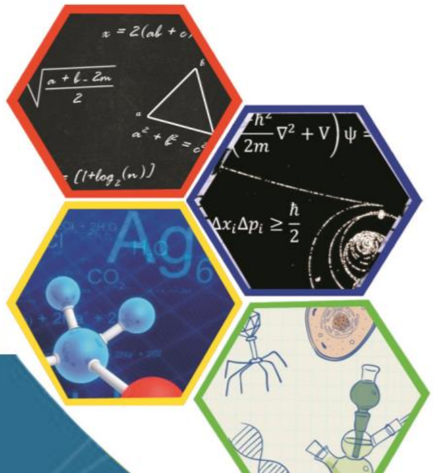
PROCEEDING

The 2nd International Seminar of Basic Science

Natural Science For Exploration The Sea-Island Resources

Ambon, May 31st 2016



Organized by
Faculty of Mathematics and Natural Science
Pattimura University

PROCEEDINGS

The 2nd International Seminar of Basic Science

"Natural Science for Exploration The Sea-Island Resources"

Poka-Ambon, 31st May 2016

Mathematic and Natural Science Faculty
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2nd edition

Welcoming Address By The Organizing Committee

Today, We have to thank the The Almighty Allah SWT for the implementation of this international seminar. This is the second seminar about Basic Science in The Faculty of MIPA Pattimura University. The seminar under the title "Natural Sciences for Exploration the Sea-Island Resources" will be carried out on May 31st 2016 at Rectorate Building, Pattimura University. There are 200 participants from lecturers, research institute, students, and also there are 34 papers will be presented.
My special thanks refer to the rector of Pattimura University and the Dean of MIPA Faculty, Prof. Dr. Pieter Kakissina, S.Pd., M.Si. I also would like to express my deepest gratitude to Prof. Amanda Reichelt-Brushett, M.Sc., Ph.D. ; Kazuhiko Ishikawa, Ph.D. ; Nicolas Hubert, Ph.D. ; Prof. Dr. Kirbani Sri Brotopuspito ; Prof. Dr. Marjono, M.Phil. ; Gino V. Limon, M.Sc., Ph.D. as the keynote speakers.

The last, We hope this international seminar usefull for all of us, especially Mollucas People and very sorry if any mistake. Thank you very much.

Dr. La Eddy, M.Si.
Chairman of Organizing Committee

Opening Remarks
By Dean of Mathematic and Natural Sciences Faculty

I express my deepest gratitude to The Almighty God for every single blessing He provides us especially in the process of holding the seminar until publishing the proceeding of International Seminar in celebrating the 18th anniversary of MIPA Faculty, Pattimura University. The theme of the anniversary is under the title "Natural Sciences for Exploration the Sea-Island Resources". The reason of choosing this theme is that Maluku is one of five areas in Techno Park Marine in Indonesia. Furthermore, it is expected that this development can be means where the process of innovation, it is the conversion of science and technology into economic value can be worthwhile for public welfare especially coastal communities.

Having the second big variety of biological resources in the world, Indonesia is rich of its marine flora and fauna. These potential resources can be treated as high value products that demand by international market. Basic science of MIPA plays important role in developing the management of sustainable marine biological resources.

The scientific articles in this proceeding are the results of research and they are analyzed scientifically. It is expected that this proceeding can be valuable information in terms of developing science and technology for public welfare, especially people in Maluku.

My special thanks refer to all researchers and reviewers for your brilliant ideas in completing and publishing this proceeding. I also would like to express my gratefulness to the dies committee-anniversary of MIPA Faculty for your creativity and hard working in finishing this proceeding, God Bless you all.

