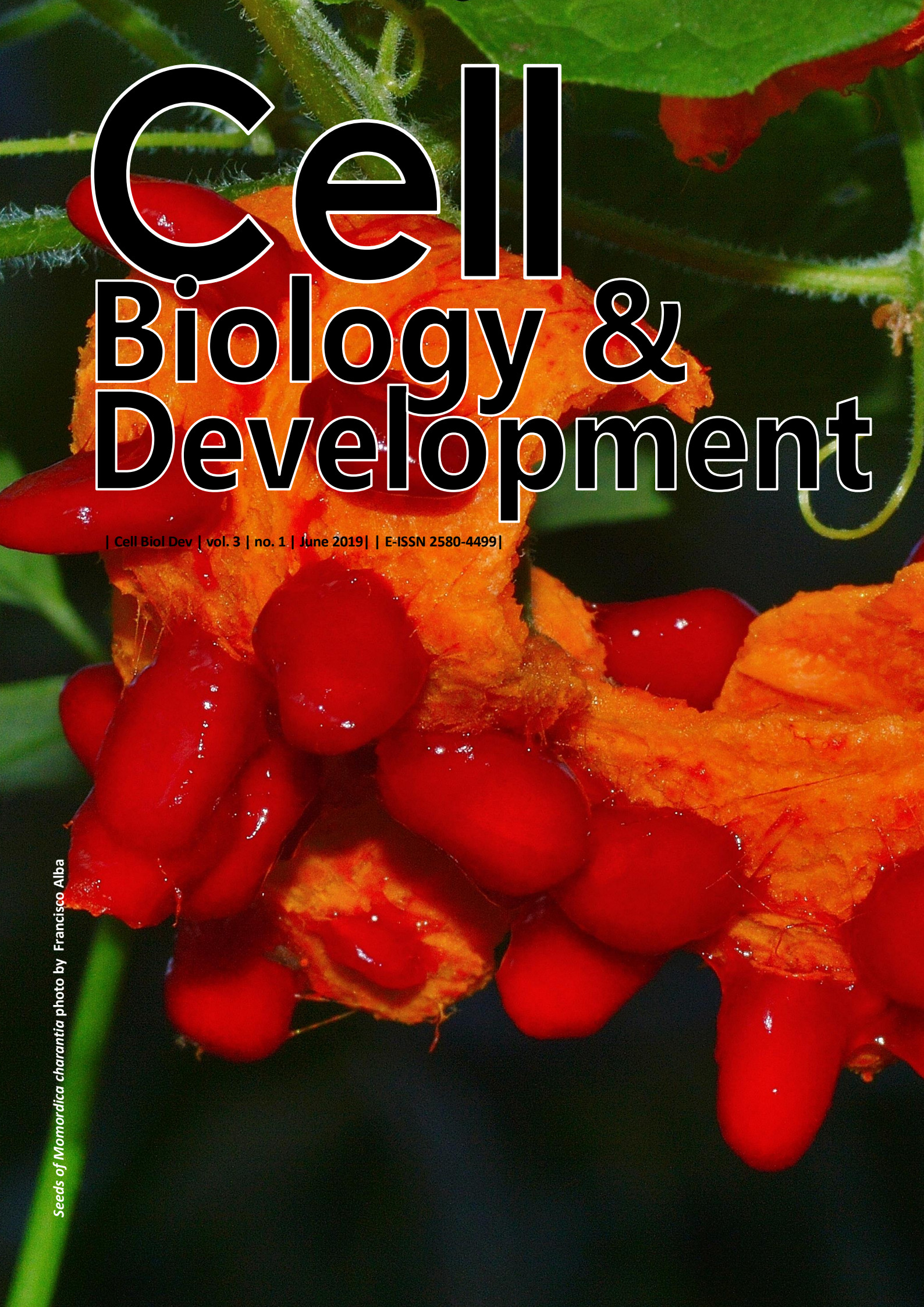

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Seeds of Momordica charantia photo by Francisco Alba

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Chapter in book:

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Biochemical characterization of *Momordica charantia* (leaf and fruit) and effect of soluble extract on MCF-7 breast cancer cell lines

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Abstract. *Ansari AA, Singh J, Aminuddin M. 2019. Biochemical characterization of* Momordica charantia *(leaf and fruit) and effect of soluble extract on MCF-7 breast cancer cell lines. Cell Biol Dev 3: 1-5.* Cancer is a serious worldwide problem targeted by various treatments, including traditional medicinal plants. One known medicinal plant is bitter gourd (Momordica charantia), which has been investigated for its anti-cancerous properties. This study was carried out to explore the biochemical analysis of different components of *M. charantia* (leaf and fruit) and the effect of alcoholic extract of *M. charantia* to investigate their potential effect on MCF-7 breast cancer cell line in comparison to cisplatin, a commercial anti-cancer drug. The different components (leaf and fruit) were separated, dried, and converted to powdered form. MCF-7 (human mammary primary epithelial cancer cells) breast cancer cell line was treated with different concentrations (8 - 800 µg/mL) of the soluble extract and cisplatin (all dissolved in DMSO and diluted in the incubating medium) for 48 hours. Initial time course experiments established that maximal cell death occurred between 24-48 hours. Cell viability (cell death) was measured using an established method. The results have shown that with the MCF-7 cell line, the extract at a high concentration (800 µg/mL) was more effective in killing the cancer cells when compared to cisplatin. The present results have clearly shown that the soluble ethanol extract of *M. charantia*, especially at high doses, can be used effectively to treat breast cancer.

Keywords: Breast cancer, cell viability, cisplatin, *Momordica charantia*

INTRODUCTION

Medicinal plants have been used to treat acute diseases like diabetes and cancer. Research has focused on using tropically grown plants like bitter gourd (Momordica charantia), which has been part of the human diet for centuries (Heinrich and Bremner 2006). Bitter gourd is cultivated throughout South America, Asia, and Africa, including Guyana, for food and medical values (Singh et al. 2004). All the components (fruit, leaves, and stem) are known for potential medical values. In recent decades, many studies have been conducted for anti-cancer, anti-diabetic, anti-viral, anti-helminthic, antioxidant, and anti-bacterial properties (Ahmed et al. 1999; Basch et al. 2003; Alessandra et al. 2008; Lee et al. 2014). The universal properties of bitter gourd may be due to the presence of many biologically active phytochemical constituents such as triterpenes, proteins, steroids, alkaloids, inorganic lipids, and phenolic compounds (Zhu et al. 1990; Murakami et al. 2001; Parkash et al. 2002; Grover et al. 2004).

Cancer is a complex disease of uncontrolled growth of cells due to signaling failure of oncogenic expressions resulting in many different types of cancers based on the origin of tumors in specific organs. Breast cancer is one of the most common cancers, emphasizing mammary gland epithelial cell cancer. The most common form of cancer affecting the human population worldwide is breast cancer, especially among females and is the major cause of mortality and is caused by aging, pollution, exposure to chemicals and ionizing radiations, genetic causes, lifestyle, and many other reasons (Torre et al. 2015; Bai et al. 2016). There are potentially many medicinal plants with therapeutic properties that have been used traditionally in many countries and are also being researched by various groups in the form of extracts against different types of cancer for possible treatments (Dandawate et al., 2016; Singh et al., 2016). *Momordica charantia* (bitter melon) is a common vegetable used as a source of food and medical values for treating many diseases as part of indigenous knowledge. All the parts of bitter melon, such as fruits, leaves, and stems, are known to have anti-cancer, antipyretic, anti-diabetic, anti-hypertensive, and multiple other positive effects on human health (Manoharan et al. 2014; Dandawate et al. 2016; Singh et al. 2016). Many researchers have investigated the effect of water, alcohol, and other organic solvent-based extracts on cancer cells that would inhibit or arrest growth by releasing cytochrome c, apoptosis induction, interference in the cell cycle, autophagy, and stem cell growth inhibition. Many bioactive compounds such as cucurbitane-type triterpenoids, triterpene glycosides, phenolic acids, flavonoids, essential oils, saponins, fatty acids, and proteins present in bitter melon may have a role in anti-cancer properties (Manoharan et al. 2014; Dandawate et al. 2016; Singh et al. 2016).

Several studies have been conducted on the effect of bitter gourd and have shown anti-cancer activity against lymphoid leukemia, lymphoma, choriocarcinoma,

melanoma, breast cancer, skin tumor, and prostatic cancer (Ganguly et al. 2000; Sun et al. 2001). The hot water extract of *M. charantia* was found to be inhibitory against inhibited uterine adenomyosis and mammary tumor growth in mice. The maximum effect was conferred by peel extract (Singh et al. 1998; Nagasawa et al. 2002). The anti-cancerous activity of water-soluble extract *M. charantia* has been reported through inhibition of DNA, RNA, and cellular protein synthesis (Licastro et al. 1980; Zhu et al. 1990; Tsao et al. 1990; Chang et al. 2008). The researchers show evidence of effective cancer treatment through *M. charantia*, and its extract controls cancer cell growth and tumor formation (Cunnick et al. 1990).

This study was designed to investigate the biochemical analysis of *M charantia* (different components-Leaf and fruit) and the anti-cancer properties of an alcoholic soluble extract of *M charantia* (different components-Leaf and fruit) on isolated breast cancer cell lines (MCF-7). In addition, the effect of cisplatin was investigated for comparison.

MATERIALS AND METHODS

Sample preparation

The leaves and fruits of *Momordica charantia* were collected locally from Guyana (Figure 1). These were shade dried and made into a coarse powder and stored in an air-tight container for biochemical analysis and testing against the growth of MCF-7 breast cancer cell lines.

Biochemical analysis of *M. charantia*

The biochemical analysis was carried out using ICAP-OES 7000 series spectrometer (inductivity coupled plasma-optical emission spectrometry) at the Chemistry lab (University of Central Lancashire, Preston, UK) from March to May 2018. 500 mg of each sample was digested with a mixture of 8 mL nitric acid and 2 mL hydrogen peroxide, and analysis was done using ICP-OES. The concentration (mg/g) of sodium, calcium, magnesium, potassium, manganese, ferrous, copper, and zinc were analyzed.

Preparation and application of *M. charantia* extract on MCF-7 breast cancer cell line

These experiments were conducted at the Biomedical Research lab (Tissue culture), University of Central Lancashire, Preston, the UK, from March to May 2018. First, 20 mg of each of the samples (leaf and fruit) of *M. charantia* was dissolved in 1 mL DMSO and 1 mL PBS by continuous stirring with a sonicator (stock solution). Next, this was followed by preparing different concentrations, namely 8, 80, and 800 µg/mL. Similarly, 5 mg of cisplatin was dissolved in 5 mL PBS and diluted to 8, 80, and 800 µL/mL concentrations. Next, the different concentration of extract in the cell medium was transferred in triplicate using a Gilson pipette to 96 well plates to give a final volume of 200 µL to the treated cell wells. Finally, the medium's equivalent volume of 200 µL was added to the control (untreated) well with cells. In this study, both time-course and dose-dependent experiments were performed. The time-course experiments were done initially for 48 hours to determine the time that produced maximal cell death. Then, dose-dependent experiments were done during the incubation period of 48 hours, either alone or combined. At the end of the treatment with either the extract or cisplatin, the viability of the cells was measured using an established fluorescent signal luminogenic ATP-assay method (Manoharan et al., 2014).

A **B**

Figure 1. *Momordica charantia.* A. Fruit and leaves, B**.** Flower

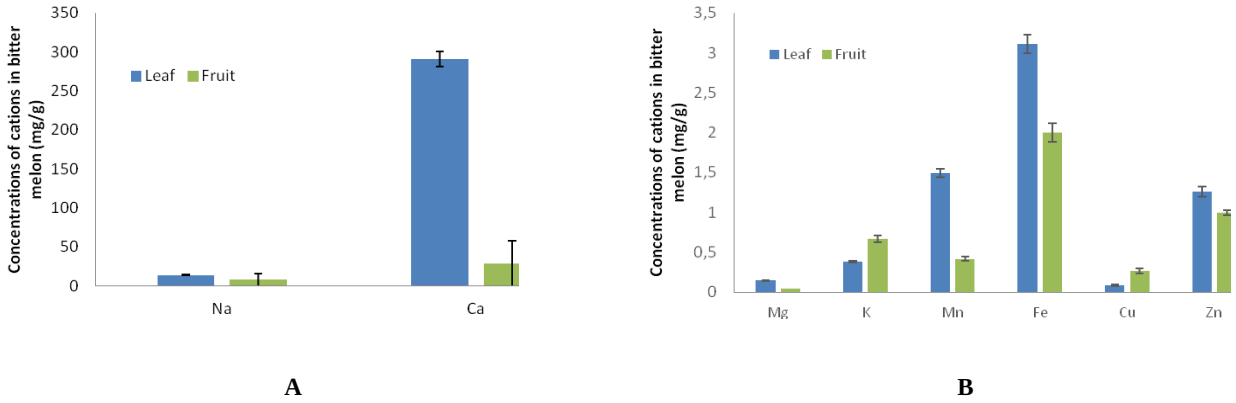

Figure 2. A. Metal analysis (sodium and calcium) of leaf and fruit samples, B. Metal analysis (magnesium, potassium, manganese, ferrous, copper, and zinc) of leaf and fruit samples

Time-course effects of the crude water-soluble extract of *M. charantia* on cell viability

The time-course effects (12, 24, 36, and 48 hours of 800 µg/mL of the extract of *M. charantia* on the viability of MCF-7 were initially done to establish the time that produced maximal cell death. From these initial time-course experiments, it was established that cell death increased to its maximal level after 24-48 hours of incubation with the alcohol-soluble extract of *M. charantia*. Therefore, the incubation time of 48 hours was employed in all dose-dependent and combined dose experiments in this study.

