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Soybean Adaptation to Water Stress on Vegetative and Generative Phases

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Abstract

Soybean is rich in protein and is one of the main sources of vegetable protein which essential in enhancing public nutrition. Climate change is the main trigger of the occurance of extreme weather events makes plants become more vulnerable to drought. Drought stress significantly affect the decline in soybean production, especially when it occurs during the reproductive phase. This research aimed to identify the response of soybean to water stress as a reference for determining the adaptive and tolerant varieties. The research was arranged in split-split plot design, with main plot was varieties (Dering and Argomulyo), the development phase (vegetative and generative phases) as the subplot, and water stress in the form of irrigation intervals (2, 5, and 10 days) as the sub-sub plots. The results showed that water stress during the vegetative phase has not statistically significant effect on soybean production. Soybean crop adapted to water stress by reducing the number of leaves, the leaf area, stomatal openings, as well as doing motion response by folding leaves. This crop adaptation mechanisms affecting the formation of dry matter quantity, seeds yield, water use efficiency, and radiation use efficiency

Keywords: adaptation response, generative phase, soybean, vegetative phase, water stress

A. Introduction

Soybean is one of the main food commodities after rice and corn that rich in protein and has diverse uses, especially as food and industrial raw materials as well as for the livestock feed industry (Zakaria, 2010). Soybean is an essential source of oil, protein, macronutrients and minerals (Clemente & Cahoon, 2009). The content of vegetable protein in soybean is essential for improving public nutrition. Vegetable protein, besides safety also relatively cheaper compared to animal protein, therefore, the soybean demand continues to increase in line with population growth.

Soybean consumption in Indonesia in 2015-2019 is expected to increase at around 2,77 million tons 2015 and 3,25 million tons in 2019. Meanwhile, projection of production for 2015-2019 based on the Ministry of Agriculture is about 93 thousand tons in 2015 and 1,22 million tons in 2019 (Bappenas, 2013). Based on these data, the soybean production is still in deficit and have not been able to filled needs of domestic consumption. To fill with domestic soybean and achieve self-sufficiency it is necessary to increase soybean planting acreage.

Potential land for soybean cultivation is quite extensive but it face difficulties, especially in the dry season is very vulnerable to drought, therefore, the water availability for the growth of soybean becomes limited. Climate change also causes extreme weather changes. A shift of season affects the planning of the agricultural activity. The planting schedule will be disturbed and decrease the production and cause crop failure. Drought reduces crop productivity especially in arid and semi-arid regions (Razzaghi, Ahmadi, Jacobsen, Jansen, Andersen, 2012). The rapid population growth also becomes a major concern, since it increases the food demand, especially soybean while the agricultural productivity decrease due to the climate change.

Drought stress during the reproductive phase causes the soybean production decreases more than 40%. In fact, soybean plantation in Indonesian is largely (65%) in the rice fields during the dry season. In this condition, the cultivation of soybean is often faced the risk of drought. The dry condition during flowering period cause flowers and young pod fall and reduce the number of pods and seed size. While, in the filling phase, the beans are not formed perfectly, that causes the soybean becomes smaller and the dry matter becomes lower, that cause the production decrease up to 40%. To anticipate the drought impact, the more adaptive variety is developed such as use old varieties of early maturing or tolerant to drought stress (BPTP, 2013). Drought has an influence in decreasing the soybean crop growth for the stems and the roots which lead to the decreasing of crop total dry matter (Hamim, Sopandie, & Jusuf, 1996). The photosynthesis rate of the crops that suffer from drought decrease sharply and is lower compared to the crops that do not suffer from drought (Liu, Jensen, & Andersen, 2004). The production of the soybeans is decreased when the water stress is increasing (Candogan, Sincik, Buyukcangaz, Demirtas, Goksoy, & Yazgan, 2013).

Plants have evolved two major mechanisms to cope with water deficit: stress avoidance and tolerance. Stress avoidance is achieved by the formation of seeds before drought conditions prevail and specialized in the plant architecture. Morphological adaptations are, for example, the development of specialized leaf surfaces to decrease the rate of transpiration, the reduction of leaf area, sunken stomata or an increase in root length and density to use water more efficiently (Ramanjulu & Bartels 2002). The objective of this research is to identify the soybean adaptation responses to water stress as a reference to determine the adaptive and tolerant varieties.

B. Methodology

The research was conducted in Cikabayan field Experiment, Bogor Agricultural University from April to June 2015. This research used experimental design of split-split plot design in three replications with the main plot was varieties (V1= Dering variety, V2= Argomulyo Variety). The subplot was the development phase (F1= stress during vegetative phase, F2= stress during generative phase). The sub-subplot was water stress in the form of irrigation intervals (I1= every 2 days, I2= every 5 days, I3= every 10 days). This research used the shade

of bamboo with a transparent plastic cover to be protected from contamination of rain to the water stress treatment.

Water stress treatment for vegetative phase was started after 2-week-after planting (Week After Planting=WAP) and stopped at the time of entering of generative phase or flowering stage and at the same time, treatment for generative phase was started and finished at harvest. At the beginning of planting until 2 WAP, all crops were irrigation every day. The crops were given the saturated water and left for some time until the condition is reached field capacity. The weighing method was done to determined water loss and soil water content during the stress period.

The variables observed in order to identify the adaptation responses to water stress are the number of leaves, the leaf area, the stomatal openings, the total dry matter, the seed yield, water use efficiency and radiation use efficiency. The number of leaves was observed every week until the age of 7 WAP. The leaf area was calculated every two weeks using the gravimetric method. The stomatal opening observations were done using samples of a replica of stomatal and observed under a trinocular microscope with 10x40 magnifying, which is equipped with Olympus DP25 digital camera types and DP2-BSW application. The dry matter was measured every 2 weeks using destructive sampling method as much as 3 crops for each treatment and oven dried at 80^{o}C for 48 hours. The seed yield was observed at harvest and oven dried at 80^{o}C for 48 hours. The data analysis was performed using F-test (ANOVA).

Water use efficiency (WUE) was calculated using the equation:

$$WUE = dW / \text{Crop water use (ETA)}$$

where, WUE is water use efficiency (kg/m^3), dW is the addition of the dry matter (kg), and ETA is crop water use (actual evapotranspiration) (m^3) The amount of water loss from the pots, represented the evapotranspiration.

Radiation use efficiency was calculated using the equation :

$$\varepsilon = dW/Qint$$

where, **ε** is globally radiation use efficiency (g MJ^{-1}), dW is the addition of the dry matter (g m^{-2}), and Qint is cumulatively of solar radiation intercepted by crop canopy (MJ m^{-2}).

Radiation interception was calculated using Beer's Law which is the function from the leaf area index, extinction coefficient and the intensity of solar radiation above the canopy. Extinction coefficient value used is the average value in various leaf area index. The intensity of radiation measurement was performed using a solarimeter sensor which has been calibrated by a standard solarimeter and connected to the data logger. The equation used is (Handoko, 1994):

$$Qint = Qo(1- e^{-k\,LAI})$$

Where, Qint is radiation interception (MJ m^{-2}), Qo is radiation above the canopy (MJ m^{-2}), k: is extinction coefficient (0,7), and LAI is leaf area index.

C. Results and Discussions

1. Relation Of Water Stress With Soil Water Content (Swc)

Water stress treatment provided information about the soil water content (SWC) on each irrigation intervals (Figure 1).

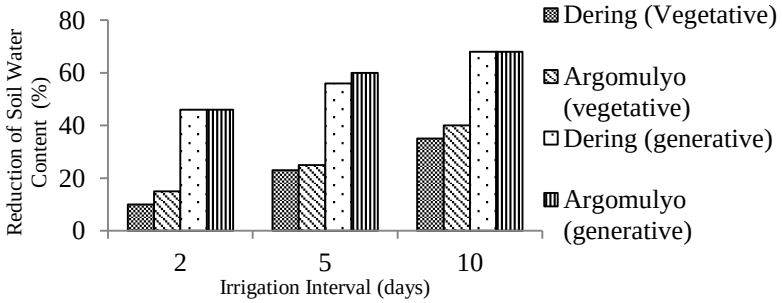

Figure 1. Percentage reduction of soil water content (SWC) on each irrigation intervals.

The reduction of SWC is not very large in the vegetative phase because crop water requirement in this phase is still relatively small, but the water requirements is rising in line with the growth of the crop, so that the SWC reduction become larger in generative phase. Water loss caused by transpiration very influenced by canopy cover stated in the leaf area index (LAI). Water loss increased with increasing value of LAI.

2. Responses of Soybean to Water Stress

Water stress at vegetative phase has not significant effect on some observation variables, meanwhile at generative phase influence all of observation variables. Period of water stress at vegetative phase is more shorter compared generative phase, thus cause moderate stress on vegetative phase and severe stress on generative phase. Short-term, moderate drought stress during vegetative growth stages generally does not impact soybean yield. Conversely, longer-term severe drought stress can cause irreversible plant cell death causing low growth yield. Soybean yield is most sensitive to water deficits during reproduction (Lenssen, 2012).

Table 1. Summary of results of ANOVA soybean responses due water stress on vegetative and generative phases.

Variables	Varieties (V)	Development Phase (F)	Water Stress (I)	Interaction (V x F)	Interaction (V x I)	Interaction (F x I)	Interaction (V x F x I)
Leaf number 4 WAP	0.524 ns	0.1099 ns	0.4822 ns	0.6355 ns	0.5493 ns	0.751 ns	0.5035 ns
Leaf number 7 WAP	0.017*	0.0004*	0.0000*	0.2522 ns	0.0026*	0.000*	0.1873 ns
Leaf area 4 WAP (cm^2)	0.031*	0.0107*	0.0025*	0.5361 ns	0.3630 ns	0.001*	0.1526 ns
Leaf area 6 WAP (cm^2)	0.036*	0.0023*	0.0000*	0.4526 ns	0.9526 ns	0.000*	0.1415 ns
Total dry matter (g)	0.254 ns	0.0009*	0.0000*	0.8070 ns	0.0510 ns	0.000*	0.4715 ns
The seed yield (g)	0.114 ns	0.0001*	0.0000*	0.2559 ns	0.0690 ns	0.000*	0.9569 ns
Stomatal opening (veg) (μm)	0.003*	0.0045*	0.0004*	0.9901 ns	0.9506 ns	0.0015*	0.7019 ns
Stomatal opening (gen) (μm)	0.464 ns	0.0098*	0.0112*	0.9955 ns	0.4495 ns	0.0077*	0.9650 ns

*) significant at P≤0.05; ns = not significant; Veg = observed on vegetative phase; Gen = observed on generative phase; WAP = week after planting.

3. Morphology Response

Leaf Number and Leaf Area

Water stress at vegetative phase has not given statistically significant effect on the number of leaves (Table 1). The stressed soybean in the vegetative phase and observed at the age of 7 WAP showed decrease in the number of leaves but not significantly different when compared to with stresses 2 days (Figure 2). This was caused by the plant ability to restore growth after passing the stress condition called recovery mechanism. Meanwhile, the stress on the generative phase gave a different effect on each variety (Figure 2). The number of leaves decreased with increasing periods of stress. Dering variety had a greater reduction than Argomulyo in the same stress conditions. The lack of leaves during stress is caused by disturbance of growth and adaptation mechanisms of plants through the defoliate of leaves to reduce transpiration.

Water stress treatment also gave different effect on the leaf area on vegetative and generative phase (Table 1). While stressed, the crop reduces leaf area to reduce the field transpiration. Leaf area decreased with increasing periods of water stress. Dering variety decreased leaf area is greater than the Argomulyo in the same stress conditions (Figure 3).

Defoliation and leaf area reduction is one of the mechanisms of plant adaptation due to water stress. This mechanism is called dehydration avoidance which is the plant ability to keep the tissue potentials by reducing the water loss. At this mechanism the plants usually have the ability to reduce in surface evapotranspiration through reduction of the leaf area and defoliation the old leaves (Fukai & Cooper, 1995). Water stress affects the growth and development of the

canopy (Stone, Wilson, Jamieson, & Gillespie, 2001). Sulistyono, Suwarno, Lubis, & Deni. (2012), stated that the first physiological processes that occur as impact of the drought is the reduction of leaf area, which can cause a decrease in stomatal conduction and photosynthesis rate. The reduction of leaf area and stomatal opening is the mechanism to avoid drought by reducing transpiration.

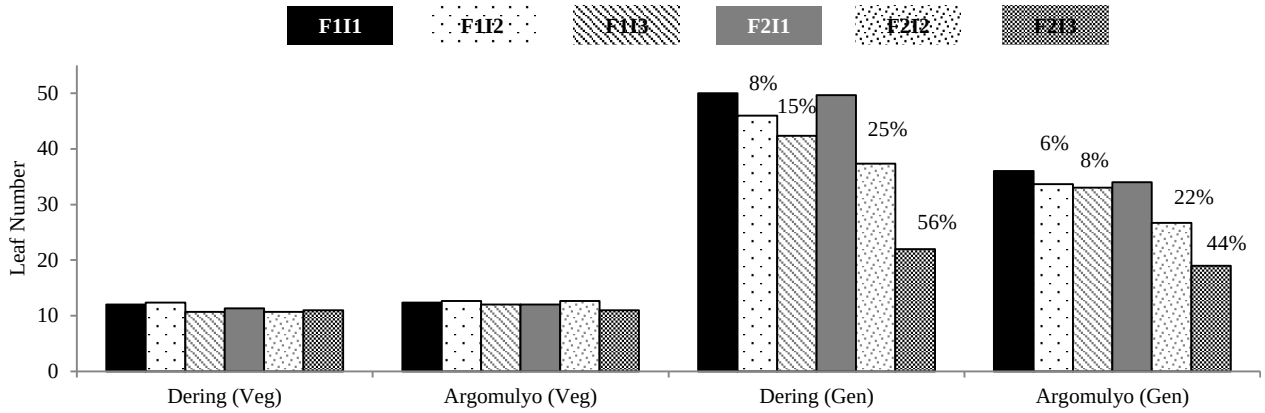

Figure 2. The number of leaves of soybean at age of 4 and 7 WAP. F1 = vegetative phase, F2 = generative phase; I1, I2, I3 = water stress by irrigation intervals of 2 days, 5 days, and 10 days. Numbers above diagram showed percentage reduction of the number of leaves by compared irrigation interval 2 days.

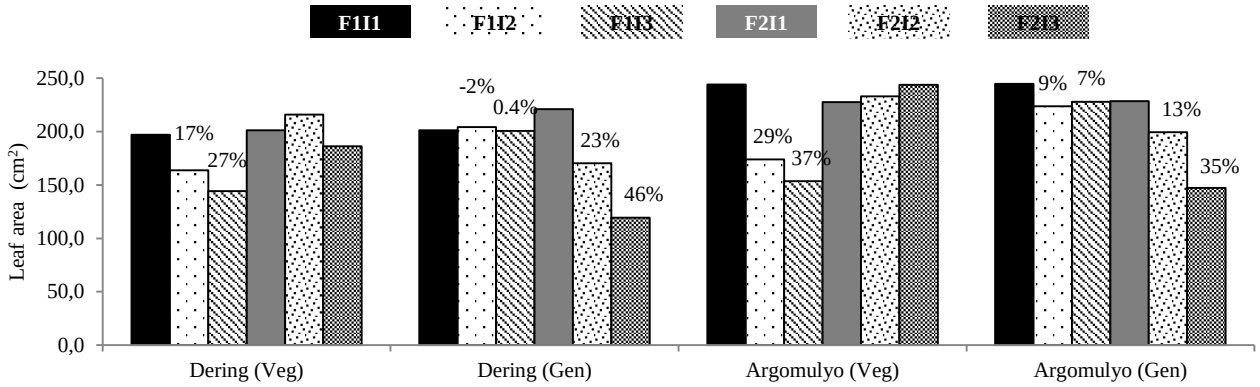

Figure 3. Leaf area of soybean at age 4 and 6 WAP. F1 = vegetative phase, F2 = generative phase; I1, I2, I3 = water stress by irrigation intervals of 2 days, 5 days, and 10 days. Numbers above diagram showed percentage reduction of leaf area by compared irrigation interval 2 days.

4. Physiology Response

Stomatal Openings

Water stress on the vegetative and generative phases gave a different effect on stomatal opening (Table 1). Water stress for 5 days and 10 days caused the crops perform adaptation response by reduced stomatal openings at vegetative and generative phases (Figure 4). Stomatal opening was decreased when the water stress is increasing. Reduction of stomatal opening is one of the mechanisms of plant tolerance to stress to reduced water loss. Argomulyo variety decreased stomatal openings is greater than Dering variety at vegetative and generative phases. Sudarsono & Widoretno (2003); Purwanto & Agustono (2010); Permanasari & Sulistyaningsih (2013) research results that the width of the stomatal openings become smaller with increasing water stress. Plants which suffer from drought will closure of stomata to reduced water loss through transpiration.