Prof. Dr. Pieter Kakisina, S.Pd., M.Si.
Dean of Mathematic and Natural Sciences Faculty

ACKNOWLEDGMENT

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"The 2nd International Seminar of Basic Science 2016"

Hotel Mutiara Ambon

Contents

THE PERFORMANCE OF MORPHOLOGICAL AND PHYSIOLOGICAL EFFECT OF THREE ACCESSIONS OF COWPEA ON DROUGHT STRESS

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ABSTRACT

The objectives of this research was to study the response of cowpea to drought through the performance of morphological characters that in turn affected plant physiological, as well as to get stress-tolerant accessions. The pot eperiment in the greenhouse used a Split Plots in a Completely Randomized Design. The main plots were the levels of drought, consisting of K0 = watering at equivalent to precipitation = 130 mm per month, K1 = 100 mm per month, and K2 = 70 mm per month; and the subplot was three accessions of cowpea, consisting of KTm16, KTm23, and a superion varieties KT6. The experiment had 3 replications. Observations on several morphological characters were done at 2, 4 and 6 weeks after planting. Data were presented in the forms of figures, and an analysis of variance was conducted on the data of growth of 6 weeks after planting, continued with Duncan difference test. The analysis results indicated the drought stress hindered plant growth at 4 weeks after planting, which became clearer at the age of 6 weeks. Results of the analysis of variance showed highly significant differences in the main plot and subplot in all growth characters, and highly significant interaction effect on the number of green leaves. Difference test results showed that the drought level K2 suppressed the growth of plant height, stem node number, branch number, total leaf number and green leaf, as compared to those of K1 and K0. The reduced growth of those characters at K2 drought level were respectively 11.9%, 20.2%, 39.1%, 33.6%, and 40.1% compared K0. Comparison of accessions showed that KTm23 gave the highest values on all vegetative variables; KTm16 showed moderate growth and higher green leaf number than KTm23; KT6 showed low growth characteristics. The interaction effects showed that local accessions especially KTm6, were more tolerant to stress level of K2 based on the number of green leaves.

Keywords: *drought, cowpea, morphological characters, vegetative.*

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is a vegetable crop and belongs to minor food legumes. However, this crop has the advantages over other legumes i.e. it is are consumed in the form of young pods as vegetables as well as in the form dry beans as staple food for a diet diversification purpose. Each 100 g cowpea dry seeds contain 22 g protein, 1.4 g fat, 60.1 g carbohydrates, 6.8 g fiber, and 3.5 g ash (Anonymous, 2008). The non-edible parts cowpea plants can be buried in the soil as manure to increase soil fertility or used to feed livestock.

Cowpea growns in tropical and subtropical regions with characteristics of low (300-600 mm) and variable rainfalls (Ndiso *et al*., 2016). Generally, cowpea is better able to adapt to drought, high temperatures and other biotic stresses than are other crop commodities (Onuh and Donald, 2009). Water is a major component in the process of photosynthesis and as a carrier of photosynthate around plant body. In addition to inhibiting photosynthesis activity, drought also inhibits the translocation of photosynthate (Yakushiji *et al*., 1998), as well as inhibits protein and cell wall syntheses (Salisbury and Ross, 1995).

It was found in our previous study local cowpea accessions that were able to grow and produce in their home area with rainfall of 66 mm per month during the dry season and 204 mm per month during the wet season (Laimeheriwa, 2007). It was yet to known how the morphological characteristics of the vegetative phase of these local accessions were in response to the drought conditions. This needed to be studied, because the ability to grow especifically vegetative growth at stress conditions determine the sustainability of crop in the reproductive phase. Results of previous studies on vegetative phase showed that drought negatively affected leaf area index, the development of new shoots, and crow-root ratio (Kramer, 1983), decreased plant height, number of nodes, root length, root and canopy dry weights (Sunaryo, 2002).

Plants are able to grow and produce at the expected yield under the stress conditions, which indicates that they have resistance mechanisms. The mechanism of drought resistance are grouped into: (1) escaping drought, (2) avoiding drought, and (3) tolerance to drought. Cowpea has avoidance mechanisms to drought (Shackel and Hall, l979), namely by reducing water loss (such as stomatal closure and reduced leaf area), by decreasing carbon assimilation due to the reduction of transfer molecules of carbon dioxide, as well as by increasing the temperature of the leaves to reduce biochemical processes, which affect negatively to the yield (Agbicodo *et al.*, 2009). According to Levit (1980), plants response to drought conditions by reducing the surface area of photosynthesis through reduced leaf expansion, faster aging and defoliation.