RESULTS AND DISCUSSION

The biochemical composition of bitter melon (*M. charantia*) suggests it is a good source of essential elements like Ca, Mg, Mn, Cu, and Zn. The results of the biochemical analysis are illustrated in Figure 2.A-B, which substantiate the vital role of bitter melon in providing essential nutrients.

The concentration of calcium (290.87±9.86, 28.96±2.2) was highest, followed by sodium (13.89±0.46, 7.76±0.52) and ferrous (3.11±0.12, 1.99±0.11) in both leaf and fruit samples respectively. The least concentration was recorded concerning copper (0.085±0.01) for leaf samples, whereas minimum magnesium concentration (0.044±0.002) was observed for fruit samples. Next, the Na, Mg, Ca, Mn, Fe, and Zn concentrations were higher in leaf than in fruit samples, whereas K and Cu were greater in fruit than in leaf samples. Single-factor ANOVA at p=0.5 suggests that the variation in concentration of metal ions in leaf and fruit samples was significant (leaf p=3.62E-23; fruit p=3.24E-17).

Figure 3.A-C shows MCF-7 cancer% cell death at different concentrations (8, 80, and 800 µg/mL) of the extract or cisplatin compared to untreated cells. The results show that low and moderate doses of the extract induce a lower death rate of the MCF-7 cells where are a high dose of 800 µg/mL) maximizes the death of MCF-7 cancer cells. Maximum cell death was recorded at all concentrations of leaf extract when compared to fruit extract or cisplatin.

At 8 mg, the death rate of MCF cells after 24 h of incubation was 0.53% with cisplatin. Further death of 8.49% was recorded after 48 h. That was less effective compared to leaf and fruit extract. With leaf extract, the death rate was 39.88% after 24 h incubation. 19.2% more cells died after 48 h. Fruit extract was less effective in controlling the growth of MCF-7 cells (2.78% after 24 h and 8.87% after 48 h).

At 80 mg, the death rate of MCF cells after 24 h of incubation was 0.69% with cisplatin. Further death of 20.4% was recorded after 48 h. That was less effective compared to leaf and fruit extract. With leaf extract, the death rate was 60.46% after 24 h incubation. 37.74% more cells died after 48 h. Fruit extract was less effective in controlling the growth of MCF-7 cells (3.73% after 24 h and 9.6% after 48 h).

At 800 mg, the death rate of MCF cells after 24 h of incubation was 7.91% with cisplatin. Further death of 29.26% was recorded after 48 h. That was less effective when compared to leaf and fruit extract. With leaf extract, the death rate was 65.9% after 24 h incubation. 55.84% more cells died after 48 h. Fruit extract was less effective in controlling the growth of MCF-7 cells (5.77% after 24 h and 14.58% after 48 h).

The highest cell death of 65.9% at 24 h and 55.84% at 48 h are observed for leaf extract at 800 mg concentration when compared to fruit extract (5.77% at 24 h and 14.58% at 48 h) and cisplatin (7.91% at 24 h and 29.26% at 48 h). Furthermore, two-way ANOVA shows statistical significance between the different treatments - cisplatin, leaf, and fruit extracts (p=0.001 at 24 h and p=0.03 at 48 h) on% cell death at different concentrations (8 to 800 mg) at 24 hours and 48 hours of the incubation period.

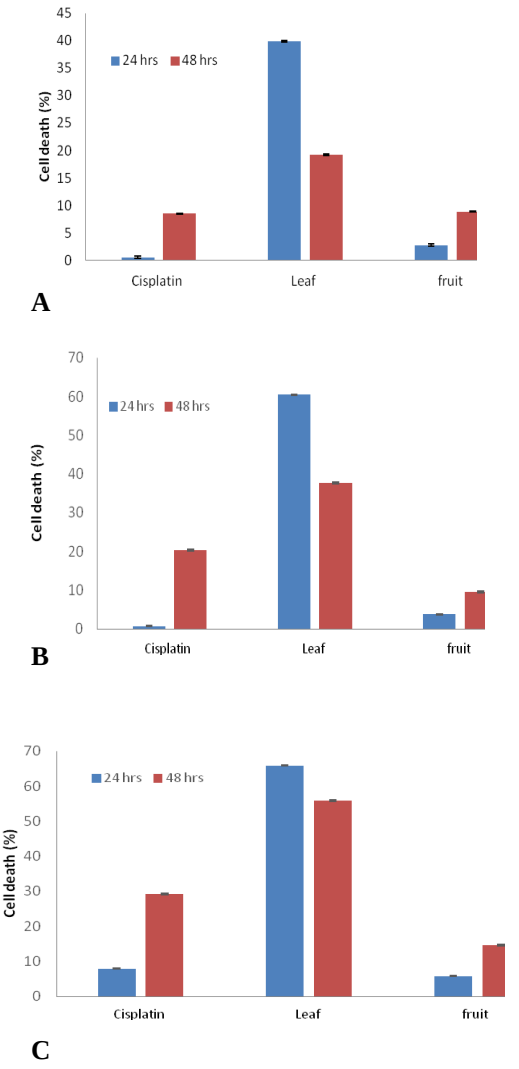

Figure 3. A. Cell death (%) at 8 mg concentration of leaf and fruit extracts, B. Cell death (%) at 80 mg concentration of leaf and fruit extracts, C. Cell death (%) at 800 mg concentration of leaf and fruit extracts

The results presented in this study have demonstrated that either the bitter melon extract or cisplatin, especially at high doses, can elicit marked and significant decreases in cell viability (cell death). Furthermore, low doses of either the extract or cisplatin seemed to cause a proliferation of MCF-7 cancer cells. In contrast, high doses, especially at 800 µg/mL, killed the MCF-7 cells compared to untreated cells (Figure 3c). While a moderate dose (80 µg/mL) of either the extract (Figure 3b) or a low dose (8 µg/mL) of cisplatin only kills about 8.49% of the MCF-7 cancer cells (Fig 3a). Reports by various researchers (Chuang et al. 2006; Nerurkar and Ray 2010; Nhiem et al. 2012; Cao et al. 2015; Bai et al. 2016) suggest that bitter melon contains cations and bio-active compounds (triterpenoids, glycosides, saponins, alkaloids, oils, protein and steroids) are probably responsible for anti-tumor effects.

In conclusion, the results indicate that cations and bio-active compounds present in leaf and fruit extracts are at desired levels and may have a potential role in effectively controlling cancer cells (MCF - cells). The different extracts of leaf and fruit components of bitter melon are more effective in controlling the growth of MCF-7 cells when compared to control-cisplatin. Furthermore, compared to fruit, the leaf was better in reducing the number of cancer cells, which supports the use of plant-based treatments for different cancers.

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Cluster analysis of *Dioscorea alata* accessions of Purwodadi Botanic Gardens (Indonesia) collection based on morphological characteristics and SSR markers

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Abstract. *Mas'udah S, Fauziah, Hapsari L. 2019. Cluster analysis of* Dioscorea alata *accessions of Purwodadi Botanic Gardens (Indonesia) collection based on morphological characteristics and SSR markers. Cell Biol Dev 3: 6-12.* The morphological characteristics of D. alata accessions show a high diversity, especially the tubers with varying form, size, weight, color, and flesh. Classification and naming of *D. alata* accessions generally use different local names in each region, causing problems. This study aimed to classify 20 accession numbers of *D. alata* collection of Purwodadi Botanic Garden collected from East Java based on tuber morphological characteristics and simple sequence repeat (SSR) molecular markers. Based on the morphological characteristic, the accessions showed high variation. However, from the seventeen characters observed, two characters were similar, and 15 others were diverse. The main morphological characteristics that contribute to the cluster are tuber skin color, tuber shape, and the color of the inner skin of the tuber. Clustering results based on DNA amplification showed different groups compared to the morphological characters cluster, although some accessions with close morphological characters were in the same molecular Group. The accession origin could not be used as the group marker. Marker E11 and A7 have the highest polymorphisms in this experiment. As conservation strategies, accessions with high-level similarity could be represented by one accession for maintenance efficiency. Conserving species until varieties level with different characters could enrich germplasm for breeding development.

Keywords: Classification, diversity, local varieties, microsatellite, phenotype, yam

INTRODUCTION

Dioscorea spp. is a tuber plant with a climbing stem; it belongs to Dioscoreaceae. The Dioscorea family comprises more than 600 species spread in the Tropical and Sub-Tropical regions. Still, less than 200 species have been identified (Ayensu and Coursey 1972), and only about 50 - 60 species have been cultivated as food and medicine (Coursey 1976; Onwueme 1996). Indonesia has many types of Dioscorea; there is *D. warbugiana*, *D. alata*, *D. keduensis*, *D. nummularia*, *D. esculenta*, *D. pentaphylla*, *D. sansibarensis*, *D. hispida*, *D. bulbifera*, etc. (Onwueme 1996; Herison et al. 2010; Trustinah 2013; Fauziah and Mas'udah 2015; Solikin 2017).

Dioscorea alata was one of the most important species, much cultivated and utilized by citizens, especially in Southeast Asia, including Indonesia (Onwueme and Ganga 1996). *D. alata* is a substitute for rice/sago as a staple food in the dry season (Sulistyono and Marpaung 2004). *D. alata* is locally known as uwi (Javanese), huwi (Sundanese), lame (Celebes), obi (Madura), and lutu (Maluku Island), which in other country known as greater yam, water yam, or ten-months yam (Onwueme dan Ganga 1996), Tuber of *D. alata* has a high content of starch and protein, vitamin C and antioxidant but low on sugar. The tuber was also a source of minerals, so *D. alata* was prospective to be functional food and supported the diver antioxidant. The tuber was the source of minerals considered functional food and supported food diversification (Hapsari 2014). Some studies of tuber processing were found, and its tuber could be used for bakery, cookies, flakes, muffins, noddle, vermicelli (Hapsari 2014); flour (Afidin et al. 2014); chips (Putri et al. 2017); *fries* (Munawaroh et al. 2018), etc.

Dioscorea alata in Indonesia showed high morphological diversity, especially in tuber shape, size, skin, and flesh color. Accessions clustering and naming of *D. alata* used different local names depending on the origin of the plant/tuber, which confused identification (Onwueme and Ganga 1996; Herison et al. 2010; Purnomo et al. 2017; Kinasih et al. 2017). Characterization and clustering of *D. alata* in Indonesia are needed as base information to make valid accession names and arrange development programs. Morphological characters and genetic markers could hold characterization and clustering. Many studies used morphological characterization to observe the diversity of plant germplasm used as base information. Furthermore, some molecular marker was developed to confirm the diversity of plant identity by morphological characters (Azrai 2005; Zulfahmi 2013)

Purwodadi Botanic Garden, Indonesian Institute of Science is an ex-situ plant conservation institute that collects *D. alata* from some region in Indonesia especially Java (Lestarini et al. 2012) some Dioscorea collected from Malang (Fauziah 2013), Nganjuk (Trimanto and Hapsari 2015) and Pasuruan (Fauziah and Mas'udah 2015). Some of

local variety was collected are Uwi Kelopo, Uwi Putih, Uwi Jaran, Uwi Sego, Uwi Perti, Uwi Bangkulit, Uwi Ireng, Uwi Alas, Uwi Klelet, Uwi Randu, Uwi Senggrani, Uwi Bangkong, Uwi Ndoro, Uwi Dusono, etc. A study of its utility and potential was needed to add *D. alata* tuber value and support the food security program. This study aimed to cluster accessions of D. alata collected in Purwodadi Botanic Garden based on morphological characteristics and molecular markers using simple sequence repeats (SSR) or microsatellites. SSR marker was a very useful genetic marker as if it is dominant, high polymorphism, and could detect variation between the population in a different region, individual variation in population and microsatellite was also cheap in an experiment (Powell et al. 1996: Siqueira et al. 2012; Tamiru et al. 2015; Vieira et al. 2016). The results of this study could be used as base information to choose a valid name for *D. alata* accessions as a conservation strategy and development program consideration.

MATERIALS AND METHODS

Area of Study

This research used 20 accessions of *Dioscorea alata* L. collection of Purwodadi Botanic Garden from Nganjuk, Malang, and Pasuruan. The research was divided into two experiments. The first experiment was morphological characters observation, and the second was a molecular analysis of the accessions. Morphological characters were observed in 2015 and 2016. Molecular analysis conducted in Biotechnology Laboratorium, Faculty of Agriculture, University of Brawijaya, Malang. Molecular analysis using fresh leaves from February-March 2015 planting and extract with Purelink® Plant Total DNA Purification Kit (Invitrogen 2012).

