geographical and sueasonal barriers. (2) the production sistem can be arranged, when neede and inthe desired quantity, so that itis close to the actual market conditionns. (3) produce more consistent quality and yields ang (4) reduce land use. Arijanti and Dwi Retno (2018) added that tissue culture can becomes abusiness opportunity. Furthermore, Indarwati et al. (2020) added that tissue culture technology has also been widely used to produce secondary metabolic compounds. Severel tissue culture methods used to produce secondary metaboliites include hairy root culture, cell suspension and callus culture. According to Wonganu (2007), callus is a tissue culture method that has hight potential in providing secondary metabolites.. Severel research reports have proven that tissue culture biotechlology has been widely used to produce secondary metabolites. The addition of 5 % sucrose can increase the production of Anthocianins in callus Populus hybrida . The additioan of 12,5 % fructose in culture media can increase the content of polyphenols and citronelol in Rose hybrida. Furthermore Suryaningsih D. R., Prakoeswa S. A.,& Eryanto A. (2021), reported the result of his research that the addition of the elicitor Saccharamyces cerevisiae to MS and VW Media incresed the papain containt in the callus of papaya leaves. Callus formed from sliced papaya leaves produces papain with a range (0,126 - 0,148 %)

The chemical industry or pharmaceutical industry in an industry that is supported bay natural compound from plants. Under certain circumstances, natural compounds from this plant cannot be repleced becaouse of their healing activity. The potencial for chemical synthesis in the plant world is enermous. More of 400 thousand plant species have identified the chemical and 10 thousand of them contain secondary metabolites which potensial as raw material for vegetable pesticed. (Saenong, M. Sudjak , 2016). Purbaningrum (2013) said, to obtain bioactive compounds in large quantities naturally, it is often difficult to deal with the supply of plants

In an effort to produce secondary metabolic compounds (anthocyanin content) which is higher in dragon fruit callus, it is tried to propagate through tissue culture and biosyntheis of Anthocyanin compounds by adding 15 % sucrose, with two kinds of explant sources and the number ef explants in culture tubes.

C. Methodology

1. Place and Time

In vitro experiment was done to produce callus as an extraction material, to determine the Anthocyanin content in callus. The study was conducted at the Tissue Culture Laboratory; Faculty of Agriculture, University of Wijaya Kusuma Surabaya

2. Material and Method

Materiaals : : explants from callus (in culture tubes) and shoots of dragon fruits plants, MS Media (tabel 1); Growth Regulators, NAA, BAP Coconut Water, Glucose, Fructose, Sucrose, 70%, and 90% Alcohol.

Equipment required during this research are: Sartorius Scales, Autoclave, Oven, LAF, pH meter, Tweezers, Scalpel, Erlenmeyer, Measuring cup, Measuring pipette; Petri dish, Dropper Pipette, tweezers, spatula, Culture tube, Magnetic stirrer, and Gas Cromatografy.

3. Research Design

The study was conducted using a completely randomized design with two factors. The Factor I: Types of Planting Material / plantlet there are 2 levels; M1 = shoot explant; M2 = callus of eksplant dragon fruit. Factor II. Number of explant dragon fruit; there 4 levels; J1 :J2 ;J3 and J4 each with 1;2;3 and 4 explant each culture tube Each treatment was repeated 4 times, with 10 replications each.

4. Culture Condition

Sterile culture tubes with Autoclave 17 psi 30 minutes essential media utilized by MS media. + 15 % sukrosa (Indarwati, *et al.* 2020) Sterile youthful shoot explants from Dragon fruit plants cut into ± 1 cmpieces and absorbed betadine, planted in culture tubes that as of now contain media as indicatedby treatment. The steril explant was planted in the MS medium under Laminar Air Flow Cabinet. In the wake of planting it is set on a brooding rack which comprises hatching stages.

