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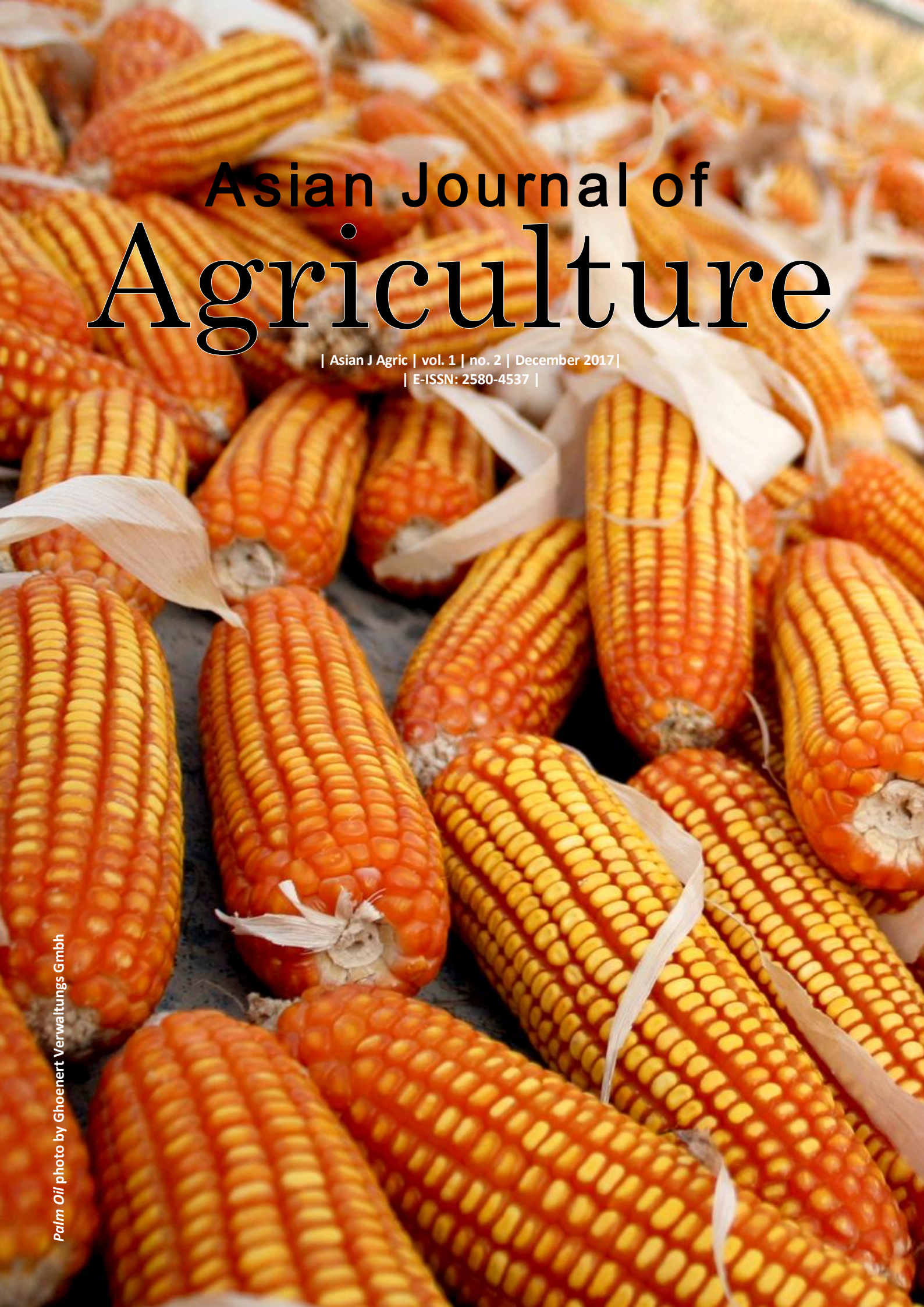

Palm Oil photo by Ghoenert Verwaltungs Gmbh

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Proceeding:

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Thesis, Dissertation:

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Analysis of flowering gene in palm oil (*Elaeis guineensis*)

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Abstract. *Faizal I, Emdi A. 2017. Analysis of flowering gene in palm oil (*Elaeis guineensis*). Asian J Agric 1: 53-58.* Palm oil has always been an important commodity in Indonesia. The most common species of palm oil is *Elaeis guineensis*. Palm oil is a monoecious plant with a tendency to be a temporal dioecious. The female flower will be the one that produces palm oil fruit, which later is treated with palm oil while the male flower only takes part in the fertilization process. To know the ratio between female and male flower trees in a plantation, this study was performed to detect a distinction between female and male flowering gene sequences from DNA samples of *E. guineensis*. Based on a previous study that managed to characterize *MADS*-box gene of palm oil, a primer was designed and named GmG (Globosa-male-Gaps). The result shows that the primer can differentiate DNA sequence female and male flower of *E.guineensis*, Palm oil. However, further studies with full sequence and more samples are needed to find distinctive results between female and male flower sequences as the GmG primer could be used to design a specific marker or primer to detect the presence of female or male flower within a tree.

Keywords: *Elaeis guineensis*, dioecious, male-female ratio, *MADS*-Box, flowering gene

INTRODUCTION

Palm oil is one of Indonesia's main commodities. It is used for many daily aspects of Indonesian life as frying oil, chocolate butter, or even biodiesel; and these days there is an increasing demand for Crude Palm Oil (CPO) in international market. Indonesian palm oil sources are mainly from the species *Elaeis guineensis*. The other species that are commonly available around the world is *Elaeis oleifera*, which has smaller palm oil yield (Durand-Gasellin et al. 2005).

Palm oil has proved itself as an important commodity, in 2012 Indonesia became the largest consumer and producer of palm oil (Obidzinski et al. 2012). Indonesian palm oil production rose to 35% of global production in 2012, and a further increase to more than 50% in 2013, moreover there was an increase in global import trend of palm oil up to 42,7 billion USD and Indonesia managed to produce approximately 17,3 billion USD which is 43% of global needs. The Indonesian government saw this trend as a beneficial opportunity, which led to a plan to increase productivity up to 33 million tons in 2015 (McClanahan 2013; Sandi 2014; Armindya 2014).

For years, the Indonesian government has tried many ways to increase palm oil production. Various studies are available and have been used to try to increase the production rate of palm oil plantations such as importing *Elaidobious kamerunicus*, a certain type of insect which was mainly used in Southern Africa to help palm oil trees to pollinate. This method has been used since 1983 in Indonesia when the insect was first time introduced (Tuo et al. 2011).

However, there are no studies to differentiate male and female flower composition in one area of a plantation, even though it is a crucial aspect of producing palm oil. Palm oil trees are a monoecious plant with a tendency to become a temporal dioecious. As a monoecious plant, a palm oil tree has two flowers: female and male type. The female flower will be the one that produces palm oil fruit which later is treated with palm oil, while the male flower only takes part in the fertilization process. Moreover, as a temporal dioecious plant, each tree has mostly just one kind of flower and to increase productivity we cannot waste our time on the trees which do not produce palm oil, so we need to have more trees with mostly female flowers rather than the male flowers. Also, to differentiate between both flowers visually the flowers need to be fully grown. However, it will often be too late since a lot of expense and time have been spent in maintaining the plantation and it's impossible to just start over again. Farmers are then forced to accept the current harvest rate (Iswanto et al. 2010; Adam et al. 2011).

A study about genes taking part in controlling and developing the flowering organ had been conducted by Adam et al. (2007), which showed that the flowering process was controlled by *MADS-box* gene. In their study, Adam et al. used cDNA samples to characterize *MADS*-box gene of palm oil. Based on their study, a primer was designed and utilized to look for the correlation between DNA sequences of female and male flowering gene samples of palm oil at Indonesia's Agency for Assessment and Application of Technology (BPPT).

The aim of this study is to detect the distinction between female and male flowering gene sequences from DNA sample of *Elaeis guineensis* and then analyze it using bioinformatics software.

MATERIALS AND METHODS

Plant materials. The plant materials included staminate flowers that were collected from one oil palm plantation in Central Kalimantan, Indonesia and Puspiptek Serpong Botanic/Experimental Gardens, Banten, Indonesia. The flowers were produced within tenera, derived from dura and pisifera.

DNA isolation. A female and male flower from two different trees were gathered as a start for DNA isolation. Cetyl trimethyl ammonium bromide (CTAB) 1%, EDTA 0.5M, Tris HCl 1M pH8, and NaCl 5 M were mixed as extraction buffer. The other materials were Chloroform: Isoamyl 24: 1, Na-acetate, isopropanol, ddH_2O, and RNAse. First, the samples were weighed and crushed with liquid nitrogen and polyvinylpyrrolidone (PVP), then the samples were moved into falcon tube and extraction buffer was added. After that, the tube was soaked in the water bath at 65°C for 30 minutes and shook every 5 minutes. Next, the samples were put at room temperature, then C: I was added as much as samples volume. Then samples were centrifuged at 15572 G, 20 minutes 4°C, the supernatant was collected, and then Chloroform: Isoamyl solution was added. After that, the samples were re-centrifuged, repeated for 3 times. After that, the supernatant was removed. The pellets were dried under vacuum pump for 90 minutes. After that, the pellets were added with TE Buffer, $NaCH_3COO$, and chilled with absolute ethanol. Then the samples were kept at -20°C overnight. The next day, the samples were centrifuged at 1846 G for 5 minutes at 4 °C and re-dried at room temperature. Last ddH_2O and RNAse were added to samples and incubated at 37°C for 1 hour.

PCR amplification. The PCR amplification was conducted using GmG primer (F RCACTAAYAGCRCA / R TCACTTARTTCPCA), PCR modified master mix, and the PCR process is prepared as shown in Tables 1 and 2.

Visualization. The DNA visualization was conducted using electrophoresis instrument. The 1% agarose gel was made with a mixture of 0.1 g agarose and 100 mL TAE buffer. The samples were mixed with Tritrack 6x Loading dye and then were injected into the well. A 1 Kb DNA ladder marker was also injected into another well. The process was run at 100 volts for 30 minutes.

Sequencing. The amplified samples were sequenced using sequencer analysis by Biotech Centre, BPPT Puspiptek Serpong.

Table 1. PCR Master Mix.

Composition	Volume for 1X Reaction (µL)
2mM dNTP mix	1
Taq polymerase	0.1
Buffer Taq 10X	1
Primer GmG F	0.5
Primer GmG R	0.5
ddH_2O	5.9
DNA Sample	1
Total volume	10

Table 2. Thermocycler setting.

Stage	Temp. (°C)	Time (sec.)	
Pre-denaturation	95	300	-
Denaturation	95	30	35 cycles
Annealing	65 (±11)	30	35 cycles
Elongation	72	60	35 cycles
Final elongation	72	300	35 cycles
Hold	4	~	35 cycles

RESULTS AND DISCUSSION

The first part was DNA isolation of both samples, and the results of DNA isolation (Figure 1) show that the total DNA from both samples was successfully isolated. All bands are shown above the highest band of marker 1Kb DNA ladder, which is 10kb. It is proven that the whole DNA had been isolated from the total genome size of palm oil tree is 1.8 GB (Singh et al. 2013).

To determine the position of Globosa gene within the genome region, analyses were conducted using PerlPrimer 1.1.21. They used Spidey algorithm to predict the gene position with mRNA sequenced, using mRNA data with accession number AF411848.1 from the study by Adam et al. (2007), and whole DNA sequence, using a genome region data with accession number NW_011550962.1 from the study of Singh et al. (2013). According to *PerlPrimer* (application for designs primers, ORF and CpG island detection algorithms) analysis of globosa gene was separated into seven exons, between each exon are introns (Figure 2.). According to next analysis using Ugene (http://ugene.net/)(application for multiple sequence alignment and genome sequencing data analysis), the amplified region of GmG primer was stretched from the 4th exon to the 7th exon as shown in Figure 3.

The amplified male and female samples (#3 and #5 of genomic DNA, respectively) size were shown in an electrophoresis result as bands between 4th and 5th band of 1 Kb DNA ladder, which is between 1000 bp to 1500 bp (Figure S1.A), when analyzed using Ugene software, the size of the amplified region is 1125 bp (Figure 3). The amplified region of GmG primer is longer than the mRNA data, which is 897 bp because in the previous study they used RNA samples to analyze the characteristic of *MADS*-box mRNA sequence (Adam et al. 2006). However, in this study, the samples were all genome or DNA samples which explains the longer size of the amplified region. The mRNA sequences were divided into several exons within the DNA genome sequences. The primer is expected to attach at a certain position within the exons, in this study the forward primer was attached at 4th exon and the reverse primer was attached at 7th exon. Thus, the amplified region included introns (Figure 3). Optimization processes were also conducted and found that the most optimal temperature for GmG primer to anneal was 58.4°C as shown in Figure S1.B.

Since the result of samples, sequencing was not fully assembled, it is presented as forward and reverse of the primer sequence. Male forward and reverse sequences, also

female forward and reverse sequences are presented in Table S1. All sequences were aligned to the amplified genomic region of GmG primer. Male and Female forward sequences were aligned using pairwise alignment against the amplified region, and the results were compared between male forward (Figure 4) and Female forward (Figure 5).

The same process was done for male and female reverse (Figures 6 and 7). Even though the samples sequences were not aligned from the first sequence of the amplified region for forwarding sequence and the last part for reverse sequence, the alignment analysis proof that the samples were indeed part of globosa sequence by Adam et al. (2006). The samples sequencing result was not shown as fully sequenced, compared to the amplified region from the database caused by the presence of a repeated sequence, which caused problems to occur within sequencing process. The problem usually occurs two-fold. First, since the repeated sequence tends to be quite long, it may result in further incorporation for further sequence runs. This problem often leads to sequence petering out in the middle of the process. Second, the enzyme used in the process will often slip for certain number of times and the enzyme will re-join in a random position that would result in the complete sequence. Moreover, palm oil is a high GC content sample, thus it poses greater difficulty in sequence process if repeated sequences occur (Low et al. 2008; Treangen and Salzberg 2011).

From the result, a few gaps were found in both male-female forward sequences, and the gaps were shown in a different position. The male forward sequence's gap was found at the 269th base, while female forward sequence's gaps were found at the 270th and 311th base (Figures 4 and 5). Gaps were also found in both male and female reverse sequences (Figures 6 and 7). However, both samples showed the same gaps position, which was at the 1065th base. It shows that the differences between male-female sequences are all in the front part of the sequence, or at 5'end since the sequencing coding starts from 5' to 3'. This base position difference is a significant marker that shows the difference between male and female sequences. Even in just one base different, at least there would be one amino acid different; and in one amino acid different, there could be a different protein conformation which results in a significant expression difference (Spindel et al. 2013).

Figure 1. DNA Isolation of male (*top*) and female (*bottom*) flower, M=DNA ladder, 1-8: samples.

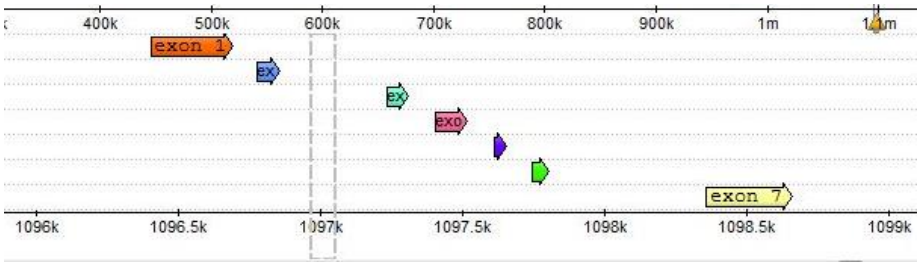

Figure 2. The exons of globosa according to PerlPrimer analysis (taken from Ugene).

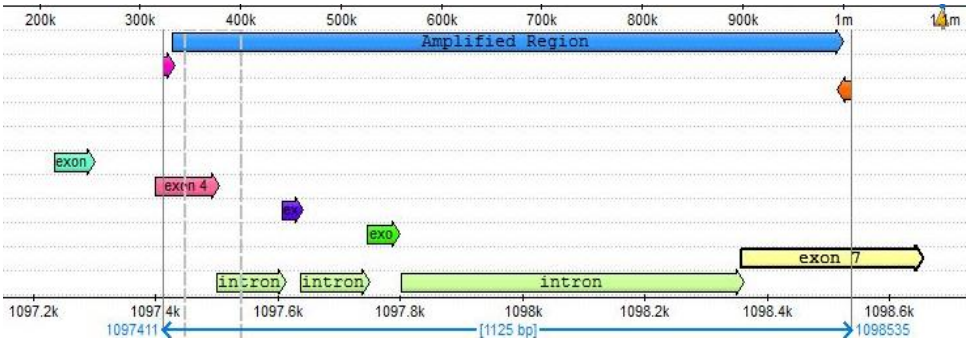

Figure 3. Amplified region of GmG primer within the genome region (taken from Ugene).

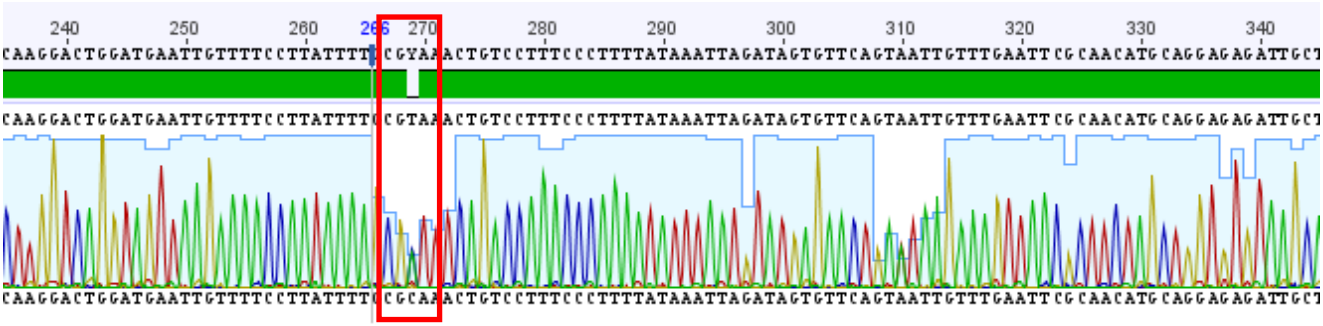

Figure 4. Male forward sequence compared to the amplified part of genome region NW_011220962 from NCBI database (red box: gap).

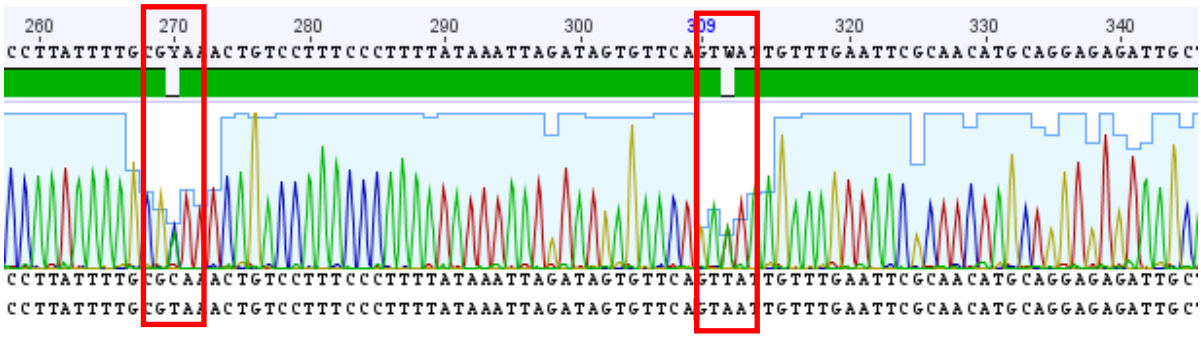

Figure 5. Female forward sequence compared to the amplified part of genome region NW_011220962 from NCBI database (red box: gaps)

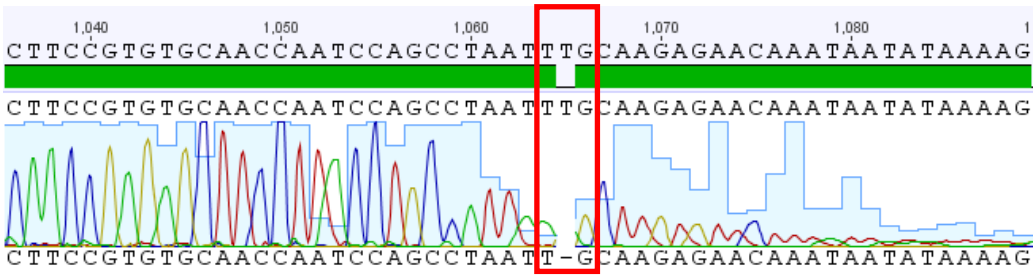

Figure 6. Male reverse sequence compared to the amplified part of genome region NW_011220962 from NCBI database (red box: gap)

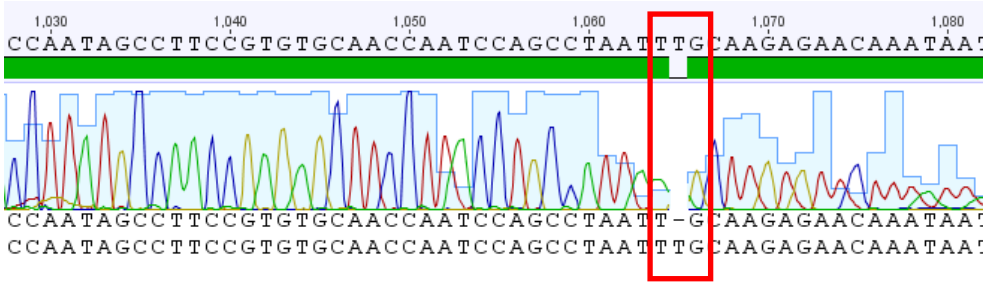

Figure 7. Female reverse sequence compared to the amplified part of genome region NW_011220962 from NCBI database (red box: gap).

These differences can be used as an initial study in determining the difference between a female and male flower. Though gaps sometimes posed as the effect of SNPs, a single nucleotide difference that usually appears in a different individual, a recent study showed that gaps could act as a marker and possess a great value for numerous applications in plant breeding and mapping. However, it would be beneficial in further studies to complete the full sequence and more samples to understand the difference between male and female flower sequences as whole, since the GmG primer could be used to design a specific marker or primer to detect the presence of female or male flower within a tree (Spindel et al. 2013).

Overall, this study shows the GmG primer can differentiate DNA sequence female and male flower of *Elaeis guineensis*, palm oil. As shown in the result of male and female forward sequences, there are gaps in both samples. However, the position of those gaps are in different positions, the male forward sequence at the 269th base and female forward sequence at the 270th and 311th base . This shows that the GmG primer has the potential to differentiate the DNA sequence of the female and male flowers. However, further studies with full sequence and more samples are needed to find distinctive results between female and male flower sequences as the GmG primer can be used to design a specific marker or primer to detect the presence of female or male flower within a tree, to increase the yield of Indonesian palm oil production.

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Table S1. Samples sequences

The following is Male forward sequence.
>JG_F
GAATCTTTCTATTGAGGTGTCCTCCAGAATGGTCTCACCAGTGT
TAGGGGCAAGCAGGTTGGTATATGTTTGTGCCTTCTTTCTGTTT
GATTGATCATGTAAATCCTGTGATATGTTTCCTTTGTGCATGGG
AATGTTACTGATTAGTTCTTTTACTATTCCAGATGGATTTCTTG
AAGAAGCTCAAGAAGAATGTATGATTCAAGGACTGGATGAATTG
TTTTCCTTATTTTGCGCAAACTGTCCTTTCCCTTTTATAAATTA
GATAGTGTTCAGTAATTGTTTGAATTCGCAACATGCAGGAGAGA
TTGCTGGAAGAGGAGAACAAGCATCTGACTTTTCTATTGGTAAT
CATCTAATCGGATGAGCTTTTGAGTTTCAAGTTATAGCAGAGGA
TGCACTTTTTATGCATGGATGGCAAAATTATAAGCATCATGATA
AACTCAACGCAGTGTGATATGTAGATAGTGATAGTAAGGAGCTA
CTTGGAGCTTTTACTTATTTGATATTTGGCACTCCACTTTACCA
TTTTTCATCTGGGACCATGCTTTTGTTGAGCCAGTTGGAGTGTT
CATTGTGATTGAAATTTTAAAGCT

The following is female forward sequence
>BG_F
CTGGAGGGATGTCCTCCAGAATGGTCTCACCAGTGTTAGGGGCA
AGCAGGTTGGTATATGTTTGTGCCTTCTTTCTGTTTGATTGATC
ATGTAAATCCTGTGATATGTTTCCTTTGTGCATGGGAATGTTAC
TGATTAGTTCTTTTACTATTCCAGATGGATTTCTTGAAGAAGCT
CAAGAAGAATGTATGATTCAAGGACTGGATGAATTGTTTTCCTT
ATTTTGCGCAAACTGTCCTTTCCCTTTTATAAATTAGATAGTGT
TCAGTTATTGTTTGAATTCGCAACATGCAGGAGAGATTGCTGGA
AGAGGAGAACAAGCATCTGACTTTTCTATTGGTAATCATCTAAT
CGGATGAGCTTTTGAGTTTCAAGTTATAGCAGAGGATGCACTTT
TTATGCATGGATGGCAAAATTATAAGCATCATGATAAACTCAAC
GCAGTGTGATATGTAGATAGTGATAGTAAGGAGCTACTTGGAGC
TTTTACTTATTTGATATTTGGCACTCCACTTTACCATTTTTCAT
CTGGGACCATGCTTTTGTTGAGCCAGTTGGAGTGTTCATTGTGA
TTGAAATTTTAAAGCTC

The following is male reverse sequence.
>JG_R
AAAGGGGCCTTTTATATTATTTGTTCTCTTGCAATTAGGCTGGA
TTGGTTGCACACGGAAGGCTATTGGAATCTGGCATGCAAAATCC
CTAGCTTTCAGAGGATCCCCAAGCTCCAATTCCCTTACATTTCC
ATTCATTGCCAATTCCTGGTGATGCTGTGAAAAGAAAGATGATT
ATTTCAGTTAAGAAAGAGAGAAAGACTGTTGAGTTTTTGGTAAT
GTCCACAATAAGCACTAACTGCACTATCTCACATTTGGCAAATT
CTTACATGGTATAGTAACCAAAATGATGCTAATATCATGACTTC
TGAGTGATACCATTACATGGAGCAACCAAAGTTATGCGTGGTGT
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ATGCACATGACCTTGATGCATCCATAGTCTAAAACTTTACAAGA
CAGTCAAATCATGGGAAAGAGTTGATAGAGCTTTAAAATTTCAA
TCACAATGAACACTCCAACTGGCTCAACAAAAGCATGGTCCCAG
ATGAAAAATGGTAAAGTGGAGTGCCAAATATCAAATAAGTAAAA
GCTCCAAGTAGCTCCTTACTATCACTAT

The following is female reverse sequence.
>BG_R
TTGGGGACTTTTATATTATTTGTTCTCTTGCAATTAGGCTGGAT
TGGTTGCACACGGAAGGCTATTGGAATCTGGCATGCAAAATCCC
TAGCTTTCAGAGGATCCCCAAGCTCCAATTCCCTTACATTTCCA
TTCATTGCCAATTCCTGGTGATGCTGTGAAAAGAAAGATGATTA
TTTCAGTTAAGAAAGAGAGAAAGACTGTTGAGTTTTTGGTAATG
TCCACAATAAGCACTAACTGCACTATCTCACATTTGGCAAATTC
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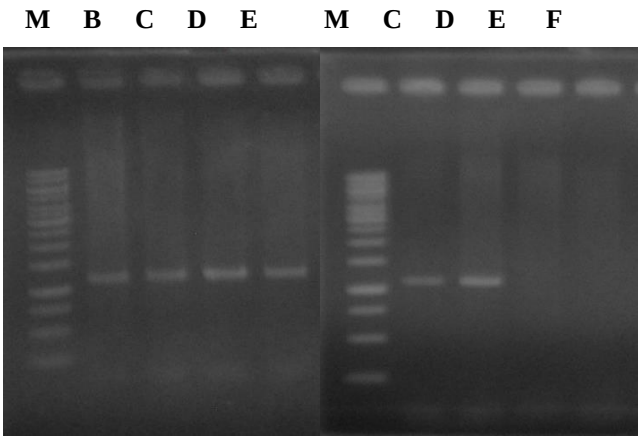

Figure S1.A. Results of PCR amplification and GmG primer temperature optimization. Male (*left*) and female (*right*) samples amplification results, the samples were labeled to represent different temperature gradient B: 55.8 °C, C: 57.1 °C, D: , 58.4 °C, and E: 59.7 °C

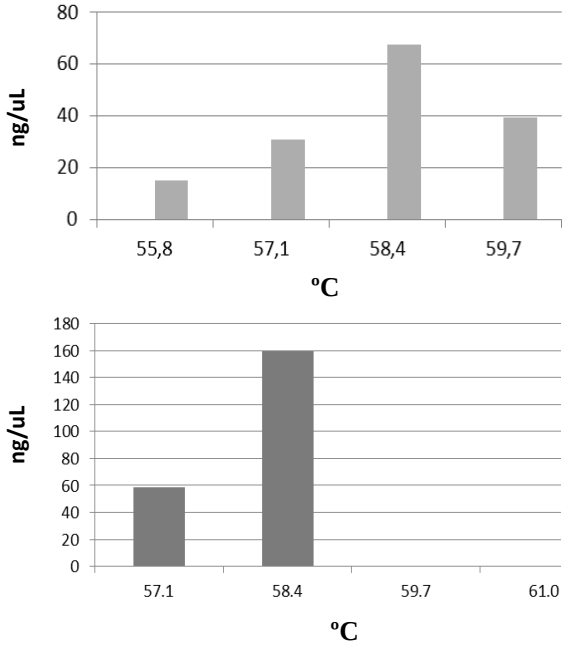

Figure S1.B. Male (*top*) and female (*bottom*) flower samples temperature optimization result.

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The color detection of two cucumber cultivars by NIR Spectroscopy

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Abstract. *Kusumiyati, Kurniasari I, Oktavia AR. 2017. The color detection of two cucumber cultivars by NIR Spectroscopy. Asian J Agric 1: 59-65.* The color of cucumber fruit can be estimated as fruit quality trait. Genotypes and cultivars affect the color of cucumber fruit. The estimation can be done quickly and without tissue damage by NIR Spectroscopy. The purpose of this study was to determine the accuracy of color quality values with NIR and conventional methods. The experiment was conducted at Horticulture Laboratory, Faculty of Agriculture, Padjadjaran University in 2013. The color components observed were L, a, b, hue and chroma. The results indicated that the predicted component of cucumber color approximates values obtained from conventional methods. Based on the data obtained, the correlation coefficient (r) of color component approaches 1.00 and the standard calibration error (SEC) is low or close to 0.00.

Keywords: Cultivar, *Cucumis sativus* L., fruit color, near-infrared spectroscopy, NIR

INTRODUCTION

One of the most important post-harvest activities on horticultural production is grouping fruit and vegetables based on certain criteria; this is referred to as grading. Grading criteria can be determined by physical appearance and internal chemical content inside the product. One of the judging criteria for the product is color. The color of the product is often associated with the level of maturity and taste of fruit, so that the criteria could be used for grading. Color pigment becomes internal content traits of the fruits and vegetables. In a simple way, green product shows high chlorophyll content, orange color shows high carotenoid, and purple-red color shows high flavonoid. The color change of the product may characterize changes in chemical content in horticultural products.

Cucumber is a popular horticultural product of Indonesia. Generally, the consumed cucumber is a young fruit and most of them will not enter the ripening phase. The cucumber fruit skin is generally green with white tinge at the lower end. The skin color of the cucumber will differ depending on the level of cucumber maturity. Cucumber fruit also changes color due to chlorophyll degradation. When associated with the color pigment, the cucumber initial color change (green on the greenish-white base toward the tip) becomes yellowish green at the base. Then the yellowish white toward the cucumber tip indicates the color degradation due to the metabolic process during storage. Visually, the color of cucumber skin can be seen directly.