Methods

Morphological observation

Observation on vegetative morphological characters was held in March-April 2015 and 2016. Tuber morphological characters were observed after harvest, during which the harvesting period was from September until October 2015 and 2016. Morphological characterization used Descriptors for Yams (Dioscorea spp) by IPGRI/IITA (1997) with modification. The result of morphologic characterization showed in Table 2

Molecular analysis

DNA extraction was taken from the fresh leaf using Purelink® Plant Total DNA Purification Kit (Invitrogen 2012). Then, DNA amplification was done using nine SSR markers (Tostain 2006) from Macrogen, South Korea. Amplification processing used Techne PCR Thermocycler. The amplification cycle consisted of 25 cycles of denaturation for 30 seconds at 95 °C, and annealing depends on the primer (Table 3) for 1 minute and extension for 30 seconds at 72 °C. The final extension was performed using 72 °C for 8 minutes, and the final result of the amplification was confirmed by electrophoresis in 4% agarose gel with 1 μg/ml ethidium bromide in TBE buffer and visualized in UV transilluminator (Bio-Rad Universal Hood II Gel Doc System).

Data analysis

Morphological data were analyzed by similarity coefficient based on Euclidean coefficient, and the result was used to construct dendrograms by unweighted pair group method with arithmetic mean (UPGMA) (Sokal and Michener 1958) using PAST 3.21 program (Hammer et al. 2001). In addition, principal component analysis (PCA) of morphological characters was conducted to identify characters with the highest contribution.

Molecular data were analyzed by DNA band scoring, which showed up in agarose gel. An SSR band in agarose gel represented an allele from each accession. Each band of markers presented shows the allele of each accession, and the allele was scored as 1 when present and 0 when absent. Polymorphism percentage was counted by polymorphism allele in each primer. Cluster analysis used UPGMA methods and similarity coefficient with Euclidean by Paleontological Statistics (PAST) 3.21 (Hammer et al. 2001).

Table 1. Twenty accessions of *Dioscorea alata* observed

Accession code	Local name	Origin
DA 23	Uwi Kelopo	Pasuruan
DA 24	Uwi Putih	Pasuruan
DA 27	Uwi Ulo	Pasuruan
DA28	Uwi Perti	Pasuruan
DA29	Uwi Ulo	Pasuruan
DA30	Uwi Perti	Pasuruan
DA31	Uwi Bangkulit	Pasuruan
DA32	Uwi Kelopo	Pasuruan
DA36	Uwi Bangkulit	Pasuruan
DA43	Uwi Bangkulit	Nganjuk
DA44	Uwi Klelet	Nganjuk
DA46	Uwi Dusono	Nganjuk
DA48	Uwi Putih	Nganjuk
DA57	Uwi Ketan Putih	Malang
DA58	Uwi Biru	Malang
DA59	Uwi Lajer	Malang
DA63	Uwi Budeng	Malang
DA64	Uwi Ulo	Malang
DA66	Uwi Legi	Malang
DA67	Uwi Sego	Malang

Table 2. Scoring of morphological characters of *Dioscorea alata*

Abbreviation	Variable	Score
R	Roots on the tuber surface	3: few, 7: many
SH	Tuber shape	1: round, 2: oval, 3: oval-oblong, 4: cylindrical, 5: flattened, 6: irregular, 10: other
CR	Cracks on the tuber surface	0: absent, 1: present
NU	Number of tubers	1: one, 2: 2-5, 3: more than 5
HD	Hardness of tuber when cutting with a knife	1: easy, 2: hard
TEX	Texture of flesh	1: smooth, 2: grainy, 3: very grainy
SK	Tuber skin color	1: light brown, 2: brown, 3: yellowish, 4: greyish, 5: maroon, 6: purple
FS	Flesh color at a central transverse cross-section	1: white, 2: yellowish white, 3: yellow, 4: orange, 5: light purple, 6: purple, 7: purple with white, 8: white with purple, 9: outer purple/inner yellowish, 10: other
SKH	Skin color at the head of the tuber	1: white, 2: yellowish white, 3: yellow, 4: orange, 5: light purple, 6: purple, 7: purple with white, 8: white with purple, 9: outer purple/inner yellowish, 10: other
G	Gum is released by cutting the tuber	3: low, 5: intermediate, 7: high
DRM	Dormancy period/sprouting after dorman	1: 0-2 months, 2: 3-4 months, 3: more than 4 months
SZ	Tuber size (weight)	1: less than 1 kg, 2: 1-4 kg, 3: more than 4 kg
SKT	Tuber skin thickness	1: < 1 mm, 2: ≥ 1 mm
OXT	Flesh oxidation time after cutting	1: < 1 minute, 2: 1-2 minutes, 3: > 2 minutes
OXC	Flesh oxidation color	1: greyish, 2: purple, 3: orange, 7: other
STC	Stem color	1: green, 2: purplish green, 3: brownish green, 4: brown, 5: purple, 7: other
WGC	Wing color	1: green, 2: green with a purple edge, 3: purple, 5: other

Table 3. SSR marker used in DNA amplification

SSR code		Primer sequence	Allele size (bp)	Annealing temp. (°C)
B5[1]	F	TTCCCTGTAGGAAAAATAGTGA	233	60
	R	CGTCCCTAGAAAATTCAACCTC		
C5[1]	F	AACCAATTACCCTTTGTCATGG	441	58
	R	GCCTTGCAAGCAATTTTGA		
E11[1]	F	ATGGTGTTCTCCCATGCTTC	291	63
	R	ACCAAAAATCAGGCTTGTGC		
H12[1]	F	TTGTAATTGGGTGTTGTATTTGC	245	53
	R	CGGCCAAAACATTTTCTGAT		
H2[1]	F	AAACCAAACAGGCAAAGCAT	331	58
	R	TGCCCTGCTTGTAAGATTGA		
E10[1]	F	GAATACTGATGATGCATAAAGCAA	284	58
	R	CCATGGTGAAGAGGATGGAT		
F1[1]	F	ATGGCTCAAGAGCACACG	403	58
	R	GGGCCTCATAAACATGCAAT		
A4[1]	F	TTCGTTCTCGATAGCGGACT	348	53
	R	CCAGTTCCCAGCCTCTTGT		
A7[2]	F	GCCCCACCTTAATTTCAT	267	52
	R	GGAATGAGATGGGACGAGAA		

Noted: [1] Siqueira et al. (2011); [2] Siqueira et al. (2012)

RESULTS AND DISCUSSION

Accession clustering based on morphological characters

The result of 17 morphologic characters on 20 accessions of *D. alata* showed three main groups of *D. alata*. Group I consisted of five accessions, Uwi Bangkulit (DA31), Uwi Dusono (DA46), Uwi Klelet (DA44), Uwi Biru (DA58) and Uwi Budeng (DA63). Flesh color (FS) and skin color at the head of the tuber (SKH) showed the character of Group I. Their flesh color is purple or contains purple. Group II was divided into 2 subgroups. Subgroup II.1 consisted of Uwi Kelopo (DA32), Uwi Ulo (DA27), Uwi Perti (DA30), Uwi Bangkulit (DA36), Uwi Bangkulit (DA43) and Uwi Ulo (DA64). Subgroup II.1 has white and yellowish-white tuber flesh color. Although Uwi Bangkulit DA30 and DA43 were in this Group, these two varieties have purple inner skin color but white until yellowish white in tuber flesh color. Genetically, there are 3 colors in the

tuber flesh color of *D. alata*, white, yellow, and purple (Purnomo and Susandari 2009). Other characteristics for Subgroup II.1 were few roots in the tuber surface, and tuber size was small or under 4 kg plant^{-1}. Subgroup II.2 consisted of nine accessions, Uwi Perti (DA 28), Uwi Putih (DA48), Uwi Lajer (DA59), Uwi Legi (DA66), Uwi Sego (DA67), Uwi Ketan Putih (DA57), Uwi Putih (DA24), Uwi Ulo (DA29) and Uwi Kelopo (DA23). Subgroup II.2 has more than 1 tuber number per plant, skin thickness >1 mm, and outer skin color of tuber was brown until dark brown. The highest Euclidean Distance, with 12.5 degrees, was between Uwi Kelopo (DA23) and Uwi Bangkulit (DA31). It showed Uwi Kelopo (DA23) and Uwi Bangkulit (DA31) have different characteristic although found in the same district. The lowest Euclidean Distance, with 2.00 degrees, was between Uwi Putih (DA48) and Uwi Lajer (DA59). Uwi Putih (DA48) and Uwi Lajer (DA59) were collected from different districts but had similar characteristics.

Purnomo and Susandari (2009) showed that *D. alata* in Yogyakarta has an oval shape, cylindrical and oblong, with white, yellow, and purple flesh. Based on this experiment, the tuber shape of *D. alata* in East Java has high variation; in another way, the diversity of *D. alata* in East Java is higher. The character variations in plants may be influenced by the gene as an internal factor and supported by environmental factors as a stimulant to express the characters (Lestari and Apriyadi 2017). The local name of *D. alata* found that given by its shape. Key characteristics of *D. alata* were on the young stem, which winged and/or spines at the base and top of the stem, while in the tuber could be seen from the shape, inner skin color, root on tuber surface, skin thickness, tuber skin color, and time of tuber oxidation (IPGRI/IITA 1997). Results showed local names based on tuber shape did not represent the same characters. Clustering based on morphological characters did not represent tuber shapes as key characters of the Group. Mwirigi et al. (2009) identified four major clusters of 43 Kenyan local landraces that used morphological characters. Still, morphological and agronomic features showed inconsistent identification and could not be used as cluster key characters. Gene expression could be seen from morphologic characters, and the influencing genes could be traced through DNA bands. Molecular analysis and morphological analysis would complement each other.

Principal component analysis (PCA) showed 7 main characters with eigenvalue > 1. The main component with the highest proportion was PC4, with 78.9%. This value showed that PC4 significantly contributed to the clustering of 20 accessions tested. Characters with positive values in PC4 were found in SK or tuber skin color. Characters with the maximum contribution to genetic material diversity are characters with the highest and positive vector value (component) (Haydar et al. 2007).

Accession clustering based on molecular marker

The molecular analysis of nine markers showed five markers had polymorphism on the accession tested and the other four markers had no band after PCR. Table 5 shows the polymorphism of the SSR marker used in the experiment and polymorphic information content (PIC). PIC showed the informativeness level of the molecular marker. PIC is used as a standard to evaluate genetic markers based on DNA band after PCR amplification. PIC value is divided into three classes, PIC> 0.5 = very informative, then 0.25> PIC> 0.5 = moderate, and PIC <0.25 = low. The results of DNA amplification show that the E11 primer has the highest PIC value of 0.55, which means that the E11 primer had a fairly high informative value. In contrast, the primers C5 and A7 have a moderate informative value.

Dendrogram based on DNA band showed 2 group clusters of *D. alata* accessions, and Group I and Group II had different characters. Group II consisted of accessions with white tuber flesh and inner skin color white until yellowish.

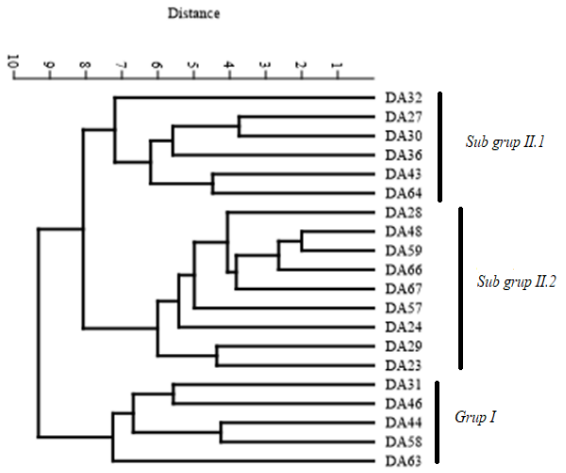

Figure 1. Clustering of *Dioscorea alata* accessions based on morphological characters

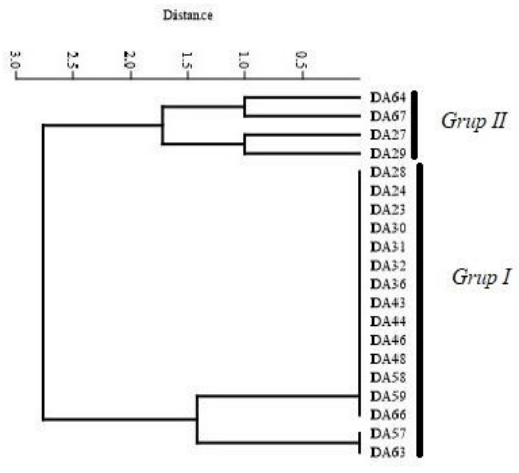

Figure 2. Clustering of *Dioscorea alata* accessions based on molecular markers

Clustering based on molecular marker analysis differed from clustering based on morphological characters. That could happen due to the gene flow between accessions as mentioned in *Manihot esculenta* (Halsey et al. 2008), *Ipomoea batatas* (Roullier et al. 2013), and *Dioscorea bulbifera* (Silva et al. 2016). The loss of genetic structure in *D. alata* is related to the behavior of farmers who often exchange planting material (in this case in the form of tubers) between farmers and even bring the planting material to other regions and causing the mixing of genotypes (Siqueira 2011; Siqueira et al. 2014). Farmers' preference to grow *D. alata* depends on the plant's usefulness to farmers. Furthermore, this affects the genetic diversity of these plants because, generally, plants that farmers and not of economic value do not sufficiently utilize will be lost from the region. The farmers selected variants/accession, maintained, and multiplied by clonal propagation. Many clonally propagated crops exhibit a mixed reproductive system in which evolutionary dynamics result from interaction. (Roullier et al., 2013) Vegetative propagation used for crops affects gene flow from experimental or commercial material. (Halsey et al. 2008). Uwi (Yam) is a dioecious plant, and crossing that occurs spontaneously contributes to some accessions, besides selection in plants that experience somatic mutations can be the main source of genetic diversity at the farm level (Obidiegwu et al. 2009).