Table 1 .Composition of Murashige and Skoog media (Arijanti and Dwi Retno S., 2018)

Ingredients	Material requirement (mg/l)
1. Macro: Nutrien	
KNO_3	1900
NH_4NO_3	1650
$CaCl_2$ $2H_2O$	440
$MgSO_4$ $7H_2O$	370
KH_2PO_4	170
2. Micro Nutrien	
$MnSO_4$ $7H_2O$	22.3
$ZnSo_4$ / $7H_2O$	8.6
H_3BO_3	6.2
Kl	0.83
$CuSO_4 . 5H_2O$	0.025
$NaMoO_4 . 2H_2O$	0.25
$CaCl_2 . 6H_2O$	0.025
$FeSO_4 . 7H_2O$	27.8
NaEDTA . $2H_2O$	37.3
3. Vitamin	
Mio-inositol	100
Thamin HCl	0.1
Nikotinik acid	0.5
Piridoksin HCl	0.5
Glisin	2
4. Carbohydrate	30.000 + 15 % Sucrose

5. Variable

a) **Callus quality**. Seen at timespans weeks outwardly utilizing scoring: 1 = no callus; 2 = reduced callus; 3 = friable callus
b) **Callus Quantity**: Observed at timespans weeks outwardly by scoring scor 1 = no callus; 2 = growing of explants; 3 = little callus (<1 times the explant size); 4 = medium callus (1-2 times the explant size); 5 = many callus (> multiple times the explant size)
c) **The content of secondary metabolites in the callus (Anthocyanin)** : Observed ruinously through Anthocyanin content examination at about two months subsequent to planting (56 days) Secondary Metabolite Analysis of the material extricated utilizing total liquor at that point broke down by gas chromatography

6. Technique of Data Analysis

The data obtained were processed using Variance Analysis (Test F) using a completely randomized design patterns at the level of 5%. If there were any real differences between treatments. If there is an influence that is a significant difference between treatments then the test is continued with a comparison test between treatments using the Least Significant Difference Test (LSD) at the 5% level.

D. Result

1. Observation of Callus Quality.

The result of observation on callus quality showed that there was no. significant different between the types of explants and the number of explants. Callus began to form at the age of 7 weeks after planting with callus quality toward compact as presented in Table 2. The observation of the growth of the quality starting from the age of 1 to12 weeks after planting can be seen in table 2.

Table 2 it can be seen that there until the age of 6 weeks the treatment types and the number of explants) had the same effect on quality of callus with score of 1. The value of callus quality began develop at week 7 tends to be slow, because for 7 weeks the explant only experienced swelling. While the number of explants one to four had no effect on the formation of callus quality. There has not been a competition for the use of nutrients in culture media nutrients due to the slow growth. It is suspected that factors from outside and from within the tissue culture environment have not provided maximum support.

Table 2. Average scoring Quality callus Dragon Fruit at Various Age Obsevation (WAP)

Treatment	1	2	3	4	5	6	7	8	9	10	11	12
M1J1	1,00	1,00	1,00	1,00	1,00	1,00	1,10	1,1	1,30	1,44	1,62	1,80
M1J2	1,00	1,00	1,00	1,00	1,00	1,00	1,04	1,04	1,12	1,12	1,14	1,30
M1J3	1,00	1,00	1,00	1,00	1,00	1,00	1,06	1,12	1,30	1,36	1,42	1,50
M1J4	1,00	1,00	1,00	1,00	1,00	1,00	1,08	1,16	1,42	1,56	1,66	1,70
M2J1	1,00	1,00	1,00	1,00	1,00	1,00	1,02	1,04	1,16	1,16	1,20	1,30
M2J2	1,00	1,00	1,00	1,00	1,00	1,00	1,02	1,04	1,16	1,16	1,20	1,30
M2J3	1,00	1,00	1,00	1,00	1,00	1,00	1,10	1,12	1,32	1,44	1,48	1,54
M3J4	1,00	1,00	1,00	1,00	1,00	1,00	1,10	1,12	1,28	1,32	1,34	1,40
LSD: 5%	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Note : NS : Non Significant ; WAP : Week after Planting

2. Observation of Callus Quality.

Analysis of callus quantity showed that there were no interactions between the single factor types of explant and the number of eksplant. The result of observations of callus quantity showed that there was No. significant difference in the treatment of explant sources and the namber of explants on quantity of Dragon Fruit Callus. The real difference only occurred in the singgle factor explant sources at weeks 2 to 5 on the quantity of callus formed as presented in table 3 below.

In table 3. Shows the quantity of callus which tends to be better in explants derived from stem than those from callus. The highest number of callus was form in the number of explants 4 although itwas not significantly different from the other treatments. It is suspected that the number of explants 4 with slow callus growth has not seen any nutritional competition for the growth and development of explants. Callus is amss of cell formed on surface of the explant or in the incision / wound. The appearance of callus on the cut surface is a protective response for plants to repair damaged tissue (Arijanti, *et al*., 2018).