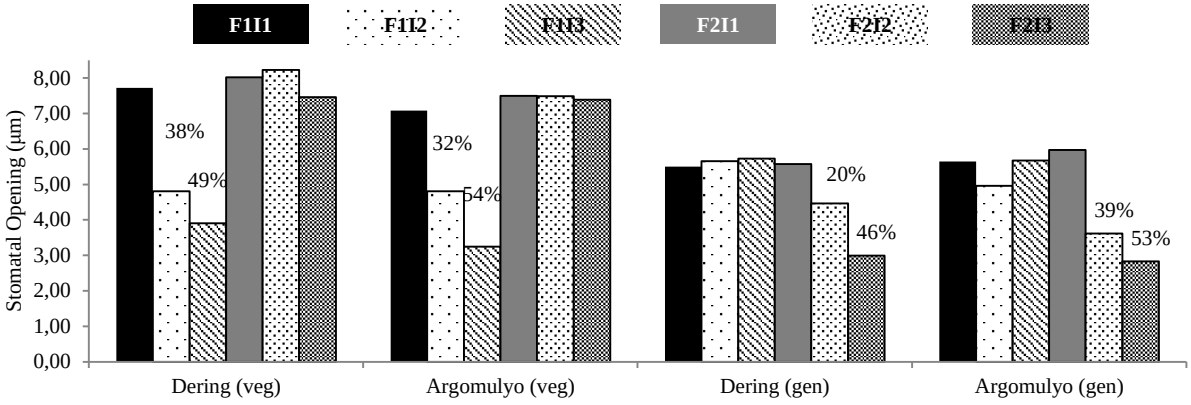

Figure 4. stomatal openings of soybean at vegetative and generative phases. F1 = vegetative phase, F2 = generative phase; I1, I2, I3 = water stress by irrigation intervals of 2 days, 5 days, and 10 days. Numbers above diagram showed percentage reduction of stomatal opening by compared irrigation interval 2 days.

5. Motion Response

Leaf Folding

One of the adaptation response of crop on water stress that the folding of leaves to reduce exposure of radiation heat on the leaves surface which can accelerate the transpiration rate. This response occurs when generative phase. In Figure 5, there was difference between the position of the leaves of crop stress and not stress. The crop control (irrigation interval 2 days) showed grew freshly and open wide the leaf surface, whereas in crop stress folding their leaves. Fukai & Cooper (1995) states that the plant will roll or fold their leaves to reduce absorption heat radiation and reduces transpiration through the leaf surface.

Figure 5. A. not stres (irrigation interval 2 days) B. folding leaves of soybean on water stress condition (irrigation intervals 5 and 10 days).

6. Adaptation Responses Influence of Production

Total Dry Matter and Seed Yield

Total dry matter is produced from the net photosynthesis. Water stress treatment at vegetative phase has not significant effect on the total dry matter (Figure 6A) and the seeds yield (Figure 6B). Meanwhile, giving stress on the generative phase affects the decrease in dry matter production and seeds yield. Dry matter and seeds yield of soybean decreased with increasing periods of water stress. Dering variety decreased production is greater than Argomulyo at the same stress conditions.

The low dry matter production at the stress conditions caused by the crop adaptation mechanism that reduced the number of leaves and leaf area so that the surface of photosynthesis is also reduced. The crops are also adapted by reduced stomatal openings and fold the leaves so that the exchange of CO_2 and H_2O in the leaves are obstructed. Mechanisms of crop adaptation to stress caused obstruction of the photosynthesis process and therefore contributes to the production of dry matter and seed yield of soybean. Based on Sopandie (2014), that the drought stress reduced the growth and photosynthesis. A decrease in photosynthesis in water stress conditions caused by stomatal closure and metabolic influences. Water deficit caused the closure of stomata which reduced the CO_2 consentration, whereas the dehydration of the leaf mesophyll cells can cause damage to photosynthesis organs. The tolerant crops are able to maintain their biological function in the low water potential condition although with limited growth.

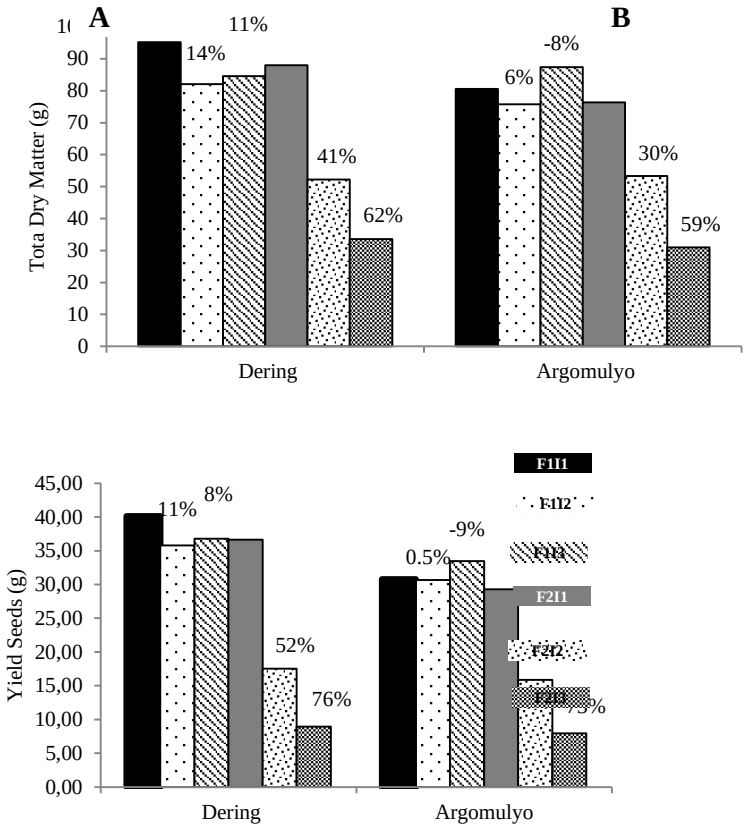

Figure 6. The total dry matter (A) and the seeds yield (B) of soybean plants on water stress condition. F1 = vegetative phase, F2 = generative phase; I1, I2, I3 = water stress by irrigation intervals of 2 days, 5 days, and 10 days. Numbers above diagram showed percentage reduction of dry matter and seeds yield by compared irrigation interval 2 days.

Harnowo (1992), stated that the water stress on reproductive phase inhibit distribution of assimilates to the reproductive parts, reducing the number of pods, seeds and seed weight per crop. Water stress also affects the decrease in the percentage of active roots, dry matter, number of leaves and pods, and plant height. The research also concluded that the stress will reduce leaf area, accelerates senescence of leaves, reduce the number of pods and seed yield per hectare. Drought stress at 50% under the condition of water available during the vegetative phase did not affect the yield. According to Pramono, Ratresni, Kamal, & Nurmauli. (1993), the effect of lack of water that occur in the generative phase is more significantly affect reducing the yield than it occur in the vegetative phase.

7. Water Use Efficiency (WUE)

Crop tolerant reduced the rate of water loss that improved the water use efficiency. Water stress affected of water use efficiency in both varieties (Figure 7). Water use efficiency decline with increasing water stress on Dering variety, whereas in Argomulyo variety have increased the efficiency. This indicates that the Argomulyo variety more efficient using water to form biomass. The value of water use efficiency for soybean based Steduto et al. (2012), is 1.2 to 1.6 kg/m^3.

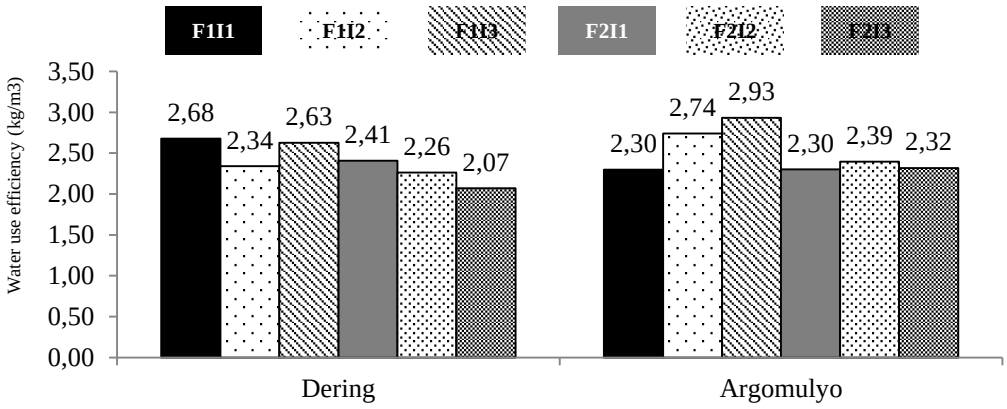

Figure 7. Water use efficiency of soybean on water stress conditions. F1 = vegetative phase, F2 = generative phase; I1, I2, I3 = water stress by irrigation intervals of 2 days, 5 days, and 10 days. Numbers above diagram showed the value of water use efficiency.

On the water stress conditions, water use efficiency were higher in genotype tolerant effect that genotypes is able to maintain their generative growth and suppress yield loss lower than the sensitive genotype (Efendi & Azrai 2008). Blum (2005); Sinaga (2008) stated that the reduction water requirement by crops is useful to increasing the water use efficiency.

8. Radiation Use Efficiency (RUE)

Water stress greatly affected the radiation use efficiency of soybean (Figure 8). The low value of the efficiency in the stress condition is caused by the reduction of photosynthesis rate due to lack of water and then it impacts on the crop growth. Rusmayadi, Handoko, Koesmaryono, Hadjar, (2008), stated that the effect of soil water deficit can be attributed directly to radiation use efficiency (RUE), crop growth and yield. Water deficit reduced the RUE due to the decrease of the photosynthesis activity. RUE measurements is helpful to understand the consequences of the drought stress for the crops and its variations based on the age. The low RUE on stress conditions caused by the interception of the radiation is strongly influenced by the structure of the canopy through the leaf area index and extinction coefficient of the canopy. Crop seized has a value of leaf area index were low because the crops folding the leaves and reduced leaf area. Salvagiotti & Miralles (2008) stated that crop production is determined by the partition and the accumulation of plants biomass. The process depends on the role of the canopy in the interception of radiation which is affected by leaf area index (LAI), the canopy structure and the process of converting the radiation into biomass accumulation. LAI decline effect on the radiation use efficiency because to the interception of the radiation is determined by LAI (Bonhomme, 2000).

RUE value based on various literatures are 1.32 - 2.52 g/MJ PAR (Sinclair & Muchow 1999), 2.04 g/MJ PAR (Singer, David, Thomas, John, & Jerry, 2010). In this reseach, the radiation use efficiency value with global radiation is quite big, it mean that the crop is very efficient in using the solar radiation to form dry matter. It is caused by the used of pots/polybags method give the optimum growing environment for the crops and minimizing the other factors other than water stress treatment. The used of pots also cause the crop competition with the surroundings can be reduced so that the crop can grow optimally with high production compared to the crops that are planted in field.

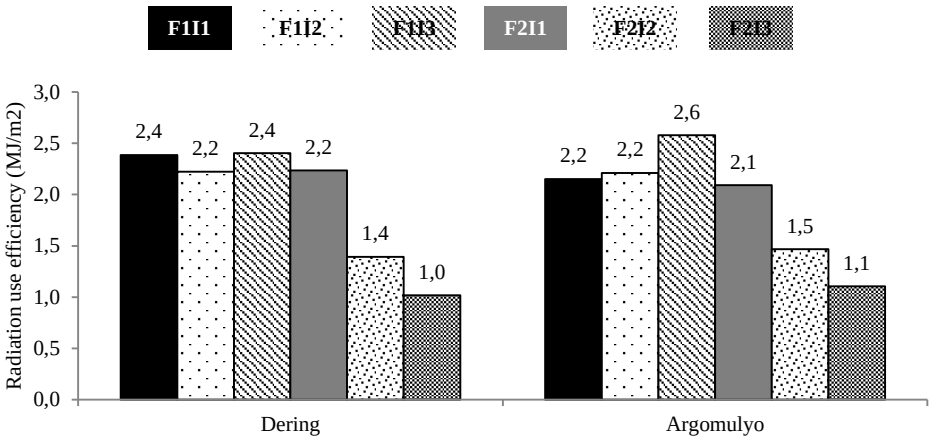

Figure 8. Radiation use efficiency of global radiation at water stress conditions. F1 = vegetative phase, F2 = generative phase; I1, I2, I3 = water stress by irrigation intervals of 2 days, 5 days, and 10 days. Numbers above diagram showed the value of radiation use efficiency.

D. Conclusion

Dering and Argomulyo adapted to the water stress by maintaining high water potential although they lack of water (dehydration avoidance) through the defoliating of leaves, leaf narrowing, reducing stomatal opening, and folding the leaves to reduced water loss through transpiration. Soybean adapted by recovery mechanism when water stress occur at vegetative phase so that it is did not significant effect on the reduce production. Based on the mechanism of adaptation both varieties it can be concluded that both soybean varieties tolerant to drought stress. Argomulyo is more tolerant because at the same water stress conditions decreased production less than Dering variety. It also improved water and radiation use efficiency on water stress conditions.

The selection of variety for cultivation in dry season is recommended to use Argomulyo because it is more adaptive and tolerant to water stress. But for cultivation in the normal condition, it is better to use dering variety because this variety have a higher production potential than Argomulyo.

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Land Use Potential on Water Balance Based on SWAT Method in Saddang Watershed in Bendung Benteng Irrigation System

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Abstract

The purpose of this study is how the Saddang watershed has the ability to store large amounts of water throughout the year. Saddang Watershed (DAS) as a large watershed in Sulawesi, rich in natural resource potential in the form of land, topography, slope, geology, soil, vegetation, climatology; rainfall, temperature, humidity, and sunshine. In maintaining and utilizing (watershed management) availability and water requirements for; humans, plants and animals. The availability and demand of water in watershed management requires the role of land in regulating agroclimatology and hydrology conditions. The water balance approach method used is SWAT (Soil and Water Assessment Tool) method of soil and water assessment tools, to determine the condition of availability and demand of water in an effort to maintain water flow conditions at all times (number and distribution) of Bendung Benteng irrigation system, which is capable of supply water for paddy field irrigation in two regencies of South Sulawesi's paddy granaries namely Pinrang Regency and Sidrap Regency. According to the Schmidth-Fergusson climate classification, the type of climate in Saddang watershed area belongs to type C climate = slightly wet area with tropical rainforest vegetation, the average amount of rainfall ranges from 2.155 mm/year. This indicates that there islarge level of rainfall every year and land use with a forest area of 676,39 or 26,41% of the watershed area, thus Saddang watershed is able to save tremendous amount of water supply. Based on the results of the water balance analysis using SWAT method, the amount of water available in the average watershed ; 3.133 mm year^{-1}, the amount of water being flowed ; 1.040,9 mm, and stored as ground water; 29,60 mm, as well as direct runoff ; 366,9 mmand flow coefficient of 0,45. Hence, there is 45% of the flow loss as surface stream and there is 55% of the flow stored in the watershed, and

the model application is categorized as good both in conducting simulations and validating the flow discharge on Saddang River. Watershed processing classified as having good watershed conditions, because one indicator of a watershed's water performance can be seen from the river dischargefluctuation. River discharge fluctuations can be seen from the river regression coefficient, which is a number that shows ratio between maximum discharge (Q_{max}) and minimum discharge (Q_{min}). The highest discharge (Q_{max}) was 30.805 m^3/sec while the lowest discharge (Qmin) was 994 m^3/sec. The regression coefficient value of Saddang River watershed was 26.650 m^3/sec.Based on the results of the 2017 data analysis, the condition of Saddang watershed provides surplus value of 1.911.986 (m^3 $year^{-1}$), out of the total water availability of 2.155.273 (m^3 $year^{-1}$) minus the total irrigation water requirement of 243.286,50 m^3 $year^{-1}$, with the pattern of planting paddy-paddy-secondary crops (palawija). Therefore, Saddang watershed has the ability to store large amounts of water throughout the year

Keywords: agricultural irrigation, SWAT, watershed management, water balance, water availability

A. Introduction

The potential that must be preserved and maintained in Saddang watershed management is how to maintain and manage the availability and water needs (number and distribution) can be available throughout the year by improving the management of land, forests and vegetation. Improving the land condition so that it does not cause a lot of surface flow, but increasing the potential of rainwater into the watershed as infiltration water, which is then stored as shallow ground water and ground water store.

Saddang watershed is a watershed that has a very wide catchment area, covering Mamuju Regency (West Sulawesi), Tanah Toraja Regency, and Enrekang Regency (South Sulawesi). Hence,Saddang watershed has very great contribution in utilizing the hydrological cycle/watershed cycle, and is able to maintain the availability and needs of water in land management, irrigation for agricultural irrigation which outlets are located in Bendung Bendeng, Pinrang Regency, and Sidrap Regency.

One method to determine the availability and needs of water of a watershed is to utilize SWAT model application, which allows a number of different physical processes to be simulated in a watershed. The utilization of SWAT model can identify, assess, evaluate the level of a watershed problem and as a tool to select management actions in controlling the problem. Thus, it is expected that SWAT model utilization can develop several scenarios to determine the conditions of the best watershed management planning.