Near-Infrared Spectroscopy (NIRS) is widely used for color measurements precisely and quickly at various fruit and vegetable products including cucumber. NIRS is a device that utilizes the near infrared wave color spectrum able to predict the internal quality of a product quickly and uniformly. Some research has been done to measure the internal quality of fruits and vegetables using NIRS such as grape (Guidetti et al. 2010), tomato (Nikbakht et al. 2011), Cavendish banana (Liew and Lau 2012), mango cv Gedong (Sugianti et al. 2012), Thai mango (Watanawan et al. 2014), apple (Eisenstecken et al. 2015). This research focused on the internal quality determination using NIRS.

This research will predict the color of the fruit of two cucumber cultivars. According to Mladenovic et al. (2013), fruit color trait is under the influence of dominant genes. Two cucumber cultivars are used in this study as a comparison. The prediction of cucumber color is done by using NIRS which will be compared with the assessment result using conventional method.

MATERIALS AND METHODS

Material sources and tools

The experiment was conducted at the Laboratory of Post-Harvest, Faculty of Agriculture, Padjadjaran University, Indonesia from June to August 2013. 300 fruits of cucumber (*Cucumis sativus* L.) cultivar "Bandana and Wulan" was used in this research. Portable near-infrared spectrometer (NirVana AG410, Integrated Spectronics Pty, Ltd, Australia), CIE L * a * b * box was used to measure the cucumber color. A set of computers for data processing using ISIS software (Integrated Spectronics Pty, Ltd, Australia), Microsoft Excel 2007 and Multivariate Unscrambler (version 9.7, CAMO, Oslo, Norway), Adobe Photoshop CS3 were used to analyze the NIRS data.

Procedures

Fruit sampling

The samples of cucumber fruit used in this study were cucumber cultivars Bandana and Wulan. They were obtained from a garden located in Curug Rendeng Village, Jalan Cagak Sub-district, Subang, West Java, Indonesia. The 7 to 14 days after flowering, cucumbers were selected

as samples. Sampling and cucumber quality testing were performed ten times and each time was done for 60 samples in each cultivar. Before the test, fruits were stored for 12 hours at room temperature.

NIRS analysis

Portable NIRS was used to obtain the predicted values of cucumber fruit color components. The NIR wavelength range was 300-1100 nm with 2 nm interval. The halogen lamp light emitted directly by fiber optics to the fruit was placed behind the handle of the instrument.

Each cucumber was given a spectrum sensor of six different points at the base, middle, and end of the fruit. The duration of the one-time spectrum measurement was 5 seconds. The cucumber was placed on black cardboard during irradiation; black cardboard is required to cover the inclusion of light during the NIR measurements on the cucumber.

Fruit color analysis

Each cucumber fruit sample was inserted into the CIE L* a* b* box. Furthermore, the fruits were photographed by a digital camera that has been placed on top of the hole box CIE L* a* b* (Yam and Papadakis 2004).

Calibration

The estimation of cucumber color was performed using partial least squares (PLS) method. Calibration was the stage to determine the relationship between the color of cucumber absorbent data predicted by NIR and the result of conventional methods.

Validation

Validation aims to test the accuracy of the predicted calibration equations that have been produced. Validation was done by inserting different data samples with calibration data samples into the calibration equation.

Data analysis

The data obtained was analyzed using Microsoft Excel 2007, ISIS (Integrated Spectronics Pty, Ltd, Australia) and Multivariate Unscrambler (version 9.7, CAMO, Oslo, Norway) software. ISIS software was used to receive NIR spectra data and transformed into a computer. Microsoft Excel 2007 was used to process raw data obtained from NIR spectra.

After the spectral data is obtained then the next spectral data will be divided into two groups namely the calibration group and the validation group with different samples. The calibration and validation models were built using the partial least squares (PLS) method. All such processes were performed by multivariate data processing software available in Unscrambler software (version 9.7, CAMO, Oslo, Norway).

RESULTS AND DISCUSSION

The color measurements of cucumber fruit using Numerical color codes are L*, a*, b*, hue, and chroma. The L* notation denotes reflected light that produces white, gray, and black achromatic color (0: black, 100: white). The a* notation is chromatic color with red and green mixture which the +a* (positive) value from 0 to +80 for red and-a* (negative) value from 0 to-80 for green. The b* notation is a mixture of blue with yellow, which the + b* (positive) value from 0 to +70 for yellow and-b* (negative) value from 0 to-70 for blue. Hue states the clarity of green color. The value of chroma expresses the gradations of moving colors from young to old (Zainal 2012).

Measurement of cucumber fruit color conventional method

The measurement of cucumber fruit color components was done by conventional method. Data quality component of cucumber test result of conventional method can be seen below (Table 1). Based on obtained data, cucumber cultivar Bandana and Wulan fruit color showed similarity. The-a* showed fruit color of both cultivars was green. The b* value was positive and lead up to yellow color. Measurements by using this conventional method fit with the cucumber color change from green to yellowish. The data presented in Table 1 shows the narrow distribution indicated by the standard deviation value. This data can be used as a comparison of cucumber fruit color prediction using NIR. Color testing using NIR aimed to obtain more objective data. Darrigues et al. (2008) stated that measuring color from digital images requires standardization and interpretation because digital devices use a color space that is not standardized, is nonlinear, and may vary between hardware devices and software applications.

Measurement of cucumber fruit color non-destructive method using Near-Infrared (NIR)

Measurement of the cucumber color component was performed with absorbance data in the 300-1100 nm wavelength range using the partial least squares (PLS) calibration method. Absorbance data is data obtained from the process of absorption of cucumber samples. Cucumber samples were divided into two groups, group one for the calibration stage and group two for the validation stage with each using different samples. The calibration regression to predict the cucumber yield quality component based on the absorbance data, is constructed by making the relation of the conventional fruit color quality component (reference data) to the NIR prediction value using the PLS method. A summary of non-destructive component cucumber component data using NIR is shown in Table 2.

The data presented in Table 2 is the color data of cucumber fruit (L * a * b * h * C *) obtained by using NIR Spectroscopy. The value obtained from the sample showed a narrow data distribution. It can be seen based on the small standard deviation value. The cucumber color component has a standard deviation value below 10 except the standard deviation value of chroma. The low standard deviation value can be a reference that the data obtained is good. The data presented in Table 2 were used to construct calibration models based on NIR reflectance data correlations and cucumber color data with image processing.

Table 1. Cucumber fruit color (*L* a* b* h* C**) test result using conventional method

Component		Sample	Min	Max	Mean	SD
Color	**L***	Bandana	71,90	91,27	84,55	2,54
		Wulan	77,13	90,67	84,90	2,02
		Combined	71,90	91,27	84,76	2,27
	a*	Bandana	-30,54	-18,22	-26,41	2,20
		Wulan	-29,61	-16,09	-24,45	2,72
		Combined	-30,50	-16,10	-25,30	2,68
	b*	Bandana	22,87	47,50	39,24	3,49
		Wulan	24,67	66,70	36,45	3,70
		Combined	22,87	47,56	37,65	4,49
	Hue	Bandana	-63,19	-45,00	-55,68	6,45
		Wulan	-64,24	-50,79	-56,13	2,23
		Combined	-64,30	-45,00	-55,90	4,65
	Chroma	Bandana	19,97	54,9	39,53	9,88
		Wulan	17,90	52,51	38,21	10,34
		Combined	17,90	54,90	38,81	10,15

Table 2. Cucumber fruit color (*L* a* b* h* C**) test result using non-destructive method using NIR

Component		Sample	Min	Max	Mean	SD
Color	**L***	Bandana	78,90	91,12	84,4	2,31
		Wulan	79,14	97,85	86,69	4,66
		Combined	79,44	89,37	84,33	1,51
	a*	Bandana	-30,74	-18,29	-26,66	2,13
		Wulan	-30,98	-19,89	-25,37	2,39
		Combined	-30,60	-15,67	26,13	2,47
	b*	Bandana	29,52	46,71	38,92	3,23
		Wulan	23,11	63,53	40,64	9,80
		Combined	25,17	46,09	37,36	4,06
	Hue	Bandana	-63,25	-52,34	-56,32	2,43
		Wulan	-62,42	-50,68	-56,31	2,14
		Combined	-61,14	-52,02	-56,14	1,84
	Chroma	Bandana	20,89	55,74	37,74	10,17
		Wulan	17,92	51,46	37,46	9,39
		Combined	18,29	56,21	39,28	9,22

The best calibration regression was seen from the amount of data considered to represent the value range of the cucumber fruit color, the correlation coefficient (r) and the standard error (SE). The value of correlation coefficient (r) is used to know the contribution of free variables to related variables. Standard error (SE) is the difference between the expected value and the true value. The best correlation coefficient (r) is a value close to 1.00 and close to 0.00 for standard error.

Comparative analysis of conventional and non-destructive measurement data using Near-Infrared

L* value prediction

The best calibration regression on the predicted L* values was obtained on both cultivars combined (Table 3). The number of samples at the calibration stage, as many as 217 samples, resulted in the value of correlation coefficient of 0.75. Standard calibration error (SEC) was 1.30 showing the calibration regression prediction is built quite well. This suggests that the approximation of the L* value by NIR is almost close to the conventional value. In the validation stage, 129 samples were used and the correlation coefficient value was 0.94 and the standard error validation value was 1.64. This proves that the L* value of cucumber fruit can be well predicted using NIR. The comparison graph of L* NIR prediction value with L* value of the reference of conventional method results in calibration and validation stage can be seen in Figure 1.

The linear lines of the calibration stage and the validation of the estimated value of L* cucumber in Figure 1 show uneven lines, but both points are in positive X and positive Y. This happens to be expected because it has a high error standard. On the other hand, graph of dispersion value of L * cucumber fruit at calibration stage for two cultivars can be seen in Figure 2. The higher L* (Lightness) value showed brighter cucumber fruit color. L* value color points distribution of two cultivars almost same, but the Wulan cultivar has higher brightness level than the Bandana cultivar.

Table 3. Calibration and validation of cucumber fruit L* value analysis result

Statistical description	Bandana		Wulan		Combined	
	C	V	C	V	C	V
Sample number	90	79	107	93	217	129
Coefisien correlation (r)	0,94	0,85	0,97	0,79	0,75	0,94
Standard error	0,82	1,51	1,12	3,09	1,30	1,64

Note: C: Calibration, V: Validation

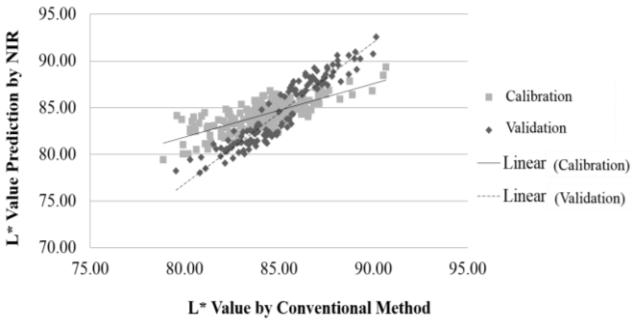

Figure 1. Comparison values of L* cucumber fruit prediction NIR with value L* conventional method reference at the calibration and validation stage

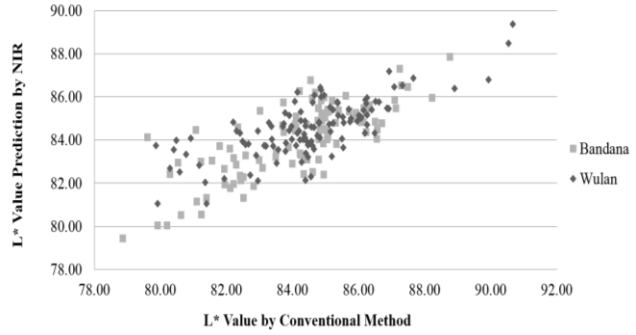

Figure 2. Dispersion value of L* cucumber fruit at calibration stage of two cultivars

a* value prediction

The best calibration regression of the calibration and validation analysis result of the estimated value of a* cucumber fruit is shown in the cumulative data of the two cultivars compared with the data of each cultivar (Table 4). In the calibration stage of the combined cultivars, there were 227 samples used. The correlation coefficient value was 0.97. Standard error calibration was obtained for 0.60. The comparison graph of a* value NIR prediction with a* value reference of the conventional method results in the calibration and validation stage can be seen in Figure 3.

At the validation stage, 121 samples were used and the correlation coefficient value for the combined data obtained value of 0.96 and the standard validation error of 0.98. Judging from the value of correlation coefficient close to 1.00 and the error value is quite low then this result proves that the estimation of a* value using NIR is quite accurate. This is in line with the research of Kusumiyati et al. (2008) which stated that high coefficient correlation (closed to 1) confirms a high validity for predicting the data in each parameter.

Figure 3 shows that the linear line of the calibration stage and the validation of the approximate value of a* cucumber fruit are almost parallel and are in the third quadrant X negative and Y negative. This shows the validation value is almost equal to the calibration value. The comparison graph of the value of a* cucumber predictive NIR with a * value of conventional reference results in the two-cultivar calibration stage can be seen in Figure 4.

Figure 4 shows that the a* value of both cultivars is negative. The a* negative value indicates that fruit color was green fruit. The greater the negative value, the greener the color of the fruit. Seen in the picture above the value of a* on cucumber cultivar Bandana has a negative value larger than the Wulan cultivar. It can be concluded that the green color of Bandana cultivar is higher than the green color content value compared to Wulan cultivar. The use of two cucumber cultivars that have different genetic properties can increase the variation of the data so that the data range gets wider. It is suggested to increase the number of samples when dealing with heterogeneous samples for calibration with a larger range of values for the characteristic to be studied to increase: the heterogeneity of the sample, and the greater the range, the better the consistency of the calibration (Machado 2012; VIAVI 2015).

b* value prediction

Estimation of best b* value calibration regression result of calibration and validation data analysis was obtained on cumulative data of both cultivars (Table 5). The number of samples used was 202 samples. The value of both cultivar correlation coefficients obtained value of 0.97, standard error calibration was obtained for 0.85. This shows that the NIR predicted value is almost the same as the conventional method. The comparison graph of the value b* NIR prediction with the value of b* the reference of conventional method. Results of the calibration and validation stage can be seen in Figure 5.

Table 4. Calibration and validation of cucumber fruit a* value analysis result

Statistical description	Bandana		Wulan		Combined	
	C	V	C	V	C	V
Sample number	147	74	164	80	227	121
Coefisien correlation (r)	0,94	0,72	0,98	0,95	0,97	0,96
Standar error	0,77	1,72	0,56	0,81	0,60	0,98

Note: C: Calibration, V: Validation

Table 5. Calibration and validation of cucumber fruit b* value analysis result

Statistical description	Bandana		Wulan		Combined	
	C	V	C	V	C	V
Sample number	92	80	110	80	202	132
Coefisien correlation (r)	0,96	0,84	0,98	0,78	0,97	0,92
Standard error	0,88	2,51	1,74	5,77	0,85	1,61

Note: C: Calibration, V: Validation

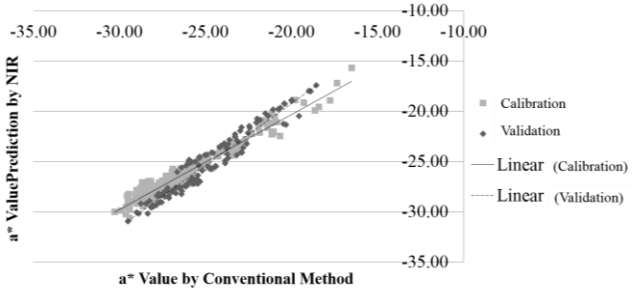

Figure 3. Graph of comparison values of a* cucumber fruit prediction NIR with value a* conventional method reference at the calibration and validation stage

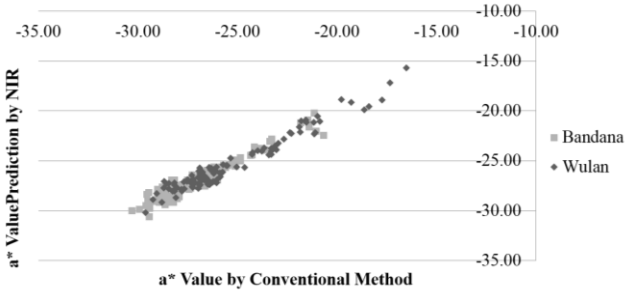

Figure 4. Dispersion value of a* cucumber fruit at calibration stage of two cultivars

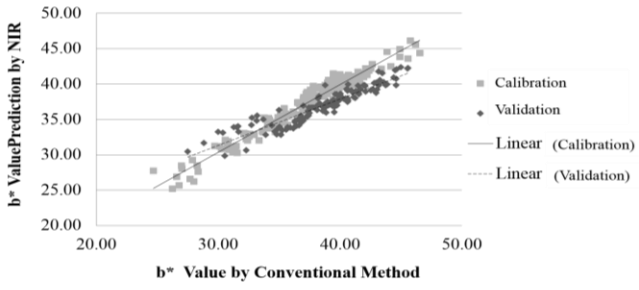

Figure 5. Graph of comparison values of b* cucumber fruit prediction NIR with value b* conventional method reference at the calibration and validation stage

132 samples were used in the validation stage. Correlation coefficient value obtained for 0.92 and the standard error validation value of 1.61. These results prove that NIRs can predict the value of b * quite accurately. Figure 5 shows that the linear lines of the calibration stage and the validation of the predicted value of b* the two cultivars are almost parallel, which means the validation value is almost equal to the calibration value. Graph of value spread of b* cucumber fruit in the calibration stage for two cultivars data can be seen in Figure 6.

Figure 6 shows the b* value of both cultivars has a positive value indicating the color of the green fruit followed by the yellow color. It is seen that the value of b* in Bandana cultivar is higher than Wulan cultivar. This shows the cucumber samples of Bandana cultivars used in this study are much older. Old cucumber fruit has a yellowish green color. The use of two different cultivars and different levels of maturity can widen the range of data. Watanawan et al. (2014) stated there was fairly high correlation between NIR and a* or b* value which was obtained from increasing harvest maturity mangoes.

Hue prediction

The best calibration regression result of calibration and validation data analysis on hue estimation is found in the data of the two cultivars combined (Table 6). The calibration stage of the number of samples used was 247 samples; the correlation coefficient value was 0.84. Standard error calibration (SEC) obtained at 1.17. Judging from the value of the correlation coefficient and the standard error obtained, it can be expected that the calibration value obtained is quite high. The number of samples at the validation stage was 134 samples obtained correlation coefficient value of 0.82 and the standard error validation value of 1.93. It can be concluded that hue value can be predicted well using NIR. The comparison graph of the hue prediction value of the NIR with the reference value of the conventional method results in the calibration and validation stage can be seen in Figure 7.

The hue estimation of cucumber fruit in the calibration stage and the validation of both cultivars have a linear line that is not parallel, but is in the same quadrant shown in Figure 7. Both cultivars have almost equal correlation coefficients of 0.84 for calibration and 0.82 for validation. Both lines are not aligned because they have a high error standard.

Graph of hue distribution of cucumber value at calibration stage for two cultivar data can be seen in Figure 8. Figure 8 shows that the hue value of both cultivars (-) is negative. The hue negative value indicates that the clarity of the green color on the cucumber fruit. The points of the two cultivars overlapped. It can be concluded that the green color found in both cultivars has almost the same clarity value.

Chroma value prediction

Based on the number of samples, the value of correlation coefficient and standard error estimation of chroma value of cucumber fruit of calibration data analysis and calibration regression validation is the best found on the data of both cultivars combined. In the calibration stage, 205 samples were used. The value of correlation coefficient of both cultivars obtained a value of 0.95 standard error calibration obtained for 2.98. The stages of validation of the number of samples of 126 samples, obtained the value of correlation coefficient of 0.90 and the standard error of validation of 5.12 (Table 7).

Table 6. Calibration and validation of cucumber fruit hue value analysis result

Statistical description	Bandana		Wulan		Combined	
	C	V	C	V	C	V
Sample number	87	82	99	88	247	134
Coefisien correlation (r)	0,92	0,85	0,95	0,87	0,84	0,82
Standard error	1,00	2,67	0,69	1,96	1,17	1,93

Note: C: Calibration, V: Validation

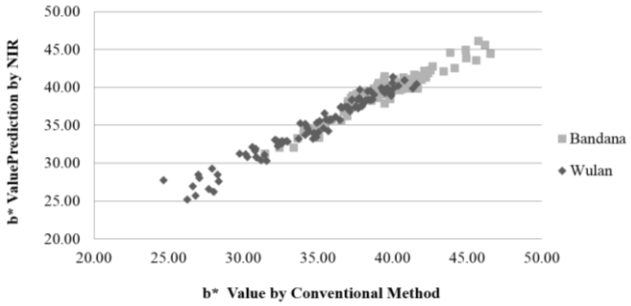

Figure 6. Dispersion value of b* cucumber fruit at calibration stage of two cultivars

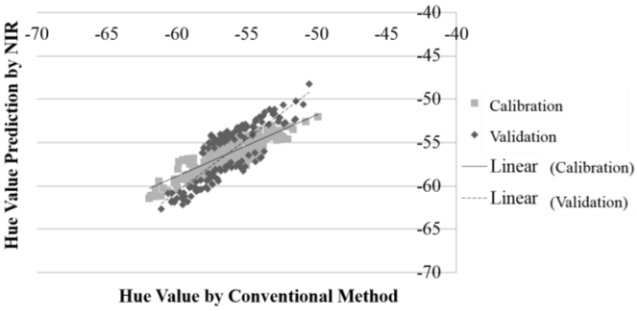

Figure 7. Hue comparison between prediction NIR with conventional method reference at the calibration and validation stage

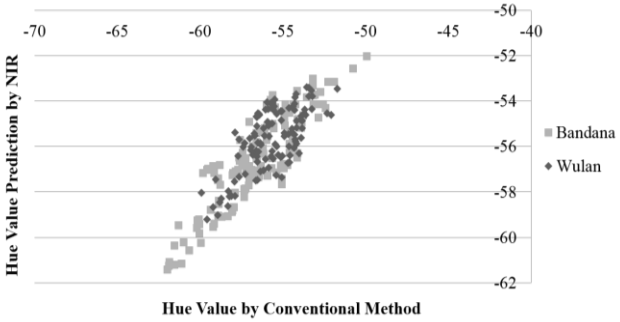

Figure 8. Dispersion hue of cucumber fruit at calibration stage of two cultivars

Table 7. Calibration and validation of cucumber fruit chroma value analysis result

Statistical description	Bandana		Wulan		Combined	
	C	V	C	V	C	V
Sample number	102	85	112	96	205	126
Coefisien correlation (r)	0.98	0.82	0.97	0.95	0.95	0.90
Standar error	1.75	5.74	2.31	4.14	2.98	5.12

Note: C: Calibration, V: Validation

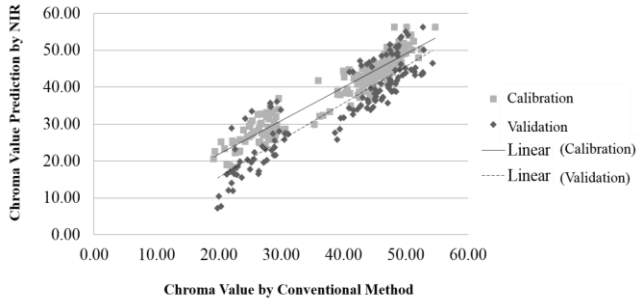

Figure 9. Chroma comparison between prediction NIR with conventional method reference at the calibration and validation stage.

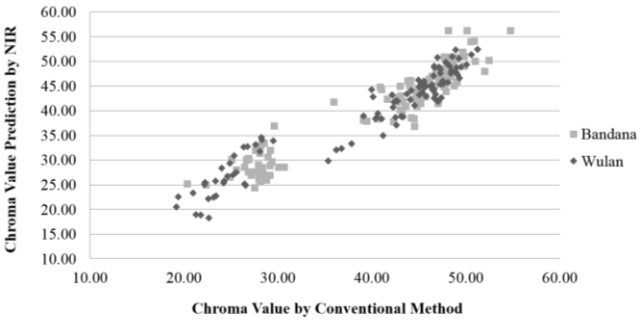

Figure 10. Dispersion of chroma cucumber fruit at calibration stage of two cultivars

Based on the high standard of the validation error, it shows that chroma value is not well predicted by NIR. Chroma predictive value comparison chart of NIR with reference value of the conventional method of chroma at the calibration and validation stage can be seen in Figure 9.

The linear line of the calibration and validation stages on the approximate value of the cucumber chroma appears parallel and positive (Figure 9). It shows that the calibration value is almost equal to the validation value. The comparison chart of the chroma value at the calibration stage for the two-cultivar data can be seen in Figure 10.

Chroma value shows gradations of green cucumber color from young to old. Figure 10 shows that there are empty spots on both cultivars. This shows that the gradations of cucumber green color from young to old in Bandana and Wulan cultivars are less visible, so the standard error obtained is quite high. Additional samples are required to fill the void of the graph above to reduce the high error standard. According to Dardenne (2009), the influence of sample number is important. The number of samples will affect error in predictions.

In conclusion, the results that have been described previously show the color component analysis of the non-destruction method using NIR and conventional method on cucumber fruit cultivar Bandana and Wulan. The L, a, b, and hue can be predicted using NIR well. Unlike other components, the chroma value in this study was not well predicted. This is certainly different from the research done by Sanchez et al. (2012) that said modified partial least squares analysis confirmed the feasibility of NIRS for predicting color-related external quality parameters (L∗, a∗, and C∗). Precisely predictions can occur because of some things such as improper absorbance values used or characteristics of the fruit itself. Based on the data, it can be concluded that the non-destructive color cucumber component using NIR has accuracy value like the conventional method. In the observed color component, the correlation coefficient (r) approaches 1.00, and the standard calibration error (SEC) is low or close to 0.00.

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Traditional knowledge on the use of local food crops by Togutil Ethnic in Halmahera Island, Indonesia

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Abstract. *Tamalene MN. 2017. Traditional knowledge on the use of local food crops by Togutil Ethnic in Halmahera Island, Indonesia. Asian J Agric 1: 66-72.* Food crop is an important component in the life system of the Togutil ethnic group in Halmahera Island. The main characteristic of the group is their nomadic life, despite the existence of some sedentary groups. Generally, the group consumes wild food plants growing in their natural ecosystem. This paper presents a selected result of food crop studies conducted in Halmahera Island, Indonesia. The botanical supply of food crops of the Togutil has high usefulness in their social and cultural activities. Data was collected through in-depth interview method with key informants chosen based on purposive sampling. Plant species were identified in the Laboratory of Herbarium Botany Bogoriense of the Biological Research Center of Indonesian Institute of Science (LIPI). 48 species of 23 families of food crops are used by the Togutil tribe of Halmahera Island, Indonesia. The food crops identified were wild plants with 45.83% growing in bushes habitat and primary forest, with 56.25% being cultivated crops planted in the garden and yard. Other uses of the food crops were identified as being medicinal plants (64.28%), house building (29.17%), and handicrafts and adhesive for animal (4.17%). In the cultural aspect, food crops were used for hunting rituals, medium of exchange (barter), welcoming guest rituals and traditional rituals. The value of Cultural Food Significance Index (CFSI) in the very high significance category was 64.58%, high significance was 27.08%, and low significance and very low significance are 4.16%.

Keywords: Local knowledge, local food crops, Togutil, Halmahera, Indonesia

INTRODUCTION

Food crops consumed by local communities in the world are those vegetable crops that grow wild and cultivated. Local communities consume food crops based on their own cultural system that have, until recently, lasted a long time. In Indonesia, local ethnic groups cultivate food crops to fulfill their life necessities. Most of the people in Indonesia work as food crops, horticultural and plantation farmers. Food crop itself consists of grains (cereals), legumes, and tubers. Food crops are the main or principal necessity to fulfill nutrition adequacy of human body for carbohydrates, proteins, vitamins, and minerals that are used for the sustainability and health of human life (Bharucha and Pretty 2010). Around 5% of total plant species in the word are functioned as being food and drink for humans (Anthony et al. 1993).

Food crops cultivated or growing wildly in the forest are used by local communities for family food security. Local food crops provide high nutrients to support family life and increase the economy (Gahukar 2015). Local food crops used by local communities are varied. Currently, local food crops that grow wild are cultivated as a rice substitute alternative food. Traditional cultivation techniques are developed by local communities to maintain the population of food crops. Non-chemical fertilizer from the traditional cultivation knowledge is used by local communities as their realization of the importance of plant crops for their life. Traditional cultivation activities are conducted for conservation purposes (FAO 1983).

The Togutil ethnical group of Halmahera Island, Indonesia consumes local food crops that are both growing wildly and cultivated. Local food consumption has lasted until recently and has deep cultural meaning. Local foods reflect the history, rich tradition, and identity of Togutil ethnic group. Local foods are important factors that are involved in every ceremony in the life cycle of the ethnic group, and are influenced by social status. Local foods are processed from food crops obtained from wood. Some of the commodities are cultivated traditionally through ways that consider sustainable cycles and use non-chemical fertilizers. Therefore, it results in superior quality, healthy, and environmentally friendly organic products.

It is important this research is done since it tries to document traditional knowledge on local food crops used by the Togutil ethnical group of Halmahera Island Indonesia . The practice of traditional food security by the Togutil is not systematically documented; therefore, the research aims to document the traditional knowledge of the Togutil on the use of food crop species based on local culture. The research will add literature in the field of ethnobotany in Indonesia. The paper also identifies other uses of wild food plants consumed by the Togutil.

MATERIALS AND METHODS

Survey technique and data collection

Information presented in the research is part of data series collected by authors in two locations: Akelamo, East

Halmahera, and Oba Tidore Islands (Figure 1) from July-September 2014 and November-December 2015. The coordinates of the research locations were 128° 40' 18'' E, and 127° 44' 43 'E. Surveys were used as the research approach to collect data on food crop ethnobotany through in-depth interviews, participant observation, and site visits (Martin 1995; Maundu 1995; Alexiades and Sheldon 1996; Cunningham 2001). An in-depth interview was conducted with key informants who were chosen based on purposive sampling (Tremblay 1987). Open questions were asked to discover a knowledge map from the key informants (Pretty et al. 1995; Kvale 1996). Informants guided the data collection process that involved a combination of observation and interview methods. Interview activity was conducted, along with observations of crops that were cultivated as well as grown wild in the wood and bushes. Informants were also asked to rank and score edible wild plants based on their own preferences.

Interviews were conducted in the local language by visiting each informant individually. Agreement was gained from each of the informants before the research was conducted referring to the ethical code of International Society of Ethnobiology (ISE 2016). The informants consisted of 84 people, as presented in Table 1. Questions related to food crop ethnobiology were asked to several age groups consisting of children (5-11 years), teenagers (12-25 years), adults (26-45 years), elders (46-65 years), and old aged (≥ 65 years). Plant specimens with unknown scientific names were collected and identified in the Herbarium Bogoriense of the Research Center for Biology of Indonesian Institute of Science (LIPI), Cibinong, Bogor, West Java, Indonesia.

Data analysis

Plant types were analyzed using a guide from Pieroni (2001) on the calculation of Cultural Food Significance Index (CFSI). The formula of CFSI has determined category index values: the index value of quotation index (QI) availability index (AI), frequency of utilization index (FUI), the plant parts used to index (PUI), the multifunctional food use index (MFFI), taste score appreciation index (TSAI), and the food medicinal role index (FMRI). Following is CFSI formula:

$$\mathbf{CFSI = QI \times AI \times FUI \times PUI \times MFFI \times TSAI \times FMRI \times 10^{-2}}$$

Research results were descriptive qualitatively analyzed using MS Excel 2010.