Table 3. Average scoring quantity callus due at Various Age Obsevation (WAP)

Treatment	1	2	3	4	5	6	7	8	9	10	11	12
M1	1,00	1,00b	1,12b	1,35b	1,76b	2,00	2,00	2,11	2,27	233	2,44	2,49
M2	1,00	1,29a	1,48a	1,88a	2,00a	2,00	2,00	2,08	2,20	2,27	2,28	2,32
LSD 5 %	**NS**	**S**	**S**	**S**	**S**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
J1	1,00	1,14	1,28	1,56	1,84	2,00	2,00	2,08	2,20	2,30	2,30	2,40
J2	1,00	1,16	1,30	1,62	1,88	2,00	2,00	2,06	2,14	2,18	2,19	2,20
J3	1,00	1,14	1,30	1,62	1,88	2,00	2,00	2,10	2,30	2,40	2,44	2,50
J4	1,00	1,14	1,32	1,66	1,92	2,00	2,00	2,14	2,34	2,44	2,48	2,52
LSD: 5%	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Note: S : Significant ; NS = Not Significant WAP : Week After Planting

Callus quantity shows the number of callus formed as a result of cell division. In general, the growth and development of callus is influenced by the elements in the media. Each explant of different species has different media requirements in this case the nutrients needed for growth. Success in plant tissue culture techniques is highly dependent on the media used. It is known that tissue culture media contain macro, micro, vitamins and carbohydrates (glucose) as carbon substitutes (Rahmawati S., 2006).

3. *Observation of Callus Weight.*

The results of the observation of callus weight at the age of 12 weeks showed a significant difference in a single factor. The average weight of callus at 12 weeks of age is presented in Table 4. From Table 4. it can be seen that the heaviest callus was produced in the treatment with explant from callus. Compared with other treatments, it was suspected that in explant one there was no nutritional competition between explants so that the allocation of nutrients was able to increase callus weigh.

Table 4. Callus Weight Effect of Treatment Types and Number of Explants

Treatment	Callus Weight (g)
M1	0.61 a
M2	0.60 b
LSD 5 %	**S**
J1	0,80
J2	0,55
J3	0,54
J4	0,51
LSD 5 %	**NS**

Note : S : Significant ; NS : Non Significant

4. *Anthocyanin Content in Callus*

The result of observasion made on the anthocyanin conten in callus aged 8 and 12 weeks showed no significant difference between the treatmant of explant sources and the number of explants as shown in table 5.

Table 5. Anthocyanin Content Effect of Treatment on Explant Material

Treatment	Anthocyanin content on callus (%)	
	8 WAP	12 WAP
M1	0,68 a	0,74 a
M2	0,65 b	0,72 b
LSD 5%	S	S
J1	0,62	0,68 c
J2	0.67	0,73 bc
J3	0.70	0,76 a
J4	0.68	0,74 b
LSD 5%	NS	NS

Note S : Significant ; NS : Non Significant ; WAP : Week after Planting

From Table 5. It can be seen that at 12 WAP, the type of explating source treatment had a significant effect on the anthocyanin content formed. Explants grown from young shoots of callus that were formed contained anthocyanins (0,74%) higher than explants from callus dragon fruit (0,72%). While in singgle factor; number of explant treatment; It can be seen that the the results of the analysis of anthocyanin content of the planted callus with the number of explants 3 and 4 explants each culture tube. Produce Anthocyanins equally well by addition of explants . In the observation of 12 WAP, it can be seen that the treatment J3 could produce anthocyaniin secondary metabolites. The results of the analysis showed that The callus formed contained the highest anthocyanin tends to be more (0,76 %); highest compared to other treatment.

E. Discussion

Callus is a selamorphous collection that occurs from dividing cells and consists of parenchyma cells (Slater, A., N. Scott & M. Fowler., 2003). From research observations on the analysis of the quality and quantity of callus in Table 2 and Table 3; it can be seen that callus of young shoot an callus explant show slow growth. Callus quality scores began to increase at week 7; while a score of 2 on the quantity of callus (growing of explants) began to appear at week 6 sedangkan score 2 pada kuantitas calus (growing of explant) mulai terlihat pada minggu ke 6. Callus formation is one indicator of the occurrence of explosive growth in tissue culture. The results showed that callus began to form at week 6. Callus began to form on young shoot incisions that were in contact with MS media. This showed that the explant is starting to adapt and begins to respond to callus growth.). In line with the the statement of Hos (2008) in callus culture there are 3 stages of induction, proliferation, and differentiation. In the induction stage, cells begin to devide; the proliverative stage (cell divition occurs very quickly); Stage of differantiation (the process of metabolism or organogenesis occurs). In the Induction stage, it begins with the absorption of water so that cell wall loosens and the cell size enlarges and actively divedes.