Cowpea is known as avoider of dehydration through strong stomatal sensitivity, and able to rapidly reduce growth (Lawan, 1983). As drought avoider, cowpea tested in this study was expected to grow well in drought conditions as indicated by the morphological performance. Thus, this study aimed to study the responses of cowpea to drought stress through several morphological behavior that affected plant physiological, as well as to get accessions of cowpea that are tolerant to drought stress.

MATERIALS AND METHODS

The experiment at the Greenhouse of the Faculty of Agriculture Pattimura University until the plant was six weeks old. The plant materials in the form of seeds of 3 cowpea genotypes. This study used Split Plot Design with the basic design of a completely randomized design, with three replicatios. The main plots were the levels of drought, consisting of K0 = watering at equivalent to precipitation = 130 mm per month, K1 = 100 mm per month, and K2 = 70 mm per month. The subplots were three accessions of cowpea consisting i.e. KTm16 and KTm23, a improved variety KT6. The PVC pipe as a water channel was installed, 25 cm in length and 1.5 cm in diameter, with several holes made around it and one end covered with plastic. The PVC pipe was placed vertically with the closed end on the bottom. Each pot (subplot) was given a pipes and a pole to plant climb. Subplot pots were placed randomly on each main plot. Fertilization with NPK fertilizer was given in an amount of 4 g per pot at planting time.

Vegetative characters which were observed included plant height (cm), the number of stem nodes, number of leaves and number of branches per plant, when the plants were 2, 4 and 6 weeks after planting. All data in the sixth week were analyzed by analysis of variance, followed difference test of Duncan multiple range test, for those treatments showing significant differences. Data of the plant vegetative growth at 2, 4 and 6 weeks after planting were presented in the form of figures.

RESULTS AND DISCUSSION

Effect of Drought on Cowpea Growth

Some vegetative characters observed in drought treatment in cowpea included plant height, number of nodes, total number of leaves, and number of branches per plant. The results showed that drought affected the growth of cowpea starting at 4 weeks after planting, and more obviously at 6 weeks after planting (Figure 1). These results suggest that cowpea was strongly influenced by the availability of water since the beginning of growth. Inhibition of growth was more evident with age of plants or the duration of plants in a state of water unavailability around their roots. Unavailability of groundwater in K2 treatment, which was equal to a rainfall of 70 mm per month, gave a greater effect on the inhibition of cowpea growth (Figure 1).