Implications in ex-situ conservation strategies

Grouping of accessions *D. alata* based on the region cannot be determined because the character possessed by accession originating in one area also appears in accessions from other regions. For this reason, conservation strategies that can be carried out in rescuing *D. alata* diversity in Indonesia must be accomplished to varieties. (local varieties). That is supported by the results of the study of Purnomo et al. (2017), which states that the *D. alata* kinship tree based on the results of RAPD molecular analysis shows no groups formed based on regional origin.

Table 4. Principal component analysis of 17 morphological characters of *Dioscorea alata* accessions

Variable	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8
R	-0.0383	0.1517	-0.3449	-0.1334	-0.0556	0.6431	0.4531	-0.1236
SH	-0.0030	-0.3363	0.2951	-0.2935	0.5527	-0.1527	0.5346	0.1709
CR	-0.0022	0.0164	0.0192	0.0435	0.0394	0.0330	0.0450	0.0071
NU	-0.0914	0.1193	-0.0269	0.1130	0.0615	0.3107	0.1696	0.1035
HD	-0.0189	-0.0311	-0.0711	-0.1470	0.0289	0.1184	0.2059	-0.0302
TEX	0.0027	0.0121	-0.0587	-0.0133	-0.0787	0.1017	0.0920	0.3952
SK	0.3009	-0.3775	0.1297	0.7409	0.2214	0.2540	0.0504	-0.1704
FS	0.5393	0.6756	-0.1276	0.0521	0.4474	-0.1368	0.0074	-0.0246
SKH	0.7690	-0.3418	-0.0442	-0.3642	-0.3105	0.1116	-0.0564	0.0827
G	0.0900	0.0884	-0.1428	0.3325	-0.4938	-0.4533	0.5964	0.0707
DRM	0.0083	-0.0059	0.1032	0.0958	-0.0330	0.0905	0.0042	-0.2067
SZ	-0.0031	-0.0019	0.0120	-0.0711	-0.0907	0.1219	-0.0385	-0.4375
SKT	-0.0199	0.0768	0.0251	0.0327	0.0311	-0.0210	0.1401	-0.0310
OXT	-0.0363	0.0329	-0.0904	0.1552	-0.0031	0.2520	-0.1864	0.6316
OXC	0.0655	0.3428	0.8403	-0.0349	-0.2798	0.2248	0.0950	0.0432
STC	0.0388	-0.0006	-0.0018	0.0894	0.0247	-0.0272	-0.0262	0.2559
WGC	0.0385	-0.0129	0.0564	0.1271	-0.0211	0.0413	-0.0160	0.2119
Eigenvalue	14.703	582.592	344.201	256.358	191.139	147.266	110.139	0.551
Proportion	45.273	17.939	10.599	78.937	58.855	45.346	33.914	16.962

Table 5. Polymorphism of SSR Marker used in this experiment

Primer	Number of polymorphism allele	Present on accession number-	Polymorphism (%)	PIC
B5	1	DA64	5	0.05
C5	1	DA27, DA29, DA57, DA63, DA64, DA67	30	0.3
E11	3	DA27,DA29, DA57, DA63, DA64, DA67	88	0.55
H12	1	DA29	5	0.05
A7	1	DA64, DAA67	40	0.1
H2	0	-	-	-
E10	0	-	-	-
F1	0	-	-	-
A4	0	-	-	-

The diversity of plant populations is needed to prepare future conservation and breeding strategies to improve crop varieties. Accessions with close similarity could be represented by one accession so that only one accession can be chosen for germplasm collection if the facilities and infrastructure are very limited. In contrast, remote-related accessions are accessions that are well used for breeding activities (Sukartini 2007). Based on the results of this study, Uwi Putih (DA24) from Pasuruan and Uwi Putih (DA48) from Nganjuk were accession with close similarities. Therefore, one of the accessions can be selected as an ex-situ conservation priority to avoid duplication and optimize facilities. Furthermore, based on the results of the grouping analysis with SSR markers, as many as 14 accessions from Group I had a large similarity distance, but it is known that there were differences in morphology, so they are still considered for conservation as the materials for further breeding and development.

In conclusion, morphological characterization of 20 access numbers of *D. alata* showed a very varied appearance. From 17 tuber morphological characters observed, there were two uniform and 15 diverse characters. The analysis of the main components of the morphological characters showed seven characters with a diversity contribution value of 17.9 - 78.9%. The character of the outer (tuber) skin color has the largest vector value and is thought to have a large contribution to the diversity of *D. alata*. In addition to the character of the skin color (outer) of the tuber, the shape of the tuber and the color of the inner skin of the tuber are characters with a large contribution to the diversity of *D. alata*.

The amplification of 9 molecular markers showed a level of polymorphism of 5 -88%. The highest polymorphism was found in E11 (88%) and A7 (40%) primers. Grouping accessions based on molecular markers SSRs give different results from grouping patterns based on morphological characters.

Conservation strategies that could be done to maintain the existence of *D. alata* and avoid duplication to streamline the use of facilities and infrastructures, namely choosing one of the accessions that have the same local name and the same morphological character (high level of similarity) and conserving at the cultivar level for accession with characters different but has a high level of similarity based on the grouping of DNA banding patterns.

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Physical characteristics of the seeds of soybean (*Glycine max*) varieties and the effect of fermentation time on the chemical characteristics of *tempeh*

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Abstract. *Suhartanti PD, Handajani S, Nandariyah. 2019. Physical characteristics of the seeds of soybean (*Glycine max*) varieties and the effect of fermentation time on the chemical characteristics of tempeh. Cell Biol Dev 3: 13-18.* Soybean is the main raw material for making *tempeh*, and so far, the raw material for *tempeh* is imported soybeans. Therefore, Indonesia needs to develop soybean varieties to overcome this matter. The soybean varieties are expected to be processed into *tempeh* with good physical and chemical properties. This study aims to determine the effect of different varieties on the physical characteristics of soybean seeds and fermentation time on the chemical characteristics of *tempeh*. The research design was a completely randomized design (CRD) with a factorial pattern consisting of two factors, namely soybean varieties (Grobogan, Argomulyo, Seulawah, Anjasmoro, Burangrang, and Galunggung) and variations in fermentation time (30, 42, and 54 hours). The results showed that the difference in varieties did not affect the color of the *tempeh*. Different varieties affect seed weight, water absorption, and swelling power of soybeans. Soybean varieties that have the best physical characteristics (highest water absorption and swelling power) are Grobogan. Variations in fermentation time affect the chemical properties of *tempeh*. Longer fermentation time will increase the tempeh's water, ash, and total protein content but decrease the fat and carbohydrate content. The soybean variety with the best chemical characteristics (highest protein content) is Galunggung. The soybean seed coat is yellow and greenish-yellow, and the color of the *tempeh* is white. The biggest weight is the Grobogan var of 24.14 g, and the largest water absorption capacity of the Grobogan var is 188%. Therefore, the biggest swelling power of Grobogan var is 150%. Based on the time of fermentation, the highest water content of *tempeh* was found in Grobogan var (54 hours), with 67.33%. The highest ash content of *tempeh* was in Anjasmoro var (30 hours), with 1.97%. The highest fat content of *tempeh* was in Galunggung var (30 hours), with 8.89%. The highest protein content of *tempeh* was in the Galunggung var. (54 hours) with 25.17%%. The highest carbohydrate content was in the Seulawah var (42 hours), with 11.43%.

Keywords: Chemical characteristics, fermentation time, *Glycine max*, seeds, soybean, *tempeh*, varieties

INTRODUCTION

According to botanists, the soybean is a plant from Manchuria and parts of China, where there are wide varieties of wild soybeans. Then it spreads to tropical and subtropical areas. After breeding, superior varieties of soybeans are produced. Soybean harvest age varies depending on the variety but generally ranges between 75 and 105 days. Regarding food and nutrition, soybean is the cheapest protein in the world and the highest protein compared to other beans (Septiani et al. 2004; Koswara 2005).

Soybean is the main raw material for making *tempeh*. So far, *tempeh* producers have met the needs of *tempeh* raw materials on imported soybeans, even though their price is increasing daily. According to Sisworo (2008), of the domestic demand for soybeans of two million tons per year, as much as 1.4 million tons are met from imports. If the world soybean price jumps above 100% from the normal Rp 2,500.00 per kg (August-September 2007), the soybean price will become Rp 7,500.00 per kg (Early January 2008).

The Indonesian developed soybean varieties to overcome their dependence on imported soybeans. The soybean varieties are expected to be processed into *tempeh* with better physical, chemical, and antioxidant activity than imported soybeans. The local soybean varieties used in this study were Grobogan, Argomulyo, Seulawah, Anjasmoro, Burangrang, and Galunggung. Indonesia has wide local soybean varieties due to individual variability. Furthermore, this individual variability is caused by internal, genetic, external, and environmental factors, such as soil conditions and types, nutrients, climate, temperature, humidity, etc. *Tempeh* is one of the authentic Indonesian foods made from soybeans by fermentation. In the *tempeh* production process, the ingredients are boiled soybean seeds and microorganisms in the form of *tempeh* molds: *Rhizopus oligosporus, Rhizopus oryzae, and Rhizopus stolonifer* (a combination of two/three species). Also, the supportive environment consists of a temperature of 30°C, initial pH of 6.8, and 70-80% relative humidity (Sarwon 1996).

According to SNI 01-3144-1992, soybean tempeh is a food product that is fermented from soybeans by certain molds, is in a compact solid form, and has a distinctive odor of white or grayish color (BSN 2010).

Tempeh is a highly nutritious food, so it has a strategic meaning and is very important for nutritional fulfillment. In addition, *tempeh* has other advantages: antioxidant content,

simple producing technology, low price, good taste, and easy to cook.

The antioxidant content of *tempeh* can counteract free radicals, including vitamin E, carotenoids, superoxide dismutase, isoflavones, and so on. Therefore, the consumption of antioxidants in *tempeh* could mobilize antioxidant activity in the body. For example, there are three antioxidant compounds in soybeans in the form of isoflavone compounds: daidzein, genistein, and glycitein. In addition, it can prevent diseases caused by free radicals in the body, such as atherosclerosis, coronary heart disease, diabetes mellitus, cancer, etc., by serving as an antidote to free radicals (radical scavenger) (Haslina and Pratiwi 1996). Moreover, several local soybean varieties are used as raw materials for making *tempeh* as an alternative to imported soybeans with the same physical and chemical characteristics with, or even better than, imported soybeans at an affordable price.

The aims of this study were: (i) to determine the effect of different varieties on the physical characteristics of the seeds of several soybeans (Grobogan, Argomulyo, Seulawah, Anjasmoro, Burangrang, and Galunggung), including weight, seed coat color, cooking quality such as swelling power, and water absorption; (ii) to determine the effect of fermentation time (30 hours, 42 hours, and 54 hours) on the chemical characteristics of *tempeh* (contents of protein, fat, water, ash and carbohydrates) from several soybeans (Grobogan, Argomulyo, Seulawah, Anjasmoro, Burangrang, and Galunggung).

MATERIALS AND METHODS

Materials

The main ingredients in the making of *tempeh* are local soybeans, namely Grobogan, Seulawah, Burangrang, and Galunggung, and the introduced varieties Anjasmoro and Argomulyo obtained from the *Balai Penelitian Kacang-Kacangan dan Umbi-Umbian* (Malang, East Java, Indonesia), and Raprima *tempeh* yeast produced by PT. Aneka Fermentasi Industri (Bandung, West Java, Indonesia).

Research design

The experimental design of this study was a completely randomized design (CRD) with a factorials pattern consisting of 2 factors repeated 2 times. Factor 1: Soybean varieties (Grobogan, Argomulyo, Seulawah, Anjasmoro, Burangrang, and Galunggung) (K1, K2, K3, K4, K5, K6), so there were 36 replications. Factor 2: Duration of fermentation (30 hours, 42 hours, and 54 hours) (P1, P2, P3), so there were 36 replications.

Physical characteristics test of soybeans

Weight analysis was carried out by weighing 100 seeds using an analytical balance in duplicate, and the seed coat color was observed visually. The observed cooking quality included (i) swelling power (Plhak et al. 1989) and (ii) water absorption (Plhak et al. 1989). Boiling quality analysis was performed by calculating each sample's weight (a gram) and volume (b mL). Furthermore, the seeds were put in a glass filled with water 10 times the volume of seeds and soaked for 12 hours. Next, the seeds were boiled with constant heat for 20 minutes. Finally, the seeds were drained, and the weight (d gram) and volume (e mL) were calculated.