The basis for selecting the research location in Saddang watershed is that the watershed is a catchment area of Bendung Benteng and its outlet includes center district of paddy granary in South Sulawesi, namely Pinrang Regency and Sidrap Regency.

This study aims to analyze the carrying capacity of Saddang catchment area for the water availability or water supply capacity, and the demand of water for agricultural irrigation (water demand capacity).

B. Literature Review

Land Use

Water and soil are very closely related, when rainwater reaches the surface of the earth, some will enter the soil (infiltration) to become part of groundwater (groundwater), while rainwater that is not absorbed by the soil will become a surface flow/run -off (Nugroho, 2015). The land needs to be well managed for development purposes, because of four things. First, land varies, both horizontally and vertically, and even between times. Second, the function of land in the context of space (and spatial distribution) is very large and touches almost all development activities in human life. Third, the body of the land and all the elements contained inside and above its surface constitute a single complex system, which is resource component (to be utilized), and an ecological/environmental component (to be conserved). Fourth, overall, the land area is fixed while the need for space/land increases in line with the increasing number of

human populations. Hence, good land use management is very necessary for the benefit of the current generation by not ignoring the interests of future generations (Baja, 2012).

Water Availability

The amount of water on earth is fixed, the changes experienced by water on earth only occur in the characteristic, shape and distribution. Water will always experience rotation and changes in shape during the hydrological cycle. The watershape changingprocess occurs with the heat of the sun during the day causing water on the earth's surface to experience evaporation and transpiration into water vapor. Water vapor will rise until it experiences condensation to form clouds. As a result of continuous cooling, the water droplets in the clouds become larger and eventually fall into precipitation. Furthermore, this rainwater will seep into the ground (become ground water) or flow into surface water which eventually forms rivers, lakes, or swamps (Pamungkas, 2012).

Water demand

The demand of water is the amount of water used for various purposes or community activities in the area. In this case the water demand that are taken into account are the water needs for paddy and secondary crops (palawija) irrigation.

Demand of irrigation water is calculated based on the area of irrigated paddy fields and analyzed by calculating 15 days Planting Pattern with the water balance method. Demand of irrigation water that are taken into account are surface irrigation water because on average farmers irrigate their paddy fields by using river water.

Water demand for river maintenance is estimated based on the multiplication of the population with the standard of water requirements for flushing (river maintenance).

According to Triatmodjo (2010), the demand of irrigation water is determined by the agricultural land area, the type of crop, the type of soil, agroclimatology and the planting pattern established, thus the water demand for irrigation can be calculated by the following equation;

$Qi = ((Etc + IR + RW + P - Re) / Ie) \times A$ ..... (1)

Description :

Qi = Demand of irrigation water (m^3),
Etc = Demand of plant consumption (mm/day) = Eto x K,
K = Plant coefficient,
Eto = Evapotranspiration (mm/day),
IR = Water demand for land preparation (mm/day),
RW = Demandof land processing (mm/day),
P = Loss due to percolation (5 mm/day),
Re = Effective rain (mm/day),
Ie = Irrigation efficiency,
A = Area of irrigation (m^2).

C. Methodology

This research analysis is based on GIS (Geographic Information System), analyzing spatial data using ArcView GIS 10.3 Software, while for non-spatial data is analyzed using Microsoft Excel. Spatial and non-spatial data processing were carried out to determine the availability and demand of water, project the data determining the availability and demand of water, and to determine the status of the carrying capacity of the land within Saddang watershed. SWAT methode is a model developed to predict the impact of agricultural land management on water, sedimentation and the amount of chemicals, in a complex area of the watershed taking into account variations in soil type, land use, and management conditions of a watershed after a long period.

The equipments used are; GPS, Softwere Arc Gis 10.3, GPS, Computers and Cameras. The material used is; land use map results of spot satellite interpretation 7.

Data analysis

Rainfall

Rainwater availability is calculated using Thiessen method. Thiessen Polygon Formation is done with the help of ArcView GIS 10.3 Software. Thiessen's coefficient is the percentage of each polygon area, which according to Soemarto (1999), the average area rainfall can be determined from the multiplication of the area of each polygon with the depth of rain on the station in the polygon (Equation 1).

$$P = \frac{P1A1 + P1A2 + P3A3 + ... + PnAn}{A1 + A2 + A3 + ... + An} \quad 1)$$

description;

P1, P2, P3, ... Pn = rainfall recorded in the rain detector post 1, 2, ..., n.
A, A, ... A = area of polygon 1, 2, ..., n, and
n = the number of rainfall checkpoints, to determine rainwater volume in each area in the Sub-watershed by multiplying the value of the average rainfall in the area with watershed area.

River Flow Discharge

The availability of river water for water utilization is determined based on the availability of the mainstay river flow discharge. The procedure for calculating mainstay discharge availability is conducted with Basic Month Planning Method. River water availability is determined based on discharge with 80% reliability (Sosrodarsono & Takeda, 2006).

Land Carrying Capacity

Determination of land carrying capacity status based on the ratio can be determined after the amount of water availability and water demand at the research location is known.

Criteria for the status of land carrying capacity based on the water balance are not enough to be stated with a surplus or deficit. But to show the relative magnitude, it is also necessary to state the value of supply / demand (Admadhani, *et al.* 2014).

Supply shows the amount of water availability in the region, namely the amount of water availability from the area's average rainfall volume and river flow discharge with 80% reliability, while demand shows the amount of water demand based on the determinants of water demand at the research site. Rustiadi, *et al.* (2010), stated that for Supply Demand Ratio> 2, the status of environmental carrying capacity is included in the safe category, while for the ratio between 1-2 is included in the conditional safe category, and for the ratio <1 is included in the unsafe category (land carrying capacity has been exceeded).

Irrigation water needs

The need for irrigation water is determined by the size of the agricultural land, the type of plant, the type of soil, agroclimatology and the planting pattern determined, so that the water needs for irrigation are calculated by the following equation:

$$Qi = ((Etc + IR + RW + P - Re) / Ie) \times A \quad .....2)$$

Description :
Qi = Demand of irrigation water (m^3),
Etc = Demand of plant consumption (mm/day) = Eto x K,
K = Plant coefficient,
Eto = Evapotranspiration (mm/day),
IR = Water demand for land preparation (mm/day),
RW = Demand of land processing (mm/day),
P = Loss due to percolation (5 mm/day),
Re = Effective rain (mm/day),
Ie = Irrigation efficiency,
A = Area of irrigation (m^2).

D. Result and Disscussion

The Schmidth-Fergusson climate classification system is based on rainfall events, namely monthly rainfall. Wet month rainfall (BB = CH> 100 mm/month, and dry month rainfall (BK = CH <60 mm/month), and rainfall with humid months is the month with rainfall between 60 mm to 100 mm/month. The results of the analysis show that the type of climate in Saddang watershed area belongs to type C climate = slightly wet area with tropical rainforest vegetation, where the average number of dry months rainfall is divided by the average number of wet months ranging from 0,85%, with the average rainfall is 2.155 mm/year.The data shows that with a large level of rainfall each year and land use with a forest area of 676,39 or 26,41% of the watershed area, Saddang watershed is able to save sufficient water availability The highest monthly average rainfall occurs in April while the lowest rainfall in August with the total rainfall of each are 578 mm and 181 mm. Annual rainfall in Saddang watershed varies greatly during the period 2007-2010, the highest amount of rainfall occurred in 2010 amounting to 3.213 mm, while the lowest amount in 2014 was 1,381 mm.

The steps taken in implementing SWAT model using ArcSWAT 10.3 software are watersheddelineation. The model automatically delines watersheds based on the DEM data entered. The method used in the delineation process is the threshold method. The size of the threshold entered will determine the number of river networks and sub-watersheds that will be generated. The topography and the area shape in Saddang watershed obtained from the slope class spatial data are automatically generated by SWAT in the DEM (Digital Elevation Model) process and analysis according to specified interval classes with 5 classes, namely 0-8% (flat), 8-15% (ramps), 15-25% (slightly steep), 25-40% (steep), and> 40% (very steep).

Table 1. Slope class of Saddang watershed

Slope Class (%)	Size of area	
	Ha	Percentage (%)
Flat (0-8)	78.964	11.87
Ramps (8-15)	60.148	9.04
Slightly steep (15-25)	114.420	17.21
Steep (25-40)	195.665	29.42
Very steep (>40)	215.837	32.45
Total	665.035	100.00

Source : Digital Elevation Models (DEM) SRTM, USGS year 2010.

This indicates that the presence of forests in Saddang watershed is very important in the arrangement and control of the water system which includes the amount, distribution and time of water supply to support the irrigation system of Benteng Bendung. The higher the rainfall, the higher the erosion produced, as well as the greater sedimentation produced (Nugroho, 2015)

Model Validation

The use of particular model in a watershed must consider the validity factor, it is due to each watershed has different characteristics. Validation aims to make the model output used has similar results close to the output of the actual watershed. This is very necessary to asssess the model suitability in watershed hydrology analysis. Validation for Saddang watershed was carried out at one outlet in accordance with the available secondary data, namely Saddang River flow dischargedata obtained from Bendung Benteng River Area Central Office. The statistical criteria used in model validation include the coefficient of determination (R^2), and the Nash-Sutcliffe (NS) coefficient.

Validation for Saddang watershed uses flow discharge data in 2010. The outlet used for calibration in the model is adjusted to the SPAS location of Bendung Benteng which is an outlet located in the downstream of Saddang River. Validation results of the daily flow discharge on

Saddang River showed the coefficient of determination (R^2) of 0.58, while the calculation for the coefficient between model discharge and observation was 0.55.

From the simulation results show there is a positive relationship between the model flow and observation flow where the percentage difference in value is very small, which means that the model discharge is almost very close to the amount of observation discharge, and the model application is categorized as good in simulating the daily flow of Saddang River. The validation results of the daily flow of Saddang River can be seen in Figure 1 and Figure 2.

Land use in Saddang watershed area will affect watershed hydrology conditions such as increased peak discharge, surface flow coefficient, and surface flow volume (Hartanto, 2009; Lipu, 2010; Emilda, 2010). Changes in the land use of Saddang watershed in 2007 and 2010 affected the Dirrect Runoff values of 268.82 mm and 465.07 mm, respectively (Table 2).

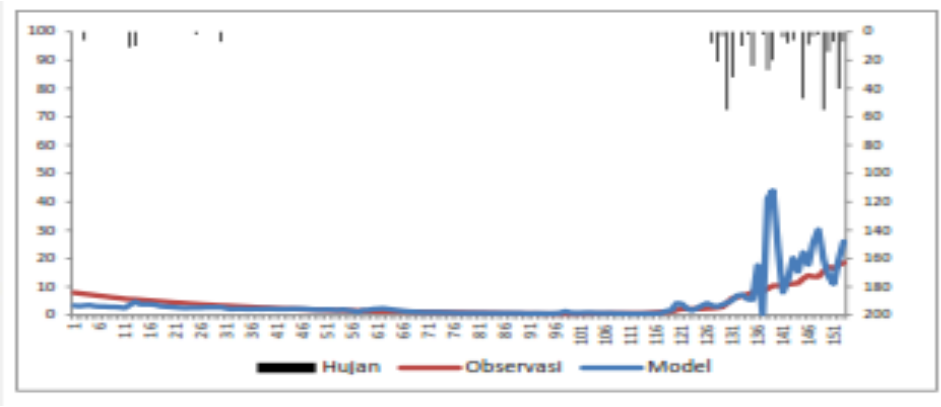

Figure 1. Validation Results of Debit Flow and Debit Model

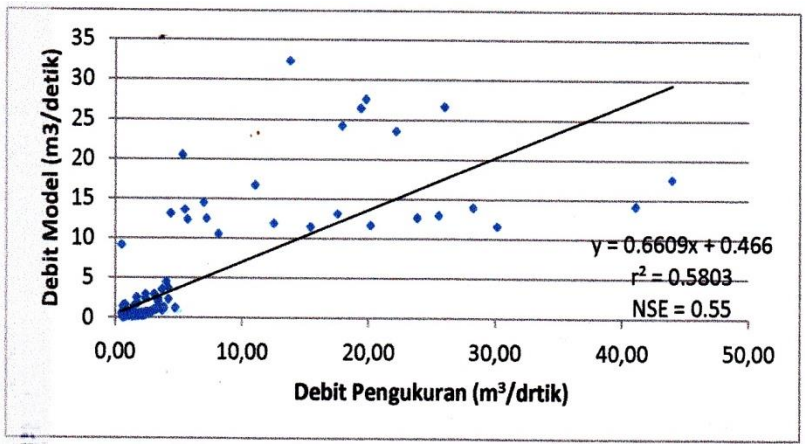

Figure 2. Regression Analysis and Validation with NS values: 0,55 and R^2: 0,58

Table 2. Effect of land use changes on water conditions of Saddang watershed

Land Use Year	Rainfall Rate (mm)	Number of streams (mm)	*Base Flow* (mm)	*Direct Runoff* (mm)	*Coefisien Runoff*
2007	3.053	596.42	18.48	268.82	0.28
2010	3.213	1.485.3	40.71	465.07	0.61

Source : SWAT Analysis 2017.

One indicator of performance, particularly the water system of a watershed, can be seen from the fluctuation of river discharge. River discharge fluctuations can be seen from the river regression coefficient, which is a number that shows a comparison between maximum discharge (Q_{max}) and minimum discharge (Q_{min}). The highest debit (Q_{max}) was 30.805 m^3/sec

while the lowest debit (Q_{min}) was 994 m^3 / sec. The regression coefficient value of Saddang watershed (KRS) 2010 value of 28,824 m^3/sec is classified as moderate or Saddang watershed condition is still good.

Table 3. Effect of land use changes on discharge fluctuations of Saddang watershed

Tahun	Q Max (m^3/detik)	QMin (m^3/detik)	KRS (Q Max/Q Min)
2007	30.805	3.708	8.308
2010	28.824	5.860	4.991

Source : SWAT Analysis 2017.

Water availability (water supply capacity)

According to the Schmidth-Fergusson classification of climate types, Saddang watershed area belongs to type C climate = the area is slightly wet with tropical rainforest vegetation, with an average rainfall of 2.155 mm/year. This indicates that there is large level of rainfall every year and land use with a forest area of 676,39 or 26,41% of the watershed area, thus Saddang watershed is able to save large amount of water supply capability.

Table 4. Calculation of Water Balance

Component	Number of units
Total Water Availability	2.155.273(m^3 $tahun^{-1}$)
Total Water Demand	243.286,50 m^3 $tahun^{-1}$
TotalSurplus	1.911.986 (m^3 $tahun^{-1}$)

Table 5. Land use of Saddang watershed

No.	Land use	Size of area	
		Ha	Percentage (%)
1.	Secondary Forest	465.372	69.97
2.	Forest	39.296	5.91
3.	Mix Garden	61.107	9.19
4.	Open Land	110.52	1.66
5.	Habitation	48.28	0.73
6.	Rice Field	272.74	4.10
7.	Shrubs	289.92	4.36
8.	Fishpond	48.69	0.73
9.	Field	19.39	2.89
10.	Water body	30.45	0.46
Total of DAS Size		665.035	100.00

Source: Results of the 2013-2016 SPOT67 Satellite Imagery Data Interpretation.

Irrigation water demand include meeting the demand of water for agricultural purposes in general. In addition to fulfill the water demand in fields, it is also needed to meet the water demand oflivestock and fisheries. The demand of water for irrigation is estimated from the multiplication of the land size area that is flowed by irrigation needs.

According to Triatmodjo (2010), the demand of water for irrigation can be calculated by the following equation;

Qi = ((Etc + IR + RW + P - Re) / Ie) x A …… .2)

Description :

Qi = Demand of irrigation water (m^3),
Etc = Demand of plant consumption (mm/day) = Eto x K,
K = Plant coefficient,
Eto = Evapotranspiration (mm/day),
IR = Water demand for land preparation (mm/day),
RW = Demand of land processing (mm/day),
P = Loss due to percolation (5 mm/day),

Re = Effective rain (mm/day),
Ie = Irrigation efficiency,
A = Area of irrigation (m^2).

Based on the results of the analysis obtained, the value of Etc was 6 mm/day, the value of the plant coefficient = 4.While evapotranspiration value was 10 mm/day. The water demand for land preparation (IR) = 4,17 mm/day, while the water demand for processing (RW) = 35,34 mm/day. Loss due to percolation (P) = 5 mm/day, effective rain (Re) = 13,93 mm/day), irrigation efficiency = 60%, area of irrigation (A) = 6.650,48 km^2. Hence, the total value of demand of irrigation water (Qi) of Saddang watershed was 2423.286,50 m^3 $year^{-1}$.