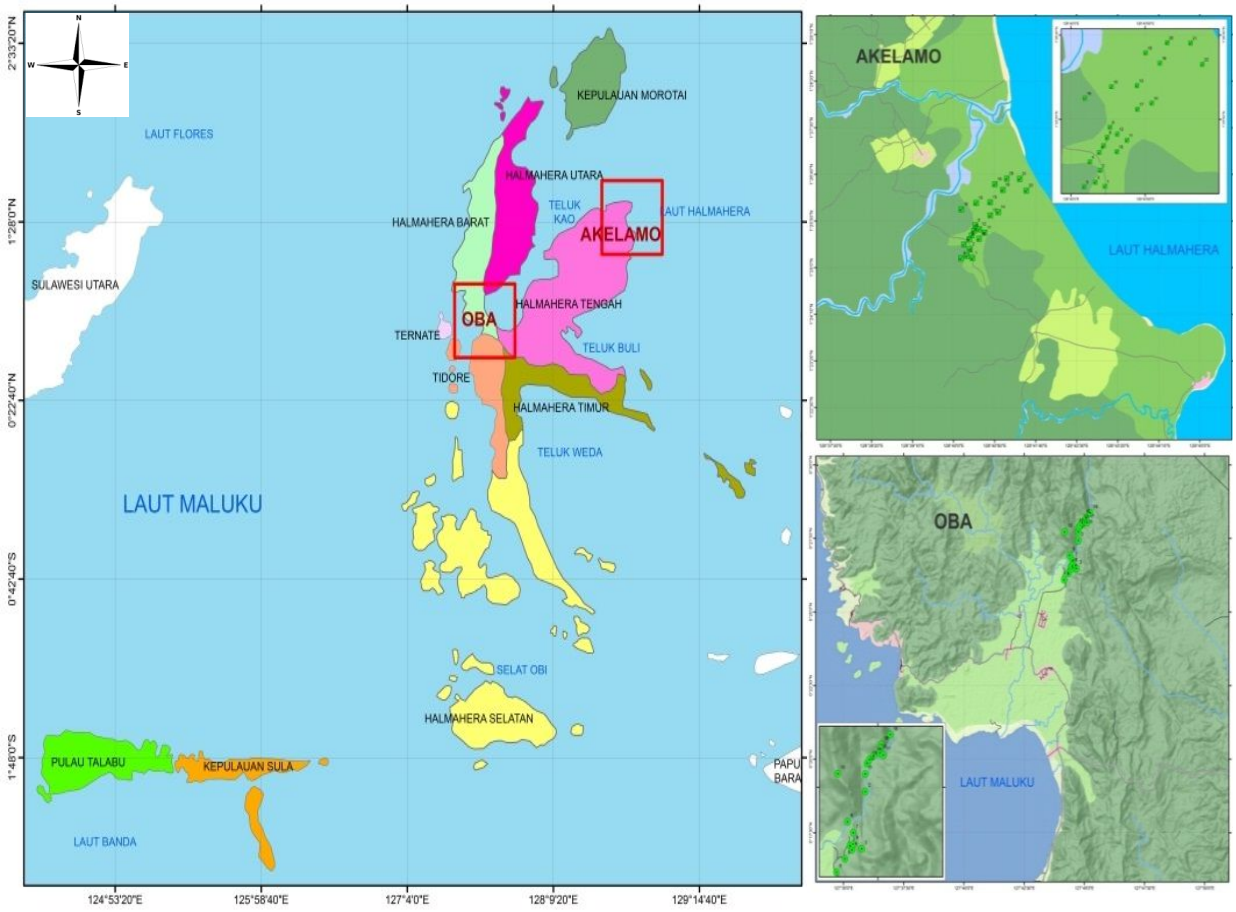

Figure 1. Study site in East Halmahera, North Maluku, Indonesia

Table 1. Demographic of interviewed informants

Category	Sub-category		Togutil ethnical group Akelamo Haltim	Togutil ethnical group Oba Tikep	Total	%
Gender	Male		23	34	57	68
	Female		15	12	27	32
Age (years)	Children	5-11	5	6	11	13
	Teenagers	12-25	10	14	14	17
	Adults	26-45	8	11	19	23
	Elder	46-65	12	11	23	27
	old aged	66 +	3	4	7	8
Marital Status	Married		27	32	59	70
	Single		11	14	25	30
Education	-		-	-	-	-

RESULTS AND DISCUSSION

There were 48 species from 23 families of food crops used by the Togutil tribe in Akelamo and Oba areas of Halmahera Island Indonesia (Table 1). Around 11 species (47.83%) of the crops came from family of Arecaceae, Zingiberaceae made up 7 species (30.43%), Myrtaceae and Poaceae were 4 of the species (17.39%), Burseraceae was 3 species (13.04%), Araceae and Moraceae were 2 of the species (8.70%), and the species with only one (4.35%) individual plant was from the families Athyriaceae, Amaranthaceae, Anacardiaceae, Bromeliaceae, Caricaceae, Combretaceae, Cucurbitaceae, Convolvulaceae, Euphorbiaceae, Gnetaceae, Lamiaceae, Moringaceae, Musaceae, Oxalidaceae, Sapindaceae and Solanaceae. The identified food crops were 45.83% wild plants from bushes and primary wood habitat, and 56.72% were cultivated crops planted in the garden and yard (Figure 2). Other uses of the crop plants included 64.28% medicinal, house building consisted of 29.17%, and handicraft and adhesive for animals was 4.17% (Figure 3). Regarding the socio-cultural aspect, food crops were also used for hunting rituals, medium of exchange (barter), welcoming guest rituals, and traditional rituals.

Wild plants, such as *Arenga undulatifolia* Becc, *Artocarpus elasticus* Reinw. ex Blume, *Arenga pinnata* (Wurmb) Merr., and *Metroxylon sagu* Rottb were the main staple foods consumed by the Togutil tribe as they contain the main carbohydrates, as both the main food source and energy source. Tuber from plant types of *Xanthosoma sagittifolium, Ipomoea batatas* (L.) Lam. and *Manihot esculenta* Crantz L. were foods that originated from plant roots and contain high amounts of carbohydrates. These types of plant became hereditary food staples consumed. Local food crops can be processed into various foods, such as *papeda*, *sagu kering*, and *sinoli* (traditional name foods of the Togutil tribe). Grains were not the main staple foods, but additional foods; these included *Canarium vulgare* Leenh, *Canarium decumanum* Gaertn, *Canarium indicum* L. *Pometia pinnata* J.R.& G.Forst, *Etlingera heliconiifolia* (K.Schum.) A.D.Poulsen, *Etlingera* sp. and *Alpinia eremochlamys* K.Schum.

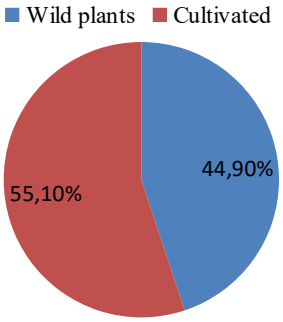

Figure 2. Percentage value of wild plants and cultivated crops

The tradition to consume staple foods and additional foods is characteristic of local wisdom that appears as a form of adaptation toward the surrounding environmental condition. Tradition to consume local food crops, as staple foods in the Togutil tribe have been running for generations. The existence of the staple foods was not only to fulfill life necessities, but also to be used in traditional ritual activities such as in paying debt if someone violated customs rules.

Multiplicity of uses, including parts used

Other uses of local food crops consumed by Togutil ethnic groups were for health, handicraft, adhesive for animal, and house building (Figure 4). The number of plants identified as having medicinal benefits was 31 species (64.28%) and the most efficacious plant was *Alpinia eremochlamys* K.Schum to protect from malaria. The number of plant species used as handicrafts was 2 (two) species (4.17%) i.e., *Saribus rotundifolius* (Lam.) Blume and *Calamus* sp and both were used to make furniture. Additional use of plants was found for house building. There were 29.17% of local food crops used for building house, such as species of *Metroxylon sagu* Rottb., *Arenga pinnata* (Wurmb) Merr., *Arenga microcarpa* Becc., *Arenga* sp., *Areca catechu* L., *Arenga undulatifolia* Becc., *Arenga brevipes* Becc. *Dendrocalamus asper* (Schult.) Backer., and *Bambusa* sp.

Based on observations, the edible parts of plants used were fruit, flower in stem, bamboo shoots, leaves and, rhizomes (Figure 3). The highest category of consumed plant parts was fruit (77.08%). The food crops were consumed raw or cooked and depended on the use of the plants. Some plants, such as *Etlingera heliconiifolia* (K.Schum.) A.D.Poulsen, *Etlingera* sp. and *Alpinia eremochlamys* K.Schum were used as seasoning. The other edible part was flower, at around 12.50%. They were consumed from the plants producing high carbohydrates and became the staple food, such as *Metroxylon sagu* Rottb. The plant was the main choice during the dry season. Bamboo shoots were used for consumption for 6.25%, and mainly from young shoots of bamboo. The part was consumed as a specific vegetable that was cooked in hunting ritual ceremonies and other traditional ceremonies. Leaves from such as *Moringa oleifiera* L., were cooked in traditional dishes and Rhizome, this part of plant was 4.17%, was being consumed not only for seasoning but also for traditional rituals such as *Alpinia galanga* (L.) Wild. and *Curcuma longa* L.

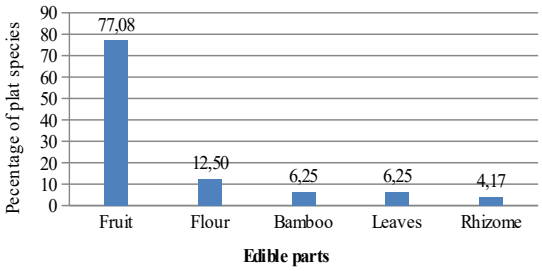

Figure 3. Edible parts of food plants

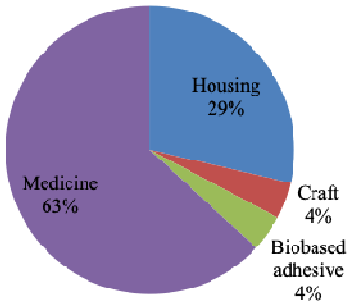

Figure 4. Additional uses of food plants

Food crops, as staple foods, and additional foods, have important benefits for the life of local communities in Indonesia as well as in various parts of the world. The food crops used in the Togutil tribe was summarized in Table 2. In addition, food crops can also be used as medicine to cure various diseases. Research shows that wild and cultivated food crops had sociocultural meaning as well as medicinal value for disease prevention. Previous research reported that food crops were useful for medicine (Tamalene et al. 2016). Plants have an overlapping use in value as not only a source of food, but also in culture and medicine. The use has been documented in various countries in Asia such as Vietnam (Ogle et al. 2003), Mediterranean (Hadjichambis et al. 2008) Inner Mongolia Autonomous Region, China (Wujisguleng and Khasbagen 2010) and Northeast Thailand (Cruz-Garcia and Price 2011).

The value of CFSI is categorized into five groups: very high, high, moderate, low, very low, and negligible (Table 4). Plants used by the Togutil tribe consisted of wild plants and cultivated plants that have a CFSI value with use value.

Food botanicals with Very High Significance values: group of plants that fell into this category consisted of plants that have a role as the main and important foods to fulfill the basic food necessity in a family. For example, the main food crops to fulfill the need for energy were *Metroxylon sagu* Rottb, *Arenga pinnata* (Wurmb) Merr, *Arenga microcarpa* Becc, *Arenga* sp. In addition, there were foods used as additional food, like seasoning or food dye, for example, *Curcuma longa* L., *Zingiber officinale* Roscoe., *Etlingera heliconiifolia* (K.Schum.) A.D.Poulsen, and *Alpinia eremochlamys* K.Schum.

Food botanicals with High Significance values: food crops that fell into this category were those with high citation index as well as high frequency of use. For example, species of *Alpinia eremochlamys* K.Schum, *Cucumis sativus* L, *Artocarpus heterophyllus* Lam, *Artocarpus elasticus* Reinw. ex Blume, *Syzygium malaccense* (L.) Merr. & L.M.Perry, *Psidium guajava* L., *Syzygium aqueum* (Burm.f.) Alston, *Psidium guineense* Sw., *Pometia pinnata* J.R.& G.Forst, *Alpinia galanga* (L.) Wild and *Alpinia eremochlamys* K.Schum. These food crops were consumed cooked, fried as well as raw.

Food botanicals with low Significance: the citation index and frequency of use of this category are low. Plant species that fell into this category were *Oncosperma horridum* (Griff.) Scheff. and *Calamus* sp. In addition, to be consumed as food, these plants were used to make handicrafts and as material to build houses as well as to be used for traditional ritual activities.

Food botanicals with very low Significance: the citation index and frequency of use of the plant that fell into this category were very low or very rare to be used. For example, species of *Saribus rotundifolius* (Lam.) Blume and *Terminalia catappa* L. In addition to be used as food, *Saribus rotundifolius* (Lam.) was used as material for hunting rituals; however, these species were very rarely used. It will only be used when it is needed most.

The higher the use value of a plant species, the higher is the value of interest of the species (Turnner 1998). Local knowledge and trust have important role in the use of plant species for biodiversity conservation and the sustainable and responsible use of various natural resources. The use value shows that the plant has high value for humans especially local people who live side by side with nature. The ICS value could change any time since the plants that are currently used were the legacy of the ancestors that still maintained up until now. If a knowledge shift occurs due to the presence of foreign culture, it is likely that every plant species would have more benefit than that found in the research. Therefore, data on the use of plant as well as ICS value has prevailed only for the current nomadic and sedentary Togutil tribe in Halmahera Island.

The study shows the important role of Togutil ethnic group in Halmahera Island-Indonesia, who used 48 species from 23 families of food crops to fulfill their life necessities. The dependency of the community was more toward the food crops that grow wildly in natural ecosystem than those of cultivated plants. From the diversity of species of food crops used as staple food, there is a very high Cultural Food Significance Index value as this contributes to the food security of a family. Food crops were used by all age groups during the seasonal changes, which include the dry season and rainy season. Some species of the food crops were also used as medicinal plants, house building, adhesive for animal, and handicrafts. The use of plant as food and medicine, can be considered very sustainable. In addition, food crops identified in the research were also used for sociocultural interest, such as hunting rituals, medium of exchange (barter), welcoming guest rituals, and traditional rituals. Therefore, specific attention should be given to those plants that are being used as staple food and additional food and efforts need to do in creating awareness on the use of the plants through local culture based sustainable conservation education.

Table 2. List of food crops used by Togutil tribe, North Maluku, Indonesia

Family name	Scientific name	Local name	Life form	Edible parts	Additional use (s)	Cultural use
Arecaceae	*Metroxylon sagu* Rottb.	Ketoko**	Tree	Flour	House building	Medium of exchange (barter)
	Arenga pinnata (Wurmb) Merr.	Hepata**	Tree	Flour and fruit	House building	Medium of exchange (barter)
	Arenga microcarpa Becc.	Baru**	Tree	Flour	House building	Medium of exchange (barter)
	Arenga sp.	Sali**	Tree	Flour	House building	Medium of exchange (barter)
	Saribus rotundifolius (Lam.) Blume	Weka **	Tree	Flour	Craft	Hunting ritual
	Areca catechu L.	Makuro**	Tree	Fruit	House building	Welcome ritual
	Arenga undulatifolia Becc.	Baru**	Tree	Flour	House building	Medium of exchange (barter)
	Arenga brevipes Becc.	Golobe**	Tree	Fruit	House building	Medium of exchange (barter)
	Cocos nucifera L.	Oigono*	Tree	Fruit	Medicine	Medium of exchange (barter)
	Oncosperma horridum (Griff.) Scheff.	Oweka weka**	Tree	Fruit	House building	Hunting ritual
	Calamus sp.	Take**	Tree	Bamboo shoots	Craft	Hunting ritual
Athyriaceae	*Diplazium esculentum* (Retz.) Sw.	Pako**	Herb	Fruit	Medicine	Medium of exchange (barter)
Amaranthaceae	*Amaranthus hybridus* L.	Bayam	Herb	Leaves	Medicine	Medium of exchange (barter)
Araceae	*Colocasia esculenta* L.	Talas*	Herb	Fruit	Medicine	Medium of exchange (barter)
	Xanthosoma sagittifolium L.	Obbete*	Herb	Fruit	Medicine	Medium of exchange (barter)
Anacardiaceae	*Anacardium occidentale* L.	Buahyakis*	Tree	Fruit	Medicine	Medium of exchange (barter)
Burseraceae	*Canarium vulgare* Leenh.	Hoburu*	Tree	Fruit	House building	Traditional ritual
	Canarium decumanum Gaertn.	Hoburu**	Tree	Fruit	House building	Traditional ritual
	Canarium indicum L.	Niara**	Tree	Fruit	House building	Traditional ritual
Bromeliaceae	*Ananas comosus* (L.) Merr.	Nanasi*	Tree	Fruit	Medicine	Medium of exchange (barter)
Caricaceae	*Carica papaya* L.	Tapaya*	Tree	Fruit	Medicine	Medium of exchange (barter)
Combretaceae	*Terminalia catappa* L.	Tiliho Maddubo**	Tree	Fruit	Medicine	No information
Cucurbitaceae	*Cucumis sativus* L.	Timu*	Herb	Fruit	Medicine	Medium of exchange (barter)
Convolvulaceae	*Ipomoea batatas* (L.) Lam.	Ogomini*	Herb	Fruit	Medicine	Medium of exchange (barter)
Euphorbiaceae	*Manihot esculenta* Crantz	Saibi*	Herb	Fruit	Medicine	Medium of exchange (barter)
Gnetaceae	*Gnetum gnemon* L.	Rukiti**	Tree	Fruit	Medicine	Hunting ritual
Lamiaceae	*Ocimum citriodorum* Vis.	Kemangi*	Herb	Leaves	Medicine	Medium of exchange (barter)
Moringaceae	*Moringa oleifiera* L.	Kelo*	Tree	Leaves and Fruit	Medicine	Medium of exchange (barter)
Moraceae	*Artocarpus heterophyllus* Lam.	Onaka*	Tree	Fruit	Glue	Hunting ritual
	Artocarpus elasticus Reinw. ex Blume	Loenge**	Tree	Fruit	Glue	Hunting ritual
Musaceae	*Musa* × *paradisiaca* L.	Gogurati*	Tree	Fruit	Medicine	Medium of exchange (barter)
Myrtaceae	*Syzygium malaccense* (L.) Merr. & L.M.Perry	Ogora*	Tree	Fruit	Medicine	Medium of exchange (barter)
	Psidium guajava L.	Gogoya 1*	Tree	Fruit	Medicine	Medium of exchange (barter)
	Syzygium aqueum (Burm.f.) Alston	Gogora 2*	Tree	Fruit	Medicine	Medium of exchange (barter)
	Psidium guineense Sw.	Gogora*	Tree	Fruit	Medicine	Medium of exchange (barter)
Oxalidaceae	*Averrhoa bilimbi* L.	Balibi*	Tree	Fruit	Medicine	Medium of exchange (barter)
Poaceae	*Saccharum spontaneum var. edulis* (Hassk.) K.Schum.	Sayur lilin*	Tree	fruit	Medicine	Medium of exchange (barter)
	Dendrocalamus asper (Schult.) Backer	Otibaha 1**	Tree	Bamboo shoots	House building	Hunting ritual
	Bambusa sp.	Otibaha 2**	Tree	Bamboo shoots	House building	Hunting ritual
	Zea mays L.	Kehetela*	Tree	Fruit	Medicine	Medium of exchange (barter)
Sapindaceae	*Pometia pinnata* J.R.& G.Forst	Omotoa**	Tree	Fruit	House building	Medium of exchange (barter)
Solanaceae	*Solanum melongena* L.	Woki-woki*	Tree	Fruit	Medicine	Medium of exchange (barter)
Zingiberaceae	*Etlingera elatior* (Jack) R.M.Sm.	Ogolobata**	Tree	Fruit	Medicine	Medium of exchange (barter)
	Alpinia galanga (L.) Willd.	Liri*	Tree	Fruit	Medicine	Welcoming guests ritual
	Curcuma longa L.	Gurati*	Tree	Rhizome	Medicine	Medium of exchange (barter)
	Zingiber officinale Roscoe	Gihoro*	Tree	Rhizome	Medicine	Welcoming guests ritual
	Etlingera heliconiifolia (K.Schum.) A.D.Poulsen	Goobe**	Tree	Fruit	Medicine	No information
	Etlingera sp.	Goloba kecil**	Tree	Fruit	Medicine	No information
	Alpinia eremochlamys K.Schum.	Goobe utan**	Tree	Fruit	Medicine	No information

Note: *cultivated and **wild plants

Table 3. The value of CFSI of food crops used by Togutil tribe of Halmahera Island, North Maluku, Indonesia

Scientific names	Botanical family	Local names	Detail of calculation of the CFSI								CFSI
			QII	AI	UFI	PUI	MFFI	TSAI	FMRI		
Metroxylon sagu Rottb.	Arecaceae	Ketoko**	84	4.0	5.0	1.5	1.0	9	2.0	10	453.6
Arenga pinnata (Wurmb) Merr.	Arecaceae	Hepata**	57	3.0	3.0	1.5	1.0	7.5	2.0	10	115.42
Arenga microcarpa Becc.	Arecaceae	Baru**	75	4.0	5.0	1.5	1.0	9	2.0	10	405
Arenga sp.	Arecaceae	Sali**	84	4.0	5.0	1.5	1.0	9	2.0	10	453.6
Saribus rotundifolius (Lam.) Blume	Arecaceae	Weka **	24	2.0	2.0	0.75	0.5	6.5	2.0	10	4.68
Areca catechu L.	Arecaceae	Makuro**	53	4.0	5.0	1.5	1.0	6.5	4.0	10	413.4
Arenga undulatifolia Becc.	Arecaceae	Baru**	84	4.0	3.0	1.5	1.0	9	2.0	10	272.16
Arenga brevipes Becc.	Arecaceae	Golobe**	41	3.0	4.0	1.5	0.5	7.5	2.0	10	55.35
Cocos nucifera L.	Arecaceae	Oigono*	84	4.0	5.0	1.5	0.75	9	3	10	510.3
Oncosperma horridum (Griff.) Scheff.	Arecaceae	Oweka weka**	36	2.0	2.0	1.5	0.5	6.5	2.0	10	14.04
Calamus sp.	Arecaceae	Take**	43	2.0	2.0	1.5	0.5	6.5	2.0	10	16.77
Diplazium esculentum (Retz.) Sw.	Athyriaceae	Pako**	64	3.0	5.0	1.5	1.5	7.5	2.0	10	324
Amaranthus hybridus L.	Amaranthaceae	Bayam	76	3.0	4.0	1.5	1.5	9	4.0	10	738.72
Colocasia esculenta L.	Araceae	Obetas*	50	2.0	3.0	1.5	1.5	7.5	2.0	10	101.25
Xanthosoma sagittifolium L.	Araceae	Obbete*	75	2.0	3.0	1.5	1.5	7.5	2.0	10	151.875
Anacardium occidentale L.	Anacardiaceae	Buahyakis*	52	2.0	2.0	1.5	1.5	7.5	3.0	10	105.3
Canarium vulgare Leenh.	Burseraceae	Hoburu*	84	3.0	2.0	1.5	0.5	10	5.0	10	189
Canarium decumanum Gaertn.	Burseraceae	Hoburu**	84	3.0	2.0	1.5	0.5	10	5.0	10	189
Canarium indicum L.	Burseraceae	Niara**	84	3.0	2.0	1.5	0.5	10	5.0	10	189
Ananas comosus (L.) Merr.	Bromeliaceae	Nanasi*	84	4.0	4.0	1.5	0.5	7.5	3.0	10	226.8
Carica papaya L.	Caricaceae	Tapaya*	84	4.0	5.0	1.5	1.0	7.5	4.0	10	756
Terminalia catappa L.	Combretaceae	Tiliho Maddubo**	24	1.0	2.0	1.5	0.5	6.5	2.0	10	4.68
Cucumis sativus L.	Curcubitaceae	Timu*	63	1.0	3.0	1.5	1.5	7.5	3.0	10	95.68
Ipomoea batatas (L.) Lam.	Convolvulaceae	Ogomini*	84	2.0	4.0	1.5	1.5	10	3.0	10	453.6
Manihot esculenta Crantz	Euphorbiaceae	Saibi*	84	4.0	4.0	1.5	1.5	10	3.0	10	907.2
Gnetum gnemon L.	Gnetaceae	Rukiti**	50	2.0	2.0	3.0	1.0	10	5.0	10	300
Ocimum citriodorum Vis.	Lamiaceae	Kemangi*	68	3.0	5.0	3	0.75	9	3.0	10	619.65
Moringa oleifiera L.	Moringaceae	Kelo*	72	2.0	3.0	1.5	1.0	9	3.0	10	174.96
Artocarpus heterophyllus Lam.	Moraceae	Onaka*	84	2.0	3.0	1.5	0.5	9	2.0	10	68.04
Artocarpus elasticus Reinw. ex Bl.	Moraceae	Loenge**	33	2.0	3.0	1.5	1.0	9	2.0	10	53.46
Musa × paradisiaca L.	Musaceae	Gogurati*	84	4.0	5.0	1.5	1.5	10	2.0	10	756
Syzygium malaccense (L.) Merr. & L.M.Perry	Myrtaceae	Ogora*	84	3.0	3.0	1.5	0.5	7.5	2.0	10	85.05
Psidium guajava L.	Myrtaceae	Gogoya 1*	84	3.0	3.0	1.5	0.5	7.5	2.0	10	85.05
Syzygium aqueum (Burm.f.) Alston	Myrtaceae	Gogora 2*	84	3.0	3.0	1.5	0.5	7.5	2.0	10	85.05
Psidium guineense Sw.	Myrtaceae	Gogora*	84	3.0	3.0	1.5	0.5	7.5	2.0	10	85.05
Averrhoa bilimbi L.	Oxalidaceae	Balibi*	84	3.0	5.0	1.5	0.75	9	2.0	10	255.15
Saccharum spontaneum var. *edulis* (Hassk.) K.Schum.	Poaceae	Sayur lilin*	75	3.0	2.0	1.5	1.0	10	2.0	10	135
Dendrocalamus asper (Schult.) Backer	Poaceae	Otibaha 1**	41	4.0	5.0	1.5	1.0	9	2.0	10	221.4
Bambusa sp.	Poaceae	Otibaha 2**	41	4.0	5.0	1.5	1.0	9	2.0	10	221.4
Zea mays L.	Poaceae	Kehetela*	84	4.0	2.0	1.5	1.0	10	2.0	10	201.6
Pometia pinnata J.R.& G.Forst	Sapindaceae	Omotoa**	84	4.0	1.0	1.5	0.5	10	2.0	10	50.4
Solanum melongena L.	Solanaceae	Woki-woki*	84	4.0	5.0	1.5	1.5	9	2.0	10	680.4
Etlingera elatior (Jack) R.M.Sm.	Zingiberaceae	Ogolobata**	84	3.0	2.0	1.5	0.5	9	2.0	10	68.04
Alpinia galanga (L.) Willd.	Zingiberaceae	Liri*	84	3.0	2.0	1.0	0.75	7.5	2.0	10	56.7
Curcuma longa L.	Zingiberaceae	Gurati*	84	4.0	4.0	1.5	1.0	10	4.0	10	806.4
Zingiber officinale Roscoe	Zingiberaceae	Gihoro*	84	4.0	3.0	1.5	1.0	10	4.0	10	604.8
Etlingera heliconiifolia (K.Schum.) A.D. Poulsen	Zingiberaceae	Goobe**	84	3.0	2.0	1.5	0.5	9	2.0	10	68.04
Alpinia eremochlamys K.Schum.	Zingiberaceae	Goobe utan**	84	3.0	2.0	1.5	0.5	7.5	2.0	10	56.7

Local wisdom-based conservation strategy needs to be optimized to reduce deforestation, habitat change, environmental degradation, and cultural transformation since these factors are the main indicators for the loss of natural vegetation. In the future, the disappearance of local wisdom will be the factor for the loss of useful plants as well as knowledge related to those plant species. Therefore, enabling a conservation strategy by involving local communities participation and strengthening custom rules could help the rehabilitation of natural environment. Despite the general benefit of local food crops as part of local vegetation, the potential and the additional use of those plants are the important part to maintain food security of the families in the research location.

Table 4. Value of CFSI of Togutil ethnic group of Halmahera, North Maluku, Indonesia

Category of *Cultural Food Significance Index* (CFSI)	∑ types of plant	%
Very High Significance [100 and over]	31	64.58
High Significance [50-99]	13	27.08
Moderate Significance [20-49]	0	0
Low Significance [5-19]	2	4.16
Very Low Significance [1-4]	2	4.16
Negligible Significance [0]	0	0
Total	48	100

Data of identified plants in the research locations highlights the importance of understanding the role of natural ecosystem in providing local food crops that have meaning in the socio-cultural life of the community. Further, research is needed about other local ethnic groups on the mapping of wild food plants in small islands that have potential in improving the community economy and as effort for local wisdom-based conservation. Finally, the research on local food crops used by the Togutil tribe of Halmahera Island is important data in understanding remote local communities in utilizing plants to survive and maintain the availability of food security for the family during dry season.

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Evaluation of the use of vermicompost on the crop production of two varieties of Pak choi (*Brassica rapa* var. *chinensis*) and on the soil structure in Suriname

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Abstract. *Ramnarain YI, Ori L, Ansari AA. 2017. Evaluation of the use of vermicompost on the crop production of two varieties of Pak choi (*Brassica rapa *var*. chinensis*) and on the soil structure in Suriname. Asian J Agric 1: 73-79.* The present research was carried out from January to May 2016 at the Anton de Kom University of Suriname, Paramaribo. The investigation consisted of both field and laboratory work to evaluate the effect of vermicompost on crop production of 2 varieties *Brassica rapa var. chinensis*, white and green Pak choi, nutrient availability in the soil after the use of vermicompost and nutrient value of the crop. The experiment was carried out (Completely Randomized Block Design) in a greenhouse for six weeks. The treatments were vermicompost (V), cow manure (S), chemical fertilizer (K) and control (C). Plant growth parameters were recorded during the experiment (plant height and number of leaves) and after harvest (root and shoot biomass, leaf area). Nutrient analysis (Ca and Fe) of Pak choi was also conducted followed by pre- and post-experiment soil analysis (pH, EC, TOC, N, P, K and C/N ratio). The results were collected and analyzed using Sigma Plot 12.0 tools. In the white Pak choi, the number of leaves, root length and weight of fresh plants showed no significant differences among the four treatments using Tukey's test ($P \leq 0.05$). In the green Pak choi, the number of leaves and root length showed no significant differences among the four treatments by Tukey's test ($P \leq 0.05$). Furthermore, the soil parameters (pH, OC, N) did not indicate a significant increase or decrease of the elements in the soil. P did decrease significantly and the K increase in the treatment in the soil was not relevant. The evaluation of the use of vermicompost on the crop production of 2 varieties of Pak choi (*Brassica rapa* var. *chinensis*) proved that the plants treated with vermicompost had similar results as those treated with cow manure and chemical fertilizer.

Keywords: Bio-fertilizer, cow manure nutrients, pak choi, soil, plant-parameters, vegetables, vermicompost

INTRODUCTION

Vermicompost is a finely divided peat-like material with excellent structure, porosity, aeration, drainage, and moisture holding capacity (Edwards 1988). The input material that is used for vermicomposting decides the nutrient content of the vermicompost. It usually contains higher levels of most of the mineral elements, which are in available forms than the parent material (Edwards and Bohlen 1996). Vermicompost serves as a nutrient rich natural fertilizer, improving the physical, chemical, and biological properties of soil (Ansari and Jaikishun 2011; Nath et al. 2009; Kale 1998) and thus reducing the use of chemical fertilizers (Chanda et al. 2011; Hernandez et al. 2010). It also increases the amount of readily available water, induction of N, P and K exchange, which results in better growth of the plants (Papafotiou et al. 2005; Manivannan et al. 2009). A samba rice cultivation study revealed that the addition of vermicompost had significant positive effects on the soil physical, chemical properties, and plant growth parameters (Tharmaraj et al. 2011). According to Ali et al. (2012), the application of vermicompost increased the soil properties such as organic matter, total nitrogen, phosphorus, potassium, sulfur, zinc, and boron contents; grain and straw yields of rapeseed also increased significantly, when increasing the dose of vermicompost. Therefore, this study focuses on the effect of the application of vermicompost in the cultivation of *Brassica rapa* var. *chinensis*, white and green Pak choi and its impact on the soil structure.