Swelling power = e-b/a *x*100%
Water absorption = d-a/a *x*100%

Tempeh making

The stages of making *tempeh* were according to Syarief's (1999) method. First, seed sorting is done traditionally by choosing good and plump soybeans. In the container, soybean seeds are mixed with dirt, such as sand or wrinkled and porous seeds (Ali 2008). The washing used clean running water. The immersion I, used clean water as much as 500 mL for 12 hours, and the boiling I, used 500 mL of clean water for 20 minutes. The immersion II used 500 mL of clean water for 12 hours. Next, stripping the epidermis was done by squeezing the soybean seeds; soybean skin was added to make tempeh. Boiling II used 500 mL of clean water for 20 minutes. Finally, draining was done by placing soybeans in a winnowing tray. Soybeans were inoculated using Raprima *tempeh* yeast, and the packaging used banana leaves. Fermentation was carried out with three kinds of treatment: 30 hours, 42 hours, and 54 hours.

Analysis method

According to Apriyantono et al. (1989), the analysis includes: (i) moisture content using the gravimetric method, (ii) ash content using a kiln, (iii) protein content using the micro-Kjeldahl method, (iv) fat content using the Soxhlet method, (v) carbohydrate content using the by difference method.

Data analysis

The research data were analyzed by SPSS, using analysis of variance (ANOVA) to determine whether there was a difference in treatment at the level of $\alpha = 0.05$. Then, DMRT (Duncan Multiple Range Test) was followed at the $\alpha = 0.05$ level.

RESULTS AND DISCUSSION

Physical characteristics

Table 1 shows that the colors of soybean seeds are yellow and greenish-yellow; moreover, Soybean seed coat color does not affect the *tempeh* color. Therefore, Rhizopus sp. needs energy and nutrients to change the yellow soybean seeds to become covered with white fungal mycelia to grow and develop.

The *tempeh* was white because fungal mycelia covered it. Good *tempeh* is characterized by a surface covered by mold mycelium (fine threads) evenly, compact and white. The soybean granules are filled with mycelium with strong and even bonds so that when the *tempeh* is sliced, the *tempeh* is not crushed.

The seed weight of 100 soybeans ranges from 7.66 g-24.14 g and varies for several soybean varieties. In general, the weight of 100 seeds was 7-10 g and was not significantly different for Argomulyo, Anjasmoro, and Burangrang varieties. The biggest weight was the Grobogan variety at 24.14 g, and the smallest was the Seulawah variety at 7.66 g. The size of the seed influences the amount of produced *tempeh*. A large seed size will produce more *tempeh*.

The highest water absorption was in Grobogan soybean with 188%, and the lowest was in Seulawah soybean with 106% (Table 1). All varieties showed significant differences. During soaking, soybeans will absorb water. The immersion temperature affects the rate of water absorption by soybean seeds. The higher the immersion temperature, the greater the rate of water absorption. On the other hand, the water absorption rate will decrease in proportion to the increase in the moisture content of soybean seeds. It seems that the absorption rate is also not significantly affected by the state of the soybean seeds.

Water absorption by legume seeds is very important concerning its utilization business. The amount of water absorption has something to do with softening of legumes. According to Kamil in Handajani and Atmaka (1993), several factors that affect the rate of water absorption are seed coat/seed membrane permeability, solution concentration, temperature, hydrostatic pressure, the surface area of seeds in contact with water, intermolecular forces, species, variety, maturity level, chemical composition, and the age of the seeds. Meanwhile, according to Bewley and Black in Handajani and Atmaka (1993), several factors that affect water absorption are seed coat anatomy, the external environment (soil, light, moisture), genetic factors, and others, including seed size.

In Table 1, the highest swelling power value was 150% of Grobogan soybeans or 1.5 times the initial volume of soybeans. Table 1 also shows that the lowest swelling power value was 82% for the Seulawah variety soybean. All varieties showed significant differences. The swelling power of legumes increases the seed volume because the absorbed air is replaced by water during water absorption. In this case, the swelling power is determined more by the swelling of the seed coat and not by the seed's flesh, so the seed's softening will occur.

The correlation between water absorption and swelling power shows that the greater the water absorption capacity, the greater the swelling power. Therefore, according to Nabessa et al. in Handajani and Atmaka (1993), seeds swell during water absorption, increasing seed volume.

Water content

Water content is one of the most important characteristics of foodstuffs because water can affect the appearance, texture, and taste of foodstuffs. The water content in foodstuffs determines the freshness and durability of these foodstuffs. High water content makes it easy for bacteria, molds, and yeasts to breed, so there will be changes in foodstuffs (Wiryadi 2007). Based on the results of the study, the water content (%) in several soybean varieties with several fermentation time treatments is shown in Table 2. The water content of *tempeh* ranges from 55.80-67.33% and varies with the treatment of fermentation time and differences in soybean varieties. Table 2 shows that the longer the fermentation time, the higher the water content of *tempeh* for several soybean varieties.

All fermentation times showed significant differences, except for the Galunggung variety at 42 and 54 hours. In the Seulawah variety, 42 hours of fermentation decreased. The decrease was caused by the water content, which was still quite large at the time of immersion, making it difficult to drain these small and hard seeds.

The highest water content was in Grobogan soybean *tempeh*, with a fermentation time of 54 hours, which was 67.33%. The lowest water content was in Burangrang soybean *tempeh*, with a fermentation time of 30 hours, 59.03% (Table 2). The water content of *tempeh* increased with increasing fermentation time. According to Sudarmadji (1977), soybeans experienced an increase in water content after 40 hours of fermentation during the *tempeh* process.

Referring to Steinkraus (1995), some water is produced from the breakdown of carbohydrates by microbes during tempeh fermentation. According to Rokhmah (2008), water is a product of aerobic fermentation, and in *tempeh* fermentation, microbes digest the substrate and produce water, carbon dioxide, and large amounts of energy (ATP). Furthermore, in fermentation, the Rhizopus mold will destroy the matrix between the bacterial cells so that the soybeans will become soft on the third day. In the next stage, the cells in soybeans are destroyed by water, which results from the breakdown of carbohydrates and causes *tempeh* to become mushy and watery (Syarief 1999).

Table 1. Physical characteristics of seeds of several soybean varieties

Varieties	Characteristics			
	Seed coat color	Weight of 100 seeds (g)	Water absorption (%)	Swelling power (%)
Grobogan	Yellow	24.14^{d}	188^{f}	150^{f}
Argomulyo	Yellow	13.44^{b}	144^{c}	110^{c}
Seulawah	Greenish-yellow	7.66^{a}	106^{b}	82^{b}
Anjasmoro	Yellow	14.02^{b}	175^{e}	132^{e}
Burangrang	Yellow	13.44^{b}	157.5^{d}	120^{d}
Galunggung	Yellow	16.01^{c}	119^{a}	100^{a}

Note: Numbers in the same column followed by the same letter show no significant difference ($\alpha < 0.05$)

Table 2. Water content (%) of *tempeh* with various fermentation time

Varieties	Water content (%)		
	Fermentation time (hour)		
	30	42	54
Grobogan	64.73^{fg}	65.65^{i}	67.33^{j}
Argomulyo	65.77^{h}	65.96^{i}	66.24^{i}
Seulawah	64.77^{fg}	62.72^{d}	64.19^{ef}
Anjasmoro	64.58^{fg}	64.92^{h}	65.94^{i}
Burangrang	59.03^{a}	62.07^{c}	63.72^{e}
Galunggung	61.16^{b}	62.12^{c}	62.15^{c}

Note: Numbers followed by the same letter show no significant difference ($\alpha < 0.05$)

Fermentation time is one of the most important factors causing the increase in water content, so with increasing fermentation time, the water content will also increase (Mulato and Widyotomo 2003; Wiryadi 2007).

Water, as one of the products of metabolism, is very influential on other components, including mold growth as microorganisms that play a role in *tempeh* fermentation (Rokhmah 2008).

Protein content

The protein content determination test was carried out using the Micro-Kjeldahl method, calculated as total N. The total protein content of *tempeh* from several soybean varieties with variations in the time of fermentation treatment can be seen in Table 3.

The protein content of *tempeh* ranges from 16.65-25.19%, varies between treatments of fermentation time and differences in soybean varieties. In all varieties and fermentation time, it tended to increase but showed no significant difference, except for Argomulyo and Galunggung varieties, 42 and 54 hours of fermentation showed significant differences.

Galunggung soybean *tempeh*, with 54 hours of fermentation time, had the highest protein content of 67.33%, but *tempeh* was in an over-fermented condition, so it was not preferred. At the same time, the lowest protein content in soybean *tempeh* Grobogan during 30-hour fermentation was 16.65% (Table 3).

Tempeh protein content increases as fermentation time increases (30, 42, and 54 hours). These results are per the opinion of Astuti et al. (2000). Due to processing soybeans into *tempeh*, the total nitrogen, cellulose, and ash content increase significantly.

Vitamin B complex formation occurs in soybean tempeh fermentation, except for thiamin, which decreases (Astuti 2000). Vitamin B_{12} is produced by the bacterium *Klebsiella pneumoniae*, a desirable microorganism that may be required in the natural *tempeh* fermentation process (Steinkraus in Steinkraus 1983). It is suspected that during fermentation, *tempeh* also undergoes the formation of vitamin $B_{12,}$ so the increase in the amount of protein is thought to come from the nitrogen in the vitamin B complex.

Many fungi are active during *tempeh* fermentation, but researchers generally assume that Rhizopus sp is the most dominant fungus. The fungus that grows on soybeans produces enzymes that can break down complex organic compounds into simpler compounds so that these compounds can be quickly used by the body (Pangastuti and Triwibowo 1996). In addition, *R. oligosporus* produces protease enzymes. The breakdown of protein complex compounds into simpler compounds is important in *tempeh* fermentation. Furthermore, it is one of the main factors determining the quality of *tempeh*, namely as a source of vegetable protein with a high digestibility value (Pangastuti and Triwibowo 1996).

Ash content

These mineral elements are also inorganic substances or ash content (Winarno 2002). According to Winarno (2002), ash is an inorganic substance from the combustion of organic material.

Tempeh ash content ranged from 0.92-1.97% and varied during fermentation. The longer the fermentation time, the ash content of *tempeh* increases. Even though the ash content of the *tempeh* samples of the Grobogan variety decreased at 42 hours of fermentation, the decrease was not significant (Table 4).

There was no significant difference in all fermentation times except for the Argomulyo variety at 42 and 54 hours. Likewise, the Anjasmoro variety at 30 and 42 hours of fermentation showed a significant difference.

The increase in ash content during *tempeh* fermentation is to Astuti et al. (2000), which stated that the total nitrogen content increased from processing soybeans into *tempeh* slightly, and the cellulose content and ash content increased significantly. This increase in ash content is probably due to the fermentation of molds producing enzymes for their metabolism. Enzymes are protein compounds containing the mineral element nitrogen (N), and the N is counted as ash.

In addition, the increase in ash content is thought to come from vitamins formed by bacteria that grow during *tempeh* fermentation, such as *K. pneumoniae* (Ferlina 2009), especially vitamin B. Astuti et al. (2000), stated that during *tempeh* fermentation, the amount of vitamin B complex increased except for thiamin. As mentioned earlier, vitamin B12 is produced by the bacterium *K. pneumoniae* in the *tempeh* fermentation process (Steinkraus in Steinkraus 1983).

Table 3. Protein content (%) of *tempeh* with various fermentation time

Varieties	Protein Content (%)		
	Fermentation time (hour)		
	30	42	54
Grobogan	16.65^{a}	17.93^{abcd}	18.61^{cde}
Argomulyo	16.8^{ab}	18.28^{bcd}	21.06^{ghi}
Seulawah	17.70^{abc}	17.75^{abc}	18.69^{cde}
Anjasmoro	19.35^{def}	19.87^{efg}	20.21^{fgh}
Burangrang	20.21^{fgh}	21.51^{hi}	22.47^{ij}
Galunggung	21.96^{i}	23.47^{j}	25.19^{k}

Note: Numbers followed by the same letter indicate no significant difference ($\alpha < 0.05$)

Table 4. Ash content (%) of *tempeh* with various fermentation time

Varieties	Ash Content (%)		
	Fermentation time (hour)		
	30	42	54
Grobogan	1.29^{bc}	1.28^{bc}	1.34^{bcd}
Argomulyo	1.45^{cd}	1.69^{de}	1.97^{f}
Seulawah	0.99^{ab}	1.06^{ab}	1.24^{abc}
Anjasmoro	0.92^{a}	1.35^{bcd}	1.52^{cde}
Burangrang	1.34^{bcd}	1.47^{cd}	1.59^{cde}
Galunggung	1.29^{bc}	1.54^{cde}	1.84^{ef}

Note: Numbers followed by the same letter indicate no significant difference ($\alpha < 0.05$)

During soybean fermentation, the increase in vitamin B_{12} levels can reach 33 times, the increase in riboflavin reaches 8-47 times, the increase in pyridoxine ranges from 4-14 times, the increase in niacin ranges from 2-5 times, the increase in biotin ranges from 2-3 times, the increase in folic acid ranges from 4-5 times and the increase in pantothenic acid reaches 2 times (Ferlina 2009). All these compounds contain the element nitrogen (N). Vitamin B_{12} also contains an atom of cobalt (Co) bonded similar to that of iron-bound in hemoglobin or magnesium in chlorophyll (Winarno 2002). Thus, the increase in ash is thought to come from nitrogen – nitrogen and cobalt (Co in vitamin B_{12}) contained in the vitamin B complex.