In line the opinion of Iwase A, M Ohme -Takagi, & K Sugimoto (2011) added that growth begins with swelling of the explant slices of stem shoots, then a wavy callus is formed (swelling) and small white granules appear to form a compact callus. Early callus formation from incision scars in contact with tissue culture media; cells undergo division followed by the proliferation process on the surface of the slice until a callus is formed. This is accordance with the opinion of Prakoeswo, S.A. Ribkahwati & D. R. Soeryaningsih, (2010) that the emergence of difference in callus quality depends on environmental condition of growth and which is influenced by explant sources. It is assumed that up to 7 weeks the planting material/plant-let that was tried was still in the stage of adapting to the media and the environment. This is in accpordance with the opinion of Purwaningsih, Y. (2013) that the use of callus explants in tissue culture shows an easily observed morphology and can produce secondary metabolites in the form of anthocyanin pigments.

From table 4 it can be seen that the source of the explant that was tried had a significant effect on the weight of the explant formed. Explant grown from young shoots showed better callus growth. Fresh tissue have meristematic properties and are actively dividing. The success of engineering the tissue culture of the plant is highly dependent on the medium used. Further Indarwati *et al.* (2020), added that MS media has a real effect and is verygood to use as a culture medium to produce metabolic sekundair (Anthocyanin) from the youngh shoot of dragon fruit stems.

The results of observation made on the anthocyanin content in callus (table 5.) showed that the type of explant and the number of explants planted on MS +15% sucrose media had a significant effect on the secondary metabolites produced. The analysis was carried out at the age of 8 weeks, the Anthocyanin content formed was in the range (0,65%-0,69%). Observations at week 12 Anthocyanin content increased in the range (0.68% - 0,76%). The accumulation of secondary metabolites is linier with the formation of the callus quantity. In a high metabolic process, it will be followed by formation of primary metabolites in greater quantities so that it can be used to synthesize the formation of secondary metabolites. In line with the opinion Pan Y, L Lin, S Xiao, Z Chen, S Sarsaiya, S Zhang, YS Guan, H Liu, & D Xu. (2020); this is due to the condition of medium containing optimal nutrients, in its body cells cleave to grow larger, elongate to form callus by using the energy contained in the culture medium to increase growth and formation of secondary metabolites. In this study callus growth was seen towards compact , followed with the formation of high Anthocyanin secondary metabolites (0,76 %).

Once of factors that determine the success of plant regeneration is the availability of carbohydrate sources. For plants bred through tissue culture, carbohydrates serve as a sources of carbon needed to produce energy. In addition the source of carbohydrates as a energi is very depent on the konsentration of carbohydrates . The addition of 15% sucrose on MS media aims to provide an additional source of energi for metabobolic processes so that it can increase the formation on secondary metabolotes. The result of analysis of Planting Material (Shoot and callus of Dragon Fruit) which were cultured on (MS Media + 15% Sucrosa) produced secondary metabolits antochyanin (0,74 % and 0,72%). Treatmant of the number of expansion 1,2,3 and 4 explant in each culture tube at week 12 callus analyzed resulted in Anthocyanins (0,68 %-0,76%).

F. Conclusion

There is no interaction between the source of explant materia and the number of explants each culture tube on the content of secondary metabolites in callus. MS Media with the addition of 15 % sucrose had a sinificant effect on increasing the anthocyanin content in the callus of Dragon fruit formed. Callus formed from Shoot Dragon fruit contain Anthocyanin 0,74 %. The Callus formed from Shoot and callus Dragon Fruits contain secondary martabolites of Anthocyanins ranging from 0,68% – 0,76 %. The highest Anthocyanin content (0,76%) was produced in treatment J3 (3 Explant each tube).