MATERIALS AND METHODS

Experimental design

This research was carried out from January to May 2016 at the Anton de Kom University of Suriname, Paramaribo. The investigation consisted of a field and laboratory study to evaluate the effect of vermicompost on crop production of 2 varieties *Brassica rapa var. chinensis*, white and green Pak choi, nutrient availability in the soil after the use of vermicompost and nutrient value of the crop. The field experiment was carried out in a greenhouse for a total of 6 weeks at the Anton de Kom University of Suriname. The experiment was performed using a Completely Randomized Block Design (CRBD) with 3 repetitions for each variety and treatment of vermicompost, cow manure, chemical fertilizer, and control (Figure 1). Each block had an area of 6.0 m^2 (4.0x1.50 m^2) and each plot in a block had a length of 1 m, width of 0.75 m and

height of 0.20 m. Each plot in the block consisted of 12 plants of Pak choi at 25x20 cm (Figure 2 and Figure 3), with a total of 36 plants per treatment.

Plant material and cultivation

The white and green Pak choi seeds of the brand TAK II SEED from Japan were used, since this is commonly grown in Suriname. Firstly, the seeds were sown in seed trays (7x15) with potting soil of the brand "Universele potgrond van Egmond" and kept in the greenhouse. The white Pak choi had a germination rate of 94% and the green Pak choi 98%. After 2 weeks, healthy seedlings were transplanted into the field. A planting depth of approximately 3 cm was utilized and plant spacing of 25x20cm. Every day the plants were irrigated, and the harvest took place when the plants were 45 days old. The entire experimental area was managed according to standard practices for nutrient, weed and pest management. The vermicompost, cow manure and chemical fertilizer were applied to the planting hole before transplanting the seedlings into the field. The second amount of fertilizer was applied to the plants in the fourth week. The total amount of added fertilizer per plant is shown in Table 1. According to Sinha et al. (2011), an application of 1 -1.5 tons/acre of vermicompost can be used for leafy vegetables. The same applies for cow manure. For chemical fertilizers, an application of 50 g / m^2 is recommendable.

Plant observation and soil analysis

During the cultivation of Pak choi (*Brassica rapa var. chinensis*) the following growth parameters were taken weekly until harvest: number of leaves and plant height (cm). Upon maturity, the plants were harvested and preserved for analysis. The following plant growth parameters were measured: root length (cm), shoot biomass fresh (weight in grams)/ dry (at 105°C), root biomass fresh (weight in grams)/ dry (at 105°C) and leaf surface area in cm^2. After harvesting, the plants were cut into small pieces and air dried for approximately 48 hours. Afterward, the plant samples were placed in an oven at 60°C for 24 hours to dry. According to the laboratory prescription at the soil laboratory of the Anton de Kom University of Suriname, the dried plant tissue was pulverized into particles smaller than 1mm and analyzed for nutrient value of Total-Ca (%) and Total-Fe (ppm). The organic fertilizers (cow manure and vermicompost) and soil samples were subjected to various chemical analyses. Before planting and after harvesting, soil samples were taken from each plot to a depth of 10 cm. The methods described according to the laboratory prescription at the soil laboratory of the Anton de Kom University of Suriname, as mentioned before; that were used to analyze the following parameters: pH-H_2O, EC, TOC, N, C/N ratio, P and K.

Data analysis

For the statistical data analysis of the *Sigma Plot 12.0* software was used. The data was processed using an Analysis of Variance of Simple Classification and differences between means (one-way ANOVA). Treatments that were significantly different, were analyzed with Tukey's hoc test. A paired-sample t-test was conducted to compare the control (initial soil) and the experiment (final soil) treatment for different chemical parameters. Significance was set at the 0.05 level.

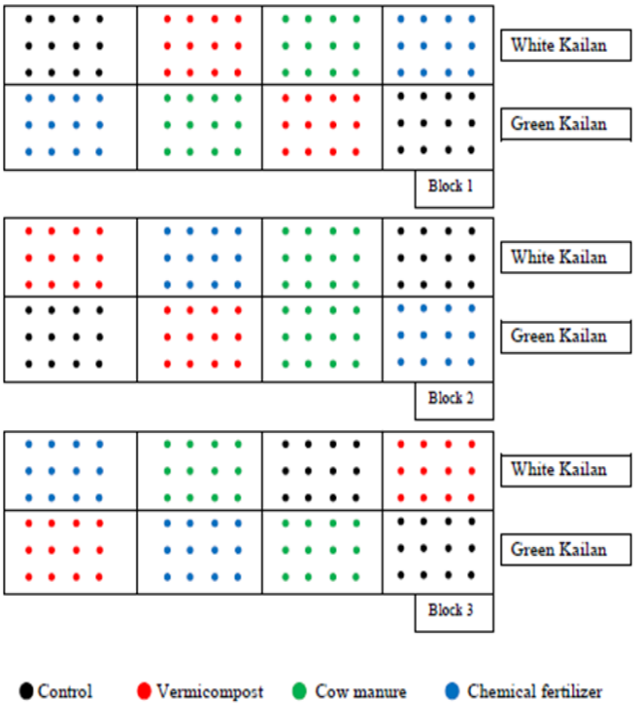

Figure 1. Randomized block design and plot 2016.

Figure 2. Site view of one plot (white Pak choi) at the greenhouse 2016.

Table 1. Fertilization treatments per plant.

Symbol	Treatments	Total amount added per plant (g)
C	Control (soil)	0
S	Cow manure	100
V	Vermicompost	100
K	Fertilizer (12-12-17)	2

RESULTS AND DISCUSSION

The research conducted on the use of vermicompost on the productivity of two varieties of Pak choi is highlighted by the impact in terms of plant growth and soil parameters. In Figure 3 the weekly average number of leaves is given and in Figure 4 the weekly average plant height (cm). It can be observed that the number of leaves and plant height did increase weekly until harvesting. There was progressively a greater number of leaves in treatment green Pak choi [K] followed by white Pak choi [C] and green Pak choi [S] (Figure 3). There was also a progressively greater plant height in treatment white Pak choi [S] followed by white Pak choi [K] and green Pak choi [V] (Figure 4).

The average values of the plant parameters of both varieties were taken on the 45th day at harvesting, which are shown in Table 2. With reference to cultivation of white Pak choi (Table 2), highest plant height was obtained by WS. The highest weight of fresh root was obtained by WS followed by WK and WV. For leaf area, there was a significant difference between WS and WC, WS and WV, WS and WK ($P \leq 0.05$). The highest leaf area was obtained by WS followed by WK and WV. With reference to cultivation of green Pak choi (Table 2), the highest plant height was obtained by GV followed by GC and GS. The highest weight of fresh plant was obtained by GC followed by GV and GK. The highest weight of fresh root was obtained by GS followed by GV and GC. The highest leaf area was obtained by GV followed by GC and GS. The results correlate with the works of Pant et al. 2011 and Archana et al. 2012.

According to Amiri Pour et al. (2013), the application of vermicompost to cabbage seedlings indicated that the effects of vermicompost on plant growth and development (leaf area, number of leaves, fresh and dry mass) not only were nutritional, but also hormonal and biochemical (Zn and auxin contents). Other studies revealed that the application of vermicompost increased leaf area and biomass in various plants such as radish, marigold, upland cress, Chinese cabbage, strawberry, and tomato (Bachman and Metzger 2008; Singh et al. 2008; Singh et al. 2010; Wang et al. 2010; Warman and Anglopez 2010). As reported by Vennila et al. (2012) vermicompost contains some plant growth stimulating substances, promotes better root growth and nutrient absorption.

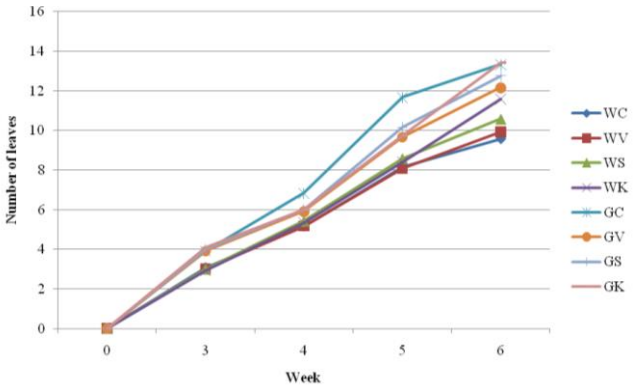

Figure 3. Growth of number of leaves.

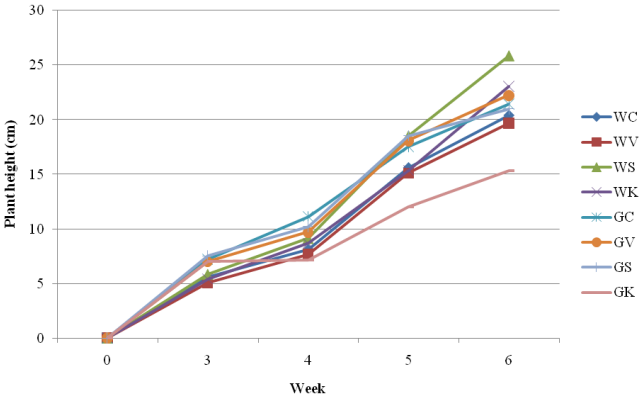

Figure 4. Growth of plant height (cm).

Table 2. Plant parameters of white and green Pak choi (Mean ± SEM).

	Number of leaves	Plant height (cm)	Root length (cm)	Weight fresh plant (g)	Weight fresh root (g)	Leaf area (cm^2)
White Pak choi						
WC	10±0.9 a	20.40±1.8 ab	15±0.8 a	109.80±26.3 a	8.30±1.5 b	181.80±17.9 b
WS	11±0.3 a	25.80±1.2 a	12.90±1.0 a	169.80±22.6 a	16.70±2.0 a	300.40±23.7 a
WV	10±0.3 a	19.70±1.5 b	12.80±1.0 a	108.40±20.0 a	9.70±1.4 b	205.30±18.6 b
WK	12±0.8 a	23±1.1 ab	13.80±1.1 a	115.30±14.9 a	10.20±1.2 b	207.10±17.0 b
Green Pak choi						
GC	13±0.7 a	21.40±0.8 a	13.50±0.7 a	117.10±13.6 a	6.70±0.6 a	141.80±11.9 a
GS	13±0.9 a	21.00±1.1 a	11.40±0.8 a	93.30±14.7 ab	9.30±2.0 a	111.60±10.0 ab
GV	12±0.7 a	22.30±1.2 a	14.00±0.9 a	110.60±18.2 a	8.40±1.1 a	156.00±15.8 a
GK	13±0.8 a	15.30±1.2 b	11.00±0.9 a	46.40±9.0 b	3.10±0.8 b	71.10±6.3 b

Note: WC: White Control; WS: White Cow manure; WV: White Vermicompost; WK: White Chemical Fertilizer; GC: Green Control; GS: Green Cow manure; GV: Green Vermicompost; GK: Green Chemical Fertilizer. *Values followed by different letters are significantly different at $P \leq 0.05$ according to Tukey's multiple range test (Tukey's test)

Earlier studies have reported a positive effect of vermicompost application on growth and productivity of cereals and legumes (Hameeda et al. 2007; Ansari and Jaikishun 2011; Fritz et al. 2012), ornamental and flowering plants (Atiyeh et al.2002; Marfá et al. 2002; Arancon et al. 2008; Chamani 2008; Lazcano and Dominguez 2010), vegetables (Atiyeh et al. 2000; Ansari 2008b; Peyvast et al. 2008; Suthar 2009; Ansari and Sukhraj 2010). Vadiraj et al. (1998) reported that application of vermicompost produced herbage yields of coriander cultivars were comparable to those obtained with chemical fertilizers, which agree with the findings of this current study, indicating that the usage of any artificial chemical input is not needed.

Nutrients in plants are an essential component for the nutritive value in diet and are indication of good plant productivity. Table 3 illustrates an overview of the nutrient value in white and green Pak choi. With reference to cultivation of white Pak choi, the nutrient analysis indicated that the highest T-Ca was observed in WK followed by WS and WC. With reference to cultivation of green Pak choi, the nutrient analysis indicated that the highest T-Fe was observed in GC followed by GK and GV.

The concentrations of total Ca in both varieties of Pak choi were in range of sufficient or normal concentrations (Table 4) of macroelements that should occur in mature leaf tissue, which is between 1 – 4 %. The concentrations of total Fe in both varieties of Pak choi were in range of sufficient or normal concentrations of microelements that should occur in mature leaf tissue, which is between 100 – 500 ppm (Munson 1998).

Table 5 provides an overview of the cow manure, vermicompost and soil measured parameters before and after harvesting of the field experiment "cultivation of white and green Pak choi". The results of the chemical analysis of cow manure and vermicompost are also shown. For each analysis, 3 samples were taken to determine the nutrient value. The pH-H_2O in the vermicompost was 6.60±0.00 and in the cow manure 6.03±0.03. The soluble salt concentrations (measured as Electric conductivity) in the resulting vermicompost and cow manure were respectively 7200±180µS/cm and 8367±300µS/cm, indicating a lower salinity in the vermicompost compared to the cow manure. The total organic carbon was 23.20±0.59% in the vermicompost and 17.69±0.72% in the cow manure. The total nitrogen was 1.60±0.04% in the vermicompost and 1.48±0.05% in the cow manure. The C/N ratio in the vermicompost and cow manure was as followed 15:1 and 12:1. Total phosphorus was 8745±604.26 ppm in the vermicompost and 9818±195.73 ppm in the cow manure. The total potassium was 7030±186.48 ppm in the vermicompost and 8255±473.30 ppm in cow manure. Vermicompost contains essential nutrients, which were within the limits as reported by earlier researchers (Ismail 1997; Ansari and Sukhraj 2010; Ansari et al. 2016).

Soil pH did not have a notable increase or decrease for all the treatments (Table 6). Soil pH is one of the most important soil properties that affect the availability of nutrients. Macronutrients tend to be less available in soils with low pH and micronutrients tend to be less available in soils with high pH (Ansari and Jaikishun 2011). According to Krogh (2005), worm cast and composts are considered to have a positive effect on soil by helping to balance the pH, retain moisture, improve drainage, and control pathogens. Vermicompost improves the pH of soil and makes the nutrient available for the crop yield (Srikanth et al. 2000).

Soil EC did increase in all the samples though not significantly, except for WS which indicated a relevant increase (Table 7). According to Atiyeh *et al.* (2002), the electrical conductivity of vermicompost depends on the raw materials used for vermicomposting and their ion concentration.

Organic carbon did not increase or decrease significantly in the soil (Table 8). According to Ansari and Jaikishun (2011), vermicompost are rich in organic carbon content and can release these into the soil very slowly and steadily, enabling the plants to absorb the available nutrients (Lalitha et al. 2000; Ansari 2008a; b).

Nitrogen did not increase or decrease notably in the soil (Table 9). The presence of nitrogen fixing bacteria in the vermicompost plays a great role in increasing the nitrogen content of the soil and mineralization (Lalitha et al. 2000; Debosz et al. 2002; Arancon et al. 2006; Ansari 2008a; b).

Phosphorous did decrease notably in the treatments WV, GC, and GS (Table 10). According to Erich *et al.* (2002), the application of vermicompost increases the bioavailability of phosphorus in the soil affecting plant growth in potato cropping.

Table 3. Nutrient value in Pak choi varieties after harvesting (Mean ± SEM).

Parameter	T-Ca (%)	T-Fe (ppm)
White Pak choi		
WC: White Control	1.94 ± 0.08 a	114 ± 6.85 a
WS: White Cow manure	1.94 ± 0.04 a	122 ± 5.36 a
WV: White Vermicompost	1.67 ± 0.03 b	123 ± 4.07 a
WK: White Chemical Fertilizer	2.00 ± 0.02 a	112 ± 6.42 a
Green Pak choi		
GC: Green Control	2.22 ± 0.06 a	146 ± 8.71 a
GS: Green Cow manure	2.12 ± 0.05 a	107 ± 6.32 b
GV: Green Vermicompost	2.14 ± 0.06 a	107 ± 2.83 b
GK: Green Chemical Fertilizer	2.27 ± 0.04 a	108 ± 4.54 b

Note: *Values followed by different letters are significantly different at P≤0.05 according to Tukey's multiple range test (Tukey's test).

Table 4. Concentration or ranges of the major elements and micronutrients in mature leaf tissue generalized as deficient, sufficient, or excessive for various plant species (Munson 1998) .

Essential elements	% Deficient	% Sufficient or normal	% Excessive or toxic
Major elements			
Calcium (Ca)	<0.50	1.00 - 4.00	>5.00
Micronutrients	**ppm**	**ppm**	**ppm**
Iron (Fe)	<50	100 - 500	>500

Table 5. Cow manure, vermicompost and soil parameters (Mean ± SEM).

Parameter	pH-H_2O	EC (µS/cm)	TOC (%)	TN (%)	C/N ratio	TP (ppm)	TK (ppm)
Cow manure	6.03±0.03	8367±300	17.69±0.72	1.48±0.05	12: 1	9818±195.73	8255±473.30
Vermicompost	6.60±0.00	7200±180	23.20±0.59	1.60±0.04	15: 1	8745±604.26	7030±186.48
Before							
Soil	7.65±0.35	369±36.50	3.87±0.76	0.25 ± 0.04	16: 1	2050±127.75	792±34.26
After							
WC	7.85±0.05	1612±498	4.52±1.44	0.25±0.12	18: 1	2813±2717	1011±410
WS	7.55±0.05	1465±114	4.41±0.40	0.31±0.06	14: 1	648±10	853±35
WV	7.60±0.00	1525±318	3.82±0.59	0.25±0.0	15: 1	276±77	725±25
WK	7.95±0.15	1202±350	2.95±0.09	0.17±0.0	17: 1	851±623	834±296
GC	7.65±0.35	1314±465	4.52±1.62	0.29±0.11	16: 1	490±186	685±15
GS	7.60±0.10	1509±711	4.35±0.11	0.32±0.04	14: 1	1049±154	996±64
GV	7.60±0.00	1164±379	5.99±0.42	0.37±0.03	16: 1	3228±2733	1103±408
GK	7.65±0.05	2045±246	4.89±0.96	0.30±0.09	16: 1	2972±2675	1046±481

Note: WC: White Control; WS: White Cow manure; WV: White Vermicompost; WK: White Chemical Fertilizer; GC: Green Control; GS: Green Cow manure; GV: Green Vermicompost; GK: Green Chemical Fertilizer; TOC: Total Organic Carbon; TN: Total-N; TP: Total-P; TK: Total-K

Table 6. Soil pH (Mean ± SEM).

Treatment	Initial soil	Final soil	Increase in pH
WC	7.65±0.35	7.85±0.05	0.20
WS	7.65±0.35	7.55±0.05	-0.10
WV	7.65±0.35	7.60±0.00	-0.05
WK	7.65±0.35	7.95±0.15	0.30
GC	7.65±0.35	7.65±0.35	0.00
GS	7.65±0.35	7.60±0.10	-0.05
GV	7.65±0.35	7.60±0.00	-0.05
GK	7.65±0.35	7.65±0.05	0.00

Note: Confidence level 95%; - indicates decrease

Table 7. Soil EC (µS/cm) (Mean ± SEM).

Treatment	Initial soil	Final soil	Increase in EC (µS/cm)
WC	369±36.50	1612±498	1243
WS	369±36.50	1465±114	1096
WV	369±36.50	1525±318	1156
WK	369±36.50	1202±350	833
GC	369±36.50	1314±465	945
GS	369±36.50	1509±711	1140
GV	369±36.50	1164±379	795
GK	369±36.50	2045±246	1676

Note: Confidence level 95%; - indicates decrease

Table 8. Total Organic carbon (%) (Mean ± SEM).

Treatment	Initial soil	Final soil	Increase in OC (%)
WC	3.87±0.76	4.52±1.44	0.65
WS	3.87±0.76	4.41±0.40	0.53
WV	3.87±0.76	3.82±0.59	-0.05
WK	3.87±0.76	2.95±0.09	-0.93
GC	3.87±0.76	4.52±1.62	0.65
GS	3.87±0.76	4.35±0.11	0.47
GV	3.87±0.76	5.99±0.42	2.11
GK	3.87±0.76	4.89±0.96	1.01

Confidence level 95%; - indicates decrease

Table 9. Total Nitrogen (%) (Mean ± SEM).

Treatment	Initial soil	Final soil	Increase in N (%)
WC	0.25 ± 0.04	0.25±0.12	0.00
WS	0.25 ± 0.04	0.31±0.06	0.06
WV	0.25 ± 0.04	0.25±0.00	0.00
WK	0.25 ± 0.04	0.17±0.0	-0.08
GC	0.25 ± 0.04	0.29±0.11	0.04
GS	0.25 ± 0.04	0.32±0.04	0.07
GV	0.25 ± 0.04	0.37±0.03	0.12
GK	0.25 ± 0.04	0.30±0.09	0.05

Note: Confidence level 95%; - indicates decrease

Table 10. Total phosphorous (ppm) (Mean ± SEM).

Treatment	Initial soil	Final soil	Increase in P (ppm)	t-test
WC	2050±127.75	2813±2717	763	NS
WS	2050±127.75	648±10	-1402	NS
WV	2050±127.75	276±77	-1774	*
WK	2050±127.75	851±623	-1199	NS
GC	2050±127.75	490±186	-1560	*
GS	2050±127.75	1049±154	-1001	*
GV	2050±127.75	3228±2733	1178	NS
GK	2050±127.75	2972±2675	922	NS

Note: Confidence level 95%; - indicates decrease; *= significant; NS= not significant

Table 11. Total Potassium (ppm) (Mean ± SEM).

Treatment	Initial soil	Final soil	Increase in K (ppm)
WC	792±34.26	1011±410	219
WS	792±34.26	853±35	61
WV	792±34.26	725±25	-67
WK	792±34.26	834±296	42
GC	792±34.26	685±15	-107
GS	792±34.26	996±64	204
GV	792±34.26	1103±408	312
GK	792±34.26	1046±481	254

Note: Confidence level 95%; - indicates decrease

Potassium increase in the treatments in the soil was not relevant, although there was a decrease in WV and GC (Table 11). The increase in potassium uptake by vermicompost application may be due to the enhancement in potassium availability by shifting the equilibrium among the forms of potassium from relatively exchangeable potassium to soluble potassium forms in the soil (Bhasker et al. 1992).

In conclusion, the evaluation of the use of vermicompost on the crop production of 2 varieties of Pak choi (*Brassica rapa* var. *chinensis*) proved that the plants treated with vermicompost had similar results as those treated with cow manure and chemical fertilizer. The plant nutrient concentrations of Ca and Fe were in range of sufficient or normal concentrations of macro and microelements that should occur in mature leaf tissue. The soil parameters (pH, OC, N) did not indicate a significant increase or decrease of the elements in the soil. P did decrease significantly and K increase in the treatments in the soil was not relevant. This research should be continued to evaluate the use of vermicompost in the production of other vegetables.

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Insecticidal selectivity of jayanti plant (*Sesbania sesban*) for integrated control of diamondback moth (*Plutella xylostella*)

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Abstract. *Suripto, Sukiman, Gunawan ER. 2017. Insecticidal selectivity of jayanti plant (*Sesbania sesban*) for integrated control of diamondback moth (*Plutella xylostella*). Asian J Agric 1: 80-84.* It has been known previously that leaves of the jayanti plant (*Sesbania sesban* (L.) Merr.) contain insecticidal ingredients. This study aimed to evaluate the insecticidal selectivity of various extract fractions of *S. sesban* leaf for the integrated control of cabbage pest, the diamondback moths (*Plutella xylostella*). Dried leaf powders from *S. sesban* were extracted in stages by using hexane, dichloromethane (DCM), ethanol, and water, successively. Each insecticidal performance of *S. sesban* leaf extract fractions were tested against two types of test insects, namely *P. xylostella* larvae as target and *Diadegma semiclausum* imago as non-target insects according to completely randomized design in the cabbage plantation at the village of Sembalun, East Lombok, Indonesia. Each mortality data of *P. xylostella* larvae and *D. semiclausum* imago was processed by *probit analysis* to determine the concentration of the death of 50% of test insects (LC_{50}) of each test extract. The results showed that the LC_{50} of *S. sesban* leaf extracts classified into four fractions, namely hexane, DCM, and water extract fractions to *P. xylostella* larvae successively was 343.71, 294.78, 29.95, and 1197.13 ppm, and to *D. semiclausum* imago row was 305.5, 121.56, 37.38, and 1043.70 ppm. The results showed that the insecticidal activity of *S. sesban* leaf ethanol extract fraction was selective because its selectivity value is 1.25. On the other hand, each insecticidal performance of three *S. sesban* leaf extracts, i.e., hexane, DCM, and water extract fractions, respectively is not selective, with the selectivity values are 0.89, 0.41, and 0.87.

Keywords: *Diadegma semiclausum*, insecticidal selectivity, *Plutella xylostella*, *Sesbania sesban*

INTRODUCTION

As a producer of vitamins and minerals, vegetables are a source of nutrients required for the human body. Many vegetables, like cabbages, are consumed by humans. One of the factors causing low production of cabbage vegetables in Indonesia is due to pests. There are two important types of pests that attack cabbage plants, namely *Plutella xylostella* L. and *Crocidolomia binotalis* Zell. As a result of the attack of cabbage worms (larvae of *P. xylostella*), it is estimated that cabbage crop production could decline by more than 90% (Verkerk and Wright 1996).

The use of insecticides to control pests that destroy cabbages, has cost more than 1 billion US $ per year (Talekar and Shelton 1993) worldwide. On the other hand, the practice of pest control with insecticides of synthetic chemicals in excess can cause problems, such as the increasing resurgence and pest resistance, and the declining population of parasitoid as a natural control agent (Coasts 1994; Suripto and Sukiman 2016).

Based on fact the seriousness of the diamondback moth pest problem, it is necessary to learn the application of natural or biological insecticides to reduce the use of synthetic chemical insecticides. Leaves of the jayanti plant (*Sesbania sesban* (L.) Merr.) have been known to have a high content of saponins, which has anti-insect activity (Mahato and Nandy 1991; Suripto et al. 2010). However, the effectiveness of the application of *S. sesban* insecticides for controlling diamondback moth, *P. xylostella* in the field is not yet known.

This research was aimed to determine the insecticidal selectivity (LC_{50}) of various extract fractions of *S. sesban* leaf against two test types of insects, namely *P. xylostella* as the target insect and *Diadegma semiclausum* as the non-target insect.

MATERIALS AND METHODS

Extraction of the active insect repellent compounds from *Sesbania sesban* Leaf

Leaves of a two-year old or more of *Sesbania sesban* (L.) Merr. species were collected. After wind drying, the leaves were milled and powdered. Later they were extracted in stages to collect the active insect repellent compounds using solvents series, which increased polarity in succession, namely hexane, dichloromethane (DCM), ethanol, and water.

Extraction was done by maceration of dry *S. sesban* leaf powders by procedure according to Harborne (1998). The solvent on each extract fraction was evaporated using a vacuum rotary evaporator and then moved into the cup resulting viscous extract condensed further in the evaporation chamber. The resulting paste form extracts were incorporated into a dark bottle before used in the bioassay.

Producing larvae of *Plutella xylostella* and imago of *Diadegma semiclausum*

Plutella xylostella insects were collected in the cabbage plantation in The Sembalun Lawang Village, Lombok Timur Regency, Nusa Tenggara Barat Province, Indonesia.

Producing Larvae of *P. xylostella* was done by using cabbage as an attractant for *P. xylostella* to lay eggs and as feed for the larvae (instar 1 to III) with the procedures according to Solichah et al. (2004) and Suripto and Sukiman (2015) until reaching a sufficient population for bioassay.

Pupa of *D. semiclausum* was collected from the cabbage plantation from the same location. Mass breeding imago of *D. semiclausum* from their pupa was carried in a nylon cage 50 cm x 50 cm x 40 cm with diameter 2 m for each mesh. By using a solution of pure bee honey as feed in accordance with the procedures according to Wing and Keller (2008) and Suripto and Sukiman (2015) to obtain a sufficient population for bioassay.

Bioassay

Insecticidal test of extract fractions of *S. sesban* leaf against *P. xylostella* and *D. semiclausum was* carried out using six concentration treatments based on the Complete Randomized Design (CRD) with the procedure of AVRDC (Khaidir and Hendrival 2013; Supartha et al. 2014; Suripto and Sukiman 2016).

The treatment on *P. xylostella larvae* mortality test was given by spraying the extract solution in accordance with the concentration of each treatment on each test cabbage leaf surface infected by larvae of *P. xylostella*. The variables measured were the percentage of the number of dead larvae after six hours of treatment.

Imago of *D. semiclausum was* released in each nylon cage and fed using a solution of pure bee honey. The treatment was done by spraying a solution of the extract according to each concentration treatment into a confinement chamber containing the test cabbage crops and *D. semiclausum* imago (40 animals per cage). The variables measured were the percentage of the number of *D. semiclausum* imago died after six hours of treatment. The work flowchart of the insecticidal selectivity evaluation of various *S. sesban* leaf extract fractions against *P. xylostella* larvae and *D. semiclausum* imago can be seen in Figure 1.

Data analysis

Each mortality data of *P. xylostella* larvae and *D. semiclausum* imago was processed by probit analysis (Busvine 1974) to produce the LC_{50} (the concentration of the death of 50% of test insects) of each test extract.

Based on the LC_{50} on *P. xylostella* and *D. semiclausum*, the value of insecticidal selectivity (IS) can then be determined by using the formula according to Wang et al. (2004) as follows:

$$IS = \frac{LC_{50} \text{ on } P. \textit{ Xylostella}, \text{ as target insect type}}{LC_{50} \text{ on } D. \textit{ semiclausum}, \text{ as non-target insect}}$$

Criteria used to determine the selectivity (IS) were as follow: (i) If IS > 1, then insecticidal selectivity of the test extract fraction is high or selective; (ii) If IS ≤ 1, then insecticidal selectivity of the test extract fraction is low or not selective.

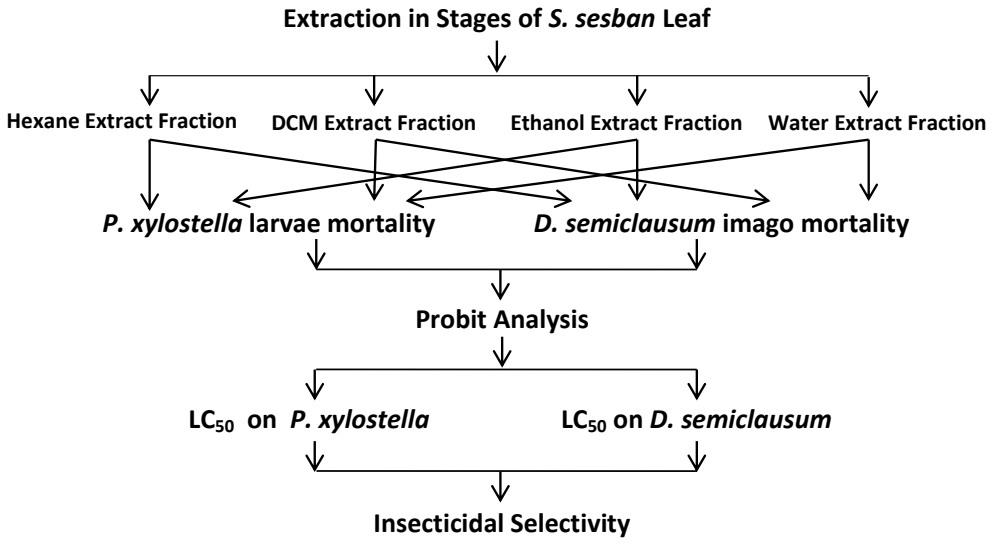

Figure 1. The flowchart of evaluating insecticidal selectivity of various *S. sesban* leaf extract fractions

RESULTS AND DISCUSSION

Four extract fractions of *S. sesban* leaf, namely extract fraction-hexane,-DCM, -ethanol, and- water were respectively lethal acute toxic against the diamondback moth (larvae of *P. xylostella*) and the parasitoid, imago of wasp beetle (*D. semiclausum*). However, toxicity of the extract fraction-ethanol of *S. sesban* leaf against larvae of *P. xylostela* was much higher than the toxicity of the other three extract factions. Mortalities of *P. xylostella* larvae and imago of *D. semiclausum* in each *S. sesban* leaf extract fraction treatment can be seen in Figure 2.