E. Conclusion

1. The large level of rainfall every year and land use with a forest area of 676,39 or 26,41% of the watershed area, thus Saddang watershed is able to save a large enough water supply.
2. There is 45% of the flow loss as a surface flow and there is 55% of the flow stored in the watershed, and the model application is categorized as good in conducting simulation and validation of the flow discharge on Saddang River.
3. The fluctuations in the flow discharge can be seen from the value of the river regression value, the number which shows the rasio between the maximum discharge (Q_{max}) and the minimum discharge (Q_{min}).
4. Based on the results of the 2017 data analysis, the condition of Saddang watershed provides a surplus value of 1.911.986 (m^3 $year^{-1}$), out of the total water availability of 2.155.273 (m^3 $years^{-1}$) minus the total demand of irrigation water of 243,286.50 m^3 $years^{-1}$, with the pattern of planting paddy-paddy-secondary crops.

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Diallel Analysis of Chili Pepper Resistance to Melon Aphid (*Aphis gossypii* Glover) Infestation in Seedling Phase

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Abstract

Aphis gossypii Glover is one of important insect pest in Indonesia. Genetic analysis of resistance to *A. gossypii* is required in plant breeding program to obtain host-plant resistance cultivar. Diallel analysis was used to estimate genetic parameters for chili pepper resistance to *A. gossypii* infestation in early generation. The objective of this research was to estimate genetic parameters of chili pepper resistance to A*. gossypii* infestation with diallel crossing design. The F1 and parent plants were arranged in randomized competed block design with three replication. Resistance lines was measured using choice test laboratory screening techniques. Two aphids were infested per plant and stopped 12 days after first infestation. Different lines respond was detected as shown by significant numbers of aphid per leaf, total aphid per plant, and total winged aphid per plant. There were no maternal effect and resistance were controlled by recessive and polygenic genes. Gene effects for resistance to aphid's infestation were additive and dominance. Dominance effect larger than additive effects. Broad-sense heritability values were high but narrow-sense heritability values were very low

Keywords: additive, dominance, half diallel, heritability, recessive

A. Introduction

The cultivation of chili pepper in lowland area has many obstacle such as high insect pest infestation. Melon aphid, *Aphis gossypii*, is one of the most important insect pest in low altitude and humid areas (Messelink, Bloemhard, Sabelis, & Janssen, 2013) and estimated can reduce ranged from 56 to 65% of chili pepper yields when no insectisides are taken (Fereres, Avilla, Collar, Duque, Fernández-Quintanilla, 1996). Cultural practices such as crop rotation, field sanitary, and delayed planting dates, are not always effective to control this pest. The development of host plant resistant remains the most effective and reliable management strategy against insect pests and may also increase the suppression of the pest development in combination with biological control (Maharijaya & Vosman, 2015).

Genetic information on resistance to melon aphid infestation in chili pepper is required to obtain high-yielding varieties with melon aphid-resistant and it can be done by estimation of genetic parameters. One of the methods used for genetic parameter estimation is the diallel cross analysis (Syukur, Sujiprihati, Koswara, & Widodo, 2013). The diallel cross method is known as a systematic and defensive genetic evaluation in view of the potential for crossing in the early generations (Johnson, 1963). Diallel mating design have been used primarily to estimate genetic variances when parents are either random individuals or inbred line from a random-mating population in linkage equilibrium (Hakizimana, Ibrahim, Langham, Haley, & Rudd, 2004).

Diallel mating design has an advantages to estimate the general combining ability (GCA), specific combining ability (SCA), additive and dominance effects, genetic variance and heritability (Roy, 2000). On the other hand, Combining ability can be analyzed by Griffing method (Griffing, 1956), while genes action, genetic component and heritability estimated by Hayman method (Hayman, 1954). GCA being a measure of additive gene action while SCA is due to non-additive (dominant or epistatic) gene action (Nsabiyera, Ssemakula, Sseruwagi, Ojiewo, & Gibson, 2013). Diallel crossing design has used in many plant species such as chili pepper (do Reˆgo, do Reˆgo, Finger, Cruz, & Casali, 2009; Daryanto, Sujiprihati, Syukur, 2010; Sitaresmi, Sujiprihati, Syukur, 2010; Syukur *et al*., 2013; Nsabiyera *et al*., 2013; Ganefianti, Hidayat, Syukur, 2015), papaya (Hafsah, Sastrosumarjo, Sujiprihati, Sobir, & Hidayat, 2007), maize (Vivek, Odongo, Njuguna, Imanywoha, Bigirwa, Diallo, & Pixley, 2010), tomato (Elsayed Ay, Henriques, Mizbuti, & Carneiro 2011; Saleem, Asghar, Iqbal, Rahman, & Akram, 2013) and wheat (Hakizimana, Ibrahim, Langham, Haley, & Rudd, 2004; Malla, Ibrahim, & Glover, 2009).

Diallel crosses have been used extensively to study the genetics of disease resistance in chili pepper such as chili resistance against Cercospora (Nsabiyera *et al*., 2013), Anthracnose (Syukur *et al*. 2013), and Begomovirus resistance (Ganefianti *et al*. 2015) but it has not been done on the evaluation of pest resistance in chili pepper. Evaluation of Alfafa resistance to *Acyrthosiphon pisum* with diallel crossing was reported by Bournoville, Carre, Julier, Landre, & Ecalle (2001) and the resistance of maize to the *Busseola fusca* reported by Beyene, Mugo, Gakunga, Karaya, Mutinda, Tefere, Njoka, Chepkesis, Shuma, & Tende, (2011).

This article analyzed gene's action, genetic components, combining ability, and heritability. of chili pepper plant resistance to aphid infestation. This study was to obtain information about the genetic parameter of chili pepper resistance to aphid infestation using half diallel analysis to found out host-plant resistence genotype. Host-plant resistance genotypes can be useful for keeping number of aphid population under economically damage level.

B. Methodology

Plant materials: The plant material used in this study was five lines genotypes which selected from 21 genotypes of chili pepper (*Capsicum annuum* L.) from previous study (Daryanto, Syukur, Maharijaya, & Hidayat, 2017). Genotype IPB 20 selected as low aphid infestation whereas IPB C3, IPB C4, IPB C5, and IPB C313 as high aphid infestation. They are collection of Bogor Agricultural University. These lines were crossed in all possible combination without reciprocals (half diallel). The plants were grown from seeds in plastic tray with 50 holes and placed in insect-tight box. The seeds were sowed on each holes of plastic tray containing a mix of growing medium (coco peat: soil: green manure; 1:1:1 v) and did not use insecticide during this experiment to avoid insecticide effects on the treatment.

Aphid colonies: Melon aphids were collected from pepper cultivation at Unifarm of Bogor Agricultural University, Indonesia followed by the identification of the species to ensure that the aphid colonies were A. gossypii Glover. The specific identification keys for A gossypii were the black cornicles, cauda lighter than cornicle, and the antennal tubercles were weakly developed.

The identification was based on the identification key guides of Blackman & Eastop (2014). Imago were cultured on susceptible pepper plants and propagated in insect-tight box, temperature of 28 ± 2oC; RH 65 ± 10% (Daryanto *et al*., 2017). Routine maintenance by moving the adult aphids to susceptible pepper plants were done when the aphid population had seen too crowded.

Choice test: Five parents and 10 F1 were conducted during the seedling phase of pepper (4-6 leaves or 5 weeks after sowing), in an insect box. Two adult wingless-aphids (apterous) were transferred with a soft brush to the leaves of the seedlings. Aphids were allowed to migrate, feed, and reproduce freely. A chili pepper resistance test to aphid infestation was conducted in Plant Breeding Laboratory, Department of Agronomy and Horticulture, Bogor Agricultural University. The experiment was designed in a randomized complete block design with pepper genotypes (five parents and 10 F1) as treatment with three replications. Observation was done at 12 days after infestation by counting the number of aphids on each genotype.

Statistical analysis: Normality test and Bartlett's test at 5% level of significance were done to meet the assumption εij ~ N (0, 2); error normal spread, the mean μ, and variance homogeneous. Furthermore, the data were tested by ANOVA (F-test), when the treatments significantly difference, genetic parameter estimation was continued using two approaches, the Hayman and Griffing methods (Singh & Chaudhary, 1979).

C. Result and Discussion

Maternal effect test (female parent) was done by comparing of morphological character and aphid's infestation on chili pepper genotype. Maternal effect used for early clarification of diallel method which need to involve reciprocal genotype (F1R) or not. The test results showed F1 population was not significantly different to F1R (Table 1 & Table 2). It's mean that the phenotype and aphid's infestation respon of F1 and F1R chili pepper genotypes was relatively similar. The absence of maternal effects indicated that the resistance of chili pepper to aphid infestation are controlled by genes in the nucleus.

The absence of maternal effects on chili pepper meet with assumption of no reciprocal effect occur, so analysis can be done by methods 2-Griffing (Singh & Chaudhary, 1979) and Hayman analysis (1954). Method 2-Griffing or half diallel is using population F1 and parents without involving F1R. The advantage of this method is result of predicting genetic parameters and combining ability as well as Method 1-Griffing, while the population used is less. Griffing's 2 method has been used in the estimation of corn resistance against stemborer (Beyene *et al*. 2011), phytopthora blight in tomatoes (Elsayed *et al*., 2011), and spotting Cercospora in chili pepper (Nsabiyera *et al*., 2013).

Table 1. Mean and homogenity of seedling leaf morphology in F_1 and F_{1R} chili pepper crossing of IPB C20 x IPB C333

Character	Genotype	Mean	Anova test	t-student [1)]
Leaf width	F_1	1.87	0.67 ns	1.34 ns
	F_{1R}	1.94		
Leaf lenght	F_1	3.88	1.35 ns	1.93 ns
	F_{1R}	4.22		

[1)] ns: not significantly different at P < 0.05

Table 2. Mean and homogenity of aphid infestation in F_1 and F_{1R} chili pepper crossing of IPB C20 x IPB C333

Aphid infestation	Genotype	Mean	Anova Test	t-student [1)]
Aphid per leaf	F_1	43.9	0.73 ns	0.38 ns
	F_{1R}	40.9		
Aphid per plant	F_1	124.0	0.39 ns	0.52 ns
	F_{1R}	142.5		

[1)] ns: not significantly different at P < 0.05

The ANOVA showed significant variation among genotypes based on analysis of variance (F-test) of melon aphid infestation on chili pepper genotypes (Table 3). Therefore, genetic parameter estimation using the diallel cross analysis can be determined for all characters.

Table 3. Analysis of variance (mean square) of melon aphid infestation on chili pepper genotypes

Aphid infestation character	Mean square [1)]
Aphid per plant	16.712**
Aphid per leaf	1.925 **
Winged aphid	0.629 **

[1)] **: significantly different at $P < 0.01$

The gene interaction can be seen from b regression coefficient (Wr, Vr) on Table 4. The result of b value for all characters were not distinct to one. Therefore, no gene interaction occurs in determining resistance to melon aphid infestation on chili pepper half diallel population. The result showed that chili pepper resistance to melon aphid infestation was caused by allelic gene that was one of diallel cross analysis assumption.

The non-additive (H1) showed dominant effect because regression coefficient (b value) known no epistasis effect. The addictive effect was showed only on winged aphid (0.30) but it was still lower than its dominance value (0.56). This suggested that the melon aphid infestation in chili pepper plants was influenced by action of dominant gene rather than additive gene. This result was consistent with the evaluation of genetic inheritance of chili pepper resistance to melon aphid infestation on six generation population (Daryanto *et al*., 2017).

The gene distribution on parents can be seen from H2 value. The genes that determine the ability of melon aphid infestation on chili pepper spread evenly within the parents on the total aphid per plant. This was reflected from not significant value of H2. This situation is in accordance with the term of the diallel analysis where the genes incorporated in the parent (Roy, 2000). Meanwhile, aphid infestation per leaf and winged aphid did not spread evenly within the parents which showed significant value of H2. Positive genes proportion will be apparent from comparison of H1 to H2 value. If value of H1 > H2, most genes were positive; on contrary, if H1 < H2 negative genes were more than the positive ones. Most genes determining susceptible to melon aphid infestation were positive gene.

Table 4. Estimation of genetic parameter of chili pepper to melon aphid infestation using the Hayman method of diallel analysis.

Genetic parameter	Aphid per plant	Aphid per leaf	Winged aphid[1)]
b (Wr, Vr)	-0.74 ns	-0.52 ns	0.92 ns
D	3.43 ns	0.23 ns	0.30 **
F	7.34 ns	0.46 ns	0.28 ns
H_1	25.93 *	2.81 **	0.56 **
H_2	22.00 ns	2.62 **	0.40 **
h^2	0.92 ns	-0.01 ns	0.02 ns
E	1.68 ns	0.19 ns	0.09 **
$(H_1/D)^{1/2}$	2.75	3.49	1.38
$H_2/4H_1$	0.21	0.23	0.18
Kd/Kr	2.27	1.80	2.06
h^2/H_2	0.04	-0.01	0.04
h^2bs (%)	76.63	77.39	67.63
h^2ns (%)	0.11	-2.18	31.78

[1)] b (Wr, Vr): Covariance-variance regression coefficient, D: Additive effect, F: Fr mean, H_1: Dominance effect, H_2: Proportion of dominance due to positive and negative effect of genes, h^2: F1 deviation from the average parent, E: Environment effect, $(H_1/D)^{1/2}$: Mean degree of dominance, $H_2/4H_1$ Proportion of dominance genes to recessive genes, Kd/Kr: The proportion of dominant to recessive genes, h^2/H_2: Number of groups of genes. h^2bs: Heritability in broad-sense, h^2ns: Heritability in narrow-sense, ns: not significantly different at $P < 0.05$, *: significantly different at $P < 0.05$ **: significantly different at $P < 0.01$.

The value of (H1/D)1/2 was indicated dominance effect level. According to Hayman (1954), if value of (H1/D)1/2 was more than one that indicates over dominance, whereas the value of (H1/D)1/2 between zero and one, indicating partial dominance (partial dominance or partial recessive). (H1/D)1/2 value of melon aphid infestation on chili pepper was more than one (2.75, 3.49 and 1.38), indicating over dominance effect (Table 4). In line with the results of the study of inheritance of chili pepper resistance against infestation of A. gossypii using six generations population (Daryanto *et al*., 2017), that susceptible over dominance to resistance

effect. So the resistance of chili pepper to melon aphd was actually recessive. The evaluation of chili pepper crossing to Anthracnose (Syukur *et al*., 2013) and inbreed tomato line against Phytophthora blight (Elsayed *et al.,* 2011) were controlled by recessive genes.

The number of dominance genes in parent genotypes reflected from the value of Kd/Kr. The value of Kd/Kr > 1, indicating dominance gene numbers were large in parent. On the other hand, Kd/Kr < 1, parent contains high recessive genes (Singh & Chaundhary, 1979). All charachter showed Kd/Kr values > 1 (2.27, 1.80, and 2.06), indicating more dominance genes in parent genotypes. This can be explainded because only the IPB C20 as a genotypes that carried the resistance character with minor recessice gene while the other four parent genotypes carried dominant genes.

Resistance to infestation of melon aphid was controlled by recessive genes. Their numbers of gene were reflected in the value of (h^2/H^2). The number of genes controlling resistance to infestation of melon aphid was one controlling groups (Table 4). The resistance estimation of broad-sense heritability ($h_2$bs) to three characters of melon aphid infestation in chili pepper genotypes were high with value 76.63, 77.39, and 63.67 but narrow sense heritability (h^2ns) was very low (Table 5). The high value of broad-sense heritability (h^2bs) have been able to explain well the genetic proportions of phenotypic-observed. However, the small value of narrow-sense heritability (h^2ns) showed that the proportion of the dominant (non-additive) genetic variation was greater than additives on the aphid infestation per plant and aphid per leaf. Daryanto *et al.* (2017) reported similar value of heritability of aphid infestation in chili pepper through a population of six generations. Non-additive gene action was reported to be characteristic on chili pepper resistance against spotting of Cercospores and bacteria (Nsabiyera *et al*., 2013).

Table 5. Variance analysis of general combining ability (GCA) and specific combining ability (SCA) of chili pepper genotypes to melon aphid infestation.

Source	df	Mean squares [1)]		
		Aphid per plant	Aphid per leaf	Winged aphid
GCA	4	1.651 ^{ns}	0.169 ^{ns}	4.620 **
SCA	10	7.148 **	0.803 **	2.187 ^{ns}
Error	28	1.644	0.178	0.083

[1)] ns: not significantly different at P < 0.05 **: significantly different at P < 0.01

Table 6. General combining ability (GCA) and spesific combining ability (SCA) values of chili pepper genotypes to melon aphid infestation.