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Stomata Density Analysis of Red Chili (*Capsicum annuum* L.) at Different Location

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Abstract

Red chili (*Capsicum annuum* L.) is a commodity of vegetable that has high economic value. Stomata are an important part of plant organs, it a gap that role in the process of photosynthesis and plant transpiration. The purpose of this study to determine differences in the stomata density of red chili (*Capsicum annuum* L.) leaves at different locations, namely locations exposed and shaded from sunlight. This study used a quantitative approach with the type of research used is experiment as by one factor is that sunlight intensity, used two paired treatments and respective of three repetition. The data collection method in this study used direct observation and used analytic method to analyze the stomata density of chili *(Capsicum annuum L)* leaves. The result of his study showed that the stomata density of red chili (*Capsicum annuum* L.) leaves differed according to the planting location in the form of locations exposed to sunlight and locations that were shaded. The stomata density planted in a location exposed to full sun had a higher stomata density at 409.9 cells/mm2, while the stomata density of red chili (*Capsicum annuum* L.) leaves planted in a shaded location had a lower stomata density at 96.9 cells/mm^2

Keywords: light intensity, red chili, stomata density

A. Introduction

Red chili (*Capsicum annuum* L.) is an annual plant belonging to the Solanaceae family, such as cayenne pepper, paprika, and so on. This plant is widely grown in Pakistan, America, Portugal, India, and Indonesia (Shaheen, N., Shahlla, I., Safia, A., Rafi, A. S., Iqbal, A., Zafar, A. M., 2018). Red chili (*Capsicum annuum* L.) is a vegetable commodity that has high economic value. Generally, its used for household consumption needs as a spice and used for herbs or traditional medicines. The chili is consumed both in fresh and as well as processed form (Fitriani, L., Toekidjo, & Setyastuti, P., 2013).

Flowering of chili is strongly influenced by light intensity for a long time (Wati, 2018). This vegetative growth requires proper nutrition, in the form of water, which is a needed component for beginning of growth to the flowers and fruits formation of red chili (*Capsicum annuum* L.) (Tonny and Laksminiwati, 2011). According to Sumarni and Agus (2005), the adaptability of red chili (*Capsicum annuum* L.) is quite extensive, both in the lowlands and highlands, up to 1.400 meters above sea level.

Vegetative growth of plants can accelerate the occurrence of better metabolic processes, especially in the process of photosynthesis (Suharso, 2017). The decrease in leaf water potential is influenced by turgor pressure changes and elasticity of leaf cell walls. This depends on external factors or environmental conditions, one of which is sunlight through the process of photosynthesis to obtain energy (Xiang, L., Wang, R.G., Mao, G., Koczan, J.M., 2006). One part of the plant whose mechanism action is strongly influenced by sunlight is the stomata (Campbell, N.A., Reece, & Mitchell, L.G., 2003). As according to Adisyahputra, Sudarsono, Kukuh, S. (2011) water loss in leaves can occur with decreasing leaf surface area and stomatal conductance. Stomata structure also affects the exchange of gases and water vapor from the leaf to the environment or vice versa. So the regulation of stomata is an important role in controlling water loss in plants.

Stomata are an important part of plant organs, in the form of gaps between the combination of two special epidermal cells called guard cells. These guard cells work to open and close according of plant transpiration (Setiawati and Inneke, 2019). Stomata are found in all plant parts that are exposed to the air, but are more commonly found in leaf organs (Kamaluddin, Gede, A. W., Muhammad, R., 2020). The mechanism of opening and closing stomata in drought-tolerant plants is to avoid water loss through evaporation.

Anatomical approach is important because environmental conditions are increasingly dynamic and each plant has a different response to sunlight stress, such as the ability to open and close stomata (Haryanti and Meirina, 2009). The research results of Haryanti (2010) show that the speed of stomata closing as a stomatal response to changes in the vapor pressure deficit is largely determined by the sensitivity of the stomata. The process of opening and closing stomata is strongly influenced by the sunlight conditions.

Sunlight greatly affects the work of stomata, especially in the opening and closing process of stomata. With the light intensity factor, plants make adaptations to support the continuity of the physiological functions of the plant. One adaptation of plant is the response of stomata formation in shaded and unshaded areas (Campbell, et al., 2003).

Based on the description above, the authors conducted a study to analyze the stomata density on red chili (*Capsicum annuum* L.) leaves at different locations, namely exposed locations and shaded locations.

B. Methology

This study uses a quantitative approach, in the data is analysis of stomatal density. The type of research used is an experiment consisting of a single factor, namely the sunlight intensity, using two treatments and three repetitions each.

Planting and sampling of this research were located in the Biology Garden and Green House of Biology Education, and stomata observations at the Laboratory of Biology Education, Muhammadiyah University of Parepare. It was held from July 17th to August 31st 2021. This study used red chili (*Capsicum annuum* L.) as the research subject. The research sample used each one of third leaf, from three red chili (*Capsicum annuum* L.) at the age 28 days after planting.