The lowest ash content was in Anjasmoro soybean *tempeh* at 30 hours of treatment with 0.92%%, and the highest ash content was in Argomulyo soybean *tempeh* at 54 hours of treatment with 1.97%.

Fat content

Table 5 shows the total fat content of *tempeh* samples of several local soybean varieties such as Grobogan, Seulawah, Burangrang, and Galunggung introduced varieties Anjasmoro and Argomulyo with variations in the length of fermentation treatment in this study ranging from 6.33-8.89%. Table 5 also shows that the fermentation treatment length affected the tempeh samples' fat content. The Grobogan, Argomulyo, and Galunggung varieties showed significant differences in all fermentation times. Meanwhile, the Anjasmoro variety showed a significant difference between 30 and 42 hours of fermentation. Meanwhile, the Burangrang variety showed a significant difference between 42 and 54 hours of fermentation. In other varieties and fermentation time, there was no significant difference.

In the Grobogan variety, fat content decreased at all fermentation times of 30, 42, and 54 hours, and all three showed significantly different. In the Argomulyo variety, fat content was decreased at all fermentation times of 30, 42, and 54 hours. At 30 hours and 42 hours of fermentation, there was no significant difference in fat content, while at 54 hours of fermentation, there was a significant difference. The decrease in fat content also occurred in the Seulawah variety at all fermentation times of 30, 42, and 54 hours but did not show a significant difference.

In the Anjasmoro variety, there was also a decrease in fat content at 30, 42, and 54 hours of fermentation. However, there was no significant difference between 42 hours and 54 hours of fermentation, while there was a significant difference between 30 hours of fermentation. The decrease in fat content also occurred in the Burangrang variety at 42 and 54 hours of fermentation (8.43% and 7.87%), and there was a significant difference. Finally, in the Galunggung variety, there was a decrease in fat content at 30, 42, and 54 hours of fermentation, but there was no significant difference.

The fat content of *tempeh* in several local soybean varieties of Grobogan, Seulawah, Burangrang, and Galunggung and introduced varieties of Anjasmoro and Argomulyo with variations in the length of fermentation treatment (30, 42, and 54 hours) in this study tended to experience an insignificant decrease (Table 5). It is because fat is not easily used directly by microbes compared to protein and carbohydrates (Ketaren 1986; Wiryadi 2007). A significant decrease in fat content occurred in Anjasmoro *tempeh* at 42 hours of fermentation. In Kasmidjo (1990), it is stated that the fat content of soybeans will decrease due to fermentation into *tempeh*. More than 1/3 neutral fat from soybean was hydrolyzed by lipase enzyme during 3 days of fermentation by R.oligosporus at 37°C. After 48 hours of fermentation, all fat will be hydrolyzed.

In this study, it was found that the highest fat content was found in samples of *tempeh* of the Galunggung variety with a fermentation time of 30 hours (8.89%), while the lowest fat content was found in samples of *tempeh* of the Anjasmoro variety with a fermentation time of 54 hours (6.33%).

Carbohydrate content

Table 6 shows carbohydrate levels in *tempeh* from several soybean varieties with variations in the length of fermentation treatment.

In the results of statistical analysis, it can be seen that the treatment of fermentation time and differences in soybean varieties have a significantly different effect on the carbohydrate content of *tempeh* samples. Furthermore, it can be seen from the different notations behind the carbohydrate content numbers. For example, the highest carbohydrate content was in the *tempeh* of Seulawah variety in 42 hours of treatment with 11.43%, and the lowest carbohydrate content was in the *tempeh* of Grobogan variety in 54 hours of treatment with 3.34%.

Table 5. Fat content (%) of *tempeh* with various fermentation time

Varieties	Fat content (%)		
	Fermentation time (hour)		
	30	42	54
Grobogan	8.22^{hi}	7.82^{f}	7.48^{e}
Argomulyo	8.31^{i}	8.19^{ghi}	7.39^{de}
Seulawah	7.24^{cde}	7.04^{bc}	6.87^{b}
Anjasmoro	8.40^{i}	6.52^{a}	6.33^{a}
Burangrang	8.43^{i}	8.43^{i}	7.87^{fg}
Galunggung	8.89^{j}	7.95^{fgh}	7.07^{bcd}

Note: Numbers followed by the same letter indicate no significant difference ($\alpha < 0.05$)

Table 6. Carbohydrate Content (%) of *Tempeh* with Various Fermentation Time

Varieties	Carbohydrate content (%)		
	Fermentation time (hour)		
	30	42	54
Grobogan	9.11^{e}	7.32^{o}	5.24^{e}
Argomulyo	7.63^{m}	5.89^{f}	3.34^{a}
Seulawah	9.29^{p}	11.43^{r}	9.02^{n}
Anjasmoro	6.77^{j}	7.39^{l}	6.00^{g}
Burangrang	10.99^{q}	6.52^{h}	4.36^{c}
Galunggung	6.70^{i}	4.92^{d}	3.76^{b}

Note: Numbers followed by the same letter indicate no significant difference ($\alpha < 0.05$)

According to Kim, Smit, and Nakayma in Kasmidjo (1990), during the soaking process, monosaccharides increased, but in soaking for 24 hours at 25°C with a seed: water ratio of 1: 3 and 1:10, there was no decrease in oligosaccharides. According to Mulyowidarso (1988), sucrose decreased by 84%, while stachyose, raffinose, and melibiose decreased by 64% from the content in the seeds during soaking.

The reduction of stachyose, raffinose, and melibiose compounds and the increase of monosaccharides provide microbiological and nutritional advantages in the manufacture of *tempeh*. However, *R. oligosporus* cannot metabolize these compounds; on the contrary, it can utilize monosaccharides well. In addition, glucose is a sugar compound that encourages the germination of *R. oligosporus* spores.

Stachyose, raffinose, and sucrose, the main carbohydrate sources in beans, are carbon sources for *tempeh* yeast to grow. Therefore, the treatment of soaking and boiling can cause a reduction in the main sugar content. The decrease in carbohydrate levels during the fermentation process is due to the use of monosaccharides by *tempeh* yeast to grow so that the fermentation process can run. Stachyoses will be reduced further during fermentation by *tempeh* mushrooms, remaining only 30% of the stachyose content of raw soybeans after 48 hours and only 7% remaining after 72 hours of fermentation. Meanwhile, the relative raffinose content will be the same during fermentation.

In conclusion, (i) The difference in varieties does not affect the color of the *tempeh* produced. However, different varieties affect seed weight, water absorption, and swelling power of soybeans. Heavy seed weight will produce more *tempeh*, and high-water absorption will increase the swelling power. The soybean variety that has the best physical characteristics is Grobogan. (ii) Variations in the fermentation time treatment affect the chemical properties of *tempeh*. The longer fermentation time will cause the tempeh sample's water content, ash content, and total protein content to increase while the fat and carbohydrate content to decrease. The soybean variety that has the best chemical characteristics is Galunggung.

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Improving the quality and quantity of hemp fiber (*Boehmeria nivea*) by giving indole acetic acid and gibberellic acid

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Abstract. *Rahman SF, Mudyantini W, Anggarwulan E. 2019. Improving the quality and quantity of hemp fiber (*Boehmeria nivea*) by giving indole acetic acid and gibberellic acid. Cell Biol Dev 3: 19-29.* Hemp plant (*Boehmeria nivea* (L.) Gaudich.) is an annual plant that is easy to grow and reproduce in a tropical region. Hemp fiber has a higher strength than cotton fiber, so it is not easily broken off. It provides less reduction than other fibers, the humidity of hemp fiber can achieve 12%, and hemp fiber has a smooth characteristic, long-lasting, and its glint is similar to silk. This research used complete random design (CRD) using two factors that were GA_3 with 3 concentration variations (G), such as 0 ppm, 175 ppm, 200 ppm, and 3 water availability variations (A), such as 50%, 75%, and 100%. The treatments were given to the rhizome before it was planted, and the water availability was given when the shoot started to form. The measured parameters were parameters of growth and fiber quality. This research concludes that GA_3 treatment influences the increase of shoot stem height, dry weight, fresh weight, and fiber pulling test (fiber's strength), but it does not influence the change of shoot number, leaf number, and elasticity of the fiber. The water availability treatment does not influence the entire parameter. The interaction between GA_3 and water availability influences hemp (*B. nivea*) fiber elasticity. The giving of GA_3 in the concentration of 200 ppm shows the best influence on the entire parameter of growth and fiber quality observed except in fresh and dry weight. Water availability treatment in SQ 100% strongly influences fresh and dry weight; in SQ 75%, it influences the fiber's elasticity.

Keywords: *Boehmeria nivea*, giberelat acid (GA_3), growth, pulling and elasticity test, water availability

INTRODUCTION

Indonesia is known for its fiber industry, consisting of natural, artificial, and filament yarn industries, as well as the spinning and dyeing industries. Indonesia is currently the world's seventh largest producer of artificial fiber, supplying 10% of the world's rayon fiber needs. Approximately half of the spinning industry's output is consumed domestically, with the remainder exported abroad (Miranti 2007). In the textile and textile product (IT-PT) industry, hemp fiber is currently limited to a mixture of cotton fibers. Because the need for hemp fiber as a supplement is not so great, which is around 11 tons per year and almost entirely met by imports from China, the length of hemp fiber is adjusted to the length of cotton fiber by cutting it first (Agriculture Department 2007).

Hemp (*Boehmeria nivea* (L.) Gaudich.) is an easy-to-grow annual that thrives in the tropics. Hemp fiber is a material that can be used to make high-quality fashion fabrics and cellulose (α-cellulose) manufacturing materials (Tarmansyah 2007). Because hemp fiber is stronger than cotton fiber, it is not easily broken. The disadvantages of hemp fiber include its lower elasticity and flexibility compared to cotton (*Gossypium* sp.). Clothing made from hemp fiber fabrics can absorb a lot of water and is easy to wash (Saroso and Sastrosupadi 2000; Brink and Escobin 2003). Hemp fiber, according to Hill (1972), is smooth, durable, and has a silky shine. Natural fibers derived from the hemp plant (*B. nivea*) have properties similar to cotton. They can be used as textile raw materials, one effort to reduce reliance on cotton (Buxton and Greenhalg 1989).

Plant growth and development are controlled by growth regulators, such as Gibberellic acid (GA_3) (Kastono 2005). According to Kusumo (1990), gibberellins aid cell division and RNA formation, allowing protein synthesis. Water is one of the most important aspects of plant cultivation because it serves as a solvent for plant nutrients in the soil and aids in the translocation f nutrients and photosynthates within the plant body (Gardner et al. 1991). The availability of sufficient water will aid plant growth; however, if there is too much or too little water, plant growth will be hampered, resulting in suboptimal yields (Levitt 1980).

The fiber's tensile strength indicates the amount of fiber strength that can be supported before breaking; the fiber's creep strength is defined as the length of the fiber that can creep before breaking (Lee 1999 in Indrawan 2007). Fiber quality can be improved using the right GA3 and enough water. Cellulose and lignin as cell wall constituents will increase as the number of phloem increases due to GA_3 administration. Cellulose influences fiber quality, whereas lignin increases fiber resistance.

With the GA_3 treatment and water availability in this study, it is expected that the tensile test, which is quite high, and elasticity, which is quite good, will be increased. The fiber produced from the first harvest of the hemp plant will have higher quality to increase the efficiency of the waiting time for harvest.

MATERIALS AND METHODS

Materials

The materials used in this study were rhizome hemp, planting media of a mixture of soil, sand, and manure, and GA_3: 175 ppm and 200 ppm. The material used in the tensile and elongation tests was hemp fiber that has been separated per strand.

Media preparation

The media was prepared by mixing soil, sand, and manure in a ratio of 1:1:1. The media mixture was weighed for each ½ kg polybag.

Preparation and planting of hemp rhizomes

Uniform rhizomes were chosen for this study and cut into 10 cm lengths, with each rhizome having one shoot. The rhizome pieces were then planted in the media in a polybag as deep as 5 cm, slightly tilted, and watered.

GA_3 administration treatment

GA_3 was administered once before planting. Each rhizome was sprayed with 5 mL of the hormone. After spraying, the plants were immediately stored in a dark and closed place before planting in polybags so that the hormones were not damaged by light and did not evaporate. Planting was done two days after treatment (Mudyantini 2008).

Determination of field capacity

The drained planting media mixture was weighed in a perforated polybag at the bottom and weighed 1/2 kg. The polybag was then watered until the water stopped dripping from the bottom hole, allowing the volume of water used for watering and its field capacity to be calculated. The following formula is used to calculate field capacity:

KL = (Weight of soil + polybag + water) – (Soil weight + polybag) (Patoni 2000).

Cultivation

Cultivation was carried out by watering once a day with various variations of water availability, including 50%, 75%, and 100% field capacity.

Growth observation

The number of shoots, shoot height, and leaves were calculated every 1 week, beginning on day 0 and continuing for 2 months. The fresh weight of the plants was determined by weighing all shoots that appeared on each rhizome at the end of the treatment. The dry weight of the plant was determined by drying all of the shoots that appeared on each rhizome and then weighing them.