Genotype	Aphid per plant	Aphid per leaf	Winged aphid
IPB C3	-0.150	0.108	-0.154
IPB C4	-0.501	-0.168	-0.135
IPB C5	0.045	-0.008	-0.010
IPB C20	-0.188	-0.130	-0.107
IPB C313	0.795	0.199	0.406
IPB C3 x IPB C4	-2.606	-0.958	-0.365
IPB C3 x IPB C5	-0.604	-0.413	0.172
IPB C3 x IPB C20	0.407	0.322	0.071
IPB C3 x IPB C313	1.137	0.714	-0.130
IPB C5 x IPB C4	3.819	1.361	0.108
IPB C4 x IPB C20	4.326	1.283	0.503
IPB C4 x IPB C313	-2.816	-0.854	-0.407
IPB C5 x IPB C20	0.093	-0.097	0.334
IPB C5 x IPB C313	1.770	0.539	0.654
IPB C20 x IPB C313	1.248	-0.978	-0.375

The selection of parents was based on high GCA values, because GCA represented the average appearance of a parent in all of its hybrid sets and was strongly associated with the action of the additive gene (Elsayed *et al*., 2011). The effect of GCA was not evident for aphid's infestation per

plant and aphid per leaf, while the winged aphid showed a very significant effect (Table 5). Generally, GCA value are indicated by large and positive values (Bournoville *et al*., 2001). However, on resistance characteristics such as to aphid infestations, the negative and large GCA values would contribute a high level of aphid resistance. Similarly reported, the negative GCA and SCA values contribute a high level of antracnose resistance in papaya (Hafsah *et al*., 2007) and high level of Wheat Steak Mosaic Virus resistence in winter wheat (Hakizimana *et al*., 2004)

Spesific combining ability (SCA) values were a reflection of the average appearance of hybrids, the crosses of two parents, strongly associated with non-additive i.e. dominant and epistasis gene effects (Elsayed *et al.,* 2011; Hakizimana *et al*., 2014). Highly significant difference were observed for SCA on character of aphid per plant and aphid per leaf while the infestation of winged aphid was not significantly different amongs hybrids (Table 5). The expected SCA value for aphid resistance character was negative as a contribution to the genotype resistance in suppressing the preferences and reproduction of melon aphid infestation in chili pepper plants. This value was in line with the low narrow sense of heritability, reflecting that the action of the dominant (non-additive) gene was more responsible for the character of aphid infestation in chili pepper plants.

The hybrid of IPB C4 x IPB C313 and IPB C3 x IPB C4 had high specific combining ability to the resistant of aphid per plant and per leaf i.e. -2.816, -2.606, -0.854 and -0.958 (Table 6). The genotypes of IPB C3 was susceptible genotype and IPB C4 was medium or low moderate category of aphid infestation in previous studies. IPB C4 was suspected has minor genes of resistance to aphid infestation so it was able to increase resistance level of IPBC3 and IPB C313 in hybrid form. IPB C4 had a good GCA on the character resistance to phytophthora blight (Yunianti, Sastrosumarjo, Sujiprihati, Surahman, & Hidayat, 2011). Performance of hybrids crossed by IPB C20 did not appear to have good SCA. It showed that aphid infestation resistant of IPB C20 controlled by recessive gene.

IPB C20 consistantly evaluated as a resistance genotypes to aphid infestation with negative GCA on all aphid infesation character, while IPB C313 consistantly susceptible to aphid infestation with positif GCA. Similar result, antixenosis test, that IPB C20 showed significantly reduced development and reproduction of melon aphid infestation and IPB C313 as suceptible genotype (Daryanto *et al*., 2017). Daryanto et al. (2010) stated that IPB C20 was not a good GCA for fruit character and chili production because IPB C20 is an ornamental chili pepper type with small fruit. Similar result was also found in the evaluation of the combined ability of corn resistance against corn stalk borer, stem borers, in which resistant maize genotype did not have a good GCA in production characters (Beyene *et al*., 2011). Futher, breeding program needs to create good ideotype on both consumption and resistance character to melon aphid.

These combining ability information indicated that resistance genes were still scattered outside of the IPB C20, so it needs to be assembled into a genotype. Convergent breeding methods can be applied to collect these minor resistance genes. One method of convergent breeding i.e, transgressive recombination can be used to collect genes that are dispersed into a genotype. This method is not much different from the pyramiding gene method for transferring some specific genes into a plant (Acquaah, 2011).

This research was the initial information about the chili pepper genetic resistance parameters of the melon aphid infestation. The experiments focused on the use of various types of chili pepper populations, Capsicum annuum species, in identifying the resistance of chilli peppers to aphid infestations. The expected output was breeding line that could be used as a resistant donor line and susceptible check for the development of chili pepper varieties.

D. Conclusion

There was no interaction between the non-allelic (epistatic) genes in the resistance of aphid infestations in chili pepper. The influence of dominance was significant on the character of aphid infestation while the additive effect was not significant. The genes that determine the character of aphid infestations dispersal evenly within the chili pepper. The dominant level that occurs was over dominance of susceptible genotype to the resistant genotype and the dominant genes were more susceptible than the resistance genes within the dialel crosses. General combining ability of the winged aphid infestation character was significantly different amongs parent genotype and IPB C3, IPB C4, IPB C5, and IPB C20 effectively becoming good GCA in suppressing the formation of winged aphid.

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F. Author Contribution

Ady Daryanto: Designed and conducted the experiment. Muhamad Syukur, Awang Maharijaya, Purnama Hidayat, supervisor and edited the manuscript.

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Genetic Variability and Heritability of in vitro Chrysanthemum on Various Combination of Plant Growth Regulators

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Abstract

Heritability is a measure that describes the magnitude of a genetic influence on a character rather than an environmental factor. The higher the heritability value of a character, the higher the genetic variability. The aim of the study was to know the interaction between the combination of growth regulators and chrysanthemum varieties that gave the best chrysanthemum growth *in vitro* and analyzed the heritability of various chrysanthemum characters. The research was carried out at the *In vitro* Laboratory of Agronomy Department, Faculty of Agriculture, Hasanuddin University, Makassar from May to September 2016. The study used a separate plot design in the group. The main plot was a combination of growth regulator consisting of 200 ml Coconut Water, 0.5 ppm BAP + Coconut Water 100 ml, BAP 1 ppm + Coconut Water 100 ml, 2,4-D 0.5 ppm + Coconut Water 200 ml, 2,4-D 0.5 ppm + BAP 0.5 ppm + Coconut Water 100 ml, 2,4-D 0.5 ppm + BAP 1 ppm + Coconut Water 100 ml. The subplots included 8 varieties of chrysanthemum (Fiji Pink, Marimar, Pasopati, Salzieta, Solida Pelangi, Limeron, Arosuka Pelangi, Yellow Tumohon). The basic media used was MS medium. The results showed that adding 200 ml young coconut to the culture media gave the best in-vitro chrysanthemum growth in terms of shoot and root formation, shoot count, root number, and shoot height in all chrysanthemum varieties; as well as all observed characters have high heritability and genetic variability

Keywords: chrysanthemum, genetic variability, growth regulators, heritability, varieties

A. Introductio

Chrysanthemum is one of the most popular ornamental plants in Indonesia. Chrysanthemum species are characterized by being decorative with beautiful colorful flowers. It has medical benefits so it is usually included in traditional medicine; moreover, it produces material which can be used as an insecticide. Worldwide, chrysanthemum varieties are highly valued as cut flowers. In general, the demand for chrysanthemums has been increased over the last years because of the availability of alternative choices and varieties of chrysanthemum; each variety has a flower with specific characteristics in terms of shape, size, color and their distinctive aroma.

There is an increase in the total harvest of chrysanthemum flower cuts from 2011 till 2015; in the same time increase in production and productivity of chrysanthemum plants were achieved. Based on data obtained from agricultural department (2015) about the production rate of chrysanthemum in South Sulawesi, in 2011 it reached 51.557 stems, in 2012 it became 54.198 stems, in 2013 the production increased heavily to reach 565.956 stems, and in 2014 the total production increased to be 640.222 stems.

The continuous increase in demand for chrysanthemums flowers raises the necessity to strive for a better system of cultivation for achieving a higher productivity of flowers and high quality seeds. Cultivars revealed that conventional propagation method of chrysanthemums is not recommended because it is time consuming and the plant would be more susceptible to diseases and pests infestations.

Vegetative propagation of chrysanthemum is rarely done because of its heterozygous nature, so the progeny seeds will not be the same as the parent seeds. Another alternative method to get chrysanthemum propagation in high scale and short time is the in-vitro propagation method.

For optimum results, the basic media and growth regulator are very important factors (Purnamaningsih & Lestari, 1998). The combination of basic media and proper growth regulator will increase the activity of cell division organogenesis stage. Growth regulators include auxin and cytokinin groups. In general, auxin is used for induction of callus formation, suspension culture and roots through the stimulation of cell division and the elongation of cambium tissue (Pierik, 1987).

The combination of cytokines with auxin can stimulate the morphogenesis process in bud formation (Flick, Evans, & Sharp, 1993). In common rue plants, the formation of adventitious shoots from the stem can be obtained using the following media: MS + BA 1.5 mg/l + 2.4-D 0.3 mg/l (Lestari & Husni, 1997).

The usage of growth regulators in tissue culture depends on the desired purpose or direction of plant growth. The growth regulator BA (benzyl adenine) is widely used to stimulate shoot bud proliferation and multiplication because of its strong, effective activity in compare with other artificial growth regulators. The addition of complex organic supplements is very important in stimulating in-vitro explant growth. Coconut water is considered as a complete organic compound that contains a variety of important nutrients which help in the growth of plant cultures, it is expected that adding coconut water to the culture can be helpful in the in-vitro propagation of chrysanthemum (Yusnida, Syafii & Sutrisna, 2006). Kristina & Syahid (2012) revealed that every 1 liter of coconut water contains 273.62 mg ZPT kinetin (cytokinin) and several other minerals (Zaer & Mapes, l982).

Based on the abovementioned information, a study was conducted regarding the in-vitro growth of different types of chrysanthemum using different combinations of growth regulators. Through this research, it is expected to determine the proper combination of growth regulators for the tested chrysanthemum varieties regarding the production of high quality seeds.

B. Methodology

This research was carried out at the Plant Tissue Culture Laboratory, plant culture division, Faculty of Agriculture, Hasanuddin University, Makassar from May till September 2016.

The tools which were used in this research include analytical scale, culture bottles, autoclave, graduated beaker glass, measuring cylinders, erlenmeyer flasks, funnels, drop pipettes, wash

squeeze bottle, pH meters, electric heater, handsprayers, Laminar Air Flow Cabinet (LAFC), forceps, benzene, scalpel, scissors, petri dishes and matches.

Materials include MS media (Appendix Table 1), sucrose, agar-agar, coconut water, aquabides, 2,4 Dichlorophenoxacetic Acid (2,4 D), Benzyl Amino Purine (BAP), alcohol 96%, alcohol 70%, eight varieties of chrysanthemums explants, aluminum foil, rubber bands, plastic wrapping, spirtus lamp and tissue.

This research was carried out based on split plot design employed in a Randomized complete block design with the main plot composed of 6 combinations of media; MS + 200 ml Coconut Water, MS + BAP 0.5 ppm + 100 ml Coconut Water, MS + BAP 1 ppm + Coconut Water 100 ml, MS + 2,4-D 0, 5 ppm + 200 ml Coconut Water, MS + 2,4-D 0.5 ppm + BAP 0.5 ppm + 100 ml Coconut Water, MS + 2,4-D 0.5 ppm + BAP 1 ppm + Coconut Water 100 ml. While the sub-plots included 8 varieties of chrysanthemums Fiji Pink, Marimar, Pasopati, Salzieta, Solida Pelangi, Limeron, Arosuka Pelangi, Tumohon Kuning. The total treatment combinations were 48. Each treatment combination had 3 replications, each replication included 2 units so the total experimental unites was 288.

Preparation

All the dissection tools (forceps, scaples, scissors and knives), culture bottles and petri dishes, were washed with soap, then rinsed with running water and then autoclaved for sterilization at 121^0C and pressure of 17-20 psi for 1 hour.

Stock solutions of MS media (1000 ml) and hormone stock solutions (50 ml) (2, 4-D and BAP) were prepared. For For culture media preparation, stock MS media was mixed with other components including sucrose, agar-agar, 2,4 D and BAP hormones, and coconut water according to a predetermined concentration. The next step was to adjust the pH of the culture media using a pH meter until reaching 5.8. Then in the final, aqua bides was added to a total volume of 1000 ml. The media was heated over the electric heater meanwhile it was stirred up to near the boiling point 98^oC. After that the media was poured into the culture bottles (±20) each and covered with aluminum foil. After that, the media was sterilized in autoclave at a pressure of 15 psi for 10 minutes.

Incubation room was sterilized by cleaning and spraying formaldehyde into every corner of the room then the room temperature was adjusted at 24^0C. In addition, 40 watts of lighting was also provided.

Culturing

Plant culturing was carried out in Laminar Air Flow Cabinet (LAFC). Chrysanthemum explants were cut ± 2 cm above petri dish using culture scissors and forceps. Then the explants were carefully transferred using sterile forceps and cultured in each treatment media.

Incubation

Maintenance was carried out by spraying 70% alcohol in culture bottles to avoid contamination in each treatment. Spraying with alcohol was performed every day. The contaminated bottles were immediately removed and discarded from the incubation chamber.

Observed parameters

Shoot and root initiation in each treatment media. Its were determined by counting the days from culturing until the first appearance of the shoot. Total number of roots and shoots in each treatment media that ware calculated in the end of the experiment. Height of shoots (cm) in each treatment media that was measured from the base of the leaf till the final height of growth in the end of the experiment.

Data Analysis

Data obtained from the observations were analyzed using one way Anova employed in a Randomized complete block design (RCB). Significantly different variables were then analyzed using LSD analysis ($\alpha = 0.05$).

Heritability

According to Stansfield (1983) the predicted value of heritability and its criteria were calculated using the following formula:

$$h^2 = \frac{\sigma^2 g}{\sigma^2 p} \times 100\%$$

Heritability value criteria:

- $h^2 > 0.5$: high heritability
- $0.2 < h^2 < 0.5$: medium heritability
- $h^2 < 0.2$: low heritability

C. Result and Discussion

Analysis of variance showed that the interaction between varieties and planting media had a very significant effect on all observed parameters. Interactions that occur between varieties and media have an effect on the growth of explants.

1. Shoot Initiation

Table 1. Average of shooting initiation rate (days) in each treatment media and Chrysanthemum varieties

Varities	Media						NPv LSD
	M1	M2	M3	M4	M5	M6	
V1	7.67^{b}_{q}	4.75^{a}_{p}	7.67^{b}_{q}	9.00^{b}_{q}	8.08^{b}_{q}	7.33^{b}_{pq}	1.72
V2	5.75^{a}_{pq}	5.58^{a}_{p}	5.50^{a}_{pq}	6.42^{ab}_{p}	7.58^{b}_{pq}	6.42^{ab}_{p}	
V3	7.17^{ab}_{q}	8.58^{b}_{q}	6.50^{a}_{pq}	21.08^{c}_{s}	10.08^{b}_{r}	10.17^{b}_{q}	
V4	7.67^{ab}_{q}	7.50^{ab}_{q}	6.33^{a}_{pq}	6.33^{a}_{p}	7.92^{ab}_{q}	9.00^{b}_{q}	
V5	4.75^{a}_{p}	6.08^{ab}_{pq}	5.42^{a}_{p}	5.83^{ab}_{p}	6.08^{ab}_{p}	7.50^{b}_{pq}	
V6	6.17^{a}_{pq}	6.00^{a}_{pq}	6.08^{a}_{pq}	13.42^{b}_{r}	13.00^{b}_{r}	7.17^{a}_{p}	
V7	5.17^{a}_{p}	4.75^{a}_{p}	6.00^{ab}_{pq}	9.00^{c}_{q}	7.75^{bc}_{pq}	7.17^{b}_{p}	
V8	5.58^{a}_{pq}	7.42^{b}_{q}	7.17^{ab}_{q}	7.50^{b}_{pq}	8.67^{b}_{qr}	7.75^{b}_{pq}	
NPm LSD	1,72						

Note: Means in the same column (abc) with different super script and in the same row (pqr) with different sub script are significantly different ($P < 0.05$).

Results in table 1 revealed that there were interaction between the media type and the plant variety regarding the shoot initiation period as the following; there were an interaction between M1 media (MS+coconut water 200 ml) and V5 (Solida Pelangi) and V7 (Arosuka Pelangi) varieties, between M2 media (MS+ BAP 0,05 ppm+coconut water 100 ml) and V1 (Fiji Pink), V2 (Marimar), and V7 (Arosuka Pelangi) varieties, between M3 media (MS+BAP 1 ppm+coconut water 100 ml) and V5 (Solida Pelangi) variety, between M4 media (MS+2,4 D 0.05 ppm+coconut water 200 ml) and V4 (Salzieta) variety and between M6 media (MS+2,4 D 0.05 ppm+BAP 1 ppm+coconut water 100 ml) and V6 (Limeron) variety producing the fastest shoot initiation which were not significantly different from the interactions between M1 media with V2, V3, V4, V6, V8 varieties, between M2 media with V4, V5, V6 varieties, between M3 media with V2, V3, V4, V6, V7, V8 varieties, between M4 media with V2 and V5 varieties, between M5 media with V4 and V5 varieties, between M6 media with V2 variety but significantly different from other treatment interactions.