The data collection method in this study was direct observation to determine environmental conditions and morphology of red chili (*Capsicum annuum* L.) leaves based on different locations, namely those exposed and shaded from sunlight, and using analytical methods to calculate stomata density of red chili (*Capsicum annuum* L.). This study used one red chili variety with two different treatments and made three observations on each treatmen.

1. Research Preparation

a. Planting preparation

Preparation for planting begins with preparing the selected seeds in advance by soaking for 3 hours, then sowing. Seeds that grow 3-4 leaves are then transferred to an exposed location and a shaded location, until the age of 28 days after planting.

b. Observation Preparation

This research prepares tools and materials in the form of a microscope, camera, lux meter (application), thermohygrometer, cover slip, object glass, transparent tape, scissors, label paper, transparent nail polish, and leaf samples from red chili plants.

2. Reserch Step

The research steps were carried out by observing the stomata density of red chili leaf (*Capsicum annuum* L.) with the replica or mold methods, that using transparent tape and transparent nail polish. After that, it is placed on a slide and then observed under a microscope.

Preparations for observation of stomata density was carried out using the mold or replica method. Where is the manufacture of stomata replicas on red chili leaves as:

a. Prepare tools and materials needed to conduct research.
b. Take a sample of leaf blades and clean it from dirt.
c. Apply transparent nail polish on the surface of the center of the leaf and allow it to dry.
d. Paste transparent tape on the surface of the leaf that has been smeared with transparent nail polish, by gently massaging the surface of the leaf using your fingers, so that the nail polish sticks well.
e. Peel off the tape slowly and stick it on the slide.
f. Give label paper to the corner of the slide and provide information.
g. Observing the density of stomata until the object can be observed clearly.
h. Make similar observations for each sample from two different locations at 11.00 AM on three repetitions, for more accurate of observation results. (Source: Humami, D. W., Puput, A. W. S., Iska, D., 2020).

3. Final Stage

The final stage in this research is to analyze data based on the stomata observations results. Stomata in the field of view of the microscope will be counted for each cell. Then it is entered into the formula for calculating stomatal density. The data obtained from observations of stomatal density were made in the graphs and explain the description. The results of this study will be drawn a conclusion and published.

4. Data Analysis Technique

Research the density of stomata on leaf blades of red chili (*Capsicum annuum* L.), then the data analysis technique used is descriptive data analysis and quantitative description, namely: presenting the results of analysis based on facts displayed with pictures (photos) and explain the description, so the results of the study can be returned directly to the data obtained.

After getting the results of descriptive data from images, then analyzing the percentage of stomata density using the formula. The formula for measuring stomatal density:

$$\text{Stomata density} = \frac{\text{number of stomata}}{\text{wide field of view}}$$

Measurement of stomatal density using 400x magnification with a broad field of view from a microscope measured by the formula:

$$\begin{aligned}\text{Area of view} &= \tfrac{1}{4}\,\pi\, d^2 \\ &= x\ 3.14 \times (0.5\ \text{mm})^2 \\ &= 0.19625\ \text{mm}^2\end{aligned}$$

C. Result and Discussion

Environmental conditions based on different planting locations of red chili, show that conditions not have high significance. Sampling was carried out at 11.00 AM, with the location for planting shading in the Biology Education Green House which only gets little sunlight intensity, while the location is exposed to sunlight which is around the Green House of Biology Education.

Observations showed that environmental conditions exposed to sunlight had an average temperature is 30^0C with 67% humidity. The results of the observation of light intensity as measured by Lux Meter, which is 65.536 Lux. In the shaded environment, the average temperature is 28,4^0C with 69% humidity. The light intensity obtained from the measurement results is 32.834 Lux. These results indicate that the higher the light intensity received by chili plants, has the higher temperature and lower the humidity.

The results from observations of red chili plants in shaded locations and exposed to sunlight, have clear morphological differences. The results of observations on red chili leaf blades in shaded and exposed locations also have differences in leaf size and leaf texture. The size of the shaded red chili leaves has an average leaf width is 1.5 cm and average leaf length 2.8 cm, while the size of the red chili leaves at the exposed location has an average leaf width is 1.7 cm and a length 2.2 cm.

The results of stomata observations of red chili (*Capsicum annuum* L.) leaves also attention to the shape and parts of the stomata. Based on the shape of the stomata found in the observations, the stomata in red chili (*Capsicum annuum* L.) belong to the Anisocytic type.