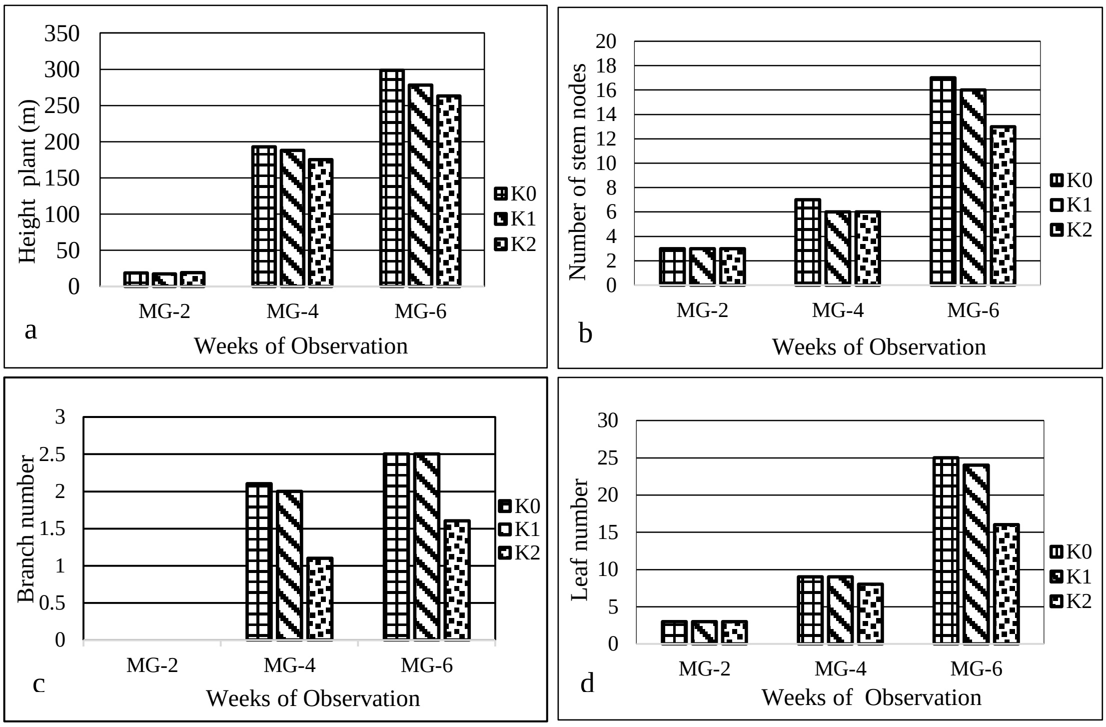

Figure 1. Cowpea Plant Growth at Drought Levels of K0, K1 and K2 Over Several Weeks of Observations. (a) Average plant height, (b) Average number of stem nodes, (c) Average leaf number, and (d) Average branch number.

F test results showed that the four growth characters, namely plant height, number of stem internotes, number of leaves and number of branches per plant were strongly affected by the drought treatment (main plot). Duncan difference test results on four characters are presented in Table 1. K2 drought stress inhibited the growth of plant height, number of stem nodes, number of branches, and total number of leaves per plant at 11.9%, 20.2%, 39.1% and 33.6%, respectively, as compared to those of the K0 drought level. These results indicate that the growth of plant organs was greatly affected by the drought compared to the increase of plant height. The increases of the numbers of plant organs as shown in this study were closely related to the number of cells in the formations of new organs.

Table 1. The Effect of Drought on Cowpea Growth at 6 Weeks After Planting

Drought Level	Vegetative Characters				
	Plant Height (cm)	Number of Stem Nodes	Branch Number	Leaf Number	Green Leaf Number
K0	298,56 a	17,00 a	2,56 a	25,44 a	17,44 a
K1	291,33 a	16,11 a	2,56 a	24,22 a	16.33 a
K2	263,11 b	13,56 b	1,56 b	16,89 b	10.44 b
Coeficient of variance	3,8%	4,10%	21,2%	12,7%	19,1%

Note: Numbers in the same column followed by the same character are not significantly different based on Duncan Multiple Range Test at) α =5%

According Hardjadi and Yahya (1988), when plants experience increasingly severe water shortages, the first to get affected is the differentiation of new organs and enlargement of the existing ones, and subsequently a reduction in the rate of photosynthesis occurs. During the vegetative growth, division and enlargement of cells occurs actively and water is needed for the process of metabolism and maintenance of cell turgidity. The reduction in plant height at the extreme drought conditions is caused by reduced turgor pressure that affects cell division and expansion (Okunlola *et al*., 2015). According to Prasad *et al*. (2008), cell expansion and division start to get affected in a mild drought conditions, before the drought level affects photosynthesis and respiration.

The results of this study also showed that cowpea was still able to grow and thrive equally well in treatment K0 and K1 which were shown through the four vegetative characters. Meanwhile, the level of water stress of K2, could be considered as the water shortage level that affected the growth of cowpea. Summerfield *et al*. (1976) reported that cowpea was very sensitive to water availabily at the vegetative growth stage. This is because water is a major component of the plant, and almost 80-90% of plant fresh weight consists of water. Water is also important in the process of photosynthesis, in addition that water is a carrier photosynthates to parts of plants and nutrients from the roots to other plant parts. According Hardjadi and Yahya (1988) water is essential for maintaining cell turgidity including in the cell enlargement, stomatal opening and maintenance of the shape of leaves. Prolonged soil water shortage can cause plant wilting, and severe water stress can cause plant death.

In this study, water shortage in cowpea, especially at a rainfall of 70 mm per month (K2), significantly inhibited the growth of plant height, number of stem notes, number of branches and number of total leaves. These results are similar to those obtained Abayomi *et al*. (2001) that water shortage during the vegetative stage of cowpea significantly reduced the number of leaves per plant, branching and plant height, as compared to the control. According to Kramer and Boyer (1995), a decrease in the number of leaves and plant growth is characterized by decreased cellular expansion due to reduced water content and turgor pressure under drought conditions.