Four extract fractions of *S. sesban* leaf, namely extract fraction-hexane,-DCM, -ethanol, and-water were respectively causing lethal acute toxicity against the diamondback moth (larvae of *P. xylostella*) and the parasitoid, imago of wasp beetle (*D. semiclausum*). However, toxicity of the extract fraction-ethanol of *S. sesban* leaf against larvae of *P. xylostela*, was much higher than the toxicity of the other three extract factions. Mortalities of *P. xylostella* larvae and imago of *D. semiclausum* in each *S. sesban* leaf extract fraction treatment can be seen in Figure 2.

Results also showed that toxicity of the extract fraction-hexane,-DCM, and -water, respectively against *D. semiclausum*, as non-target insect higher than to against *P. xyloxtella*, as target insect. Thus, each of three extract fractions is considered to have properties that are not a selective insecticide for control of diamondback moth.

Unlike the other three extract factions, the extract fraction-ethanol of *S. sesban* leaf showed lower acute lethal toxicity to *D. semiclausum* imago compared to *P. xylostella* larvae. Thus, the extract fraction-ethanol of *S. sesban* leaf can be considered to have insect repellent properties which are selective for controlling diamondback moth, because it is very toxic to *P. xylostella* larvae as a target insect and it's toxicity is very low or not toxic to the parasitoid, *D. semiclausum* imago as non-target insects. Comparison of LC_{50} (Concentrations of death of 50% of test animals) of four extract factions of *S. sesban* leaf against *P. xylostella* larvae and *D. semiclausum* imago and insecticidal selectivity values, results of probit analysis can be seen in Table 1.

Table 1. LC_{50} (in ppm) of various extract fractions of *Sesbania sesban* leaf against *Plutella xylostella* larvae as target insects and *Diadegma semiclausum* imago as non-target insects

Extract Fraction	*P. xylostella*	*D. semiclausum*	Selectivity
hexane	343.7101	305.5448251	0.888961
DCM	294.7871	121.5585483	0.41236
ethanol	29.94509	37.37949173	**1.248268**
water	1197.129	1043.699945	0.871836

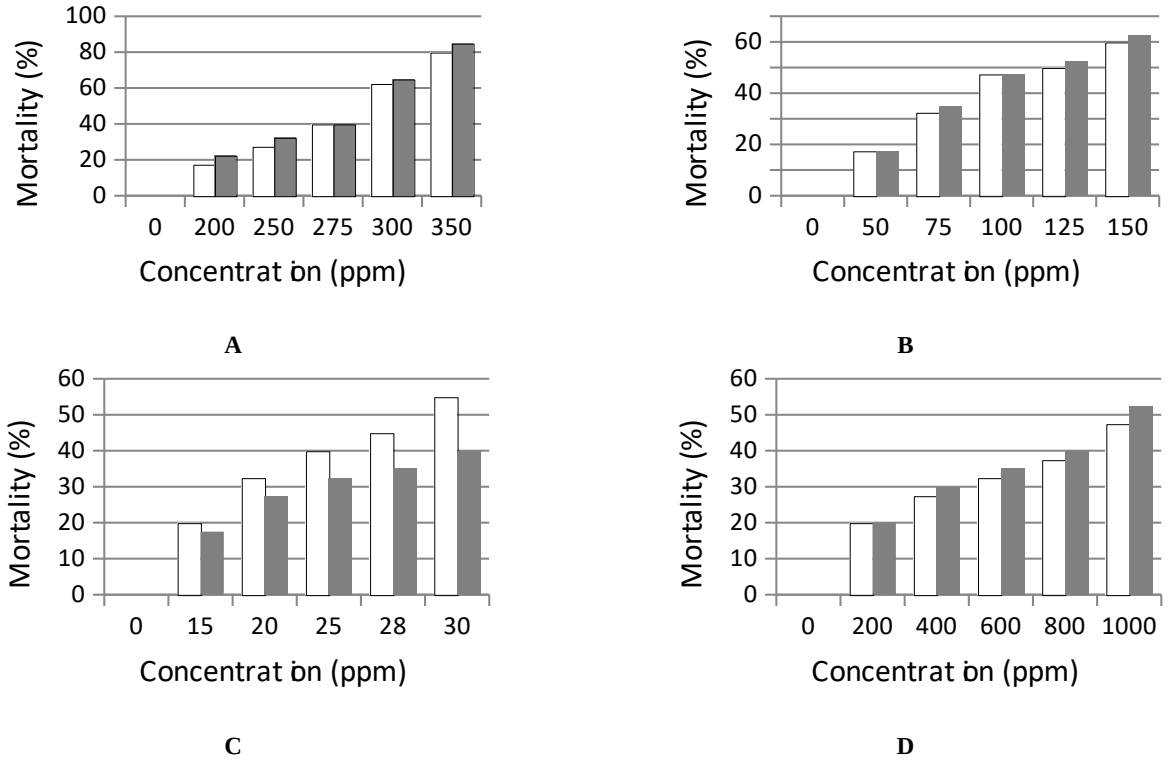

Figure 2. Mortality of *Plutella xylostella* larvae (☐) dan *Diadegma semiclausum* imago (■) in various *S. sesban* leaf extract fraction tretments: (a) extract fraction-hexane, (b) extract fraction-DCM, (c) extract fraction-ethanol, dan (d) extract fraction-water

Table 2. Acute lethal toxicity of *Sesbania sesban* leaf extracts to larvae of *Plutella xylostella* and imago of *Diadegma semiclausum* and their selectivity (Suripto et al. 2010)

Solvent for extraction	LC_{50} 24 hours (ppm)		The value of selectivity (S)
	P. xylostella	*D. semiclausum*	
Hexane	343,71	305,54	0,8890
DCM	134,77	121,56	0,9020
Ethanol	29,62	37,39	1,2619
Water	5071,55	2689,61	0,5303

Each *S. sesban* leaf extract, obtained by using a single solvent such as hexane and DCM are shown to have lower selectivity for the control of caterpillar cabbage, using *P. xylostella* larvae as target animal and imago *D. semiclausum* as non-target animal, compared with ethanol extract (Table 2) (Suripto et al. 2010).

It is known that the active component of anti-insects from *S. sesban* leaf is a class of saponins (Suripto et al. 2010). The active ingredient in the form of group saponins from *S. sesban* leaf extract can affect the permeability of cell membranes, including nerve cells in the larvae of *P. xylostella* treated with the extract. According to Francis et al. (2002), changes in the nerve cell membrane permeability can interfere with the transmission of nerve cells, and one consequence is the removal of acetylcholine too fast. This incredible accumulation of acetylcholine causes muscle spasms quickly followed by swelling, paralysis and subsequent death of the larvae of *P. xylostella*.

Because of the insecticidal ingredient of the *S. sesban* leaf, in this case extract, fraction-ethanol has a very high lethal acute toxicity against *P. xylostella*, but not toxic or very low toxicity to the parasitoid, *D. semiclausum*. The use of natural insecticide from *S. sesban* plant can be considered quite effective and efficient for controlling diamondback moth. With the LC_{50} is 29.95 ppm, and when each 10 mL of the extract solution is sprayed enough for each plant cabbage, then for the applications to 100 thousand cabbage plants required only 29.95 g of the extract fraction-ethanol of *S. sesban* leaf. In this study, the mass of extract fraction-ethanol produced by 20% of the dry weight from *S. sesban* leaf powders extracted. Therefore, the mass of 29.95 g of the extract fraction can be obtained from the extraction of approximately 150 g of *S. sesban* leaf dried powders.

Saponins of *S. sesban* plant may not be harmful to plant cabbage. The content of bioactive form of saponins from leaves of *S. sesban* is also harmless to animals and humans because this is only lethal acute toxic when it is administered intravenously, but very low toxicities when administered orally (Francis et al. 2002; Quetin-Leclerq et al. 1992). It is possibly safe as a use of insecticide, containing an active ingredient extracted from *S. sesban*. This is also based on the fact that leaves of *S. sesban* were often used by farmers as green manure (Heyne 1998) and were also frequently used as a livestock feed mixer (Shqueir et al. 1989).

Altogether, *S. sesban* plants can be developed as a source of natural insecticide for integrated control of diamondback moth, which is feasible for farmers and secure environment. This is in accordance with the criteria for selection of plants as a source of natural insecticide according to Hamburger and Hostettmann (1991) and Schmutterer (1997), i.e., the high toxicity against target insects but very low toxicity to natural enemies or non-target insects.

Conclusion

One of four leaf extract fractions of Jayanti plant (*S. sesban*) studied, the extract fraction-ethanol has the highest acute lethal toxicity against *P. xylostella* larvae, but its toxicity is very low to the parasitoid, *D. semiclausum*. Insecticidal activity of the extract fraction-ethanol of *S. sesban* leaf is considered selective for integrated control for diamondback moth. On the other hand, the other three extract fractions i.e. extract fraction-hexane, -DCM, and water have unselective insecticidal activity for controlling diamondback moth, because each of their toxicity to *P. xylostella* as a target insect was higher than to *D. semiclausum* as a non-target insect type.

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Genetic engineering of sweet potatoes (*Ipomoea batatas*) using isopentenyl transferase gene for enhanced drought tolerance

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Abstract. *Nawiri SO, Oduor RO, Jalemba AM. 2017. Genetic engineering of sweet potatoes (*Ipomoea batatas*) using isopentenyl transferase gene for enhanced drought tolerance. Asian J Agric 1: 85-99.* Approximately 70% of yield crop reduction worldwide is caused by drought. Due to severe drought, which happens often as a result of climate change, substantial yield deprivation is usual among the major cereals such as maize, wheat, and barley. Therefore, drought tolerant crops that still yield amidst erratic climatic phenomena are greatly needed. Due to its capability to produce high yield in a short period, sweet potato is suitable for cultivation in regions with limited or erratic rainwater supply where other food crops cannot grow easily. Nevertheless, its sensitivity to water deficit may lead to adverse crop growth and yield. By conventional hybridization method, sweet potato is tried to be improved, but it gives unsatisfied results due to its high male sterility, sexual incompatibility and hexaploidy nature of its genome. The aim of this study, therefore, is to develop new varieties of sweet potato with improved tolerance to water-deficit stress for sustainable production of sweet potato under water-limited conditions. Three sweet potato genotypes: Jewel, Kemb36, and Ksp36 were transformed using *isopentenyl transferase* gene (*IPT*) that delays drought-induced senescence via up-regulation of cytokinin biosynthesis, under the control of a water-deficit responsive and maturation specific promoter (PSARK). The PNOV-IPT gene construct was introduced into sweet potatoes to evaluate their transformability and regenerability. It was done via *Agrobacterium tumefaciens* strain EHA101 and the plants subsequently regenerated via somatic embryogenesis. Jewel genotype recorded the highest transformation and regeneration frequency, followed by Kemb36 and KSP36. Calli were cultured on media supplemented with various mannose concentrations to evaluate the suitability of mannose as a selectable marker for sweet potato, and it was discovered that 30 g/L concentration was optimal for selection of transformed events. At the time of PCR analysis, Jewel had the highest transformation efficiency followed by Kemb36. At the time for evaluation on drought tolerance under controlled conditions, the sweet potato showed delayed senescence and greater drought tolerance under water deficit conditions in the glasshouse. These plants exhibited better growth, higher yield, higher water status maintenance, higher chlorophyll content, and thus higher photosynthetic rates under reduced water conditions in comparison to wild-type. These results, therefore, indicated that expression of *isopentenyl transferase* gene in sweet potato significantly improves drought tolerance. Therefore, *IPT* gene should be used to transform other economically important food crops to delay drought-induced senescence and enhance drought tolerance.

Keywords: Drought tolerance, genetic engineering, *Ipomoea batatas*, isopentenyl transferase gene

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) is a perennial, herbaceous dicotyledonous species belonging to the order Polemoniales and the family Convolvulaceae (Mervat et al. 2009). The crop is ranked as the world's seventh most important food crop after wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize (*Zea mays*), potato (*Solanum tuberosum*), barley (*Hordeum vulgare*), and cassava (*Manihot esculenta* Crantz.) (Kim et al. 2010). Sweet potato is grown in more than 110 countries, on an area estimated at 8.5 million hectares (Placide et al. 2013). In Africa, the area under sweet potato cover is estimated at 1,714,000 hectares. The annual global production of sweet potato is estimated at 106.5 million metric tonnes and out of this, 15% of the annual production is from Eastern and Central Africa. China is the highest producer of sweet potato at 80% of the total of the world annual production, followed by Nigeria and Uganda (FAOSTAT 2010).

Sweet potato is an economically important crop in tropical, subtropical, and warm temperate regions (Mervat et al. 2009). It is a valuable source of food, animal feed and industrial raw material (Anwar et al. 2010). The crop is known as "the crop of the twenty-first century" because it represents a potential new source of energy. The storage roots of sweet potatoes have high a carbohydrate content that ranges between 80-90% of their dry weight. The carbohydrates consist mainly of starch, sugars, and a low quantity of pectin, hemicelluloses, and cellulose (Lebot 2009). The high energy levels of sweet potato, make it an attractive industrial raw material for biodegradable plastics and for biofuel production (Placide et al. 2013).

The presence of trypsin inhibitor is found in sweet potato, and thus has several medicinal benefits, along with the major storage root of protein. Trypsin inhibitor has antioxidant activity; it inhibits angiotensin converting enzyme activity, growth, and induction of apoptosis in NB4 promyelocytic leukemia cells (Chen et al. 2010). The antioxidant compounds such as anthocyanin, carotenoids, and vitamin C (Teow et al. 2007) have attracted special attention because they can protect the human body from oxidative stress reactants, which may promote aging and many diseases including cancer (Halliwell 2007; Luceri et al. 2008).

Facing a global undersupply of water, appropriate action should be done to overcome this problem, especially on the sweet potato plantation. This plant is relatively drought tolerant, but at the stage of growth and development, this plant is susceptible to drought. Conventional sweet potato proliferation can improve drought tolerance, but requires a long time to select suitable plants. In recent years, with the rapid development of plant gene engineering, it has become feasible to improve crops using transgenic technology (Liu et al. 2008; Wang et al. 2008; Cao et al. 2009). Several attempts have shown that an enhanced tolerance to drought in sweet potato can be achieved through introduction of genes that encode antioxidant enzymes (Cu/Zn superoxide dismutase [CuZn SOD] and ascorbate peroxidase [APX]) and kinase (nucleoside diphosphate kinase 2 [NDPK2]) (Kim et al. 2009; Lu et al. 2010).

Genes encoding transcription factors have received much attention in recent years, due to their ability to activate stress tolerance genes under drought conditions (Jiang et al. 2010). The DREB/CBF class of transcriptional factors have been found to be effective in conferring drought tolerance (Umezawa et al. 2006; Shinozaki and Yamaguchi-Shinozaki 2007; Yang et al. 2010). Besides progress being made in expressing stress-related transcription factor genes in transgenic plants, several reports indicate that changes in hormone homeostasis, brought about by the expression of *isopentenyl transferase* (*IPT*) a key enzyme in the biosynthesis of cytokinin, under the control of a maturation-and stress-induced promoter (PSARK), resulted in enhanced drought tolerance (Rivero et al. 2007; Rivero et al. 2009; Rivero et al. 2010).

To regenerate *IPT* transgenic sweet potato lines, *Agrobacterium tumefaciens*-mediated transformation was used in this study. Overexpression of *IPT* gene in sweet potato led to significantly enhanced drought tolerance, showing the potency to develop drought stress-tolerant cultivars of this crop. The pathway involves the transfer of isopentenyl group from the dimethylallyl diphosphate (DMAPP) to the N6 of AMP resulting in the production of isopentenyl adenosine-5-monophosphate (iPMP). This reaction is catalyzed by DMAPP: AMP isopentenyl transferases (Kakimoto 2001).

The objectives of this research were (i) To transform and regenerate sweet potato varieties Kemb36, KSP36 and Jewel for enhanced drought tolerance using *isopentenyl transferase (IPT*) gene. (ii) To evaluate the effect of explant used on transformation and regeneration. (iii) To evaluate effectiveness of phosphomannose isomerase as a selectable marker for generation of transgenic sweet potato events. (iv) To evaluate the performance of *IPT* expressing transgenic sweet potato varieties under drought stress.

MATERIALS AND METHODS

Source of explants

This study used two Kenyan sweet potato cultivars, i.e., Kemb36, KSP36 obtained from Kenya Agricultural Research Institute (KARI) and one non-African cultivar, i.e., Jewell obtained from International Potato Centre (IPC) after virus indexing as observation materials. These plants were grown under greenhouse conditions at Kenyatta University. The plants were allowed to grow for two months before being used as stock plants for subsequent regeneration experiments.

Surface sterilization

Stem cuttings were accumulated from healthy vines from the potted sweet potato in the glasshouse and the leaves were removed. The stem pieces were then cut into 2 cm long nodal cuttings, each having a bud (Figure 1.A). The vines were cleansed thoroughly under running water to remove any loose dirt on the surface. This was followed by soaking them in 70% (v/v) ethanol for 3 min and then finally soaking in 2.5% (v/v) sodium hypochlorite (JIK) containing 2-3 drops of Tween 20 for 20 minutes with occasional shaking under sterile conditions in the laminar floor (Njagi 2004). To remove the sodium hypochlorite, the stems were rinsed in sterile distilled water then blot dried using Whatman filter papers (CAT No. 1001 090 Whatman International limited England). The damaged parts were removed using a sterile scalpel and were now ready for propagation experiment.

In-vitro propagation of sweet potato

In-vitro plants were cultured on sweet potato propagation media, as per the procedure described by Luo et al. (2006). Two sterile nodal segments, with at least two nodes, were cultured in universal culture bottles containing 80 mL of sweet potato propagation medium (Figure 1.B). The cultures were incubated in the growth room at 28°C, 16/8 h light/dark photoperiod, 70% relative dampness and 3,000 light intensities supplied by fluorescent bulbs for 3-5 weeks. The cultures were kept in the growth room as in-vitro source of clean explants for regeneration and transformation experiments (Figure 1.C) and were subcultured after every 5 weeks to keep an adequate supply of clean mother plants (Figure 1.D).

Determination of optimal mannose concentration for selection of transformed sweet potatoes

Four-week-old leaf explants from the in-vitro plants were used to evaluate the impact of mannose on callus proliferation and embryo development on callus induction medium supplemented with different carbohydrate sources. First, the explants were put on callus induction medium to initiate calli. Upon callus formation, the young calli were displaced to selection medium containing varying concentrations of mannose. The callus induction medium was divided into four equal parts and different concentrations (1%, 2%, 3%, and 4% w/v) of mannose added. Callus induction medium with 30 g/L of sucrose was used as positive control while one without sucrose was used as a negative control. Cultures were maintained in the dark at 27±1°C for 12 weeks then subcultured on the same substrate medium after every two weeks. During subculturing, callus proliferation and aspect (color, consistency, and hydration) were qualitatively evaluated.

Figure 1. In-vitro propagation of sweet potato. A. Jewel nodal stem before propagation. B. KSP36 nodal stems in sweet potato propagation media 3 days after incubation. C. Fully established Jewel in-vitro plant. D. Kemb36 in-vitro plant after 4weeks on propagation media (bar = 2cm).

Bacterial strains and plasmid

Agrobacterium tumefaciens strain EHA 101 harboring binary plasmid vector pNOV-IPT was used in this study. The pNOV-IPT binary vector contained *IPT* gene driven by SARK promoter. This gene construct also harbored a *PMI* gene as a selectable marker gene encoding phospomannose Isomerase enzyme that confers resistance to mannose in positively transformed tissues. The IPT gene was a kind donation by Dr. Edwardo Blumwald from the University of California Davis, USA.

Maintenance of *Agrobacterium tumefaciens* strain EHA101 harboring PNOV-IPT gene construct

Agrobacterium tumefaciens strain EHA101 carrying the *IPT* gene construct was initiated form stock plates stored for less than two weeks at 4ºC and maintained on LB medium (Duchefa, Netherlands) supplemented with 100m g/L kanamycin and 100m g/L spectinomycin (Figure 2). The plasmids were refreshed every three weeks.

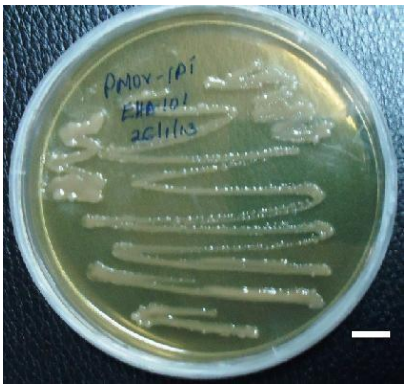


Figure 2. Maintenance of plasmid gene constructs bearing PSARK-IPT gene on LB media (Bar =1 cm)

Infection and Co-cultivation of sweet potato explants

A loopful of *A. tumefaciens* strain EHA101 harboring pNOV-IPT gene construct from 48-h cultured LB agar plates was used to inoculate 50 mL of standard LB liquid media containing kanamycin at 50 m g/L and spectinomycin at 50 m g/L. These were then put on a shaker (200 rpm) at 28°C for 48 h to grow. A 2 mL aliquot of the 3-day bacterial culture was displaced into a 50 mL falcon tube with 20 mL LB media containing kanamycin and spectinomycin. This was put on a shaker (200 rpm) at 28°C until 0.4-0.6 at 600 nm optical density of culture was obtained. Bacterial cells were then centrifuged at 3,000 rpm for 10 min at 4°C to obtain the bacteria pellet. The supernatant was poured out and the pellets resuspended with 20 mL of bacterial infection media (MIB). Before infection of the explants, the leaf and stem explants were first put on a preculture media and incubated at 28°C for 2 days (Figure 3.A). For infection, leaf and stem explants were submerged in 10 mL of MIB with the strain EHA101 and kept for 2 h in the dark at 28°C without agitation. Finally, the infected explants were removed from the infection media, blot dried using sterile filter papers and displaced to a co-cultivation media (Table 1) at 28°C for 3 days in the dark (Figure 3.B).

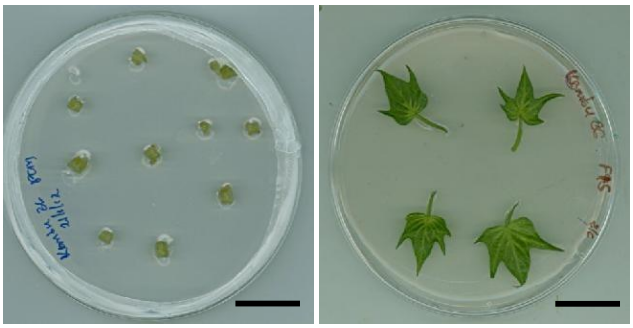


Figure 3. Sweet potato transformation. A. Kemb36 stem explants on preculture media 12 h before infection. B. Kemb36 leaf explants from the in-vitro cultures on co-cultivation media after infection (Bar =2cm).

Table 1. Composition of culture media and transformation and regeneration protocol of sweet potato.

Media preparation	Stage	Basic composition	Hormones and antibiotics	Concentration m g/L	Treatment period
EPM	Preinfection	LS, sucrose, MES (0.5 g/L), pH 5.5	NAA GA3 BAP	0.1 0.01 1	12 hrs
BIM	Infection	LS, sucrose, acetosyringone (20 m g/L), pH 5.5	N/A		2 hrs
SCM	Co-culture	LS, sucrose, glucose (20 g/L), MES (0.5 g/L, pH 5.5			2 days in dark
F15	Callus induction	LS, sucrose, pH 5.7-5.8	Zeatin riboside 2,4-D Mannose Cefotaxime	0.2 0.05 30 200	5-6 days then continue with F9
F9	Callus induction	LS, sucrose, pH 5.7-5.8	Zeatin riboside Mannose Cefotaxime	0.2 30 200	Until embryo form
G24D*	Embryo development	LS, sucrose, pH 5.7-5.8	GA3 2,4-D Mannose Cefotaxime	0.01 0.05 30 200	2-4 months*
ABA	Embryo maturation	LS, sucrose, pH 5.7-5.8	Abscisic acid** Cefotaxime Zeatin riboside	1 200 0.2	1-2 months
F9	Shoot induction	LS, sucrose, pH 5.7-5.8	Cefotaxime Zeatin riboside	200 0.2	until shoots form

Selection and regeneration

After the 3 days of co-cultivation, the infected explants were cleansed adequately with antibiotic wash solution. The explants were then dubbed on sterile filter papers and positioned on F15 media supplemented with 30 g/L mannose and 250 m g/L cefotaxime for callus induction for 3 days then transfer to F9 media for callus proliferation for 28 days. The explants were displaced on fresh F9 media once after every two weeks (Kreuze et al. 2008). The resultant embryogenic calli surviving the selection pressure were displaced to G24D media for embryo development and initiation of shoots. At this stage, not all calli were displaced to G24D medium, the calli were subdivided and some put on F25 medium. This followed a preliminary observation of root emergence before shoot regeneration on G24D medium. Fully developed shoots were multiplied on hormone-free sweet potato propagation media before they were displaced to peat moss for hardening.

Molecular analysis of transgenic plants

The presumed transgenic plants were screened for the presence of *IPT* genes using PCR with gene-specific primers. With a modified cetyltrimethylammonium bromide (CTAB) method as described by Zidani et al. (2005), the genomic DNA was extracted from the leaf tissues of presumed transgenic as well as the non-transgenic (control) plants.

DNA extraction from presumed transgenic plants

About 3g of freshly freeze-dried leaf tissues from the presumed transgenic plants were crushed using a pestle and mortar and 9 mL of CTAB extraction buffer (100 mM Tris-HCL, 1.4M NaCl, 20 mM EDTA) at 65°C. The liquid mixture was displaced to a 50 mL falcon tube and incubated at 65°C in a water bath for 30 min. Four mL of chloroform isoamyl alcohol (24: 1) was added to the samples and inverted twice to mix. The mixture was centrifuged at 5500 rpm for 10 min and after that 1 mL of the aqueous layer was carefully displaced to 2 mL Eppendorf tube. To precipitate DNA, 0.7 mL of isopropanol (stored at-20°C) was added to each sample and inverted twice to mix. The tubes were chilled at-20°C for 20 min and then centrifuged at 5500 rpm for 15 min. The supernatant was discarded, and 1 mL of 70% v/v ethanol was added to cleanse the DNA pellet and centrifuged at 12000 rpm. The supernatant was discarded, and the pure DNA pellet air dried for 30 min, resuspended in 100µl of sterile water and stored at-20°C.

DNA quantity estimation through gel electrophoresis

The concentration of the isolated plant genomic DNA was estimated by running on 0.8% (w/v) agarose gel where 5 µL of the genomic DNA was mixed with 1 µL of 6 times loading dye (New England Biolabs, Ipswich, USA) and 1 µL of sybr green (Invitrogen, USA) before loading for electrophoresis against 5 µL of 1 kb ladder (New England Biolabs) and run at 100 volts for 30 min using 1 time TAE buffer. The level fluorescence of the sample DNA bands

against the 1kb ladder was used to determine the amount of the DNA when visualized using gel documentation equipment and recording the gel.

Polymerase Chain Reaction assay

The PCR fragment containing the coding regions of *IPT* gene was amplified using its gene-specific primer. Forward primer; 5'-CCAACTTGCACAGGAAAGAC and Reverse primer; 5'-CTAATACATTCCGAACGGATGAC. The results of the PCR were determined through agarose gel electrophoresis where 5 µL of the DNA was mixed with 1 µL of 6 times gel loading dye (New England Biolabs, Ipswich, USA) and 1 µL of sybr green gel loading dye (Invitrogen, USA) before loading. Electrophoresis was done at 100 volts for 40 min using 0.8% w/v agarose in 1 times TAE buffer and 1 kb ladder (New England Biolabs) as the standard. Gel documentation equipment was used to visualize and record the gel.

Experimental set up for drought stress of sweet potato plants

Pot experiments were performed to evaluate the drought stress of transgenic sweet potatoes and non-transgenic sweet potatoes. The experiments were conducted in a greenhouse at the Kenyatta University plant transformation laboratory. Vine cuttings of about 4 cm were obtained and planted in 30 cm plastic pots containing 15 kg of homogeneous soil which were completely air dried with 1 plant per pot. All plants were watered sufficiently every two days and allowed to grow for 4 weeks before they experienced drought stress experiments. Plants at the same stage of development and the same size were then faced with drought stress for 96 days by withholding irrigation. Treatments were a combination of three irrigation regimes; severe drought stress (water withheld) moderate drought stress (irrigated 50% every 2 days) and control (irrigated 80% every 2 days) for both transgenic and non-transgenic. Rewatering was conducted at the end of the growth phase, after every two days until the plant fully recovered. Treatments were arranged in a completely random design with 3 replicates of 4 pots per treatment. This was performed according to the protocol by Saraswati et al. (2012).

Measurement of physiological and morphological characteristics

Data on physiological and morphological characteristics (relative water content, chlorophyll content and growth parameters) were collected from mature, healthy, and fully expanded third, fourth and fifth leaves of 96-day old plants at day 42, 63 and 96 and on the 1st, 5th, and 9th day of recovery. Sampling of control, stressed and recovered plants was done simultaneously.

Leaf relative water content

To minimize the age effects, the leaves were collected from the mid-section of the vine. It is used to monitor the relative water content. Individual leaves were first removed from the stem and then weighed to obtain the fresh weight (FW). After fresh weight determination, leaves were floated in distilled water for 24 h obtaining the turbidity weight (TW). Dry weight (DW) of leaves was determined by oven-drying the leaves at 80°C for 72 h and then the weight was determined. All mass measurements were taken using an analytical scale with precision of 0.0001g. Values of FW, TW, and DW were used to count the leaf relative water content (RWC) using the formula:

$$RWC\ (\%) = [\ (FW-DW) / (TW-DW)] * 100$$

Leaf chlorophyll concentration

Three plants per replicate were used for chlorophyll determination. Ten fresh leaf discs from the third fully expanded leaf were sampled, chlorophyll was extracted using 100% v/v acetone by crashing the leaves using a mortar and pestle. The chlorophyll was obtained from the leaf debris by centrifugation at 5000rpm for 10 minutes and the supernatant was pipetted and displaced into clean 2 mL Eppendorf tubes. The chlorophyll concentration was measured using a spectrophotometer at 662, 645 and 470 nm wavelengths and the readings at these absorbance levels were used to count chlorophyll a, chlorophyll b and carotenoid concentration. Measurements and calculations were performed according to Lichtenthaler (1987).

Chl a = 11.75A662-2.350A645 Chl b = 18.61A645-3.960A662

C x+c = 1000A470-2.27Ca-81.4Cb/227

Plant growth parameters

Vine length, internode length, branch number, leaves per vine, leaf width and leaf length were measured on 4 randomly sampled plants per treatment a week before stress treatment, at 42, 63 and 96 days after planting (DAP). The increment in each of these traits was determined by the difference between the measurements at 96 DAP and 42 DAP, stated as a percentage.

Data analysis

Transformation frequency, transformation efficiency and regeneration frequency differences between genotypes were analyzed using ANOVA at 95% confidence interval. Transformation efficiency (TE) was counted as affirmed presumed transformants divided by the number of initially infected explants stated as percentage whereas the transformation frequency (TF) was determined by dividing the number of presumed transformed callus by the number of infected explants stated as a percentage. Regeneration frequency was counted as the number of shoots formed over the number of explants used stated as percentage. On drought stress experiments, experiments on growth parameters (leaf length, width, internode length, vine length, leaves per vine and number of branches per plant) and relative water content were conducted in a completely randomized fashion and data collected analyzed with statistical analysis system (SAS). Treatment means were compared using Turkey's tests at the 5% level of significance.

RESULTS AND DISCUSSION

In-vitro response of sweet potato to tissue culture

Explants from three sweet potato varieties (Kemb36, KSP36 and Jewell) produced shoots within 10 days of culture. Kemb36 explants were the first to shoot in 4 days while KSP36 and Jewell explants took 5 and 7 days, respectively to shoot (Figure 4). These sweet potato varieties were cultured and maintained under in-vitro conditions devoid of any contamination.