Figure 1. Stomata Observations of Red Chili (*Capsicum annuum* L.)

The observations results of stomatal density obtained from the calculation of stomata and the surface area of the microscope's field of view, were then analyzed for each sample. The results of observations of stomatal density can be seen in Table 1.

Table 1. Stomata Density Observation Results

Treatment	Stomata Density Observation Result		
	Sample	Stomata Result	Stomata Density (cells/mm2)
Shaded	A1	19	96.9
	A2	14	71.4
	A3	24	122.4
Exposed	B1	88	448.9
	B2	82	418.3
	B3	71	362.5

The results showed that the stomata were in two different locations, namely shaded and exposed to sunlight. In samples A1, A2, and A3 are the shaded leaves conditions. Sample A1 had 19 stomata with a stomata density of 96.9 cells/mm2, sample A2 had 14 stomata with a stomata density of 71.4 cells/mm2, sample A3 had 24 stomata with a density of 122.4 cells/mm2. In samples B1, B2, and B3 are the exposed leaves to sunlight. Sample B1 had 88 stomata with a density of 448.9 cells/mm2, sample B2 had 82 stomata with a density of 418.3 cells/mm2, and sample B3 had 71 stomata with a density of 362.5 cells/mm2.

The average density of stomata in shaded locations is 96.9 cells/mm2, while the average density of stomata in exposed to sunlight area is 409.9 cells/mm2. The results of the analysis of stomatal density at the shaded location included the category of low stomatal density. Meanwhile, at the exposed location has moderate of stomatal density analysis.

Based on the paired sample test (Paired sample test) with SPSS as shown in Table 4.3. obtained the value of Sig ≠ 0 (0.013), it can be concluded that H0 is rejected and H1 is accepted. Therefore, it was concluded that light intensity had an effect on stomatal density.

1. *Stomata Condition of Red Chili Leaves (Capsicum annuum L.)*

Stomata sampling on red chili (*Capsicum annuum* L.) leaves was carried out at 11.00 AM, and stomata observations were immediately carried out using the replica method. So that it is possible that there is no stomata damage that occurs in the leaf samples to be observed. Stomata observations of red chili (*Capsicum annuum* L.) leaves were carried out to determine the parts of the stomata and the characteristics of the stomata types (Figure 4.3.). The difference in the light intensity of received also affects the temperature and humidity. The sunlight intensity of received in the shaded area is lower, so it has a lower temperature and high humidity. Meanwhile, in areas exposed to sunlight, the temperature is high and the humidity is low.

Based on the shape of the stomata found in observations, the stomata in red chili (*Capsicum annuum* L.) belong to the *Anisocytic* type. As found that has neighboring cells that are not the same size. *Anisocytic* or *Cruciferous* type, each guard cell is surrounded by 3 neighboring cells of unequal size, found in *Crucifera* and *Solanaceae* family (Kamaluddin, et al., 2020).

Structure and distribution of stomata in each plant is different. Likewise, the distribution of stomata on red chili (*Capsicum annuum* L.) leaves in shaded locations and exposed to sunlight, has the same distribution pattern, which adjusts to the morphology of the pinnate leaves, so that the distribution pattern is also spread out.

2. *Stomata Density in Red Chili (Capsicum annuum L.) Leaves*

The level of stomatal density is influenced by environmental factors such as the sunlight intensity, water availability, temperature and humidity. The increase in light intensity is proportional to the increase in environmental temperature, namely the red chili (*Capsicum annuum* L.) planting location exposed to full sun has high temperature with low humidity. Otherwise, in shaded planting location, it will receive a little light intensity, so that it has a low temperature with a high level of humidity (Meriko and Abizar, 2017).

3. *Shaded Location*

Observations of the environmental conditions that are shaded location still receive exposure to the morning sun, namely at 07.00 until 09.00 AM. Sampling was carried out at 11.00 AM, with received sunlight intensity of 32.834 Lux. The location of planting red chili (*Capsicum annuum* L.) in the shade showed the number of stomata in the microscope field of view, namely 19.14 and a maximum of stomata cells is 24. This is a stomata response to the environment, because of the shade which results in the low intensity of sunlight obtained, so the number of stomata is small.