Results in table 2 revealed the presence of interaction between M1 media (MS + coconut water 200 ml) with V6 (Limeron) and V7 (Arosuka Pelangi) varieties, between M4 media (MS+2,4 D 0.05 ppm+coconut water 200 ml) with V3 (Pasopati) variety which were significantly different from other treatment interactions.

2. Roots Initiation

Table 2. Average of root initiation rate (days)

Varietes	Media						NPv LSD
	M1	M2	M3	M4	M5	M6	
V1	10.25^{a}_{qr}	90.00^{c}_{t}	90.00^{c}_{p}	27.67^{b}_{s}	90.00^{c}_{q}	90.00^{c}_{p}	2.42
V2	12.17^{a}_{r}	42.50^{c}_{s}	90.00^{d}_{p}	37.33^{b}_{u}	35.08^{b}_{p}	90.00^{d}_{p}	
V3	10.75^{a}_{qr}	90.00^{b}_{t}	90.00^{b}_{p}	11.17^{a}_{p}	90.00^{b}_{q}	90.00^{b}_{p}	
V4	9.50^{a}_{q}	22.67^{b}_{q}	90.00^{c}_{p}	23.58^{b}_{r}	90.00^{c}_{q}	90.00^{c}_{p}	
V5	9.67^{a}_{q}	90.00^{c}_{t}	90.00^{c}_{p}	31.08^{b}_{t}	90.00^{c}_{q}	90.00^{c}_{p}	
V6	5.08^{a}_{p}	10.58^{b}_{p}	90.00^{c}_{p}	8.75^{b}_{p}	90.00^{c}_{q}	90.00^{c}_{p}	
V7	5.83^{a}_{p}	37.67^{c}_{r}	90.00^{d}_{p}	19.08^{b}_{q}	90.00^{d}_{q}	90.00^{d}_{p}	
V8	12.42^{a}_{r}	90.00^{c}_{t}	90.00^{c}_{p}	27.50^{b}_{s}	90.00^{c}_{q}	90.00^{c}_{p}	
NPm LSD			2.33				

Note: -Means in the same column (abcd) with different super script and in the same row (pqrstu) with different sub script are significantly different (P < 0.05), Number 90 means there is no root formed.

3. Total number of roots

Table 3. Average of total number of roots

Varieties	Media						NPv LSD
	M1	M2	M3	M4	M5	M6	
V1	12.25^{a}_{q}	0.00^{c}_{s}	0.00^{c}_{p}	5.33^{b}_{r}	0.00^{c}_{q}	0.00^{c}_{p}	
V2	8.92^{a}_{s}	1.83^{c}_{r}	0.00^{d}_{p}	3.17^{b}_{s}	3.67^{b}_{p}	0.00^{d}_{p}	
V3	7.50^{a}_{t}	0.00^{c}_{s}	0.00^{c}_{p}	3.33^{b}_{s}	0.00^{c}_{q}	0.00^{c}_{p}	
V4	12.75^{a}_{q}	2.08^{c}_{r}	0.00^{d}_{p}	7.08^{b}_{p}	0.00^{d}_{q}	0.00^{d}_{p}	0.70
V5	11.17^{a}_{r}	0.00^{c}_{s}	0.00^{c}_{p}	3.75^{b}_{s}	0.00^{c}_{q}	0.00^{c}_{p}	
V6	10.50^{a}_{r}	7.42^{b}_{p}	0.00^{c}_{p}	6.17^{b}_{q}	0.00^{c}_{q}	0.00^{c}_{p}	
V7	14.17^{a}_{p}	3.00^{c}_{q}	0.00^{d}_{p}	5.08^{b}_{r}	0.00^{d}_{q}	0.00^{d}_{p}	
V8	12.33^{a}_{q}	0.00^{c}_{s}	0.00^{c}_{p}	7.17^{b}_{p}	0.00^{c}_{q}	0.00^{c}_{p}	
NPm LSD			0.68				

Note: - Means in the same column (abcd) with different super script and in the same row (pqrst) with different sub script are significantly different (P < 0.05)

Results in table 3 revealed the presence of interactions between M1 media (MS + coconut water 200 ml) and V7 (Arosuka Pelangi) variety producing the highest number of total roots that was significantly different from other treatment interactions.

4. Total number of shoots

Results in table 4 revealed interactions between M2 media (MS+BAP 0.05 ppm+coconut water 100 ml) and V3 (Pasopati) variety, between M3 media (MS+BAP 1 ppm+ coconut water 100 ml) and V2 (Marimar) variety, between M5 media (MS+ 2.4 D 0.05 ppm+ BAP 1

ppm+coconut water 100 ml) and V3 (Pasopati) variety producing the highest total number of shootings that were significantly different from other treatment interactions.

Table 4. Average of total number of shoots in each treatment media and Chrysanthemum varieties

Varieties	Media						NPv LSD
	M1	M2	M3	M4	M5	M6	
V1	1.58^{bc}_{p}	1.67^{b}_{p}	2.33^{a}_{q}	1.08^{c}_{q}	1.42^{bc}_{pq}	1.33^{c}_{p}	
V2	1.25^{c}_{q}	1.67^{b}_{p}	2.92^{a}_{p}	1.50^{bc}_{p}	1.67^{b}_{p}	1.17^{c}_{pq}	
V3	1.33^{b}_{pq}	1.83^{a}_{p}	1.83^{a}_{r}	1.00^{c}_{q}	1.67^{a}_{p}	1.17^{bc}_{pq}	
V4	1.42^{a}_{pq}	1.00^{b}_{q}	1.17^{ab}_{t}	1.00^{b}_{q}	1.00^{b}_{r}	1.08^{b}_{pq}	0.27
V5	1.00^{a}_{q}	1.17^{a}_{q}	1.00^{a}_{t}	1.00^{a}_{q}	1.00^{a}_{r}	1.00^{a}_{q}	
V6	1.42^{a}_{pq}	1.17^{ab}_{q}	1.25^{a}_{s}	1.00^{b}_{q}	1.33^{a}_{q}	1.00^{b}_{q}	
V7	1.00^{a}_{q}	1.00^{a}_{q}	1.00^{a}_{t}	1.00^{a}_{q}	1.08^{a}_{qr}	1.00^{a}_{q}	
V8	1.42^{a}_{pq}	1.00^{b}_{q}	1.00^{b}_{t}	0.83^{b}_{q}	1.00^{b}_{r}	1.00^{b}_{q}	
NPm LSD			0.26				

Note: - Means in the same column (abc) with different super script and in the same row (pqrst) with different sub script are significantly different (P < 0.05).

5. Shoot Length

Table 5. Average of final shoot length in each treatment media and Chrysanthemum varieties

Varieties	Media						NPv LSD
	M1	M2	M3	M4	M5	M6	
V1	19.67^{a}_{p}	3.45^{c}_{q}	2.83^{cd}_{q}	5.87^{b}_{q}	2.47^{d}_{pq}	2.88^{cd}_{p}	
V2	9.15^{a}_{s}	2.23^{c}_{rs}	2.09^{cd}_{r}	3.47^{b}_{s}	1.96^{cd}_{q}	1.46^{d}_{q}	
V3	11.42^{a}_{qr}	1.97^{c}_{s}	2.55^{bc}_{qr}	2.79^{b}_{t}	1.42^{cd}_{qr}	0.79^{d}_{r}	
V4	8.04^{a}_{t}	1.75^{d}_{s}	2.00^{d}_{r}	3.75^{b}_{s}	2.83^{c}_{p}	2.42^{cd}_{p}	0.65
V5	12.04^{a}_{q}	4.96^{c}_{p}	4.67^{c}_{p}	6.04^{b}_{q}	1.25^{d}_{r}	1.17^{d}_{qr}	
V6	7.29^{a}_{u}	4.83^{b}_{p}	2.38^{c}_{qr}	4.71^{b}_{r}	0.67^{d}_{r}	0.67^{d}_{r}	
V7	11.21^{a}_{r}	2.07^{c}_{rs}	1.82^{c}_{r}	3.67^{b}_{s}	0.88^{d}_{r}	0.75^{d}_{r}	
V8	8.19^{b}_{t}	2.67^{cd}_{r}	2.25^{d}_{qr}	8.93^{a}_{p}	1.88^{d}_{qr}	3.05^{c}_{p}	
NPm LSD			0.67				

Note: - Means in the same column (abcd) with different super script and in the same row (pqrst) with different sub script are significantly different (P < 0.05).

Results in table 5 revealed interaction between M1 media (MS+coconut water 200 ml) and V1 (Fiji Pink) variety, between M4 media (MS + 2.4 D 0.05 ppm +coconut water 200 ml) and V8 (Tumohon Kuning) variety producing the highest shoots which are significantly different from other treatment interactions.

6. Heritability

Table 6. Heritability value in each treatment media and Chrysanthemum varieties

No	Character	h^2(%) value	Degree
1	Shoot Initiation	91.10	High
2	Root Initiation	99.04	High
3	Total number of roots	93.29	High
4	Total number of shootings	88.70	High
5	Shooting length	95.69	High

Note: $0 < h^2 \leq$ (low), $21 < h^2 \leq 50$ (medium), $51 < h^2 \leq 100$ (high)

Results of heritability analysis in table 6 showed that all the evaluated 5 parameters have high heritability scores. The highest heritability score was revealed in the plantlet formation rate parameter which has heritability score equal to 99.26 %. Such results confirm that the influence of genes on chrysanthemum plants dominates when compared with the influence of environment.

D. Discussion

The research results showed that media without the addition of 2, 4-D (M1, M2, and M3) provide faster sprout growth in compare with adding 2, 4-D (M4, M5, and M6). The rapid growth of shoots in M1, M2 and M3 media were caused by the addition of coconut water and BAP as a cytokinin hormone which accelerated cell division and the growth of explants, and stimulated cytokinesis process that caused the cells to grow long and cell differentiation, such a differentiation make those cells to undergo functional specialization that initiate the organogenesis process and lead to buds/shoots formation (Suryowinoto, 1996).

The formation of shoots within short time leads to a fast shoot length, the highest shoot length produced in this investigation was found in pink Fiji variety which was cultured in M1 media that contain 200 m.L-1 (M1) (Table 5). It could be because the coconut water is a complex organic compound containing vitamins, ZPT, macro and micro nutrients, and high sugar content as an energy source for growth of shoots and leaves (Vigliar, Sdepanian, & Fagundes-Neto, 2006).

The highest shoot formation would lead to the formation of auxin in the tip of the shoot, then the produced auxin translocate to the base of stem, and causes auxin concentration to be increased until the stimulation of faster organogenesis process in the root area and the increased number of grown roots. In MS+air kelapa 200 $m.L^{-1}$ (M1) the growth rate was faster and the total productivity was more (Table 2 and 3). Such results are in line with the findings of Bey, Syafii, & Sutrisna, (2006), who revealed that adding coconut water at a concentration of 250 $m.L^{-1}$ gave faster orchid growth (*Phalaenopsis amabilis* BL.).

Nevertheless, the total number of shoots was higher in case of combining coconut water with air BAP in MS like in M3 media (Table 4). According to Parera (1997), adding 20% coconut water in the media of orchid plants lead to a significant increase in the total number of shootings if compared with other concentrations 30%, 40%, and 50%, but not significantly different with 10%.concentration. The higher the concentration of coconut water the less the number of shoots produced and this shows a quadratic tendency. Thus, decreasing the concentration of coconut water to 100 mL^{-1} combined with BAP 1 ppm gave the highest number of shoots in Fiji Pink (V1) and Marimar (V2) varieties. Such results were in agreement with Sompotan & Tilaar (2008) who revealed that adding BAP to MS media with concentration 1 - 5 ppm lead to the growth of new shoots. Moreover, Tilaar, Rantung, & Tulung (2013) had an investigation about in-vitro propagation of kulo chrysanthemum buds explants in media containing NAA and BAP, the highest number of new shoots (8.2 shoots) were observed when adding 1 ppm BAP.

The presence of hormones in the culture media is considered as an important factor that impact on the growth rate of explants. The in-vitro organogenesis and growth rate of the

explants are usually controlled by the balance and interaction of the plants' hormones (Kasli, 2009). Basri & Muslimin (2001) revealed that part of the hormone in the culture media will infiltrate the plant cell through diffusion or active absorption, that lead to the change in the internal hormone balance.

Such a change can be obviously revealed from the results of heritability of each evaluated variable. In this research, the results revealed that all the evaluated variables have high heritability score. That led to the assumption that the growth and organogenesis rates were mainly affected by the genetics (75.16% - 99.26%) rather than the environment. therefore, chrysanthemum has a high genetic variability and incase of performing genotypic selection, and better types could be improved. Rasyad (1996) suggested that the coefficient value of genetic variability was high, so genetic factors had a great influence on traits expression compared to the environment effects.

E. Conclusion

Based on the results obtained, then the conclusion is as follows:

1. The fastest in-vitro organogenesis of chrysanthemum Fiji Pink varieties could be achieved by using the following culture media formula; MS + 2.4-D 0.5 ppm + BAP 1 ppm + coconut water 100 ml (M6), callus formation occurred in just 11.5 days.
2. The best in-vitro organogenesis of chrysanthemum for shoot, root formation, total number of shoots, total number of roots, root length, shoot height, total number of leaves and plantlet formation could be achieved by using the following culture media; MS + coconut water 200 ml (M1).
3. All the observed variables have high heritability and genetic variability.

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Effectivity of Manure at Various Dose Added Agrobost to The Growth of Mango Seedlings

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Abstract

One of the efforts to increase mango production in Southeast Sulawesi is to produce high quality and high quantity mango seeds. Manure contains macro and micro nutrients that are important for plant growth and development as well as play a role in maintaining nutrient balance in the soil to improve soil properties. The aim of this study was to obtain the best manure doses applied with agrobost in improving the growth of Arumanis mango seedlings. This research was conducted in Kemaraya Sub-District of West Kendari District, Kendari City. The study design used was a randomized block design (RBD) with 6 treatments, namely: control (T_0), 100 g (T_1); 150 g (T_2); 200 g (T_3); 250 g (T_4); and 300 g (T_5) of manure per polybag. Each treatment was added with agrobost of 17 ml/polybag and replicated 5 times. The variables observed were plant height, stem diameter; number of leaves, and leaf area, at ages 1, 2, and 3 months after planting. The results showed that the manure added agrobost influenced the growth of Arumanis mango seedlings. The manure treatment with a dose of 150 g per polybag (T_2) was the best treatment in improving the growth of Arumanis mangoes at 1, 2 and 3 months after planting. This was indicated by the plant height of 28,40; 30,94; and 32,82 cm, stem diameter of 0,44; 0,70; and 0,99 cm; and number of leaves 6,60; 7,20 and 9,00 strands were significantly higher compared to other treatments during the study

Keywords: agrobost, arumanis seedlings, manggo, manure doses

A. Introduction

Mango plants (*Mangifera indica L.*) are annual fruit plants originating from India, then spread to Southeast Asia including Malaysia and Indonesia. Mango is a type of tropical fruit that is loved by the world community and is a trade commodity between countries. These horticultural commodity has good prospects if it is developed intensively and on an agribusiness scale. From year to year the demand for tropical fruit is increasing at home and abroad, both in the form of fresh and processed products.

Mango is very good to consume because the nutritional content is quite complete as a source of vitamins and minerals. Nutrients contained in Arumanis cultivar mangoes: calories, protein, fat, calcium, phosphorus, iron, vitamins A, C, B_1, and water (BPTP, 2010).

In Indonesia, mango is the second largest fruit commodity after bananas. Even Indonesia is the fourth largest mango producer in the world after India, China and Thailand with production of 2.4 million tons $year^{-1}$. This potency has still big opportunity to be developed because currently the planting area is only 165.000 ha, while the land area for the development of mango plants is still available (Hardiyanto, 2017). However, this plant can only produce well in areas that have certain dry season.

Southeast Sulawesi Province does not include as the mango production center in Indonesia. Southeast Sulawesi mango production is still low at 14.769 tons (BPS, 2014). The low production of mangoes in Southeast Sulawesi is caused by among others; mango cultivation is still a non-commercial plant, various varieties or cultivars, poor quality seeds, less intensive cultivation, cultivated in soil types dominated by soils that have developed further with low soil fertility conditions and inappropriate cultivation techniques application.

One of the efforts to increase mango production in Southeast Sulawesi is to produce high quality and high quantity mango seeds. Seeds are one of the determinants of successful plant cultivation. Plant cultivation has actually begun since the selection of plant seeds because the seeds are the main object that will be developed in the next cultivation process. In order to produce high quality mango seedlings in land classified as marginal soils with low soil fertility, it is necessary to add technological inputs in the form of manure added agrobost.