Plant Morphological Performance and Physiology Effect Under Drought Stress

The results showed the occurance of significant inhibition of leaf production and leaf aging (senescence) followed by defoliation on K2 drought treatment compared to those of K1 and K0 (Table 1) in 4 weeks after planting (Figure 1). The total number of leaves in K2 treatment was 17 per plant, and only 59.9% leaves were still green, or 6 leaves had fallen during the active growth of vegetative parts; whereas in K0 treatment 8 leaves had fallen out of 25 leaves formed. These results indicated that the physiological changes due to drought stress in cowpea were shown very clearly through the character of leaf number by inhibiting the production of leaves and leaf defoliation. Even in drought stress treatment categorized as a humid month with 100 mm rainfall and a wet month with 130mm rainfall, defoliation also occured. According to Alves and Setter (2004), leaf area expansion was a growth process that was very sensitive to drought. In mild drought conditions, leaf expansion and cell division were affected first before photosynthesis and respiration (Prasad *et al*., 2008). It was also found in this study that although the number of leaves was small in K2 treatment, but in this treatment it was not followed by extreme defoliation when compared to that in K0 treatment.

The data in Table 1 shows that the number of green leaves was highest in K0 and K1 drought treatment as compared to K2. This can be explained that in the conditions of K0 and K1 the available water was more likely for leaf production (Table 1), despite the availability of water in these treatments was not sufficient for the actual need by cowpea, so that their leaves turned yellow and then fall at about 31-32% or 8 out of 25 leaves (Figure 1). Meanwhile, in the K2 drought level, less number of green leaves were formed (Table 1), which showed a drought avoidance mechanism. The small number of leaves in this treatment was more likely to be maintained. The remaining green leaves could contribute to providing photosynthates for further growth of the plants.

At the age of 6 weeks or during plants in active growth, approximately 31-38% of leaves fell at all levels of drought (Table 1). It showed a mechanism of drought avoidance in cowpea by reducing the number of leaves (defoliation) and low production of leaves, as well low vegetative growth at all drought levels. Some researchers have demonstrated the effects of drought on the decline of the growth of some vegetative characters, which included leaf number, plant height, branch numbers and stem node numbers (Hiler *et al*., 1972; Summerfield *et al*., 1976; 1993; Karamanos, 1980; Chung, 1997; Abayomi *et al*., 2001; Sunaryo, 2002; Riduan *et al*., 2005; Prasad *et al*., 2008; Abayomi and Abidoye, 2009).

Cowpea is one of the plants have a mechanism of drought avoidance through leaf area reduction, lowering stomatal activity and the change in the orientation of trifoliate leaflets (Shackel and Hall, l979; Reddy *et al*., 2003). A decrease in the total leaf area per plant due to drought is indicated by: (1) a reduction in leaf area, meaning that the leaf size is smaller (Hsiao, 1973; Alves and Setter, 2004), (2) fewer number of leaves produced (Abidoye, 2004; Karamanos, l980; Abayomi *et al*., 2001), and (3) senensence (aging) and defoliation (Karamanos, l980; Abidoye, 2004; Gwathmey *et al*. 1992;Okunlola *et al*., 2015). The decrease in the total leaf area is a mechanism to reduce the extent of transpiration so that the leaf water potential can be maintained. The following result is a reduction in the photosynthate production area which in turn will have an impact on the growth and productivity of plants. Stomatal closure is also a mechanism resulted from the lower leaf water potential, which at the same time become a means of reducing transpiration, and results in inhibition of CO_2 absorption for plant photosynthesis (Hardjadi and Yahya, 1988).