The planting location has an influence on stomata conditions, namely the average stomata density obtained is 96.9 (cells/mm2). Red chili (*Capsicum annuum* L.) plants planted in shaded locations will get less light intensity, so the stomatal density found is in the low category according to (Table 1) indicating the stomatal density category. This is due to the physiological adaptation of plants under shade, causing the distribution of stomata on the leaf surface to scatter so that the received sunlight can be distributed efficiently for their survival. As explained in research of Martins, S.C.V., Jeroni, G., Paulo, C.C., Lucas, F.P., Marilia, C.V., Fabio, M.D. (2014), that plants grow in shaded areas will make adaptations by making chlorophyll enveloped, thus providing a wide distance between stomata to optimize their metabolic processes.

At least the intensity of sunlight received by red chili (*Capsicum annuum* L.), will affect performance and processes that occur such as photosynthesis and transpiration (Haryanti, 2010). The results of observations on the morphology of red chilies showed that the average leaf condition was 1.5 cm wider and leaf length 2.8 cm compared to chili leaves at the exposed location. This is due to the adaptation of red chili leaves to the received light intensity by increasing the leaf surface area in order to avoid light deficits, so that can receive light optimally for the photosynthesis process.

Leaves of red chili (*Capsicum annuum* L.) planted in shaded locations were thinner and greener (Table 1) than those in exposed locations. This is because the light intensity received is lower, resulting in reduced mesophyll tissue in the leaves in order to optimize light transmission in the leaves. That conditions of low light intensity make plants adapt by reducing the formation of mesophyll cells which results in a decrease of leaf thickness. While the color of the leaves of red chili (*Capsicum annuum* L.) in the shaded location is greener, due to the low intensity of sunlight affecting the increase in chlorophyll in the leaves. As according to Yustiningsih (2019), a high chlorophyll will also increase the optimization of the photosynthetic process if plants are able to adapt of shaded conditions.

4. Exposed Location

Locations exposed to sunlight have environmental conditions that receive full sunlight from 08.00 to 17.00 AM without any barrier or shade. At the time of sampling, which was conducted at 11.00 AM, the light intensity was 65.536 Lux. The planting location has an influence on the condition of the stomata, which has a fairly high number of stomata in the microscope field of view, namely 71, 82, and a maximum of 88 stomata cells. This is because the red chili leaf surface exposed to more intense sunlight will significantly increase the number of stomata, due to an increase rate of CO_2 diffusion.

The results of the analysis of the total of stomata in field of view on a microscope found that red chili planted in exposed locations had an average stomata density of 409.9 (cells/mm2). The high level of stomata density found in red chili (*Capsicum annuum* L.) leaves grown in exposed locations was included in the medium density category according to (Table 1) the category of stomata density level. These results indicate that the high intensity of sunlight received makes the leaves adapt by increasing the amount of stomata which is in line with the increase the level of stomatal density. As the research results of Yustiningsih (2019), plants that are able to adapt to the environment will produce structures, morphology, and physiology that are suitable for the environment in which they grow. This allows plants to makes specification to adjust the environmental differences, including the intensity of sunlight received.

Red chili leaves (*Capsicum annuum* L.) planted in sunlight exposed locations have thicker leaf morphology, small surface area, and yellowish color. The high intensity of light received makes the leaves thicken because it increases the number of mesophyll cells, and the small leaf surface area because the leaves have received the maximum light intensity make the leaves no longer cover the surface to reception streamline of sunlight. Plants that receive high of light intensity produce leaves that are smaller, thicker, more compact with a cuticle layer and thicker cell walls, smaller intercellular spaces, and hard leaf texture.

Yellowish color occurs on the leaves of red chili (*Capsicum annuum* L.) due the possible influence of the presence of weeds around the planting site exposed to sunlight. Explained by Zulkarnain (2013) that the growth of red chili (*Capsicum annuum* L.) can be disrupted due to lack of hydronation and aeration during its early growth period, as well as disturbances from pests and weeds. The optimal soil conditions for planting red chili are as long as the soil has good drainage and aeration, as well as available water and sufficient amounts during the plant growth period (Zulkarnain, 2013).

D. Conclusion

Based on the results of the analysis and discussion, it was found that the planting location of red chili (*Capsicum annuum* L.) in a place that was shaded and exposed to sunlight had a significant effect on differences in stomatal density. Differences in stomata density of red chili (*Capsicum annuum* L.) leaves grown in exposed full sun locations had higher stomata density. Meanwhile, the stomata density of red chili (*Capsicum annuum* L.) leaves planted in shaded locations had a lower stomata density

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