Fiber tensile strength and creep test

Hemp fiber was separated into strands of ±10 cm in length. The media was made of cardboard (thick paper) and measured 10 cm by 2 cm. A rectangular hole with a length of 5 cm and a width of 1 cm was perforated in the center of the paper. The fibers separated by the strands are pasted in the center of the perforated paper media. The fiber ends were glued to the media with insulating tape and glue before being tested on the Tenso Lab tool, which will automatically display the tensile and elongation strength figures in statistical values (Textile Evaluation Laboratory 2008).

Data analysis

The obtained quantitative data were tested using analysis of variance (ANOVA) for the initial treatment/one treatment; GA_3 (ANCOVA) for continuous treatment; variation of water availability; and Univariate General Linear Model (GLM) for two treatments; and fiber quality analysis. The Duncans Multiple Range Test (DMRT) at the 5% test level was used to determine the true difference between treatments.

RESULTS AND DISCUSSION

Plant growth

Number of shoots

The results of the average number of shoots of *B. nivea* with GA_3 treatment are presented in Table 1. The analysis of variance (ANOVA) revealed that the GA_3 treatment did not affect the number of shoots that appeared. In Table 1, the highest number of *B. nivea* shoots were obtained in treatment G_0 (control), with an average of 4 pieces, while the lowest number of shoots were obtained in treatments G_{175} and G_{200}, with 2 and 1 fruit, respectively. This result is lower when compared to the control. This demonstrates that each plant requires the proper concentration for growth. Insufficient concentrations will inhibit rather than promote growth. According to Rahman et al. (2006), administration of GA_3 at a concentration of 250 ppm stimulated the growth of *Allium sativum* L. by 31.67%, but only 10.00% at a concentration of 500 ppm.

This minor effect was caused by the number of shoots that appeared, determined by the number of buds already present on the rhizome. The distance between the segments on the rhizome determines the number of buds in each piece of the rhizome, which is an internal factor of the hemp plant. As a result, even if the buds that have appeared have been cut before treatment, there can be a difference in the number of buds between pieces of rhizome that are the same length. Furthermore, according to Wahid (1990) in Hidayanto et al. (2003), the carbohydrate content of the cutting material, namely rhizomes, is a major factor in the development of shoot and root primordia.

Table 1. The average number of shoots of *B. nivea* with GA_3 treatment at 32 days after planting

GA_3 treatment	Number of shoots
G_0	4
G_{175}	2
G_{200}	1

Notes: G= Co. Theon of GA_3 (ppm), G_0= 0, G_{175}= 175, G_{200}= 200

Table 2. The average number of shoots of *B. nivea* with GA_3 treatment and variations in water availability 2 months after planting

GA_3 treatment	Water availability			Average
	A_1	A_2	A_3	
G_0	-	-	5.67	5.67
G_1	2.67	5.33	6.00	4.67
G_2	3.33	3.00	4.33	3.56
Average	3.00	4.17	5.33	

Notes: Concentration of GA_3 (ppm), G_0=0, G_1=175, G_2=200; Water availability (%), A_1= 50, A_2= 75, A_3= 100

According to the results of the analysis of variance (ANCOVA) in Table 2, the GA_3 treatment, as well as the water availability treatment, did not have a significant effect on the number of shoots of *B. nivea.* Treatments G_1A_3 and G_0A_3 (control) had the highest average number of shoots (6 shoots), while treatments G_1A_1, G_2A_1, and G_2A_2 had the lowest average (3 shoots). Table 3 shows the average weekly increase in the number of shoots.

The number of shoots was counted every week. The table on the increase in the number of *B. nivea* shoots shows a weekly increase. Beginning in week 7, there was a decrease in G_1A_2, G_2A_1, and G_2A_3, followed by an increase in G_2A_2 in week 8. Figure 1 compares the increase in the number of shoots of *B. nivea* with GA_3 treatment and variations in water availability.

The concentration of GA_3 used in this study wwas0 ppm, 175 ppm, and 200 ppm. Of the three treatments, the highest number of *B. nivea* shoots were produced at a concentration of 0 ppm, while the lowest number of shoots were produced at 200 ppm. This demonstrates that each plant requires the proper concentration for growth. Insufficient concentrations will inhibit rather than promote growth.

According to Wareing and Phillips (1981), administration of IAA compounds at optimal concentrations causes meristematic cell division, increasing the number of shoots. Giving GA_3 to hemp rhizomes, on the other hand, did not affect the number of shoots produced because it did not increase the number of buds on the rhizomes. GA_3 is more effective at stimulating cell elongation, while IAA is more effective at stimulating cell enlargement (Davies 1995).

Variations in water availability in this study include 50%, 75%, and 100% KL. The highest number of shoots of *B. nivea* was produced at 100% KL treatment, while the lowest number of *B. nivea* shoots was produced at 50% KL treatment (Table 2).

The increasing availability of water causes the number of plant shoots to increase; if water availability decreases, the number of shoots will decrease. Fitter and Hay (1998) stated that water affects cell growth. The lower the availability of water, the lower the turgor pressure. This causes a decrease in the growth rate.

Water stress causes changes in the type and amount of carbohydrate compounds in plants. Plants that experience water stress experience a decrease in flour and an increase in sugar content. Research by Kramer (1977) in Islami and Wani (1995) shows that an increase does not always follow a decrease in flour content in sugar content. Even in bean (*Phaseolus* sp.) and tomato (*Lycopersicon* sp.) plants, continuous water stress reduced to flour, sugar, and total carbohydrate levels in chickpeas (*Phaseolus* sp.) and tomatoes (*Lycopersicon* sp.). The effect of water stress on carbohydrate and nitrogen metabolism can inhibit the formation of auxin in plants suffering from water stress. This activity was followed by a decrease in auxin transport to the cambium resulting in modification of the cambium activity. Water stress also causes a decrease in cytokinin activity and the supply of gibberellins to stems (Islami and Wani 1995). According to Mullet and Whitsitt (1996), the main effect of lack of water is a lower stem growth rate due to the accumulation of abscisic acid (ABA).

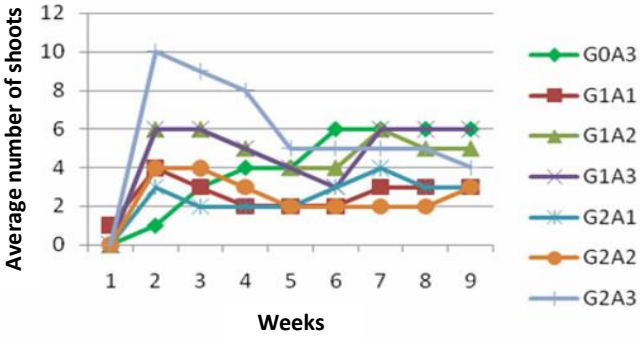

Figure 1. The average increase in the number of *B. nivea* shoots with GA_3 treatment and variations in water availability every 1 week. Notes: Concentration of GA_3 (ppm), G_0= 0, G_1= 175, G_2= 200; Water availabiity (%), A_1= 50, A_2= 75, A_3= 100

Table 3. The average number of shoots of *B. nivea* with GA_3 treatment and variations in water availability once a week (cm)

GA_3 and water treatment	The average number of shoots in the following week								
	1	2	3	4	5	6	7	8	9
G_0A_3	0	1	3	4	4	6	6	6	6
G_1A_1	1	4	3	2	2	2	3	3	3
G_1A_2	0	6	6	5	4	4	6	5	5
G_1A_3	0	6	6	5	4	3	6	6	6
G_2A_1	0	3	2	2	2	3	4	3	3
G_2A_2	0	4	4	3	2	2	2	2	3
G_2A_3	0	10	9	8	5	5	5	5	4

Notes: Concentration of GA_3 (ppm), G_0=0, G_1=175, G_2=200; Water availability (%), A_1=50, A_2=75, A_3=100

Shoot length

The results of the average shoot length of hemp plants with GA_3 treatment are presented in Table 4.

The analysis of variance (ANOVA) showed that GA_3 treatment significantly affected the shoot length of *B. nivea* plants. The average shoot length always increased with increasing GA_3 concentration. The growth of shoot length is accelerated by, among other things, the appropriate use of the GA_3 hormone. This result was per Sumiasri and Priadi's (2003) statement that the growth of sungkai branch cuttings (*Peronema canescens* Jack) at an optimum concentration of GA_3 5 mg/l increased the shoot height of the sungkai.

Based on the results of this study, the highest average shoot length was obtained in the G_{200} treatment, which was 22.92 cm, and the lowest average shoot length was in G_0 (control), which was 6.12 cm. This shows that every plant requires the appropriate concentration of GA_3 for growth. Inappropriate GA_3 concentrations will not stimulate growth but can inhibit growth. Salisbury and Ross (1995) state that active growth substances at low concentrations stimulate growth to a certain extent. According to Gul et al. (2006), administration of the hormone GA_3 300 ppm in *Araucaria heterophylla* (Salisb.) Franco affects weight. Aisyah (2004) also stated that applying GA3 to *Allium cepa* L. by immersion increases plant height and GA_3 concentrations up to 10 ppm. However, at concentrations below and above, it was even lower.

A common response in plants treated with GA_3 is stem elongation due to cambium activity in the internodes, causing the plant to grow taller than normal. Stem elongation is influenced not only by cambium activity but also by increased mitosis in the stem's subapical meristem area, which increases the number of cells in each internode. A higher cell count causes faster stem growth, resulting in a longer stem. This response in the trunk usually only results in increased length and does not result in an increase in increase formed (Wareing dan Phillips 1981).

According to the results of the analysis of variance (ANCOVA) in Table 5, the GA_3 treatment, as well as the water availability treatment, did not have a significant effect on the shoot length of *B. nivea*. The G_1A_1 treatment had the longest shoot length, with an average of 16.35 cm, while the G_1A_3 treatment had the shortest, with a shoot length of 4.89 cm. With an average shoot length of 7.48 cm, this result is lower than G_0A_3. This is related to the reduced cell elongation process caused by water stress.

Water availability variations in this study include 50 percent, 75 percent, and 100 percent KL. The *B. nivea* produced the longest shoot length at 50% KL treatment, while *B. nivea* produced the shortest shoot length at 100% KL treatment. Cell growth is a plant function that is affected by water scarcity. During the day, the meristem tissue water potential value frequently causes a decrease in turgor pressure below that required for cell development. This reduces protein synthesis, cell wall formation, and cell development, resulting in slower growth (Gardner et al. 1991). According to Dewi's (1993) research on two soybean cultivars (*Glycine max* (L.) Merry Willis and Lompo Batang), after 47 days of age under the most severe water stress, plant height decreased by nearly 50%, and stem diameter decreased by 47.7% for Willis and 42.14 percent for Lompo Batang. Anggarwulan et al. (2008) found that the 60% water availability treatment resulted in the best growth of kimpul (*Xanthosoma sagittifolium* (L.) Schott) at all shade levels. Table 6 shows the average increase in shoot length every week.

Table 4. The average shoot length of *B. nivea* with GA_3 treatment is 32 days after planting (cm)

GA_3 treatment	Shoot length (cm)
G_0	6.12^{a}
G_{175}	18.13^{ab}
G_{200}	22.92^{b}

Notes: G= Concentration of GA_3 (ppm), G_0= 0, G_{175}= 175, G_{200}= 200; Numbers followed by the same letter in the same column indicate no significant difference in the DMRT test at the 5% level

Table 5. The average shoot length of *B. nivea* with GA_3 treatment and variations in water availability 2 months after planting (cm)

GA_3 treatment	Water availability			Average
	A_1	A_2	A_3	
G_0	-	-	7.48	7.48
G_1	16.35	9.87	4.89	10.37
G_2	8.04	10.99	10.97	10.00
Average	12.20	10.43	7.78	

Notes: Concentration of GA_3 (ppm), G_0= 0, G_1= 175, G_2= 200; Water availability (%), A_1= 50, A_2= 75, A_3= 100

Table 6. The average increase in shoot length of *B. nivea* with GA_3 treatment and variations in water availability once a week (cm)

GA_3 and water treatment	The average number of shoots in the following week								
	1	2	3	4	5	6	7	8	9
G_0A_3	0	0.56	2.06	4.58	5.23	3.95	5.00	5.79	7.48
G_1A_1	0.6	2.24	6.04	11.44	15.87	15.22	15.32	15.91	16.35
G_1A_2	0	1.18	15.22	8.32	10.6	11.35	8.65	9.51	9.87
G_1A_3	0	1.22	2.82	4.39	5.97	7.92	4.11	4.44	4.89
G_2A_1	0	3.11	10.88	14.89	16.23	12.34	9.87	8.23	8.04
G_2A_2	0	1.79	6.96	7.58	16.32	17.82	15.47	13.27	10.99
G_2A_3	0	1.04	4.67	6.02	11.74	14.05	13.58	7.42	10.97

Notes: Concentration of GA_3 (ppm), G_0= 0, G_1= 175, G_2= 200; Water availability (%), A_1= 50, A_2= 75, A_3= 100

The length of the shoot is calculated once a week. The table of *B. nivea* shoot length increases shows that growth increases every week. There was a decrease beginning in the sixth week, except for which continued to increase until the ninth week. Figure 2 compares the increase in shoot height of *B. nivea* with GA_3 treatment and variations in water availability.

The concentration of GA_3 used in this study was 0 ppm, 175 ppm, and 200 ppm. *B. nivea* produced the longest shoot length at concentrations of 175 and 200 ppm, while the shortest shoot length was produced at a concentration of 0 ppm (Table 5).