Growth Performance of Three Cowpea Accession Under Drought Conditions

The results of the analyses of variance for the accessions of cowpea (subplot) showed a very significant effect on several growth characters, except for branch number per plant. Further test of Duncan showed that the KTm23 accession had the highest growth for all of the characters (Table 2). This accession had an average plant height of more than 3 m, stem node number of 18, trifoliate leaf number of 25. Another local accession KTm16 showed a moderate growth and tended to have high numbers of total leaves and green leaves compared accession KTm23 and KT6. Meanwhile, in this drought trial the high-yielding variety (KT6) showed lower growth performances. Vegetative growth performances, such as greater plant height and leaf number, were also shown by the local cowpea accessions compared to the superior variety KT6 (Hetharie *et al*. 2015). According Esquinas-Alcazar (1993), local traditional varieties are varieties that grow on the local areas, and are able to adapt to the unfavorable environment with a stable production.

Table 2. Plant Growth Performance of Cowpea Accessions Under Drought Stress Conditions

Accession	**Vegetative Characters**			
	Plant Height (cm)	**Number of Stem Nodes**	**Total Leaf Number**	**Green Leaf Number**
KTm16	278,60 b	13,89 b	21,33 ab	17,11 a
KTm23	319,40 a	17,67 a	24,78 a	15,67 a
KT6	255,00 c	15,11 b	19,44 b	11,44 b
	KK=3,49	KK=6,7%	KK=9,3%	KK=6,9%

Note: Numbers in the same column followed by the same character are not significantly different based on Duncan Multiple Range Test at) α =5%

Table 3. The Interaction of Drought Stress Treatment and Cowpea Accession

Treatments	Character of Green Leaf Number (%)		
	K0	K1	K2
KTm16	19,67 a	19,00 a	12,67 b
KTm23	20,00 a	18,33 a	8,67 cd
KT6	12,67 b	11,67 bc	10,00 c
	KK=6,7%		

Note: Numbers followed by the same character are not significantly different based on Duncan Multiple Range Test at) α =5%

Three accessions tested has shown growth response in drought conditions through senensens leaves are the leaves turn yellow and fall on the plant age of 4 weeks. Leaf is one of the organs of plants that are more sensitive to the effects of drought. In this study it was demonstrated through the reduction of leaf area, primarily by a decreased production of leaves and defoliation during the active growth of plants. Accession KTm16 produce leaves tend to be high, including the number of green leaves is higher than the other accessions. These results indicate KTm16 in drought stress conditions more tolerant to drought level K2 compared accession KTm23 and superior varieties (KT6).

The test results on a the variable of green leaf number showed only an interaction between drought treatment and accession but not on the other variables. Duncan difference test results (Table 3) shows that each cowpea accession had a different interaction with the level of drought stress. KTm16 and KTm23 accessions gave more green leaf number than the high-yielding varieties (KT6) at K0 and K1 drought treatment. But at K2, KTm16 accession had a higher green leaf number than those of the other two other accessions. These results indicate that KTm16 was more tolerant based on the indicator of green leaf number under a rainfall of 70 mm per month. There was a mechanism of tolerance shown by the local accession through the performance of the leaves. According Ndiso *et al*. (2016), in response to drought stress cowpea vary with variety, plant part of the economic value, the stage of development when stress occurs, and the duration of the stress.

CONCLUSION

1. Drought stress treatment by provision of water equivalent to a rainfall of 70 mm per month (K2) strongly inhibited the growth of cowpea than 100mm per month (K1) and 130mm per month (K0). A decline in plant growth occurred in K2 treatment based on plant height, number of stem nodes, number of branches, number of total leaves, and number of green leaves, by 11.9%, 20.2%, 39.1%, 33.6%, and 40.1 %, respectively, compared to K0.
2. Two morphological characters as indicators of the sensitivity of the drought stress in K2 were the low production number of leaves and defoliation as indicated by the number of remaining green leaves. The behavior of the two characters contribute for transpiration reduction as well as the reduction of CO_2 absorption for photosynthesis.
3. KTm23 accession had the highest growth based on all vegetative variables; KTm16 showed a moderate growth but it had the same or even more number of green leaves as KTm23; meanwhile, KT6 showed a low growth character. Local accession KTm6 especially tended to be more tolerant under K2 drought stress as shown by the number of green leaves that was greater than those of KTm23 and KT6.

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