Callus induction

Plant explants from all the sweet potato cultivars induced embryogenic and/or non-embryogenic callus. Callus formation started 3 weeks after incubating the explants on F9 callus induction medium. Several calli formed on the leaf lamina and petiole of the explants (Figure 5.A), but only those emerging from the base of the petiole survived repeated subculture and formed embryogenic callus (Figure 5.B) on selective medium supplemented with 30 g/L mannose.

Optimization of mannose concentration for selection of transformants

The PNOV-IPT gene constructs harbored the *PMI* gene as a selectable marker gene which was used to select for putatively transformed calli and plantlets. An experiment to establish the optimum mannose concentration to be used in selection of transformed callus/cells was conducted at varying levels of mannose (0, 10, 20, 30, and 40) (Figure 6.) using leaf and stem explants in 3 replications. After 6 weeks on selection media, it was observed that a concentration of 30 g/L of mannose was optimum to select for transformed calli (Figure 7.A). The non-transformed explants began to turn brown on the selection media and gradually died on the medium. Moreover, all the calli died on the medium with mannose above 30 g/L (40 g/L) (Figure 7.C). However, the number of calli surviving on 30 g/L mannose concentration was remarkably high (Figure 7.D).

Regeneration of embryogenic calli into plantlets

Green calli were harvested after 8 weeks of growth on F9 medium and displaced to G24D embryo induction medium for somatic embryogenesis (Figure 8.A). Embryogenic structures formed on G24D after 1 month, and were displaced to F25 embryo maturation medium. The mature embryos appeared after 1 month on F25 and were displaced to hormone-free F9 medium to allow development into shoot. At this stage, it was observed that most embryogenic calli that did not form shoots just proliferated in roots (Figure 8.B). The shooting process began with the formation of dome-shaped structures that later formed primordial leaves (Figure 8.C). The primordial leaves further grew to form shoots and whole leaves (Figure 8.D). The first fully developed plants were obtained after about 4 months from the beginning of the transformation experiment. However, more plants continued to form until end of the 8th month. In all experiments performed (approximately 120 explants each), only 14 and 8 independent events were regenerated for Jewel (non-African cultivar) and kemb36 varieties respectively. Though KSP36 variety performed well during callus induction and embryo formation, the variety just proliferated into roots on the G24D media but did not give any plant.

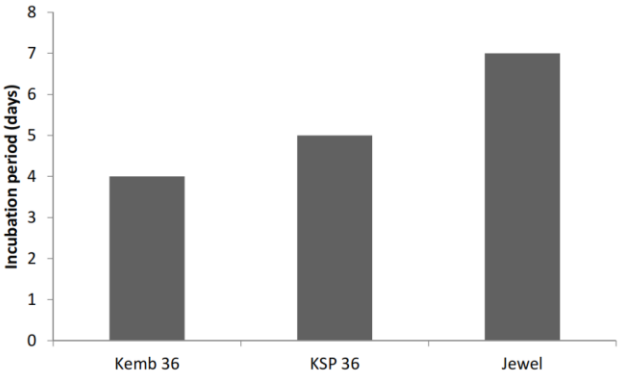


Figure 4. In-vitro micro-propagation of sweet potato. Number of days taken for shoots to emerge in-vitro from stem nodes of 3 sweet potato varieties after initiation in tissue culture.

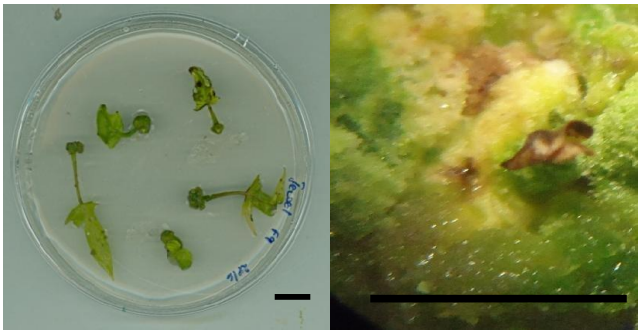


Figure 5. Callus proliferation on callus induction medium. A. Whole leaf explants form calli at the base of the leaf petiole. B. Callus proliferation after excising it from the base of the leaf petiole (Bar =2 cm).

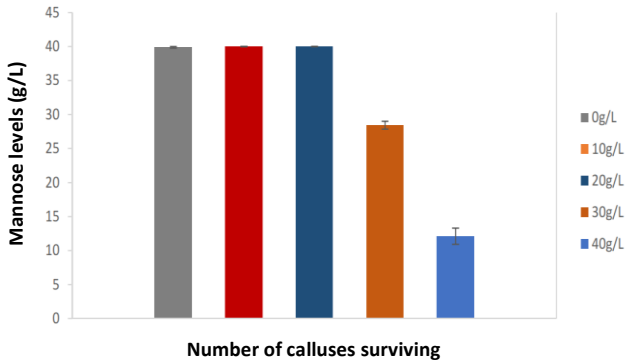
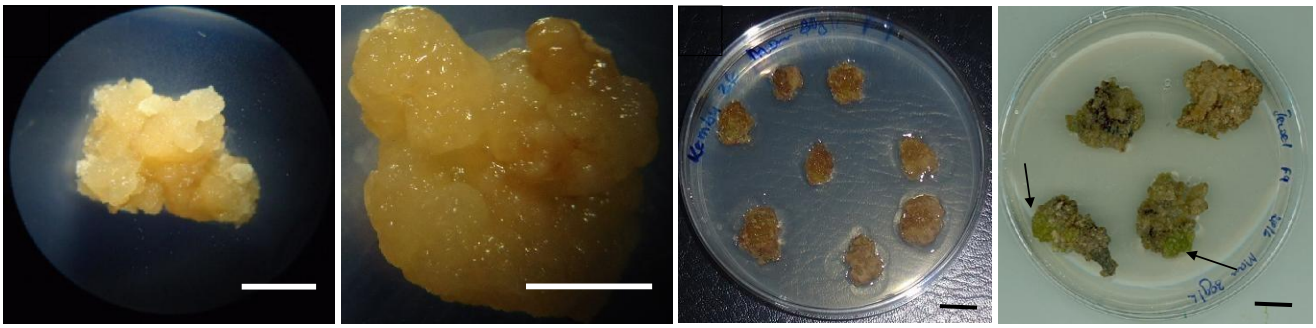
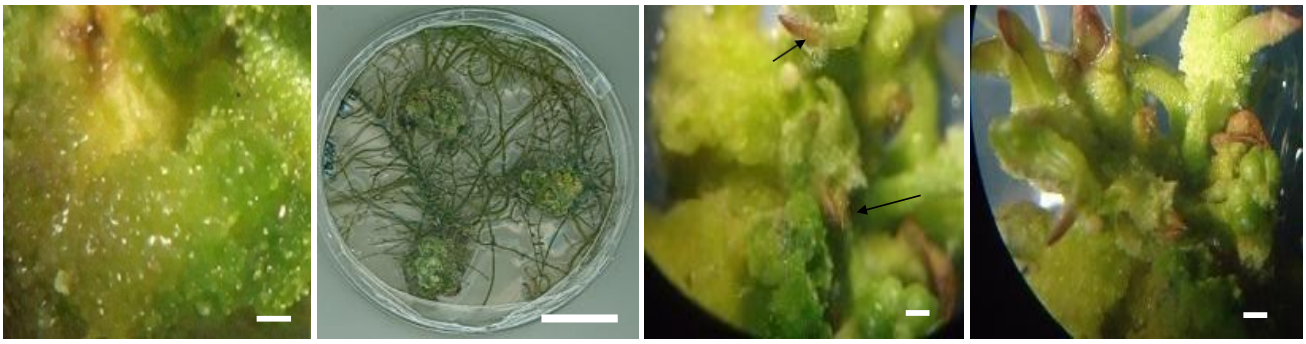


Figure 6. Effect of mannose concentration on the survival of the sweet potato calli. The concentration of 20 g/L and below did not have any effect on callus proliferation. Calli on the concentration above 30 g/L registered massive deaths thus a concentration of 30 g/L selected to the optimal selection pressure.



A **B** **C** **D**

Figure 7. Stages of callus development on selection media containing mannose. A. Well-established callus 4 weeks on callus induction media. B. Kemb36 callus during early stage of embryogenesis (note the browning of the callus caused by mannose pressure). C. Callus greatly reduced 10 wks on selection media containing 40 g/L of mannose. D. Jewel callus succumb to mannose pressure 12 wks on selection media (arrows show the transformed portion of the calli) (Bar = 1cm).



A **B** **C** **D**

Figure 8. Stages of sweet potato regeneration from embryogenic calli. A. Calli at early stages of embryogenesis (Bar = 0.1 cm). B. Proliferation of roots on G24D medium (Bar = 1 cm). C. Onset of primordial leaf formation (Bar = 0.1 cm). (The arrows show the young leaf primordia). D. Embryogenic calli forming shoots after incubation on regeneration medium (Bar = 1 cm).

Hardening and acclimatization of presumed transgenic plants

Fully developed presumed transgenic plants (Figure 9.A) were displaced to autoclaved peat moss in small pots for hardening and acclimatization. The plants were first carefully removed from the culture media to avoid breaking the roots. The roots were cleansed with sterile water to remove any media remnant attached and subsequently displaced to peat moss. The pots were covered by polythene bags for 3 days to avoid excessive evapotranspiration (Figure 9.B). Three days later, the polythene bags were removed (Figure 9.C), then plants were displaced to larger pots with normal soil (Figure 9.D) where they grew vigorously.

Molecular analysis of presumed transgenic sweet potato

Genomic DNA was extracted from presumed sweet potato leaves and quantified by running on an agarose gel (**Figure 10**). The good quality DNA with high concentration was further used for molecular analysis. PCR analysis was carried out using *IPT* gene specific primers on the regenerated plant to determine the presence of *IPT* gene and 5 out of 7 plants tested were positive (**Figure 11**). Even though stems explants regenerated into plants, it was interesting to note that only 2 of these regenerated plants were PCR positive.

Response of the sweet potato varieties to transformation and regeneration

To evaluate the response of the varieties on transformation, the leaf and stem explants from the three varieties were tested for their response to selection pressure (Table 2). There was a significant difference observed in transformation frequency among the three sweet potato varieties at 95% confidence interval using one-way ANOVA. However, although mannose tolerant calli were observed in all three varieties, only explants from two varieties Kemb36 and Jewel gave putatively transgenic plants. Of the two explants analyzed, the leaf explant responded better to transformation and regeneration (Table 3).

The values shown are means and standard errors of transformation frequencies (TF), regeneration frequencies (RF), regeneration efficiencies (RE), transformation

efficiencies (TE), and embryogenic calli formation of the three selected sweet potato varieties. Figures with the same letter in the same column are not significantly different. (0.000-implies no plant regenerated).

The data shows how the two explants responded to transformation and regeneration. The values are means and standard errors. Different letters in the same column mean there is significant difference between the 2 explants in terms of response to transformation and regeneration.

Plant growth response to drought stress

Under well-watered conditions, before water stress treatment, both transgenic and wild-type plants did not show any visible difference in growth. All the plants grew vigorously with a lush green color (Figure 12).

After 14 days of drought stress, the wild type started to wilt, and leaves drooped while the transgenic plants showed normal growth. After 63 DAP and as drought stress becomes more severe, senescence began to be manifest in the leaves which wilted, turned yellow then brown before finally falling off the plant. Compared to the transgenic plants, (Figure 13.A) senescence was more pronounced in wild-type (Figure 13.B) than the transgenic. During rewatering, the transgenic plants (Figure 13.C) recovered much earlier compared to wild-type (Figure 13.D) that remained chlorotic even after 9 days of rewatering.

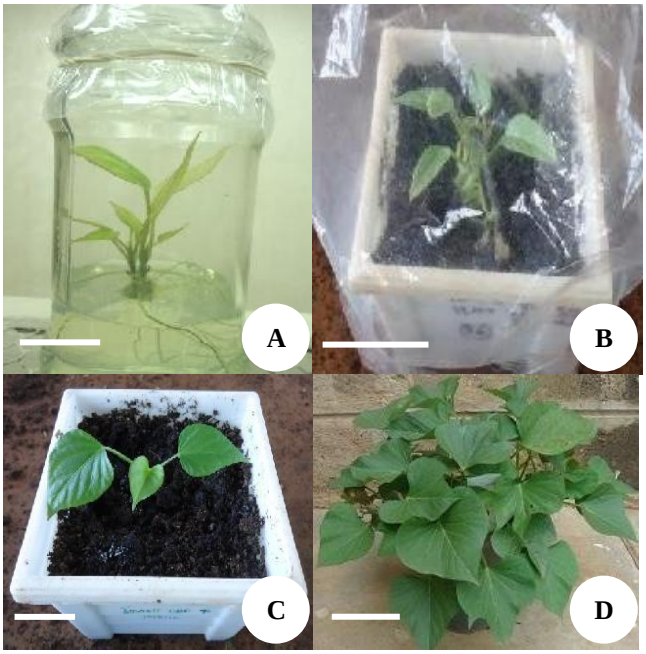


Figure 9. Hardening and acclimatization of presumed transgenic plants. A. Putative regenerant from embryogenic call. B. Jewel cultivar shoots after transfer from the medium. C. Regenerated plant during hardening. D. Completely grown and established plant in the soil.

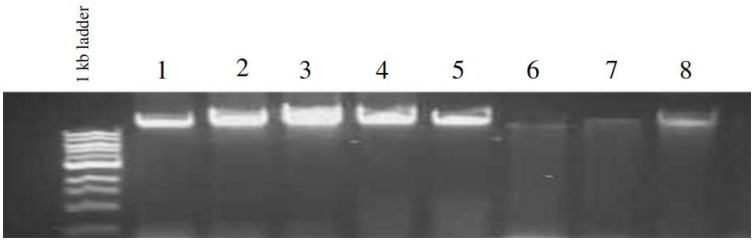


Figure 10. Gel electrophoresis showing purified genomic DNA from pSARK IPT sweet potato plants. Lane 1-6; DNA from the Jewel sweet potato variety. Lane 7-8; DNA from Kemb36 sweet potato variety.

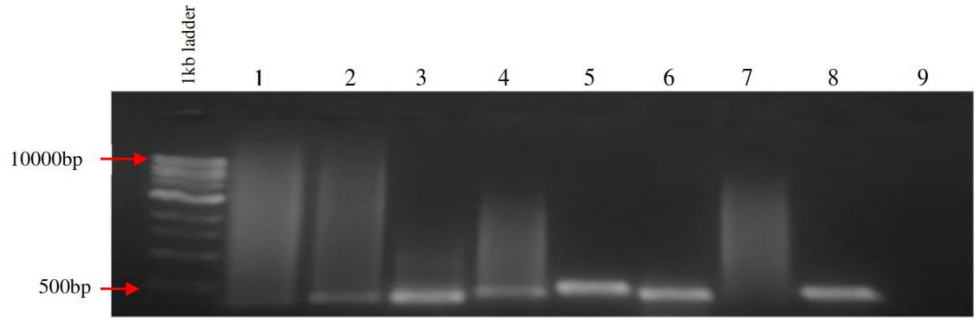


Figure 11. PCR analysis of T0 plants using *IPT* gene-specific primers on sweet potato genomic DNA. Lanes 1 and 2; presumed transgenic Kemb36. Lanes 3-7; presumed transgenic Jewel. Lane 8; +ve control (PNOV-IPT plasmid), Lane 9;-ve control.

Table 2. Transformation frequency, regeneration frequency, transformation efficiency and regeneration efficiency of 3 sweet potato varieties transformed using pSARK-IPT gene.

Variety	Means of embryogenic calli	Means of TF (%)	Means of RF (%)	Means of TE (%)	Means of RE (%)
KSP36	17.667 ±1.745a	44.167 ± 4.362a	0.000 ±0.000	0.000±0.000	0.000 ± 0.000
Kemb36	20.000±2.251ab	50.000 ±5.627ab	0.833 ±0.833a	0.833 0.833a	5.522 ± 2.162a
Jewell	22.833 ±0.946b	57.083 ± 2.364b	2.083 ±1.193a	2.083±1.193a	12.808 ±2.748a
LSD (P>0.05)	5.071	12.678	2.755	2.755	7.111

Table 3. The overall effect of explants used on transformation frequency (TF), regeneration frequency (RF), and regeneration efficiency (RE).

Explant	Means of embryogenic calli	Means of TF (%)	Means of RF (%)	Means of TE (%)	Means of RE (%)
Leaf	22.67 ± 3.393a	56.667 ± 8.483a	1.944 ± 1.843a	1.944 ± 1.843a	7.884 ± 4.758a
Stem	17.667 ± 3.393b	44.167 ± 8.483b	0.000 ± 1.843b	0.000 ± 1.843b	4.336 ± 4.758b
LSD (p>0.05)	3.393	8.483	1.843	1.843	4.758

Table 4. Effect of moderate and severe drought stress on the morphological characteristics of transgenic (T) and non-transgenic (N) sweet potato.

	Treatment					
Parameters	**TC**	**NC**	**TM**	**NM**	**TS**	**NS**
Vine Length	28.700 ± 0.404a	28.533 ± 0.291a	28.900 ± 0.586a	17.667 ± 0.657c	21.667 ± 0.267b	12.956 ± 0.267d
Internode Length	1.133 ± 0.067a	1.100 ± 0.058a	1.133 ± 0.067a	0.867 ± 0.033ab	0.967 ± 0.088a	0.607 ± 0.007b
Branches/ Vine	2.667 ± 0.333b	3.333 ± 0.333ab	5.000 ± 0.577a	2.000 ± 0.577bc	2.667 ± 0.333b	0.333 ± 0.333c
leaf Area	80.400 ± 0.300a	79.223 ±1.772ab	75.403 ± 1.043b	41.823 ± 0.939d	49.300 ± 0.681c	24.833 ± 0.601e
No. Leaves/Vine	40.000 ± 1.155a	39.000 ± 2.082a	34.667 ± 0.882a	22.000 ± 1.155b	21.333 ± 0.882b	15.333 ± 0.667c

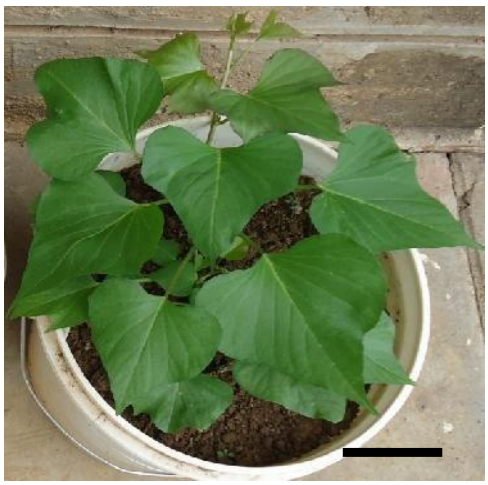


Figure 12. Morphological appearance of the plants a day before the onset of stress experiment. 28 days after planting (DAP) (Bar= 4cm).

Effect of drought stress on vine length, internode length and number of branches

Plants responded differently to different drought stress treatments. Moderate drought stress relatively increased branching in transgenic stressed plants compared to non-transgenic stressed plants (Table 4). However, both transgenic stressed and non-transgenic stressed plants were heavily affected by severe drought stress. Compared to the controls (C), moderate drought stress decreased both vine length and the internode length in transgenic stressed and non-transgenic plants. The average vine length and internode length reduction (P<0.05) in transgenic stressed was 21.67cm and 0.97cm respectively and corresponding decrease in wild-type was 12.27cm and 0.61cm. As the days progressed and drought intensity increased, both transgenic plants and non-transgenic (wild types) showed a further decrease in both vine length and internode length, but this was more severe in wild types (Table 4).

Effect of drought stress on leaf area and number of leaves per vine

Under well-watered conditions, both transgenic and wild-type plants showed no significant difference in leaf area and number of leaves per vine (Table 4). However, further drought stress had a great effect on both leaf area and number of leaves per vine. Compared to the control, the transgenic plants showed a higher leaf area (P<0.05) and leaves per vine under severe stress of 71.17cm2 and 21.33cm respectively. The corresponding leaf area and number of leaves per vine in wild-type were 24.83cm2 and 15.33cm respectively.

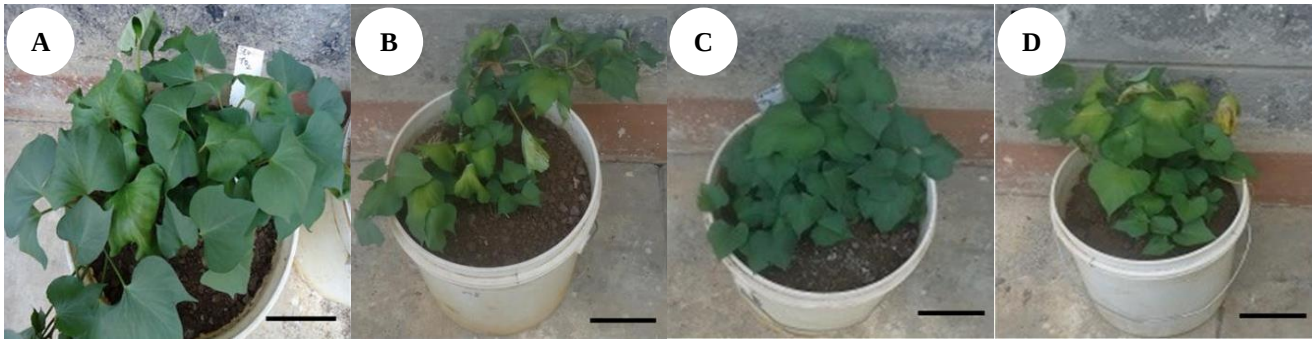


Figure 13. Effect of drought stress on transgenic and wild-type sweet potato growth. A. Transgenic Jewel variety 35 days (63 DAP) after onset stress treatment. B. Wild-type Jewell variety 35 days (63 DAP) after the start of stress treatment. C. Transgenic plant at 9 days after rewatering. D. Wild-type plant 9 days after rewatering (Bar = 4cm).

Values are means and SE of three replicates collected at day 63 after induction of drought stress. Means with the same letters in a raw are not significantly different. Plants under well-watered conditions served as control. TC-transgenic control; NC-non transgenic control; TM-transgenic moderately stressed; NM-non transgenic moderately stressed; TS-transgenic severe stressed; NS-non transgenic severe stressed.

Changes in leaf relative water content under drought stress and re-watering conditions

The relative water content (RWC) was measured one day before drought stress, on day 21 and 63 after imposing drought and then finally on the 1st, 5th, and 9th day after re-watering. Two weeks after withholding water, there was a significant decrease in the leaf RWC of both transgenic and wild-type plants. The reduction was more profound under severe drought stress conditions. Compared to the wild type, the transgenic plants registered a higher RWC under both moderate (Figure 14.) and severe drought stress (Figure 15.) than their wild-type counterparts.

Re-watering had a great effect on RWC after drought stress. In overall, the transgenic stressed plants showed better recovery than the wild type in that they recovered faster compared to wild-type. However, neither of the plants recovered fully from severe drought stress.

Effect of drought stress on total leaf chlorophyll content

Under sufficient water treatment, both transgenic and wild-type had similar total chlorophyll content. However, there was a significant reduction in total chlorophyll content 21 days after imposing drought stress. The total decrease in transgenic plants was 36.9% and 27.7% under moderate and severe stress respectively. The corresponding decrease in wild-type was 27.3% and 23.0% under similar conditions. As the drought treatment progressed both transgenic and wild-type plants showed a further reduction in the total chlorophyll content, but this was more severe in wild types.

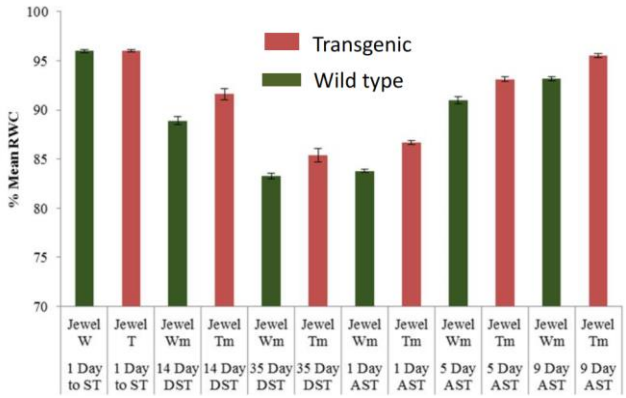


Figure 14. Relative water content in wild-type (W) and transgenic (T) sweet potato during moderate drought stress and recovery. W-wild type; T-transgenic; WM-wild type under moderate stress; TM-transgenic under moderate stress; ST-stress treatment; DST-during stress treatment; AST-after stress treatment.

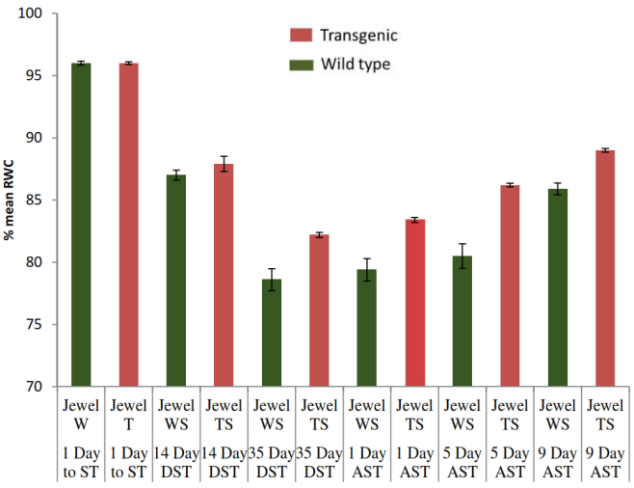


Figure 15. Relative water content of wild-type (W) and transgenic (T) sweet potato during severe drought stress (S) and recovery. W-wild type; T-transgenic; WS-wild type severe stressed; TS-transgenic severe stressed; ST-stress treatment; DST-during stress treatment; AST-After stress treatment.

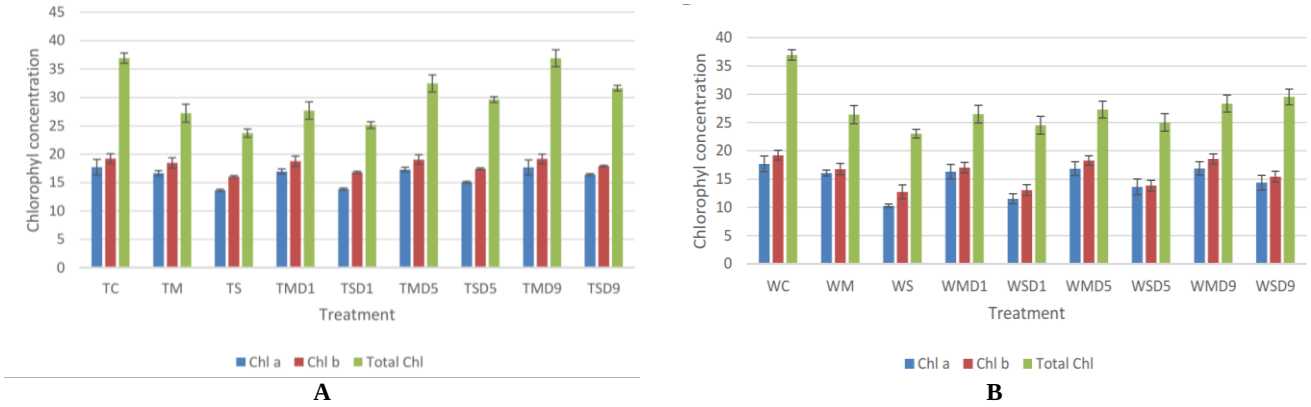


Figure 16. Effect of drought stress on total chlorophyll content. A. Chlorophyll content of Jewell transgenic variety under moderate and severe drought stress. B. Chlorophyll content of the Jewell wild type variety under moderate and severe drought stress.

Figure 17. The effect of drought stress on visual rating of plants growth and storage root formation in transgenic pSARK-IPT and the non-transgenic sweet potatoes. A. Non-transgenic tuber formation under control conditions. B. Transgenic pSARK-IPT tuber formed under control condition. C. Non-transgenic tuber formed under severe drought stress. D. pSARK-IPT tuber formed under severe drought stress.

Upon re-watering, the moderately stressed plants recorded a drastic increase in total chlorophyll content within the first 3 days then gradually till they reached the control level after 9 days (Figure 16.). On the other hand, chlorophyll content of severe stressed transgenic plants increased slowly within the first 3 days then rapidly, whereas the severe stressed wild type did not show signs of recovery even after 9 days.

Effect of drought stress on tuber and root formation

Under normal conditions, there was no phenotypic difference in the tuber formation between the wild-type (Figure 17.A) and the pSARK-IPT transgenic plants (Figure 17.B). However, under reduced irrigation the wild-type plants were greatly affected by drought stress and hence a serious impact on tuber formation (Figure 17.C). The transgenic plants, on the other hand, had visibly bigger and more tubers (Figure 17.D) compared to the wild type under the same conditions.

Discussion

Sweet potato transformation and regeneration

Through the *Agrobacterium*-mediated gene transfer method, the conversion of two varieties of transgenic sweet potatoes was successful. In the test of transformability and regeneration ability, two varieties, namely Jewel (non-African varieties) and Kemb36, produced transgenic plants, whereas one variety (KSP36) regenerated no plant, even though this variety performed well during callus formation after transformation. This is consistent with the findings by Mervat et al. (2009) who established that different cultivars show a different response to in-vitro regeneration. In the in-vitro regeneration process, growth regulators as parameters play an important role. A balance between the auxins and cytokinin, therefore, is important for conversion of somatic embryos into shoots (Santa Maria et al. 2009). In this study, this regeneration variation among genotypes could be attributed to significant variability in genotype response to growth regulator combination and genotype dependency to in-vitro regeneration.

In this study, for introduction of pSARK-IPT genes into sweet potato cells which later tolerate drought in transgenic events, *Agrobacterium tumefaciens* was used. Meanwhile, regeneration method in this study used somatic embryogenesis method because it produced high-frequency selection during transformation since each plant comes from a single modified cell (Sefasi et al., 2012). Two major factors were studied for their effect on transformation, embryogenic callus formation and hence regeneration of selected sweet potato varieties. This included the type of explants and the type of variety used.

To understand their effect on transformation, embryogenic callus formation and regeneration, leaf and stem explants were compared. Although the type of explants used did not affect the frequency of the total callus formed, the type of explants had a significant effect on the quality of the induced callus. Leaf explants induce embryogenic calli at the highest frequency of 22.67±1.354 compared to stem explants frequency of 17.67±1.213. Most importantly, the highest number of shoots regenerated emerged from embryogenic calli of leaf explants. These findings conform to the report by Sefasi et al. (2012) who reported high frequency of embryogenic calli formation from leaf with petiole explants.

The frequencies of transformation and regeneration were obtained when the two types of explants were evaluated differently and significantly. Leaf explants responded well to both regeneration and transformation. This variation could be seen in the difference in the morphological structures and stages of maturity of the explants (Anwar et al. 2010). Before infection and co-cultivation, explants were punctured with forceps to improve the infection process. When punctured, the explant stem produced latex which may have inhibited the effective infection by *Agrobacterium*. The morphological nature of sweet potatoes was generally regarded as a recalcitrant species for regeneration and transformation. Although methods to generate transgenic sweet potato plants have been reported, transformation efficiencies are generally low, and success is limited to a small number of varieties (Kreuze et al. 2008). Transgenic sweet potato plants were obtained from the Jewel (non-African) variety and Kemb36 by *A. tumefaciens*-mediated transformation and somatic embryogenesis regeneration. Initial transformation was done using pSARK-IPT gene construct.

Embryonic structures appeared in embryo maturation media, but interestingly, at this stage over-proliferation of roots from some callus material that did not form embryogenic tissues was noted. This could be attributed to the presence of high levels of auxins, specifically NAA in the explanted organs (George et al. 2008). Somatic embryos regenerated into shoots at a frequency of 2.083% for Jewel variety and 0.833% for Kemb36 variety. The difference in regeneration frequency between the Jewel variety and Kemb36, is clearly genotype dependent as earlier reported by Kreuze et al. (2008); Santa Maria et al. (2009); Anwar et al. (2010) and Sefasi et al. (2012).