Manure is fertilizer originating among others from; cows, goats, and chickens, in the form of solid feces which mix with food waste or urine. Manure contains macro and micro nutrients that are important for plant growth and development as well as play a role in maintaining nutrient balance in the soil. While Agrobost fertilizer is a biological fertilizer which contains several types of important microbes that function to improve soil properties. These microbes include: *Azospirillum*, *Azotobacter*, phosphorus solvent microbes, *Lactobacillus*, cellulose degrading microbes, *Indole Acetic Acid* (IAA), and cellulase enzymes. These types of microbes and enzymes can work optimally in improving soil properties, hence savings in the utilization of chemical fertilizers (Hamzah, S., S. Utami & M.A. Cholik. 2011). Based on the benefits of the two fertilizers in supporting the availability of high quality mango seedlings, it is necessary to do research on the growth of mango seedlings with the treatment of manure added agrobost.

Objectives and Benefits

This study aims to determine the growth rate of mango seedlings in various doses of manure added agrobost. The results of this study are expected to be a reference for those who want to cultivate mango nurseries commercially, also expected to be a source of information and as a comparison for the further study.

B. Methodology

The study was conducted in Kemaraya, West Kendari District, Kendari City for 5 months. The materials used in this study were black polybag size 20 cm x 30 cm, Arumanis mango seedlings,

Ultisol soil from Nanga-Nanga, sand, rice hulls, manure, and Agrobost. The tools used were hoes, machetes, sand sieves (3 mm), scales, caliper, ruler, sprayer, string, camera and stationery.

The design used in this study was a randomized block design (RBD) with one treatment factor, namely manure doses added agrobost (17 ml per polybag). The dosage of manure (T) consists of 6 treatments, namely : without manure (T_0), 100 g (T1), 150 g (T_2), 200 g (T_3), 250 g (T_4), and 300 g (T_5) manure per polybag. Each treatment was replicated 5 times, so there were 30 experimental units.

Research Implementation

The land was cleaned and blended. The soil was mixed with sieved rice hulls and sand, then manure was added according to the treatment dose and put into each polybag. Mango seeds were selected based on relatively uniform seed height criteria. Seeds were planted as deep as 15 cm and 1 seed per polybag. Then proceed with the administration of agrobost (15 ml of agrobost dissolved in 500 ml of water) sprayed into the mango seedlings with dose of 17 ml per polybag each application. Plant maintenance in the form of watering and weed control. Watering was carried out every morning with a volume of 250 ml per polybag and weed control was done twice.

Components observed include soil and plants. Land variables include: Nitrogen-Total (H_2SO_4 wet ashing method), Phosphat-Available (Olsen method), and Potassiun-Available (25% HCl Method). Assessment criteria for the results of soil analysis (BPT, 2009 BPT) are VL = very low; L = low; M = medium; H = high and VH = very high. All plant populations were used as sample. Observations were made at 0, 1, 2, and 3 months after planting (MAT). Observation variables were : plant height (cm) is measured from the base of the stem to the edge of the growing point; stem diameter (cm) was measured on the lower stem about 5 cm from the ground using caliper; the number of leaves (strands) was calculated as the total leaf; leaf area (cm^2) by measuring the length and width of the leaf, leaf length (cm) was measured from the base of the leaf to the tip of the leaf, leaf width was measured on the widest width of the leaf. Leaf area is calculated by the formula:

Leaf area = leaf length x leaf width x constant (α = 0.6)

The constants obtained from sampling some leaves are then drawn on graph paper (Sitompul & Guritno, 1995).

Data were analyzed using variance, if F-count is greater than F-table, then proceed with least significance difference test (LSD) at the 95% confidence level.

C. Results and Discussion

1. Results

Soil Analysis

The results of soil analysis at the beginning of the study are presented in Table 1.

Table 1. Results of soil analysis at the beginning of the study

Treatment	N-total (%)	Phosphorus (P) (mg/100 g)	Potassium (K) (mg/100 g)
T_0	0,07 (VL)	10,83 (VL)	0,012 (VL)
T_1	1,00 (H)	64,17 (H)	0,525 (H)
T_2	0,53 (H)	21,25 (M)	0,451 (L)
T_3	0,13 (L)	25,83 (M)	0,390 (L)
T_4	0,40 (M)	11,33 (VL)	0,401 (L)
T_5	0,07 (VL)	14,50 (VL)	0,281 (L)

Description: VL = very low; L = low; M = medium; H = high and VH = very high

In Table 1, it can be seen that manure treatment can increase the levels of N-total, P-available and K-available, starting from very low to high for total N (0,07 – 1,00%) and P- available (10,83 – 64,17 mg/100 g), and from very low to high for K-available (0,012 - 0,525 mg/100 g).

Growth of Mango Seedlings

The results of observations and variance showed that manure added agrobost had a very significant effect on plant height, stem diameter, leaf number, and leaf area during the study except for the number of leaves significantly affected at 1BST. The recapitulation of the results of variance in all observation variables is presented in Table 2.

Table 2. Recapitulation of results of variance (anova) on effect of manure added agrobost to all variables of mango seedling observation

No.	Time of observation	Variables of observation	Description
1	1 BST	Plant height	**
		Stem diameter	**
		Number of leaves	*
		Leaf area	**
2	2 BST	Plant height	**
		Stem diameter	**
		Number of leaves	**
		Leaf area	**
3	3 BST	Plant height	**
		Stem diameter	**
		Number of leaves	**
		Leaf area	**

Description: * = significant effect,
** = very significant effect

Mango seedlings height

The results of variance showed that the dose of manure added agrobost had a very significant effect on the height of mango seedlings during the study. The avergae plant height at ages 1, 2, and 3 BST and the least significance difference test (LSD) results are presented in Table 3.

Table 3 Average height of mango seed (cm) at ages 1, 2, and 3 BST

Treatment	Average height of mango seedlings at x month observation time		
	1	2	3
T0	25,82 b	26,98 b	28,42 b
T1	24,46 b	26,06 b	27,60 b
T2	28,40 a	30,94 a	32,82a
T3	24,74 b	26, 34 b	27,60 b
T4	23,54 b	25,24 b	26,98 b
T5	24,20 b	25,58 b	26,62 b
LSD 5%	2,46	2,86	2,53

Description: Numbers followed by the same letter in the same column was not significantly different at the 95% confidence level

Based on further tests (Table 3), the best dosage of manure is T_2 treatment (150 g of manure + 17 ml of agrobost per polybag) both at age 1, 2, and 3 months after planting with average height of mango seedlings 28,40; 3,.94; and 32,82 cm, respectively. The height growth dynamics of mango seedlings are presented in Figure 1.

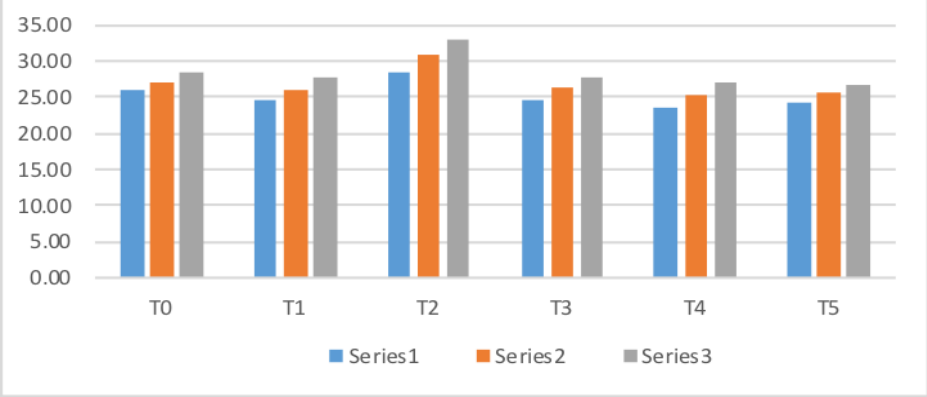

Figure 1. Height growth dynamics of mango seedlings at the age of 1,2 and 3 BST

Figure 1 shows that the average height of mango seedlings at observations 1, 2, and 3 months after planting was obtained at T_2 treatment followed by T_0, T_1, T_3, T_4, and T_5.

Stem diameter of mango seedlings

The results of variance showed that the dose of manure added agrobost had a very significant effect on the stem diameter of mango seedlings during the study. The average stem diameter of mango seedlings at ages 1, 2, and 3 BST and least significance difference test (LSD) results are presented in Table 4.

Table 4. Average stem diameter of mango seedlings (cm) at ages 1, 2, and 3 BST

Treatment	Average stem diameter of mango seedling at x month observation time		
	1	2	3
T_0	0,41 ab	0,53 bc	0,66 c
T_1	0,38 bc	0,60 b	0,74 bc
T_2	0,44 a	0,70 a	0,99 a
T_3	0,35 c	0,53 c	0,68 bc
T_4	0,35 c	0,59 bc	0,79 b
T_5	0,35 c	0,53 c	0,71 bc
LSD 5%	0,05	0,07	0,11

Description: Numbers followed by the same letter in the same column was not significantly different at the 95% confidence level

Based on further tests (Table 4), the best dosage of manure is T_2 treatment (150 g of manure + 17 ml of agrobost per polybag) both at 1, 2 and 3 months after planting with the average stem diameter of mango seedlings 0,44; 0,70; and 0,99 cm, respectively.

a. Number of leaves of mango seedlings

The results of variance showed that dosing of manure added agrobost effect was significant on the number of leaves of mango seedlings at 1 BST and very significant at ages 2 and 3 BST. The average number of leaves of mango seedlings at ages 1, 2, and 3 BST and least significance difference test (LSD) results are presented in Table 5.

Based on further tests (Table 5), the best dosage of manure is T_2 treatment (150 g of manure + 17 ml of agrobost per polybag) both at 1, 2 and 3 months after planting with the average number of leaves of mango seedlings 6,60; 7,20; and 9,00 strands, respectively. The dynamic growth in the number of leaves of mango seedlings is presented in Figure 2.

Table 5. Average number of leaves of mango seedlings at age 1, 2 and 3 BST

Treatment	Average number of leaves of mango seedlings at x month observation time		
	1	2	3
T_0	5,60 ab	6,60 a	7,40 b
T_1	5,60 ab	6,60 a	7,60 ab
T_2	5,60 ab	7,20 a	9,00 a
T_3	3,80 b	5,00 b	5,40 c
T_4	6,00 a	7,00 a	7,00 b
T_5	4,20 ab	4,60 b	5,20 c
BNT 5%	1,84	1,47	1,53

Description: Numbers followed by the same letter in the same column was not significantly different at the 95% confidence level

Figure 2 shows that the average number of leaves of mango seedlings in observations 1, 2, and 3 months after the highest planting was obtained in T_2 treatment followed by T_1, T_0, T_4, T_3 and T_5.

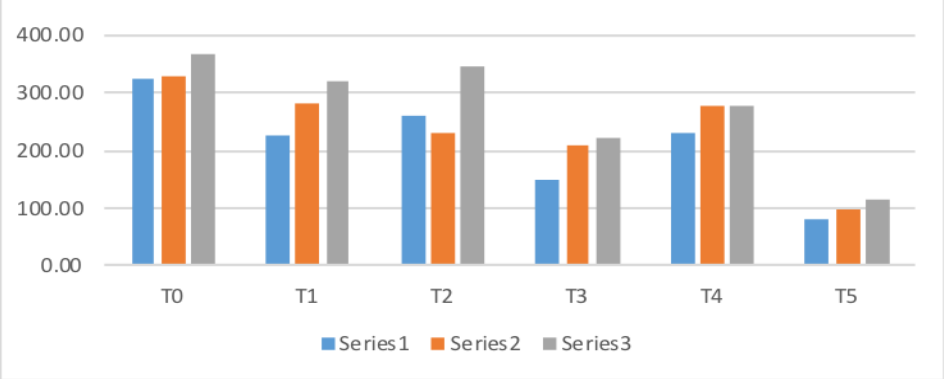

Figure 2. Growth dynamics in the number of leaves of mango seedlings at the age of 1.2, and 3 BST

Leaf area of mango seedlings

The results of variance showed that dosing of manure added agrobost effect was very significant for the leaf area of mango seedlings at ages 1, 2 and 3 BST. The average leaf area of mango seedlings at ages 1, 2, and 3 BST and least significance difference test (LSD) results are presented in Table 6.

Table 6. Average leaf area of mango seedlings (cm^2) at ages 1, 2, and 3 BST

Treatment	Average leaf area of mango seedlings at x month observation time		
	1	2	3
T0	322,46 a	325,97 a	365,08 a
T1	227,18 ab	280,05 ab	319,07 ab
T2	258,31 a	288,83 ab	346,97 ab
T3	150,40 cb	209,10 b	223,39 c
T4	229,60 ab	277,78 ab	278,18 bc
T5	82,28 c	96,58 c	115,11 d
LSD 5%	96,55	100,67	81,04

Description: Numbers followed by the same letter in the the same column was not significantly different at the 95% confidence level

Based on further tests (Table 6), the best dosage of manure is T_0 treatment (without manure + 17 ml agrobost per polybag) both at 1, 2 and 3 months after planting with the average leaf area of

the mango seedlings 322,46; 325,97; and 365,08 cm^2, respectively. The growth dynamics of leaf area of mango seedlings is presented in Figure 3.

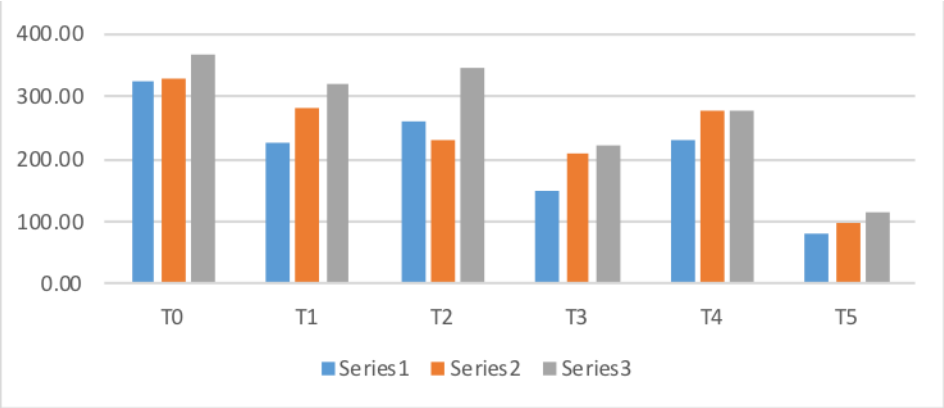

Figure 3. Growth dynamics of the area of mango seedlings at 1, 2 and 3 BST

2. Discussion

Manure can increase nutrient availability such as nitrogen, phosphorus and potassium (Table 1). These nutrients will be absorbed by the plant roots and used to stimulate photosynthesis. The results of photosynthesis will be translocated to all parts of the plant for plant growth and development (Gardner, Pearce & Mitchell, 1991).

The role of nitrogen for plants is to stimulate overall growth, particularly the stems, branches and leaves; plays an important role in the formation of chlorophyll which functions in photosynthesis process; forming proteins and enzymes, fats and various organic compounds (Lingga, 2007; Damanik, Hasibuan, Fauzi, Sarifuddin & Hanum, 2010). as well as cytokine and auxin hormones (Lakitan, 1993). Phosphorus for plants is useful for transporting metabolic energy, stimulating root formation; stimulates plant cell division and enlarges cell tissue; as an ingredient for certain proteins formation, supporting assimilation and respiration; and nucleic acid components (DNA and RNA) (Lingga & Marsono, 2005). Potassium plays an important role for plants because the K function is; helps translocate sugar in proteins and carbohydrates formation; help the process of opening and closing the stomata; expand root growth; plays a role in strengthening the plant's body, thus the stems and leaves are strong and is a source of strength for plants to deal with drought, pests and diseases (Marsono, 2007). Potassium in photosynthesis directly increases leaf growth and leaf area index, thereby increasing CO_2 assimilation and improving photosynthetic translocation into phloem (Gardner *et al*., 1991).

According to Novizan (2004), manure is fertilizer derived from animal manure mixed with food waste and urine which contains nutrients N, P, and K which can be utilized to improve soil fertility. Furthermore Winarso (2005), explained that the provision of manure will improve soil structure, increase water holding capacity, and improve soil biological life. The selection of manure type that will be used as organic material can be determined by the nutrient content. The value of nutrient content of cow manure is relatively better compared to chicken manure.

Manure has natural properties and does not damage the soil, provides macro elements (nitrogen, phosphorus, potassium, calcium and sulfur) and micro (iron, zinc, boron, cobalt, and molybdenum). In addition, manure functions to increase water resistance, soil microbiological activity, value of cation exchange capacity and improve soil structure. The effect of giving manure indirectly makes it easier for the soil to absorb water. The utilization of cow manure can increase the permeability and organic matter content in the soil, and can reduce soil erodibility value which ultimately increases soil resistance to erosion (Santoso, Purnomo, Wigena, & Tuherkih, 2004).

Manure can increase the pH of the acidic soil, thus it increases cation exchange capacity (CEC). Thus nutrients released into soil solutions are quickly absorbed by plants, particularly during the vegetative growth phase. This is proved by the increase in plant height, stem diameter, number of leaves, and leaf area. In the vegetative growth phase, plants need nutrients, namely protein sourced from nitrogen to support plant growth, therefore in the vegetative phase plants need N in sufficient quantities. The main role of nitrogen is to stimulate vegetative growth of plants (Marsono, 2007).