In all transformations, 30 m g/L mannose was added to the embryo maturation medium to prevent regeneration of untransformed events. The addition of mannose caused evident stress to the embryos but still allowed for recovery of presumed transgenic lines. Two presumed lines were recovered, and PCR was used to confirm presence of the *IPT* gene. Transformation efficiency, estimated as independent transgenic lines obtained from the total number of explants infected with *Agrobacterium* was 2.083 ± 1.193 and 0.833 ± 0.833 for Jewel and Kemb36 genotypes respectively.

Genotype-dependence for regeneration and transformation in sweet potato has previously been reported (Anwar et al. 2010; Sefasi et al. 2012). An efficient selection method is therefore required to produce transgenic plants. In previous reports, either neomycin phosphotransferase (*npt)* II or hygromycin phosphotransferase (*hpt)* (Newell et al. 1995; Gama et al. 1996; Moran et al. 1998; Otani et al. 1998) or even a combination of the two (Km-Hyg) (Song et al. 2004) have been used as the selectable marker genes for selecting transformed sweet potato cells. Choi et al. (2007) successfully regenerated herbicide resistant transgenic sweet potato plants through *Agrobacterium-mediated* transformation system.

The gene construction marker in this study is the phosphomannose isomerase (PMI) gene. Until now, the use of PMI genes as markers in sweet potatoes has not been found in any published literature. The determination of optimal mannose concentration to be used for the selection of presumed transformants was done by experimenting on sweet potato callus derived from leaf explants with various mannose concentrations, and it determined that the concentration of 30 g / L mannose was optimum to select for altered cells. At this concentration, some non-altered calluses of all the 3 varieties of sweet potato did not grow and then subsequently died on the medium. During the study, callus cultured on mannose showed moderate proliferation, brown color, and reduced mass after a long (3 month) culture period compared to the untreated controls. However, there is no clear evidence of a deadly outcome that could be apparent. In addition, among the three varieties of sweet potatoes, different levels of sensitivity to mannose were documented with KSP36 as the most sensitive variety and Kemb36 as the most tolerant.

To clearly know the effect of mannose on altered sweet potato cells, it is recommended to use a subculture of transformed cells on a medium equipped with 30 g / L mannose and performed at all stages of regeneration until plant buds. Vaccari and Martinelli (2009) also reported a similar effect of mannose in three important stages of development of transgenic grape. Although 14 presumed transgenic plants were successfully regenerated, only 6 were PCR positive so that high numbers escaped.

Response of sweet potato to drought stress

Enakayake, (1990) previously established that the effect of drought treatment on sweet potato is dependent on the growth stage that it coincides. Moreover, Placide et al. (2013) established that sweet potato is sensitive to drought stress during growth establishment, vine development and storage root initiation. Hence, to evaluate the effect of applying drought stress on the physiological and

morphological parameters and yields of sweet potatoes, the application of drought stress was held to concur with the critical development stage and extended to harvest time.

Generally, all growth parameters in wild-type plants decreased significantly due to drought stress to confirm the vulnerability of sweet potato plants to high intensity of drought stress during the critical stage of plant formation and root initiation. It also shows that in the growth and development of sweet potatoes, water is very important because it serves as a solvent and a medium of chemical reaction, organic and inorganic dissolved transport, cell turgidity, transpiration, and photosynthesis. The results have shown that underwater deficit conditions, PSARK-IPT expression increases drought tolerance in transgenic sweet potatoes. This finding is consistent with the findings by Rivero et al. (2007) and Peleg et al. (2011) who demonstrated that *PSARK: : IPT* enhanced drought tolerance in transgenic tobacco and rice respectively. Under normal conditions, morphological and physiological differences are not clearly demonstrated in both transgenic and wild species. This indicates that under normal conditions, the introduction of PSARK-IPT gene has an insignificant effect on the growth and development of normal plants. However, all growth parameters gain significant influence when in drought stress. Of which, the development of vines was impaired and the leaves turned yellow and finally fell off the plant. According to Lim et al. (2007), leaf yellowing is a convenient and visible indicator of leaf senescence, and it reflects chloroplast senescence of mesophyll cells which is the first step in senescence associated programmed cell death. This study shows the emergence of signs of aging in wild plants that are under heavy pressure two weeks after the onset of drought treatment. Leaves that begin to wither and withered leaves that turn yellow appear on wild plants while in transgenic plants are not found for the leaves remain green and look healthy. Delayed senescence of transgenic plants may be associated with expression of IPT genes by transgenic plants regulating cytokinin biosynthesis, thus delaying senescence due to drought and sustaining growth.

Along with the ongoing handling of drought, both transgenic and wild plants showed decrease in length of vine, length of internode, canopy (number of leaves and leaf area), the size and number of tubers and number of roots compared with well-watered controls. In the previous study, it was observed that water deficits reduced the number of leaves and tubers, the size and composition of roots and vines, and the acquisition of dry weight from shoots and roots (Pardales et al. 2000). Similarly, these results are consistent with findings by Saraswati et al. (2012) who observed that biomass and morphological properties decreased in response to drought stress.

Relative water content is a measure of water content in plants and delineates metabolic activity in tissues, so this is an important index for dehydration tolerance. In the early stages of leaf development, RWC of leaves is higher and decreases due to dry matter accumulation and leaves maturity (Anjum et al., 2011). Under normal conditions (without stress), the plants maintained high level of water content. Under condition of drought stress, RWC decreased significantly. In moderate water stress, RWC of tested plants declined slightly and continued to decline under severe drought stress. However, the reduction of RWC in transgenic plants is less than in wild-type plants. During re-watering period, transgenic crops recover much faster than wild species. Therefore, these results suggest that this increase might be caused by transgenic line leaves that express IPT genes.

Phytohormones play an important role in the growth and development of plants (Walter 2009). Plants have several hormones such as auxin, gibberellin, cytokinin, ethylene, brassinosteroid, jasmonate, abscisic acid (ABA), and salicylic acid. These hormones are thought to play a major role in water deficit stress. Subject to drought stress conditions, elevated ABA levels lead to stomata closure thus preventing water loss and regulating the expression of many genes associated with the stress response. Hormones such as brassinosteroids and jasmonates act in conjunction with ABA in promoting premature senescence and programmed cell death, whereas hormones like cytokines, auxins, and ethylene act conversely in response to water deficit deficits (Pinheiro and Chaves 2011). The level of cytokinin decreases in response to water deficit (Xu et al., 2012). Reduced levels of cytokines are accompanied by breakdown of proteins and destruction of photosynthetic machinery, which causes cell senescence and programmed cell death. In this study, transgenic plants showed an increase in all growth parameters compared to wild-type which indicates that IPT gene expression may have increased cytokinin availability so that overall growth of plant growth can be accomplished. This can happen because cytokinins play important roles such as controlling cell proliferation, shoot formation and branching as well as delaying senescence.

Chlorophyll is one of the main components of chloroplasts and plays a very important role in photosynthesis. Relative chlorophyll content has been used to determine the rate of photosynthesis in plants during the water deficit period. The decrease in chlorophyll levels under drought stress is considered a typical symptom of oxidative stress and this is because of photo-oxidation of pigment and chlorophyll degradation (Anjum et al. 2011). The performance of photosynthesis was analyzed by determining chlorophyll degradation under drought stress and normal conditions to evaluate the effect of water deficit on photosynthesis in PSARK-IPT transgenic plants. Under normal conditions, transgenic plants showed significant differences in chlorophyll a, b, and total chlorophyll. During this study, it was found that moderate stress had no serious effect on the photosynthesis activities on both transgenic crops and wild species. Plants under moderate drought stress maintained a high total chlorophyll content and chlorophyll b. This observation is consistent with findings by Van Heerden and Laurie, (2008) who reported that chlorophyll content was barely affected by moderate stress. The content of chlorophyll in both transgenic and wild species came to near normal level, after the watering was redone, although wild species reached this level 2 days later. The most likely reason for this was that the water potential did not fall below a sustainable level and thus the

functional activity of the plant photosynthesis engine was not affected. Similarly, under a moderate groundwater deficit, photosynthetic depression might be caused by stomatal closure or restriction, but not because of biochemical reactions. Therefore, the plants recovered to the level of control after the stress condition was removed.

Severe and prolonged drought stress on wild species caused transgenic PSARK: IPT to show relatively higher level of chlorophyll content. This happened because transgenic plants effectively protected photosynthesis by efficiently scavenging for ROS so that during water deficit pressures, water could be used efficiently. After the watering was redone, stressed transgenic plants, and stressed wild plants could not be fully recovered from severe drought conditions even after 9 days of watering. It was possible that they needed more time to recover or the dissipation of PSII heat energy was very limited, due to PSII structure and functionality damage.

Effect of drought stress on root and tuber formation

This study has shown that pSARK-IPT gene expression under maturation and promoter controls activated enhanced drought tolerance promoters and therefore increased yields in transgenic sweet potatoes. This is consistent with reports from Peleg et al. (2011) and Qin et al. (2011) about rice and peanuts respectively. Compared to wild species, excessive sweet potatoes exposed to excessive IPT are found to be in larger and higher size under less irrigation. This significant difference will be associated with higher level of photosynthesis in transgenic sweet potatoes under limited water conditions due to conserved photosynthetic equipment and higher relative water content. Therefore, the higher yields are because of the increase in photosynthetic rate, stomatal conductance and transpiration thus backing up the presumed cytokinin-mediated protection of photosynthesis in transgenic plants.

Conclusions

The experiments conducted in this study were able to produce change and regeneration in the varieties of sweet potatoes. However, the change and frequency of sweet potato regeneration and efficiency through somatic embryogenesis still depend on genotype. This confirms previous findings by Anwar et al. (2010) that the sweet potato [*Ipomoea batatas* (L.) Lam] is recalcitrant to change and regeneration so that the existing regeneration and transformation protocols are highly-dependent in-vitro response genotypes. Of the two explants tested, leaf explant provides better response to change and regeneration than in other parts so that the best way of regenerating sweet potato is through somatic embryogenesis. This study shows that sweet potato tissue is quite sensitive to mannose as a source of carbohydrates, but the deadly effects of mannose cannot be clearly noted during the early stages of development and to observe the effects of mannose then a long period of culture is required. This research succeeded in developing transgenic sweet potato plant PSARK-IPT. Plants are shown to slow down aging due to drought, and to maintain relatively higher water content, chlorophyll content, good tuber formation and, generally to maintain better growth than wild types under drought stress conditions.

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The impact of large-scale agriculture on forest and wildlife in Diga Woreda, Western Ethiopia

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Abstract. *Jaleta OG, Jebessa H. 2017. The impact of large-scale agriculture on forest and wildlife in Diga Woreda, Western Ethiopia. Asian J Agric 1: 100-113.* Large-scale agriculture uses agricultural machinery to mechanize the practices of agriculture. It is one of the leading causes of the loss of forest and wildlife in many countries including Ethiopia. Information on forest cover change that occurred from 1986 to 2006 in Diga Woreda/district (Woyessa Dimtu, Bekiltu Gudina, and Melka Beti Jirma Kebeles) was compared with the present time using Geographic Information System (GIS). The objective of this study was to investigate the impact of large-scale agriculture on forest cover change by using the satellite image of the study area and other data collecting methods such as interviews, KI, FGD and observation (survey) to detect its effect on wildlife. The study employed both qualitative and quantitative data as well as primary and secondary data sources to collect necessary information. The information providers were purposively selected from sample 'kebeles' based on their age and experiences, that is to get detail and accurate information from elders and experts who have lived in the area for many years and who know more about how and when the Hanger-Didessa state farm had established. The state farm covered a large area, including four districts as Sasiga, Diga, Arjo, and Guto Gida. For this study, Diga was selected because of its socio-economic characteristics, deforested (degraded) area, local loss of larger mammals and forest cover changes observed in the district. The descriptive research method was used to assess community's knowledge, perception, skill, and feeling about the impact of large-scale agriculture (LSA) on forest and wildlife in the area. Land cover change analysis for 1986 to 2006 showed that the land cover of the study area is classified as grazing, wood, agricultural, settlement and degraded lands. The result of the analysis showed that agriculture, settlement, and degraded lands increased from 19.68% to 32.72%, 12.12% to 26.85% and 2.76% to 4.72% respectively in an expense of a decrease in the grass (grazing) and woodlands. Therefore, LSA is the primary cause for the loss of forest and wildlife in the study area.

Keywords: Deforestation, Didessa State Farm, forest, large scale agriculture, monoculture, wildlife

INTRODUCTION

Agriculture is the cultivation of plants, fungi and other life forms for food, fiber, biofuel, medicine, and other products, and the raising of livestock that are used to sustain and enhance human life (ILO 1999). Agriculture is the deliberate effort to modify a portion of earth's surface through the cultivation of crops and the raising of livestock for sustenance or economic gain (Rubenstein 2003). Agriculture is the systematic raising of useful plants and livestock under the management of man (Rimando 2004). All forms of farming have major impacts on forest and wildlife especially when the new land is brought into cultivation and habitats are destroyed, this can lead to the loss of native species of plants and animals.

Large-scale agriculture is the process of using agricultural machinery to mechanize the work of agriculture, greatly increasing farm worker productivity. In modern times, powered motors have replaced many jobs formerly carried out by manual labors or by draft animals such as oxen, horses, and mules (Altieri 2010). Mechanization involves the use of an intermediate device between the power source and the work. Large-scale agriculture includes the use of tractors, trucks, combine harvesters, airplanes, helicopters, and other vehicles for different purposes. Large-Scale Agriculture (LSA) increases production, reduces dependence, decreases the cost of crops, and increases multiple cropping patterns which need quick land preparation, planting, weeding, harvesting, and processing (Altieri 2010). Even though large-scale agriculture increases production, it has a high impact on the forest, wildlife, soil, water, and the climate. Intensive monoculture depletes soil and leaves it vulnerable to erosion. It affects the distribution and abundance of wildlife in the area because large-scale agriculture is associated with practices such as deforestation and monoculture. The demand for agriculture, as well as the technological change in agriculture, significantly impacts the mode and rate of transformation of the forested area, which leads to the loss of wildlife habitat and the loss of animals from the field (Angelsen and Kaimwitz 2001).

In present time Ethiopia, forests are being destroyed at an alarming rate and the area covered by forest is only less than 2.4 percent compared to the estimated 40 percent before one hundred years initial coverage (Berhe 2004).

Even though there is a long list of activities that affect forest and wildlife, in this study a great deal of emphasis was given to assess the impact of large-scale agriculture on forest and wildlife in Didessa Valley, Diga Woreda, Eastern Wollega Zone, Oromia Regional State, Western Ethiopia. Didessa Valley was selected because there was a dense forest with a variety of mammal species before the

establishment of Didessa State Farm in 1974, which is now wholly deforested and has lost many of its plant and animal species.

The aims of this research were (i) To assess the way how large-scale agriculture caused an impact on forest and wildlife in the study area. (ii) To examine the major problems that the wildlife and the local society faced due to the loss of forest in the study area. (iii) To identify emerging ecological issues associated with the new settlement in the study area. (iv) To map Land Use/Land Cover (LULC) change detection of the study area. (v) To identify the cause of the loss of forest and wildlife in the study area.

MATERIALS AND METHODS

Description of the study area

Location

The study was conducted in three kebeles located in Diga Woreda, East Wollega Zone, Oromia Regional State, Western Ethiopia. The study area is found 388 km west of Addis Ababa and 60 km west of Nekemte town, which is the center of East Wollega Zone. Geographically, it is positioned between 9^0 0' 0" N to 9^0 11' 0" N latitude and 36^0 9' 0" E to 36^0 31' 0" E longitude (Figure 1). Diga is bordered by Sasiga in the North, Guto Gida in the East, Leka Dulacha in the South, Illu Aba Bora (Chawaka) in Southwest, West Wollega Zone and Benishangul Gumuz Regional State in the Northwest. The Didessa River runs on the western side of Diga Woreda.

Topography

The main landform of Diga Woreda is nearly a slope having 0-55% with small undulating and sloping relatively steep. The elevation of the area in general ranges from 1200 to 2220 m a.s.l (DAO 2016) and comprises two agro-ecological zones, the lowlands which account 51.4 % and the midlands which account 48.6%.The midlands are steep and formerly forested terrain, which now is rapidly cleared of trees. Scattered communities tend to cultivate tops and bottoms of slopes, because the slopes are too steep; therefore, it is exposed to soil erosion. The lowland bordering the Didessa River is less steep than the midlands comprising more rolling trains.

Climate

The study area exhibits two ecological zones, the mid-land (medium temperate) which accounts for about 48.6% and lowland which accounts for approximately 51.4 % of climatic conditions with a yearly rainfall ranging from 1200-2100 mm. Peak rainfall occurs between June and September which is the long rainy season, and the smaller rainfall occurs between March and April. The average minimum and maximum annual temperature of the area was 18°C and 32º respectively (DAO 2016).

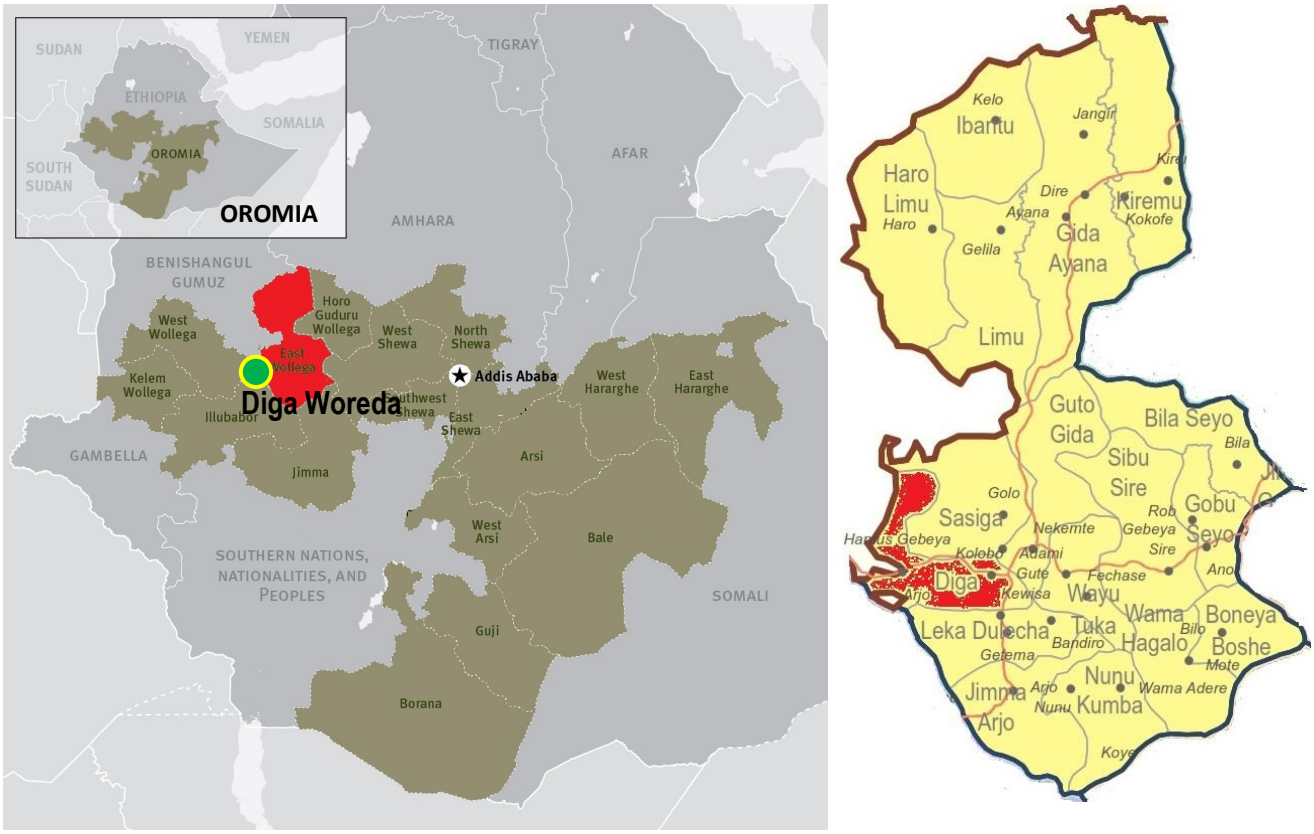

Figure 1. Map of Diga Woreda, East Wollega Zone, Oromia Region, Ethiopia

Soil

Based on FAO (2007) classification of the world soil, there are three types of soils in Diga Woreda. District nitisols (one of the best fertile soils) account for about 54,744.7 ha of the Diga Woreda. District gleysols and orthic acrisols constitute the remaining areal share of the Woreda. The dominant soil color was red in the midland whereas black in the low land (Biranu 2012 *in* Wudneh et al. 2014).

Water sources

Diga Woreda was rich in rivers and streams which served the community for drinking, irrigation, and other purposes. At present, Didessa River serves as a recreational area, with Maka and Dimtu Rivers being used for small-scale irrigation and a source of sand for Nekemte town with Chancho River serving Nekemte town for drinking. There are also other rivers such as Gulufa, Kiki, Bareda, Sororo, and others. All these rivers are tributary of the Didessa river (DAO 2016 and local elders survey 2016). But in the study area, there are only seasonal streams and two rivers Gebo and Fitesha which are used for small-scale irrigation and drinking.

Land use and land cover of the study area

Currently, Ethiopian flora has been already mapped using Geographic Information System (GIS), and remote sensing technologies and this region are in B3 on the atlas of the potential vegetation map of Ethiopia (Friis et al. 2011). The study area has been covered with dense native vegetation types before clearing or deforesting the area for Local Study Area (LSA), that is, for the "Didessa State Farm" which was established in 1974. Before the establishment of this farm, the vegetation types found in the Didessa Valley were important habitats for various wild mammals, birds, reptiles, amphibians, insects, and other organisms. At present, most of the study area is converted to bare land and agricultural land, with a few remaining scattered native plant species and fragmented plantations of non-native plant species such as eucalyptus and mango trees. Even though the vegetation of the area is at risk, there is continuous deforestation of trees by new settlers for agricultural activities.

Wildlife

The lowland areas of Diga Woreda previously were covered with dense forest in which different plant and wild animal species were living before it was cleared for the establishment of the Didessa State Farm. According to the community elders, the local people had moved to this area to hunt large mammals such as lions, buffaloes, leopards and African wild dogs for trophies and braveness. Along with hunting warthog, bush pig, zebra, and others for their meat. But, at present these larger mammals are not found in the study area because they lost their natural habitat by LSA. As information was gathered from elders, DA, community leaders and household during the field survey the most notable mammal species found in the study area at present are Spotted hyena, Anubis baboon, Grivet monkey, bush duiker and Menelik's bushbuck (Table 1) but other larger mammals were lost from the area with the loss of forest (Table 2).

Socio-economic and demographic features

The total population of Diga Woreda was 80099. Out of the total population, 39249 of them were males and 40856 of them were females (CSA 2007). The population of the households (Households) in the Woyessa Dimtu, Bekiltu Gudina, and Malka Bayeti Jirma was 500, 506 and 664 respectively.

Therefore, the total population of the study area was 1670. Based on the number of communities in each kebeles, the sample household's number was purposively determined. Accordingly, 15, 15 and 20 households were selected from Woyessa Dimtu, Bekiltu Gudina, and Melka Beti Jirma Kebeles, respectively. The people in the study area depended on mixed agricultural activities using oxen and their family's labor. Some families engaged in trade and crafts work. The communities come together and help each other during house building, harvesting, trashing, and farming. In the low land area where there are no oxen, some families use a hoe to plow small amounts of land and cultivated crops and garden root crops. The crops were cultivated using rain and some streams for irrigation. The major crops grown in the midland area include maize, onion, potato, beans, peas, barley, and wheat (Source: own survey 2016 and Key-Informants). The lowland area produces corn, sorghum, oil crops, banana, sugar cane, tomatoes, nuts, avocados, and mango. The major livestock raised in the area include cattle, goats, sheep, mules, donkeys, and fouls (Source: Local elders, community leaders, and DA). However, the production was not satisfactory because the local people used primitive methods of production (DAO 2016).

Table 1. The list of some non-native plants observed along the roadsides and in some villages during the field survey.

Scientific name	Found in
Eucalyptus camaldulensis	All three kebeles
Gravillea robusta	All three kebeles
Jacaranda mimosaefolia	All three kebeles
Juniperus spp	All three kebeles
Mangifera indica	All three kebeles
Spathodea campanulata	All three kebeles

Source: survey April 2016

Table 2. The list of some wild animals found at present in the study area

Local name	Common name	Scientific name
Dhaddee	Porcupin	*Hystrix cristata*
Jaldeessa	Olive baboon	*Papio anubis*
Qamalee	Grivet monkey	*Chiorocebus aethiops*
Quruphee	Bush duiker	*Sylvicapsa grimmia*
Waraabessa	Spotted hyaena	*Crocuta*
Bosonuu	Menelik's bushbuck	*Teragelaphus seriptus meneliki*

Source: Community elders, key-informants, and development agents

Diga Woreda/district was organized into 21 rural and 3 urban kebeles. There are also other organizations such as 63 Rural Development Zones, 422 Kebeles Development Groups, 2100 the Five Rural Development (locally known as "*Shanee Misooma Baadiyyaa"*). In the study area, there are also many traditional social organizations like Dabo, Edir, Eukub, and others. Dabo is applied during crop harvesting and production, house construction and generally when the household head cannot accomplish the work by him/herself. Both *Edir* and *Eukub* are used to strengthen the more social relationship among local societies and to save money that will be used during some ceremonies like weddings, burials and to help one another (DAO 2016, community leaders, and elders).

Research methods

The qualitative research method was used to gain an understanding of underlying reasons, opinions, and motivations that cannot be measured.

Design of the study

The study design selected for this research included a descriptive survey method, land-use change mapping and comparison, and direct observation was used. Descriptive research can only describe a set of observations, or the data collected, (Jackson 2009). The descriptive method was used to describe the data and characteristics of what was being studied. After the participants answered the questions, the researcher explained the responses given. The method of sample selection used for three kebeles and farmer households was purposive sampling.

Materials used for the study of LULC change of the study area

The materials and software used for this research were:

ArcGIS 10.2,

Earth Resource Data Analysis System (ERDAS) IMAGINE 2010

Global Positioning System (GPS),

Digital Camera

Other software (www.socscistatistics.com/tests/ztest) was used

Data collection

Appropriate data types were gathered from their respective sources to conduct the study on land cover change of the study area. A pilot survey was done in the Didessa Valley in November 2015. During the pilot survey, 10 households were randomly selected and interviewed. The primary purpose of the pilot survey was to evaluate the questionnaire whether it was suitable or not in the study area.

Types and sources of data collection

Both primary and secondary data sources were used in this study. Farmers were the primary sources of primary data. As part of the primary data information was also collected from Woreda Agricultural Experts, kebele leaders, community elders and water resource experts. Secondary data sources were obtained from published materials and internet/websites (Table 3).

Table 3. Data types and their sources as used in the current study

Type of data	Source
Satellite imageries and their date of acquisitions: Thematic mapper (TM) Date of acquisition 22/11/1986, 30 m resolution Enhanced Thematic Mapper (ETM+) Date of acquisition 26/11/2006 and 30 m resolution	Downloaded from internet
Demography data	Diga Woreda, and Central Statistical Agency (CSA 2007)
Soil type	Ministry of Agriculture &Rural Development
GPS ground-truthing	Own measurement
Information as to conservation & support practices	Woreda Rural & Agriculture Sector

Sampling techniques and sample size

In Diga Woreda, there are 24 kebeles. From these, three kebeles were purposively selected for the study because they were relatively more deforested for LSA and have lost vegetation cover. The total number of households in the three kebeles was 1670, of which 3% were included in the study because of the homogeneity of the population in ethnicity, language, culture, occupation, and location. Therefore, this sample size can be enough to represent the whole community of the study area. Generally, the sample size adds up to 70 including those selected as key informants and focus group discussion participants.

Data collection methods

Four types of data collection methods were used to conduct this study. These are a households interview, focus group discussion, critical informant interview and direct observation. Secondary data from the internet and written documents were also used to collect information about the impact of LSA on forests and wildlife. The questionnaire was prepared in English and translated to the local language Oromifa for communication convenience and responses were converted to the English language during analysis.

In-depth (households) interview

The in-depth interview was one of the qualitative research methods which served to conduct an extensive individual interview with a small number of respondents to explore their ideas or perspective (Turner 2010). An in-depth interview is applied to investigate comprehensive information about participants life experiences, attitudes, and viewpoints of a particular problem. Therefore, this detailed study discussion was used to assess the impact of LSA on forests and wildlife in the Didessa Valley. Selection of the interviewees was conducted based on purposive sampling techniques. To get detailed information from the local community, priority was given to the elders and those who have good experience and information about the Didessa State Farm.

Key informant interview

To supplement the information collected using the questionnaire and to have detailed insight into the impact of LSA on forest and wildlife in the study area, key informant interview was used as means of the data collection. Key informant interviewees were selected from Diga Woreda in three kebeles namely Woyessa Dimtu, Malka Bayeti Jirma and Bikiltu Gudina. The participants were selected purposively based on their age, experience, and involvement in different activities and practices in the Didessa State Farm. 10 key informant members were elected and interviewed.

Focus group discussion

Focus group discussion (FGD) is one of the major qualitative research tools used to examine peoples thoughts and feelings (Figure 2). It is mostly conducted by inciting 10 to 12 participants to explore information on specific issues (Flick 2007). FGD was employed by selecting a sample of people from the study kebeles. The samples were purposively selected based on their previous experiences with the problem. Both men and women were included in the selection.

Three FGDs were conducted in the study area (one for each kebele). The FGDs consist of 10 members (that is, 3, 3 and 4 members in Woyessa Dimtu, Bekiltu Gudina and Melka Beti Jirma respectively). The members of the FGD were purposively selected from community leaders, elders, agriculture experts, water resource sector, health sector and senior farmers who were known to have good knowledge about the issue.

Direct field observation

Direct observation is one of the data collecting tools that was used in this study. Field observations were made in the three kebeles to document the impact of LSA on forest and wildlife. Field observations started in November 2015 and continued throughout the whole process of data collection to make sure the validity of acquired information.

Figure 2. Focus Groups during Discussion in Melka Beti Jirma, Ethiopia

The methodology used to study LULC changes of the Didessa Valley

This section deals with the different activities postulated to attain the stated objectives of this study. Methods and techniques used to study LULC of the study area included:

Desktop review

A literature review was conducted to substantiate this study. Extracting the shapefile of the survey through clippings from a map of Ethiopia to delineate the study area. This included clipping of rivers and roads from an Ethiopian map that covered the study area and downloading Landsat images from GLCF website.

Data collection

Necessary information about demography, topography, climate, soil type, water source, of the study area was gathered from Diga Woreda Agriculture Sector, water source office and household interview, KI and FGD were conducted on a pre-set checklist questionnaires with the purposely selected informant groups of the three kebeles. Using Garmin Global Positioning System (GPS), the truthing ground record was carried out.

Data compilation

Conducted classification based on the ground truth. Change detection between 1986 and 2006 satellite imageries were made. The rate of change was calculated for each land use/land cover using the formula:

Rate of change in ha/year= (X-Y)/Z

Where:
X = Recent area of land use/land cover in ha.
Y=Previous area of land use/land cover in ha
Z= Time interval between X and Y in years

Data analysis

Data compilation and presentation

The frequency, cumulative frequency and percent of the relevant data were analyzed using statistical package for social science (SPSS 20). Data collected from different sources (Households; KI, FGD, GPS, GIS, and digital camera) were organized into meaningful facts and were explained in detail. The compiled data was presented in the form of percentages, tables, charts, graphs, and textual descriptions and photos to examine the extent to which LSA caused loss of forest in the study area. Finally, conclusion and recommendations are formulated based on the findings.