Provision of cow manure on acid soils can increase Ca availability in the soil so that Ca availability in the soil increases and subsequently functions to accelerate plant growth and development. Buckman & Brady (1982), stated that the administration of cow manure with agrobost contained nutrient balance is suitable to the vegetative and generative growth of the mango plant, therefore the photosynthate produced could be translocated well throughout the plant body. The addition of organic material in the form of manure has significant effect on the growth of mango plants because there are compounds that influence biological activity, namely growth stimulating compounds (auxin) and vitamins (Atmojo & Wongso, 2003).

Based on the results of variance showed that manure added agrobost had a very significant effect on plant height, stem diameter, number of leaves, and leaf area at 1, 2 and 3 months after planting. However, the addition of manure doses above 150 g per polybag did not increase the vegetative growth of Arumanis mangoes. Observations on vegetative growth of plants showed that the treatment of manure with a dose of 150 g per polybag + Agrobost 17 ml per polybag (T_2) was the best treatment in increasing Arumanis mango seedlings growth during the study with the highest plant height, stem diameter and leaf number (Table 2, 3 and 4), while the widest leaf area was obtained in treatment T_0 but not significantly different from T_1 and T_2 (Table 5). This finding shows that with the manure treatment at a dose of 150 g per polybag + Agrobost 17 ml per polybag (T_2), it is sufficient for the availability of macro and micro nutrients that are required for the growth and development of mango seedlings. Prolongation and enlargement of the stem of the mango seed results in the plant to grow higher, more and more segments are formed, thus the number of leaves is increasing and the leaf area is wider. The more leaves are formed, the more active leaf surface photosynthesis is, because intercepted radiation by the leaves increasing as well. Thus, if it is supported by sufficient nutrient uptake by plants, the photosynthesis process will take place smoothly.

The addition of manure to 300 g per polybag did not improve the vegetative growth of Arumanis mangoes. This is presumably because the higher dose manure is given, the higher the drainage (excess) so that nutrients are carried by drainage water.

D. Conclusions

Based on the results of the study, it can be concluded as follows: (a) Manure added agrobost treatment had a very significant effect on vegetative growth, particularly in plant height, stem diameter, and number of leaves of Arumanis mango seedlings and (b) Manure treatment with a dose of 150 g per polybag + 17 ml agrobost (T_2) was the best treatment to improve Arumanis mango seedlings growth during this study, this was indicated by plant height of 28,40; 30,94; and 32,82 cm, stem diameter of 0,44; 0,70; and 0,99 cm; and the number of leaves of 6,60; 7,20 and 9,00 strands were significantly higher compared to other treatments during the study.

Further research is recommended to conduct research on a field scale with the aim of providing quality seeds on commercial scale. The dosage of manure is recommended only 150 g + 17 ml agrobost per polybag.

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The Role of Customary Leaders on the Decision Making Process in the Ethics of Land Clearing and Harvesting Process in Rice Farmers in Puday Village, Wonggeduku Barat Sub-District, Konawe District

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Abstract

Customary leaders have dominant roles and influences, these facts are shown in the land clearing process (*monahundau*) and the harvesting process (*meolu*) of paddy rice crop. These processes begin with the opening ceremony or in the form of a small ritual carried out by customary leader numbering one person called *Puusara*or*mandaranosara*. The purpose of this study is to find out how the role of customary leaders in decision-making in the ethics of land clearing and harvesting processes in paddy rice farmers. Analysis of the data used in the form of qualitative descriptive analysis, where the data obtained is then described qualitatively using the Miles and Huberman model which consists of three stages, namely (1) Data Reduction, (2) Data Presentation, (3) Conclusion Drawing / Verification. The results of this study indicated that land clearing (monahundau) and the harvesting process (meolu) were influenced by hereditary customs in Puday Village, Wonggeduku Barat Sub-District, Konawe District. *Monahundau* is a land clearing process which is the first step to open a new planting season every year. *Meolu*is a harvesting process that is carried out in the same steps as the process of land clearing (observation, reciting intentions and praying silently for the best wishes desired in the harvesting process), and the final step is done by investigating at the rice fields who first turned yellow (ripe), then the rice fields that will be harvested first, the rice begins to be harvested when there is already a statement then the other farmers' land that has yellowed (ripe) may be harvested by themselves (meoludowo). Customary leaders are people chosen by the people of Puday village (*puusara*or*mandaranosara*) and have an influnce on decision making in the process of land clearing (*monahundau*) andharvesting (*meolu*)

Keywords: customary leaders, decision making, harvesting, land clearing

A. Introduction

Southeast Sulawesi is one of the provinces in the Republic of Indonesia with diverse ethnicities and customs; it is one of the identities of life of a heterogeneous region. These ethnicities include Tolaki, Muna, Buton, and Wawonii. The existence of these ethnicities will be one of the heterogeneous unifying lives. One of the dominant forms of action or value is the value of customs that binds and regulates human life in various aspects.

Custom is a hereditary habit of a group of people based on the cultural values of the community concerned. Custom has regulated how humans relate to other communities and humanbeingswith nature or the environment. As a cultural system, culture provides a set of models for behavior that originate from vital values and ideas. This main value and idea will be materialized in an ideological, social and technological system (Esten, 1992).

Customs typically consist of cultural values, life views and ideas, norms and laws, and knowledge and beliefs, while cultural values are concepts of what lives in the mind. Most of the community knows what they consider valuable, meaningful and important in life, therefore can function as a guide that gives direction and orientation to the lives of certain community (Koentjaraningrat, 1990).

Syani (1995), suggests that customs are behaviors and regulations that have stricter sanctions. Community members who violate customs will get legal sanctions both formal and informal. For example in rice harvesting activities, a farming community always performs it together alternately from one field to another. Farmer who does not cooperate in rice harvesting activities will get social sanctions in the form of exclusion, where if the farmer will carry out harvesting activities then no other farmer community will help him.

Customary life system is led by a customary leader who is a formal leader and involved in official government, their opinions and recommendations are accepted and obeyed by the community. A customary leader of a village does not recognize the line of his superiors and subordinates, they only recognize the circle of power (influence). The influence size of a customary leader can be seen from the extent of influence circle, they recognize the line and followers. The followers of an informal leader are horizontal; they are not limited to certain village areas but can be wider; vertically, not only in the highest layer. One of the facts that customary leaders have a big role is shown in customary leaders in Puday Village, who play a role and influence the decision making in the process of land clearing and harvesting.

Puday Village is one of the rice-producing villages in Konawe District. This village is one of the villages that adhere to customs in the life of the community and makes it a living guide in carrying out an activity whether it is social, religious, agricultural, and other activities that are conducted for together benefit. In the application, custom values certainly require a person who better understands the ethics and orientation of a value, called Customary Leader (*Puusara*). In the field of agriculture, customary leaders have dominant roles and influences. One of that facts showing the role of customary leaders is shown in the process of land clearing and harvesting in paddy rice crops.

The role of customary leaders is still needed in the process of land clearing (*monahundau)* and the harvest process (*meolu*) to determine the ethics of land clearing and harvesting processes. The activity begins with an opening ceremony or a small ritual conducted by costumary leaders. Customary leaders in the process is one person called *Puusara* or *Mandaranosara*, who will be replaced by someone else when the customary leader has passed away.

The aim of this study is to find out how the role of customary leaders in decision making in the ethics of land clearing and harvesting processes in paddy rice farmers atPuday Village, Wonggeduku Barat Sub-District, Konawe District.

B. Methodology

The population in this study were 125 farmers who cultivated paddy rice, customary leaders in Puday Village is only 1 person who was elder or trusted to become a customary

leader, so the sample of customary leader is 1 (sampling for customary leader is census of which the entire population was sampled). Sampling on farmers was done by taking 15% of the population (Arikunto, 2008) then the number of research samples was 19 people.

The variables in this study consisted of variables related to the role of customary leader in decision making and variables related to the ethics of farmers in the process of land clearing and harvesting. Variables related to the role of customary leaders in decision making are the personality of the customary leader which consists of credibility, knowledge, and level of figure character. While the variables related to the ethics of farmers in the process of land clearing and harvesting are land clearing time or land preparation, procession or ritual preparation procedures, post-harvest (ethics of storing crops).

Data analysis used in this study was qualitative descriptive; the data obtained is described qualitatively using the Miles and Huberman model which consisted of three stages, namely (1) Data Reduction, (2) Data Presentation, (3) Conclusion Drawing / Verification (Sugiyono, 2009). Data is collected in a reasonable condition (Natural Settings) in the life of an object (according to conditions and facts at the research location). Therefore, qualitative research is inductive; meaning it does not start from a hypothesis as a generalization. This study used a qualitative approach with data available in the form of written or verbal words or behavior that can be observed through interviews, observation, and documentation, so that may be analyzed by qualitative methods.

Qualitative research also intends to understand what phenomenons are experienced by research subjects such as behavior, perception, motivation, actions, and others holistically by a description in the form of words and language; and also in a specific natural context by utilizing various natural methods (Moleong, 2007).

C. Result and Discussion

Custom Structure of the Community in Puday Village

The customs of the people of the Puday Village area were influenced by homogeneous ethnic groups. They live together, harmoniously, peacefully and respect each other of their longstanding norms. The ethnic in the Puday Village area is *Tolaki*. Customs are rules or regulations that have sanctions. Community members who violate customs will get sanctions both formal and informal.

The customary life system is led by a customary leader who is an informal leader that is not involved in the official government, but his opinions, recommendations and ideas can be accepted by the community. The size of the influence of a customary leader in a community sphere in Pudayvillage can be seen from the extent of the circle of influence, they are familiar with the lines and followers. Followers of an informal leader of rural communities in a horizontal direction are not only limited to certain village areas or wider area. Vertically, the effect is not only on the lower and middle layers but also reach the high layers.

One of the facts that customary leader have a significant influence can be seen in customary leaders in Puday Village (*puusara* or *mandaranosara*). The customary leader has an influence on decision making for a process of land clearing (*monahundau*) and the harvesting (*meolu*). Before the customary structure was formed in Puday Village, the main institutional actors were formed (in this case farmer groups), in detail the members' data included in the institutional main actors (member's of farmer groups) who were sampled in this study that can be seen in detail in Table 1.

As for the structure of the customary life of the Puday Village community in decision making related to ethical issues of land clearing and harvesting processes.

Table 1. Member's Data of Farmer Groups

No.	Member's Nama	L/P	Cultivated Commodities	Land Area (Ha)
1	Muhlar	L	Rice	1
2	Sasbi Aden L	L	Rice	2
3	Risal	L	Rice	1
4	Wonggi	L	Rice	0,50
5	Astibar	L	Rice	1
6	Baudi	L	Rice	0,50
7	Tely	L	Rice	0,50
8	Sartina	P	Rice	3
9	Unding	L	Rice	3
10	Ramli	L	Rice	2
11	Jamal P	L	Rice	2
12	Kammi	L	Rice	1
13	Burhan	L	Rice	1
14	Sarman	L	Rice	1
15	Umar Dema	L	Rice	1
16	Musriakton Maladeni	L	Rice	1
17	M. Aslan	L	Rice	1
18	Baruga	L	Rice	1,50
19	Oktavina	P	Rice	3,50

Custom, is a behavior and regulation in society to conduct daily interactions and is habits inherited from ancestors. Custom is a hereditary habit of a group of people based on the cultural value of the community. Furthermore, it was also stated that custom has regulated how humans relate to other communities, humans and their nature or environment. In accordance with the opinion of Esten (1992), as a cultural system, custom provides a set of models for behavior that originates from the main system of values and ideas. The value and core idea are materialized in ideological, social and technological systems.

1. Customary leaders are the people chosen by Puday Village community, the person was chosen based on several criteria from the member of the community (interview with Mr. Muhlar, 50 years old). Community leaders are informal leaders in a society who know a lot about the ins and outs of their society.
2. Farmers are people who cultivate paddy rice in Puday Village.

The process of implementing the customary law in the local community is basically carried out by the customary leaders themselves. Customary leaders act as stakeholders or implementers of customary laws will decide on various problems in terms of agricultural activities. Farmers carry out the process of land clearing (*monahundau*) and the harvesting process (*meolu*) are influenced by the decisions of customary leaders. Although farmers have known the ethics of land clearing and harvesting, the processes are carried out still based on the intervention of customary leaders which based on the prevailing customs.

Decision Making in Land Clearing (*monahundau*) and Harvesting Process (*meolu*)

Decision-making process about the ethics of land clearing and harvesting in the local people, based on the results of interviews with customary leaders (*puusara* or *mandaranosara*) namely Mr. Drs. Abdul KaharLiambo (67 years), stated that "*the decision on the ethics of land clearing and harvesting processes is influenced by the decisions of the customary leaders themselves*". The results of the interview illustrated that there is a significant influence from customary leaders in taking and determining a decision. In the ethics of land clearing and harvesting, the determined decision related with determining the day (time) and

procedures, which are carried out based on the results of deliberations between farmers and customary leaders (based on interviews with farmers namely Mr. Sasbi, 50 years and Mr. Risal , 48 years). The decision-making process is conducted by thinking or analyzing and using feeling, which is in accordance with the opinion of Aryanto (2003) who suggested that two ways of making decisions are:

1. *Thinking*, one method for making decisions is to use the power of reasoning; by thinking, usually we will estimate the logical consequences of an action or choice taken. If we make that decision objectively based on cause and effect, after going through the analysis or basis of facts and existing data, then we will make decisions in accordance with the logical consequences that occur, even though there may be things that are not comfortable. They are generally very adept at analyzing what is right and what is wrong.
2. *Feeling,* Another way to make decisions is to use feelings. Feelings that are intended do not mean emotions but tend to consider the impact of a decision on ourselves or others; what are the benefits for ourselves and or others (without requiring beforehand that must be logical). Decision making on the basis of this feeling is based on personal values or norms rather than referring to actions called emotional. When we make a decision based on feelings, we will inquire how far we personally will involve ourselves directly and also how far we feel responsible for the impact of the decisions taken, both on ourselves and others.

***Monahundau* (Land Clearing)dan*Meolu* (Harvesting Process)**

Land clearing activityare influenced by customs or habits that have been hereditary done, it began in the early new planting season on paddy rice, *monahundau* activities are intended as a first step to open a new planting season each year (interviews with customary leaders / Drs Abdul KaharLiambo, 67 years old). Based on the results of the interview, it was also stated by customary leaders, there are several steps taken in the process of land clearing (*monahundau*) which are:

1. Observing a land that represents the land clearing and will collect all paddy rice fields.
2. Reciting intentions or intent silently about the expectations in the monahundau process (land clearing).
3. Praying.

Puday villagers, in general, are one of the social groups who still uphold the values of customs that existedin their environmental areas, this can be seen from one of the rice farming activities through land clearing and harvesting processes. Based on the results of interviews with customary leaders and rice farmers, the harvesting process or *meolu* is carried out in the same steps as the process of land clearing (observation, reciting of intentions and prayingsilentlyabout the best wishes in the harvesting process), then the final step is done by investigating at the paddy fields who firstly yellowing (ripe) that furtherly will be first harvested field, after began to be harvested; if there is already a statement about that condition then the other farmers who have yellowed (ripe) rice crops are allowed toconduct self-harvested (*meoludowo*).

Each tradition has a number of conventions that serve as guidelines, whereby violations of conventions mean violations of custom. This is in accordance with the opinion of Moertjipto (2002), which states that violating custom means violating the provisions and beliefs that stand in the community. Community members who violate customs will get informal sanctions. For example in rice harvesting activities which are carried out gradually and alternately from one to anotherland, farmers who do not cooperate in harvesting activities will get sanctions in the form of exclusion. In addition, the customs that have become a tradition or the habits of the farmers are how they treat the harvested product; that is storing a portion of their harvest for household consumption needs, it is in accordance with the opinion by Pranoto (2016), that storing crops has become community tradition or farmer characteristics.

D. Conclusion

Based on the description of the research results, it can be concluded that land clearing (*monahundau*) and the harvesting process (*meolu*), are influenced by hereditary customs in Puday Village, Wonggeduku Barat Sub-District, Konawe District. *Monahundau* is a land clearing process which is the first step to open a new planting season every year. Meolu is a harvesting process carried out with the same steps as the land clearing process (observation, reciting intentions and praying silently for the best wishes desired for the harvesting process)and the final step is conducted by investigating at the paddy fields who firstly yellowing (ripe) that furtherly will be first harvested field, after began to be harvested; if there is already a statement for that condition then the other farmers who have yellowed (ripe) rice crops are allowed to conduct self-harvested (*meoludowo*).

Customary leaders are people chosen by the Puday Village community (*puusara* or *mandaranosara*) based on several criteria by each community. Customary leaders have an influence on decision making in the process of land clearing (*monahundau*) and the harvesting process (*meolu*). Community leaders are informal leaders in a society who know a lot about the ins and outs of society.

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