Image processing and classification

Image processing. Digital image processing involves the manipulation and interpretation of digital images with the help of a computer (Lillesand and Kiefer 1994). Satellite imagery must be well processed before use for further applications. It is, in fact, essential to rectify the raw satellite image under the pre-processing stage such as geometric and radiometric correction. Image restoration also involves the correction of distortion, degradation, and

noise introduced during image processing. Image restoration produces a corrected image that is as close as possible, both geometrically and radiometrically, to the radiant energy characteristics of the original scene. To adjust the remotely sensed data, internal and external errors must be determined (Jensen 1996). In pre-processing phase, it is usually necessary to geo-reference the images on projection and datum that Ethiopia has already selected, UTM projection and Adindan datum. In this respect, all the images used which are in the WGS84 forecast have been re-projected into the country's datum and projection. This is mainly because datum and projection conflict would undoubtedly limit the use of various themes (layers) at a time. If remotely sensed data are to be used in association with other data within the context of a geographic information system, then the remotely sensed data and the products derived from such data would need to be expressed with reference to the geographical coordinates that are used for the rest of the data in the information system.

Histogram equalization is used to apply a nonlinear contrast stretch that redistributes pixel values so that there is approximately the same number of pixels with each value within a range. Haze and noise reductions with the view to overall reduce the amount of haze and noise from an input image were in general done to enhance the interpretability of the images. However, those enhancement techniques did not bring as such significant change consequently; spatial enhancement of resolution merge to increase the spatial resolution of the multi-spectral image was also carried out. Concerning bands selection, all the bands that are present in each image are not used for land use/land cover classification. Depending on the nature of each band's application, some bands were selected. After attempting different band combinations by considering their specific applications, the false color composite of band 2 (green), 3 (red) and 4 (blue) of TM and ETM+ were applied to classify the study area.

Image classification. The overall objective of image classification procedures is to automatically categorize all pixels in an image into land use/land cover classes or themes (Lillesand and Kiefer 1994). Remotely sensed data of the earth might be analyzed to extract useful thematic information. Notice that data are transformed into information. Multispectral classification is one of the most often used methods of information extraction (Jensen 1996). In classifying the images, both unsupervised and supervised image classifications techniques were applied, for the latter case training site was established based on the ground truth taken during fieldwork. The unsupervised image classification was done before fieldwork. Among different algorithms in the drop-down lists of supervised classification, maximum likelihood image classification was utilized. By applying the techniques of image classification, LULC types have been identified so that to use the classified images for change detection and recognizing the impact of large-scale agriculture as well as deforestation on forest and wildlife. With the help of visual interpretation elements and the different reflection characteristics of the features in the satellite images of 1986 and 2006, the study area has been classified into five LULC classes: Agricultural area, Settlement area, grazing land, woodland cover, and degraded land.

Agriculture land: is an area of land plowed or prepared for growing different types of crops.

Settlement land: refers to the action of people coming together to live in an area. Is a general term used in archaeology, geography, history, and other subjects for a place where people live (either permanently or temporarily). When they come from far away, it is sometimes called a colony. Settlement, locality, or populated place is a community in which people live. A settlement can range in size from a small number of dwellings grouped to the largest of cities with surrounding urbanized areas. Settlements may include hamlets, villages, towns, and cities.

Grazing land: land used for grazing animals.

Woodland cover: this class corresponds to plants that have undergone modifications from man's influence. It is composed predominantly of secondary vegetation indicative of a recovery stage from past disturbance. It occurs mostly near farmland and around settlements. Generally, it is an area covered with scattered wood.

Degraded/bare land: this type of land system has been the last category classified from both satellite images which are the most deteriorated or exhausted merely entirely. Due to its unfortunate situation, an effort is being made along the roadside to restore it. Figure 3. shows the extent of the present land degradation in some parts of the study area.

Post classification change detection

In accordance with Lillesand and Keifer (1994), change detection involves the use of multi-temporal data sets to discriminate areas of land cover change between dates of imaging. Moreover, ideally, change detection procedures should affect; data acquired by the same or similar sensor and be recorded using the same spatial resolution, viewing geometry, spectral bands, and time of day.

One way of discriminating changes between two dates of imaging is to employ post-classification comparison. This kind of change detection method identifies and provides where and how much change has occurred. It also provides to and from information and results in a base map that can be used for the subsequent year. In this approach, two dates of imagery are independently classified and registered. Then an algorithm can be employed to determine those pixels with a change in classification between dates. When evaluating the change detection made in this research against the ideal scenario, the requirements stated by many authors are least met owing to unavailability of satellite images that fulfill the standard. Change detection was carried out between the TM 1986 image with 30m spatial resolution, four spectral bands, varying radiometric resolution and ETM+ 2006 having a 30m spatial resolution, eight bands including panchromatic only for ETM+.

As the process progressed to finalize change detection, basic steps such as having identical LULC classification categories in their order, adjusting varied pixel size into 30m were done. Upon completion of all the necessary steps, the two classified images were taken into GIS analysis and calculated area coverage of the LULC classes between 1986 and 2006.

Figure 3.A. Woodland in Bekiltu Gudina Kebele, Ethiopia, May 2016

Figure 3.B. Degraded land in Bekiltu Gudina Kebele, Ethiopia, May 2016

Figure 3.C. Degraded land in Melka Beti Jirma Kebele, Ethiopia, May 2016

RESULTS AND DISCUSSION

This section describes the results obtained through demographic characteristics, data processing, and analysis techniques, that is, it deals with the results obtained from the survey as well as the analysis of the remotely sensed data of post-classification comparison of land use and land cover changes between 1986 and 2006.

Response rate of respondents

Out of the total 50 households respondents that have been interviewed during the survey, all of them (100%) were answered the questionnaires. In addition to this, 10 key-informants and 10 focal groups responded to and discussed the interview questions. This implies that the proposed sample size fully participated in the study, which helped the researcher to find out the problems caused by LSA on forest and wildlife.

Demographic data of the respondents

Based on the sample farmers, the household's survey results show that the average family size of the study area is 6.5 persons per household head ranging from 1 to 14 (Table 4). This implies that there is a high rate of population growth in the study area. As a result of the demand for household heads and his or her family's to use firewood and the land become increase, this indicates that population growth has an impact on forests and wildlife.

Table 4. Sex, age, level of education, marital status, family size, duration in the residential area and occupation of the respondents

Item	Characteristics	Frequency	%
Sex	Male	47	94
	Female	3	6
Age	30-39	3	6
	40-49	5	10
	50-59	9	18
	60 & above	33	66
Level of education	Illiterate	29	58
	Elementary (1-8)	13	26
	Secondary (9-12)	8	16
	Above 12	-	
Marital status	Married	45	90
	Single	2	4
	Divorce	3	6
Family Size	1-3	6	12
	4-6	14	28
	7-9	19	38
	10 & above	11	22
Duration in the residential area	10-20 years	1	2
	21-30	7	14
	31-40	13	26
	41 & above	29	58
Occupation	Farming only	17	34
	Raring livestock only	2	4
	Mixed farming	31	62
	Trading	-	-

Out of the 50 household heads who participated in the survey, 29 household heads (58%) were illiterate, 13 (26%) were elementary, 8 (16%) of them were secondary school. This shows that most of the household heads were illiterate because they were daily laborers in the Didessa State Farm and paid a few birrs; most of them came from different regions of the country and belonged to needy families who had not got an opportunity of education, lack of education forced them to engage themselves in clearing forest for different purpose that causes land degradation. As shown in (Table 5) the number of households that joined secondary school was 8 (16%) this indicates that there is no secondary school in the area, therefore after completing elementary school they drop out of school and become a farmer who participated in different illegal activities such as deforestation, hunting, and burning grasslands.

Exactly more than half of the households involved in the survey, 66 % were 60 years old and above, this shows that most of them are under poverty, so they could not improve their economic status and actively participated in clearing forests to increase farmlands. As a result, they cause land degradation and wildlife loss. But they were purposively selected for this study because they have useful information about the study area.

Most of the people in the study area were engaged in mixed agriculture (farming and rearing livestock). As (Table 5) shows 66% of the respondent's livelihood depended on mixed agriculture, 34% of them were practicing only farming, but only 4% of them were involved in raising livestock. This shows that the area was affected by agriculture and grazing animals‟ According to community elders, key-Informants and Development Agent (DA) of the study area when the farmland lost its fertility farmers were clearing trees remaining in some areas to hold another farm. This result shows that agricultural intensification leads to land degradation and loss of wildlife. Almost all (94%) of the respondents were males, and 6% of them were females, this shows that men are mostly involved in agricultural activities.

Forest cover in Didessa Valley before 1974

Before the establishment of the Hanger-Didessa State Farm the Didessa Valley was covered with dense forest where different plant and animal species were found (Source: community elders, DAO, and local society). Didessa Valley was inhabited by various native plant species that provided the wildlife with shelter, food, and other uses. It was also the area where people found cultural medicines, timber trees, firewood and different wild animals that were used as a source of food for the local society. People from different areas used to go to the Didessa Valley for hunting larger mammals such as buffalo, warthog, bush pig, antelope, and others for their meat, and hunting favorite dangerous mammals such as lion, leopard, and African wild dog for braveness (Source: Local elders). At present, there is no dense forest in the study area because of deforestation for LSA during the establishment of the Didessa State Farm in 1974 (Source: Elders and current survey).

Ranking the percent of forest cover in Didessa Valley before 1974

Most (70%) of the household heads respond that the study area was covered with a very high percentage of the forest, 26% households acknowledge the presence of top present of forest and only 24% of them replied that the presence of medium forest coverage in the study area before 1974. Therefore, based on the result and the information gathered from KI, FGD and community elders the area was covered with dense forest where different species of wild animals were living. During the survey, few remaining native plant species were observed that support the information gathered from various sources (Figure 4 and 5; Table 5). In addition to this, some non-native plant species were found during the survey (Table 5).

Figure 4. *Manilkara butuji* and *Pouteria adolfi friederici* in Didessa Valley, Ethiopia

Figure 5. *Syzygium* species in Bekiltu Gudina, Ethiopia

The cause for the loss of forest and wildlife

The topmost reason that is associated with the loss of forest and wildlife in the Didessa Valley was the establishment of the Didessa State Farm in the area. As the listed causes compared to respondents of the study area, there was a significant difference between them ($p<0.05$, (0). Therefore, the topmost reason for the loss of forest and wildlife in the Didessa Valley was Didessa State Farm (Table 6). There are also other factors such as settlement and wildfire which cause damage to forests and wildlife in the study area. Generally, there are multiple factors such as agriculture, deforestation, population growth, fuelwood collection, illegal logging, expansion of towns and road construction that cause loss of forest.

Size of farmland of household heads in hectares

Based on the response given by respondents of the study area there was a significant difference between the farmland size of the household at $p<0.05$, that is, the p-value is Zero (0). Occupation, the size of farmland in the study area, was different. When the Didessa State Farm was dismantled some daily laborers hold wider area of cropland whereas others were leaving the area and large area of the former farmland was remaining without landowner (Source: community elders). In 2002 the government set settlement program in the area and translocated people from Harege to Didessa Valley. As shown in (Table 7) 70% of the households hold 1to 2 hectares because the government equally gave 2 hectares of farmland for each household during the new settlement in the area, 26% of the household respond that they hold 3 to 4 ha and the remaining 4% owning 5 to 6 hectares. This shows that the indigenous people of the area were carrying more massive hectares of land than the translocated people into the area by the settlement. Therefore, the destruction of the forest is related to the size of farmland.

The present status of forest covers in the study area

As it was observed during the survey, there was no dense forest cover in the study area. The households also responded that there is no dense forest in the area. This shows that the dense forest was cleared away for LSA. As a result, most wild animals emigrated from the area.

Effects of forest cover change

Forests are one of the natural resources that assist in maintaining the biodiversity of the ecosystem. It is home to wildlife; it holds and improves soil fertility, filters water, prevents soil erosion and removes toxic gasses and maintains environmental quality. Therefore, forest cover change is associated with many effects. As indicated in (Table 8), 44% of the households responded that wildlife was immediately affected by forest cover change, 30% of the households answered that the rate of land degradation increases with the loss of forest, 14% of the households replied that poverty is another condition that appears with forest cover change and 12% of households responded that climate change could appear with the loss of forest. This implies that loss of forest is associated with loss of wildlife, land degradation, climate change and poverty. Therefore, the effect of forest cover change on wildlife and climate is significantly different at $p<0.05$ ($p=0.00038$).

Table 5. The list of some indigenous (native) plants observed during survey

Local name	Scientific name	Observed place (Kebele)
Buttujjii	*Manikara butugi*	Melka Beti Jirma
Dambii	-	Melka Beti Jirma
Goosuu	*Syzygium* spp	Bekiltuu Guddina
Makkaannisa	*Corton macrostachyus*	Bekiltuu Guddina
Mukaa Arbaa	*Albizia gummifera*	In all three kebeles
Odaa	*Ficus* spp	Woyessa Dimtu
Qararoo	*Pouteria adolfifriederici*	Melka Beti Jirma
Harbuu	*Ficus sur*	Melka Beti Jirma
Laaftoo	*Acacia abyssinica*	In all kebeles

Source: Survey April 2016

Table 6. The topmost cause for the loss of forest and wildlife in the Didessa Valley, Ethiopia

Item	Frequency	Percent
DSF	31	62
Settlement	17	34
Wildfire	2	4

Table 7. Farmland size of the households in hectares

Item	Frequency	Percent
1-2	35	70
3-4	13	26
5-6	2	4

Table 8. The immediate effects of forest cover change

Items	Frequency	Percent
Climate change	6	12
Loss of wildlife	22	44
Land degradation	15	30
Poverty	7	14

Difference between LSA and subsistence farming

Large Scale Agriculture is a mechanized farming practice that is associated with different machines to cultivate large hectares of land. It is characterized by deforestation and spraying of chemicals that can cause several environmental problems (pollutions) and loss of the biodiversity of the area.

It also encourages monoculture whereas small-scale farming promotes biodiversity by growing different varieties of crop plants. (Table 9) shows that 34% of the households respond that LSA is identified from subsistence farming by deforestation of large area of land for intensification of agriculture, 26% of the households acknowledge that it is encouraging monoculture plantation,

24% of them respond that is aimed at holding large area of farmland and the remaining 16% were replied that it uses excessive chemicals that pollute the environment. Even though LSA increases crop production, it has an environmental effect by polluting the environment because cultivators use different chemical substances such as fertilizers, herbicides, fungicides, and pesticides. Hanger-Didessa State Farm covered about 5000 hectares (Diga Woreda Agriculture Sector). At present some portion of this area is occupied by monoculture forestation of *Eucalyptus* species and *Mangifera indica* with the land coverage of 1,100 ha and 241 ha respectively, and settlement. Therefore, both monoculture forestation and settlement caused an effect on the biodiversity of the Didessa Valley.

Table 10 shows 48% of the respondents replied that the government was more responsible for the loss of forest and wildlife in the area, 24% of them answered that the drivers of the bulldozers were accountable and other responsible bodies like kebeles leaders and the local society account about 18% and 14%, respectively. In case of taking responsibility for the loss of forest and wildlife, there is a significant difference between the government and the local society at $p< 0.05$ ($p=00024$). Therefore, the study result has indicated that the government had set a program for Large Scale Agriculture in the Didessa Valley that deforested (cleared away) the dense forest which was the leading cause for the loss of many species of plants and wild animals from the area, drivers of the bulldozers removed trees with their roots and roaring (sound) of the bulldozers and other machines frustrated the wild animals which forced them to migrate from the area. Kebele leaders were involved in providing information to the local government about the importance of the former DSF for settlement which was used as a primary case for relocating the Harer people into the old Didessa State Farm who engaged in clearing of grasslands and wood trees to increase their farmland, to make charcoal and to collect firewood which increased loss of wood plants and land degradation. Some people in the local society are also involved in hunting wild animals, clearing wood plants, and destroying grasses by overgrazing system which deflected ecological succession and cause land degradation.

Distance of firewood from the households

Almost all (98%) of the respondents indicated that the local people travel up to 10 km to collect fuelwood. This result shows that the local society traveled a long distance to receive firewood because of deforestation for agriculture. As it was observed during the field surveys, a few native plant species such as *Manikara butugi*, *Pouteria adolfi friederici*, *Syzygium* spp, *Albizia gummifera* and others were found in some degraded lands of the Didessa Valley (Table 5). This indicates that the area had been covered with different native plant species before deforestation for large-scale agriculture. In the study kebeles there is a shortage of firewood. Therefore, some households have collected a pile of crop residue as a source of firewood to overcome the lack of firewood (Figure 6).

Table 9. Effects of LSA on the wildlife and local society in the Didessa Valley

Items	Frequency	Percent
intensification of farmland	12	24.0
Monoculture planting	13	26.0
deforestation of a large area	17	34.0
Use of chemicals	8	16.0

Table 10. The responsible bodies for the loss of forest and wildlife in the study area

Item	Frequency	Percent	Cumulative percent
The Government	24	48	48
Kebele leaders	9	18	66
Drivers of the bulldozers	10	20	86
Local society	7	14	100

Shortage of pure drinking water in the study area

All (100%) of the respondents indicated that there is a shortage of pure drinking water. This suggests that loss of forest in the area caused land degradation and soil erosion which led to the lack of water sources. During the field survey, the scarcity of pure drinking water was more observed in Bekiltu Gudina and Melka Beti Jirma Kebeles because these kebeles are highly populated due to settlement and destroyed the woody plants that are used for water retention and infiltration. In Bekiltu Gudina and Melka Beti Jirma, the stream named Laga Taba uses as a point source of drinking water for most villages of the two kebeles and their livestock. Laga Taba stream is found in the most degraded area of the Melka Beti Jirma Kebele bordering Bikiltu Gudina (Figure 7).

As shown in Figure 7, there is a scarcity of water during the dry season in the study area. As a result, the local people must travel more than 2kms to arrive at a point source of drinking water. This stream was used for drinking both for people and livestock. Therefore, it is more contaminated and is a source of bacterial and protozoan diseases (DHS 2016).

Figure 6. Agricultural land in Melka Beti Jirma April 2016

Figure 7. People fetching impure water from Laga Taba stream May 2016

Factors that cause the scarcity of water in Melka Beti Jirma, Bekiltu Gudina, and Woyessa Dimtu kebeles are population growth, deforestation, agriculture, overgrazing by livestock, land degradation and lack of soil conservation (Diga Woreda Agriculture Expert, Health Sector, and Key informants). Before deforestation of the Didessa Valley for DSF the local society found water and firewood from nearby sources (Source: Community elders).

Human-wildlife conflict because of deforestation in Didessa Valley

All (100%) of the respondents indicated that there was a human-wildlife conflict in the study area. As a result, many more significant wild animals faced several problems. The major cause for the appearance of conflict between humans and wild animals was the destruction of wildlife habitat by deforestation for large-scale agriculture.

As the listed wild animals compared to the bases of human-wildlife conflict, there is a significant difference between buffalo and leopard at $p<0.05$ ($p=0$) (Table 11). From this result, it can be concluded that buffaloes were more attacked by humans because of the deforestation of the dense forest and the roaring of bulldozers they flee from their natural habitat and stormed human dwellings and were killed by hunters. People hunt buffaloes for their meat and braveness. A person who killed a buffalo or lion would have proper recognition in the local society. Therefore, the result shows that all wild animals faced conflict with humans, but the degree of conflict varies from animal to animal.

Table 11. The large mammal more encountered conflict with human

Item	Frequency	Percent	Cumulative frequency
Lion	9	18	18
Leopard	3	6	24
Buffalo	27	54	78
Warthog	11	22	100

Table 12. Problems faced by wild animals during migration due to loss of habitat

Item	Frequency	Percent	Cumulative percent
Exposure to predators	13	26	26
Killing by hunters	12	24	50
Death by starvation	7	14	64
Conflict with humans	18	36	100

Table 12 shows that 36% of the households replied that loss of habitat and food sources due to deforestation for LSA wild animals encountered conflict with humans. Herbivores animals fed on crop plants and carnivores animals decided to eat livestock, 26% of them replied that some wild animals were eaten by predators, 24% of them answered that some were killed by hunters and 14% of them respond that wild animals died by starvation before arriving at their new habitat. When the problems faced by wildlife during migration were statistically tested, there was a significant difference between human-wildlife conflicts and death by starvation at $p<0.05$ ($p=0.01108$). Therefore, deforestation in the study area for LSA causes a series of problems on wild animals living in the area, because the forest is a place where wild animals live, find their food sources, breed, and care for their young. Most wild animals were migrated from the study area because of deforestation and habitat disturbance. When they relocated from one area to another area, they encountered several problems because some were pregnant, some might have fragile young, some were aged (old), and others might be sick. Therefore, they can be exposed to any attack.

Relationship between forest and wildlife

All (100%) of the respondents replied that there is a relationship between forest and wildlife. That is, forests provide wild animals with food, nesting place, cover (shelter), shade and oxygen. Animals offer plants carbon dioxide and nutrients during death and excretion.

Access to different public services to local society, because of settlement in Didessa Valley

Most (90%) of the households answered that the local society had not got access to no pre-existing services such as electric light, secondary school, road, and pure drinking water, 10% of the households respond that there are some improvements regarding elementary school, rural road, and public health sector. Therefore, in the study area there are only three elementary schools, three public clinics, and simple rural highway (Source: own observation during survey 2016), there is no secondary school, electric light, pure (pipe)water and fixed line telephone. Most of the people in the study area were in poverty they walk by barefoot.

Land use/land cover changes

Land cover changes from 1986 to 2006 for Melka Bayeti Jirma, Woyessa Dimtu, and Bikiltu Gudina kebeles are presented in (Table 13). Also, the statistics of LULC

were computed and Change in LULC in general and land cover would have either direct or indirect impact on the extent and conditions of wildlife.

The changes could be the transformation of land cover to land use or vice versa. However, it is tough to generalize whenever there is a change in land use/land cover; the change could be eventually followed by deforestation which may have been replaced by large-scale agriculture, settlement, and bare land which together led to the loss of forest and wildlife from an area. The areal extent of LULC of the study area was summarized to detect the nature of major changes that occurred between 1986 and 2006.

According to the result of this computation, woodland and grazing lands revealed a negative rate of change which implies the decrease in areal coverage while agricultural land, deforest/barren land, and settlement were able to increase their areal extent at the expense of the others (Table 13). From the 1986 land use/land cover classes, about 38.34 % was devoted to grazing land, and 27.01% was covered by woodland and other vegetation whereas 19.68% was agricultural land of different crops. The smallest share went to settlement and degraded lands which have 12.12% and 2.76% respectively. In 2006, settlement, agricultural and degraded/bare lands revealed an increase in areal coverage due to the increased population which settled in that area and used the land cover through cultivation. However, grazing land and woodland were liable to be converted into other land use/land cover unit depicted the change in decline/decrease in aerial coverage. Degraded/ bare land, agriculture land, and settlement lands coverage have got an increment of 1.96%, 13.04% and 14.73 respectively.

The following two classified maps have been used for post-change detection comparisons of the three kebeles of Diga Woreda (Figure 8 and 9).

Table 13. Summary Statics of LULC of the study area from 1986 to 2006

Land use/land cover classification	1986		2006		Rate of Change in hectares per-year
	Area (ha)	%	Area (ha)	%	Area (ha)
Grazing land	2838.33	38.34	1997.25	26.99	-42.054
Woodland	2004.47	27.10	645.02	8.72	-67.972
Agriculture land	1456.28	19.68	2421.84	32.72	48.278
Degraded/Bare land	204.12	2.76	349.09	4.72	7.248
Settlement land	896.81	12.12	1986.81	26.85	54.5
Total	7400.01	100	7400.01	100	

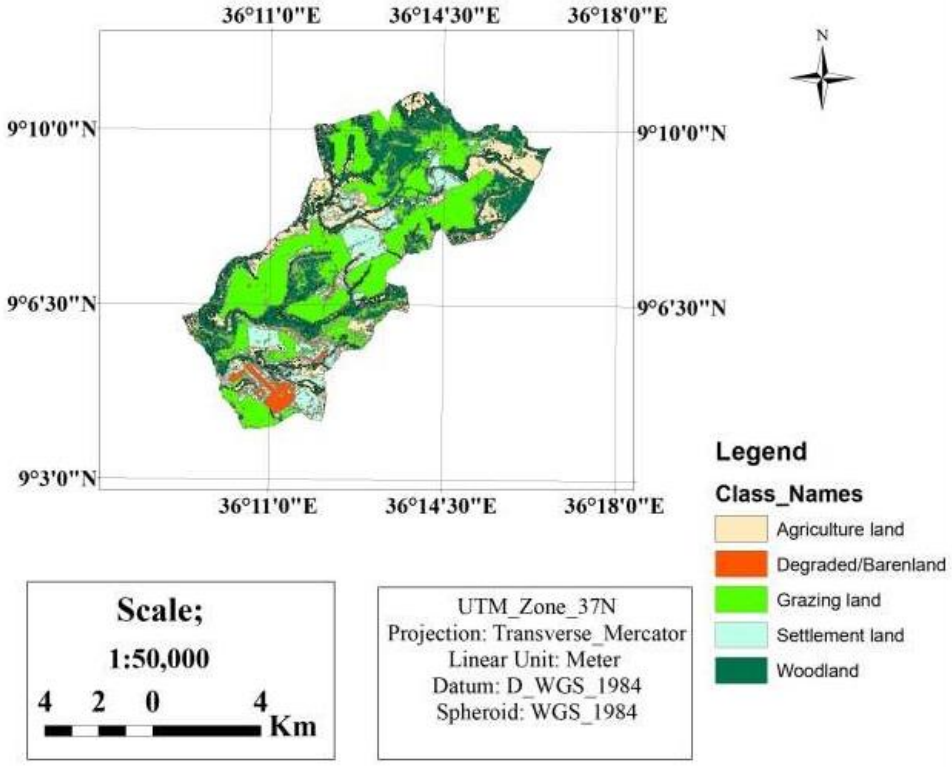

Figure 8. 1986 LULC classes of Malka Bayeti Jirma, Bikiltu Gudina and Wayesa Dimtu Kebeles

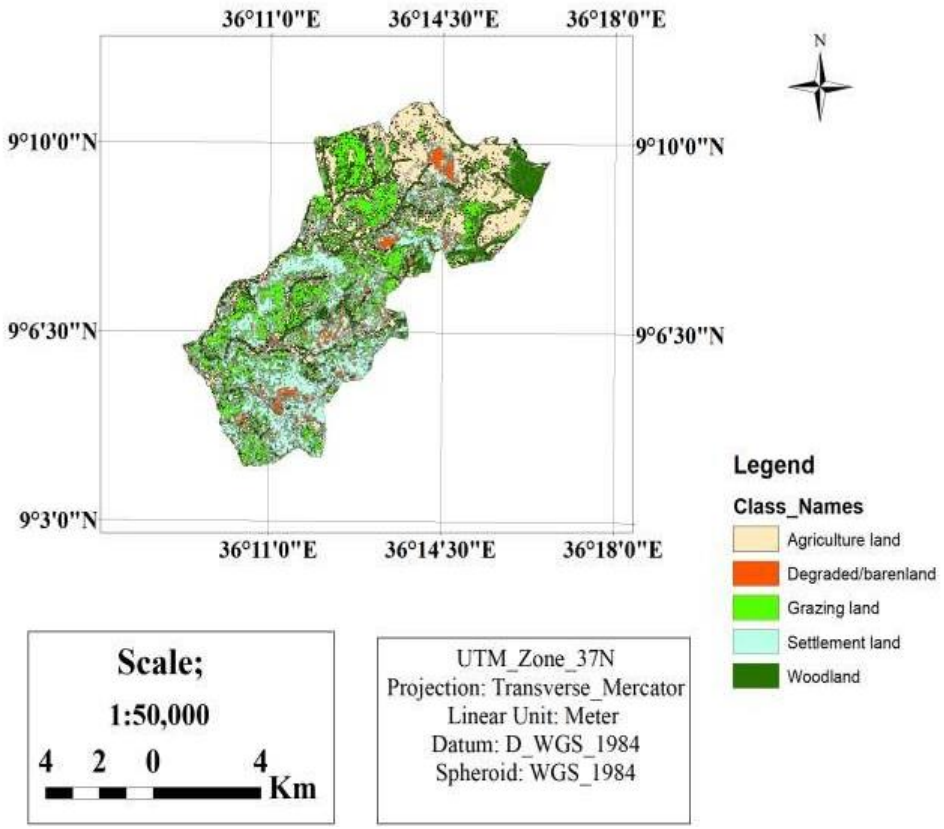

Figure 9. 2006 LULC classes of Malka Bayeti Jirma, Bikiltu Gudina and Woyessa Dimtu Kebeles

Land cover analysis for 1986

The land cover analysis of 1986 from aerial photos (Figure 8, Table 9) showed that most of the study area was under grazing (grass) land accounting to 2838.33 ha (38.34%), while woodland, agriculture land, settlement land and degraded land accounted to 2004.47 ha (27.10%), 1456.28 ha (19.68%), 896.81ha (12.12%)and 204.12 ha (2.76%), respectively. These land cover classes took about 100 percent of the study area. There was no dense forest cover. The dense forest that covered the area was deforested entirely since the establishment of the Hanger-Didessa State Farm in 1974, which was the main cause for loss of forest and wildlife in the study area.

Land cover analysis for 2006

The land sat. ETM^{+} imagery of 2006 (Figure 9) showed that much of the study area was covered by agricultural land accounted to 2421.84 ha (37.72%) while grazing land, settlement land, woodland, and degraded land accounted for 1997.25 ha (26.99%), 1986.81 ha (26.05%), 645.02 ha (8.72%) and 349.09 ha (4.72%), respectively. This shows that as the demand for more agricultural land due to settlement (population growth) increases both grassland and woodlands decrease, leading to an increase in degraded land. Much of the change from the base year (1986) occurred on agricultural land where it changed from 1456.28 ha (19.68%) to 2421.84 ha (32.72%) in 2006. This increase in agricultural land was at the expense of grazing and woodlands which decreased from 2838.33 ha (38.34%) to 1997.25 ha (26.99%) and from 2004.47 ha (27.10%) to 645.02 ha (8.72%), respectively. Other land cover classes such as settlement land and degraded land increased.

Therefore, agriculture and population growth are the major factors for deforestation and land degradation which are the cause for the loss of forest and wildlife, scarcity of water, soil erosion and shortage of energy sources (firewood) in the study area.

Conclusion

Large Scale Agriculture is the major factor that negatively affected the forest coverage of the study area. Large Scale Agriculture is linked with deforestation of large areas of forest land which are the habitat of wildlife. When forests are cleared away, wildlife that has been living in the forest lose cover (shelter) as well as food sources and decide to leave the area. When wild animals are migrating from one area to the other area, they might be killed by poachers, eaten by predators, then become extinct or reduced in number (endangered).

As information was gathered from household, elders and focal persons during survey, different native plant species such as *Ficus sur* (local name: harbuu), *Syzygium* spp (goosuu), *Manikara butugi* (buttujjii), *Ficus vasta* (qilxuu), *Cordia africana* (waddeessa), *Albizia gumifera*

(muka arbaa), *Pouteria adolfi-friederici* (qararoo) and others were found in the dense forest of the study area before deforestation. According to LULC analysis made between periods of 1986 and 2006 the land use and land covers of the study area were classified as: Agriculture land, woodland, grazing land, settlement land and degraded land. Therefore, the LULC changes over twenty years were evaluated from the land satellite images. Then, the extent and trends of LULC differences were shown. Grassland and woodlands were decreased whereas agricultural land; settlement land and degraded land were increased. During the establishment of Didessa State Farm vast hectares of forest lands were deforested for the implementation of LSA. This type of agriculture encourages monoculture, leaving mixed farming behind and reducing the biodiversity of the area. Monoculture is associated with the use of different chemicals to increase production, but it can cause environmental damage.

Deforestation leads to the loss of wildlife, that is, there is a direct relationship between forest and wildlife. The second change was observed on wood and grasslands which were brought about by intensification of agricultural land and settlement (population pressure). Population growth is associated with clearing forests for many purposes such as agriculture, charcoal, firewood, construction, fencing, and others. As it was observed during the field, survey large areas of land were left bare land without vegetation cover and exposed to soil erosion that caused a shortage of drinking water. Only a few native plant species were found around the stream, and where the land is not suitable for agriculture, this indicates that the area was covered with indigenous plant species before deforestation for agriculture. Therefore, in the case of this analysis, the dominant driving force for the loss of forest and wildlife in the study area was Large Scale Agriculture.

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