Exogenous gibberellins that can be transported to the crown apex stimulate crown apical division. Gibberellins can stimulate cell division by increasing the hydrolysis of starch, fructan, and sucrose into glucose and fructose molecules. Gibberellins have a stronger influence on cell division by increasing cell wall plasticity, which leads to stem elongation, stem development, and young leaf development (Salisbury and Ross 1995). Taiz and Zeiger (1998) support this by stating that GA_3 plays a role in cell division, cell expansion, cambium activity, RNA formation, and protein synthesis, all of which cause an increase in stem height.

The increase in growth rate and plant height caused by GA_3 is explained by the physiological role of this growth substance, which supports cell wall development and stimulates cell elongation due to starch hydrolysis. It supports the formation of amylase enzymes, which can accelerate cell development (Wattimena 1998). Wuryaningsih and Sutater (1993) found that applying GA_3 25 ppm resulted in a significant difference in stem height and faster flowering. This is consistent with Sanjaya's (1991) study, which found that applying GA_3 at the optimum concentration of 25 ppm twice, at the ages of 6 and 8 weeks after planting, can increase plant height and significantly affect the length of the chrysanthemum flower stalk.

According to Weaver et al. (1982), the use of GA_3 promotes the formation of proteolytic enzymes that release tryptophan, an auxin precursor. This means that the presence of gibberellins increases the amount of auxin. Another mechanism proposes that gibberellins stimulate cell elongation by promoting the formation of -amylase through the hydrolysis of starch produced by gibberellins. As a result of this process, the sugar concentration rises, causing the osmotic pressure inside the cell to rise, causing the cell to grow (Weaver et al. 1972 in Abidin 1990).

Excessive water availability in the soil causes anoxia / reduced oxygen in the area around the roots, which can interfere with plant root absorption of nutrients from the soil (Pezeshki 1994). According to Suyana and Widijanto (2002), too much water in the soil can cause nutrient leaching, decreasing soil fertility. Water leaches nutrients from the surface of the adsorption complex and soil solution, depleting the soil.

Number of leaves

The results of the average number of leaves of hemp plants with GA_3 treatment are presented in Table 7.

The analysis of variance (ANOVA) results revealed that the GA_3 treatment did not affect leaves. Treatment G_0 (control) had the highest mean number of leaves (7 pieces), while treatments G_{175} and G_{200} had the lowest average number of leaves (5 and 3 pieces, respectively). When compared to the control, these results are lower. This demonstrates that each plant requires the proper concentration for growth. ConcentrationInsufficient concentrations rather than promote growth. The number of leaves is also affected by the occurrence of leaf shedding. Older leaves no longer active in photosynthesis will wither and fall, reducing the total number of leaves. According to Aisyah's (2004) study, soaking the tubers of *A. cepa* seeds with GA_3 does not increase the number of leaves and even tends to inhibit because all yields are under control. This is due to nutrient competition, gibberellins' interaction with other reproductive organs, and genetic or other unsuitable environmental factors. Gardner et al. (1991) stated that genetic and environmental factors influence the number and size of leaves. Genetic factors control the position of the leaves on the plant, and this leaf position influences the rate of leaf growth.

GA_3 is known to stimulate plant growth, including the growth of leaves and roots. If GA_3 is administered to transport it to the crown's tip, cell division and growth will increase, resulting in stem elongation and (in some species) the development of young leaves (Salisbury and Ross 1995). According to Anwarudin et al. (1996), GA_3 does not affect mangosteen growth.

Table 7. The average number of *B. nivea* leaves with GA_3 treatment 32 days after planting

GA_3 treatment	Number of leaves
G_0	7
G_{175}	5
G_{200}	3

Notes: G= concentration of GA_3 (ppm), G_0= 0, G_{175}= 175, G_{200}= 200

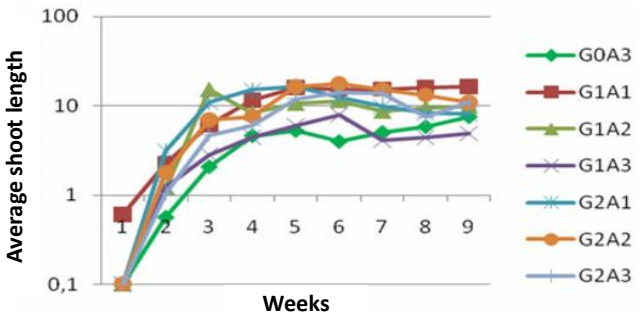

Figure 2. The average increase in shoot length of *B. nivea* with GA_3 treatment and variations in water availability once a week (cm). Notes: Concentration of GA_3 (ppm), G_0= 0, G_1= 175, G_2= 200; Water availability (%), A_1= 50, A_2= 75, A_3= 100

According to the analysis of variance (ANCOVA) in Table 8, the GA_3 treatment and variations in water availability had no significant effect on the number of *B. nivea* leaves. The most leaves were found in treatment G_0A_3 (control), which had 29 leaves, while the fewest were found in treatment G_1A_1, which had 5 leaves. Table 9 shows the average increase in the number of leaves per week.

The number of leaves was counted once a week. The table on the increase in the number of *B. nivea* leaves shows a weekly increase. There was a decrease beginning in the seventh week, except for continued to increase until the ninth week. Figure 3 compares the increase in the number of *B. nivea* leaves with GA_3 treatment and variations in water availability.

Leaves will be able to play an optimal role in photosynthesis if they have access to water, light, and sufficient nutrients. The roots will absorb water and nutrients. Auxin promotes cell division, which leads to cell enlargement and the formation of leaf primordia (Loveless 1991; Salisbury and Ross 1995). One of GA_3's properties is that it promotes the formation of proteolytic enzymes that release tryptophan as an auxin precursor, increasing. The concentrations of GA_3 used in this study were 0 ppm, 175 ppm, and 200 ppm. The highest number of *B. nivea* leaves were produced at a concentration of 0 ppm, while the lowest number of *B. nivea* leaves were produced at 200 ppm (Table 8).

The highest number of *B. nivea* leaves produced at 100% KL treatment were 29 strands. This is because, in these conditions, the plants have sufficient water availability besides increasing the number of branches will also increase the number of leaves. The availability of sufficient water will support the increase in leaf area so that it is related to the level of plant production (Sulistyaningsih et al. 1994).

The lowest number of leaves in the 50% KL treatment was 5. In this condition, there is a loss of water (transpiration) that is not matched by a sufficient water supply, inhibiting plant growth. Water stress will result from insufficient absorption rates to compensate for water loss due to transpiration (Islami and Wani 1995). According to Fitter and Hay (1998), water influences cell growth; the lower the availability of water, the lower the turgor pressure. This results in a decrease in growth rate, as the number of leaves produced is low.

Fresh weight

The results of the average fresh weight of hemp plants from this study are presented in Table 10.

According to the analysis of variance (ANCOVA) results in Table 10, the GA_3 treatment significantly affected the fresh weight of *B. nivea*, with a significance value of 0.00. The availability of water did not affect the fresh weight and did not affect the treatment. Table 10 shows that the control treatment (G_0A_3) has the highest results (24.54 g), while the other treatments have lower results when compared to the control. The G_2A_1 treatment produced the lowest yield of 5.40 g. This demonstrates that each plant requires the proper concentration for growth. Inadequate concentration will not stimulate or even inhibit growth. Figure 4 compares the fresh weight of *B. nivea* with GA_3 treatment and variations in water availability.

Table 8. The average number of *B. nivea* leaves with GA_3 treatment and variations in water availability 2 months after planting

GA_3 treatment	Water availability			Average
	A_1	A_2	A_3	
G_0	-	-	29.33	29.33
G_1	5.00	19.33	17.33	13.89
G_2	10.33	12.33	17.00	13.22
Average	7.67	15.83	21.22	

Notes: Concentration if GA_3 (ppm), G_0= 0, G_1= 175, G_2= 200; Water availability (%), A_1= 50, A_2= 75, A_3= 100

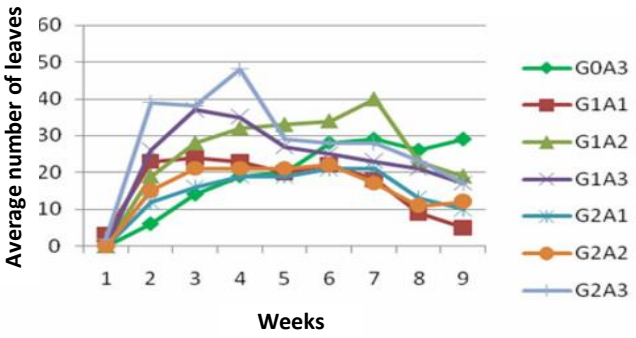

Figure 3. The average increase in the number of *B. nivea* leaves with GA_3 treatment and variations in water availability weekly. Notes: Concentration of GA_3 (ppm), G_0= 0, G_1= 175, G_2= 200; Water availability (%), A_1= 50, A_2= 75, A_3= 100

Table 9. The average increase in the number of *B. nivea* leaves with GA_3 treatment and variations in water availability every 1 week

GA_3 and water treatment	The average number of leaves in the following week								
	1	2	3	4	5	6	7	8	9
G_0A_3	0	6	14	19	20	28	29	26	29
G_1A_1	3	23	24	23	20	22	18	9	5
G_1A_2	0	19	28	32	33	34	40	23	19
G_1A_3	0	26	37	35	27	25	23	21	17
G_2A_1	0	12	16	19	19	21	21	13	10
G_2A_2	0	15	21	21	21	22	17	11	12
G_2A_3	2	39	38	48	29	28	28	23	17

Notes: Concentration of GA_3 (ppm), G_0= 0, G_1= 175, G_2= 200; Water availability (%), A_1= 50, A_2= 75, A_3= 100

Table 10 (Figure 4) depicts that the highest fresh weight of *B. nivea* was produced at a GA_3 concentration of 0 ppm, while the lowest fresh weight of *B. nivea* was produced at a GA_3 concentration of 200 ppm. For the water availability in this study, *B. nivea* produced the highest fresh weight at 100% KL treatment and the lowest fresh weight at 50% KL treatment. The water content in the tissue influences the plant's fresh weight. Because of the presence of cell enlargement, the new cell is larger than the parent cell. Increased cell size leads to increased tissue and organ size, ultimately increasing plant body and weight. A greater number of cells result from increased cell division. The increased cell count, including in leaf tissue, allows for more carbohydrate-producing photosynthesis, which can affect plant weight (Wareing and Phillip 1981; Salisbury Ross 1995).

Water is an essential component of plant growth. Growth is a process that uses appropriate substrate inputs to produce growth products. Organic matter and other elements absorbed by plants from the environment, such as carbon dioxide, nutrients, water, and sunlight, are processed into organic materials that can be measured by adding the plant's overall weight (Sitompul and Guritno 1995).

Water stress will result in inhibition of cell multiplication and enlargement. This is related to the effect of cell turgor pressure. Furthermore, a lack of water will disrupt cell metabolism, including the process of photosynthesis. Photosynthesis will be hampered if the main ingredient, water, is only available in limited quantities, resulting in lower photosynthesis results. Photosynthate production will also be hampered in its circulation to all parts of the plant, potentially reducing plant weight (Harjadi and Yahya 1988). The number of leaves will increase as the number of branches increases. The fresh weight of the plant increases as the number of leaves increases. According to Kusumo (1990), species with rapid and abundant leaf development will increase the photosynthesis rate overnight.

The results of the average dry weight of hemp plants from this study are presented in Table 11.

The analysis of variance (ANCOVA) results in Table 11 show that the GA_3 treatment significantly affected the fresh weight of *B. nivea*, with a significance value of 0.00. In the treatment, the availability of water did not have a significant effect on the fresh weight of *B. nivea*.

The dry weight reflects the accumulation of organic compounds synthesized by plants from inorganic compounds, particularly water and CO_2. Plants can effectively use the intensity of sunlight to increase the formation of carbohydrates used for growth. The absorption of nutrients and the availability of abundant water will contribute to an increase in plant dry weight. The highest results are shown in Table 11 for the control treatment (G_0A_3) of 5.46 g, while the other treatments are lower when compared to the control. The G_1A_3 treatment produced the lowest yield of 1.86 gr. This demonstrates that each plant requires the proper concentration for growth. Inadequate concentration will not stimulate or even inhibit growth.

The increase in dry weight is caused by an increase in protoplasm, which occurs as cell size and number increase. Changes in water, carbon dioxide, and inorganic salts into living materials result in protoplasm addition. This process includes photosynthesis, absorption, and metabolism, which produces carbohydrates and increases the plant's dry weight (Harjadi 1993; Lakitan 1996). Figure 5 compares *B. nivea* dry weight with GA_3 treatment and variations in water availability.

Table 10. The average fresh weight of *B. nivea* with GA_3 treatment and variation of water availability 2 months after planting (g)

GA_3 treatment	Water availability			Average
	A_1	A_2	A_3	
G_0	-	-	24.54	24.54[b]
G_1	5.84	9.56	5.48	6.96[a]
G_2	5.40	6.40	6.80	6.19[a]
Average	5.61	7.98	12.27	

Notes: Concentration of GA_3 (ppm), G_0= 0, G_1= 175, G_2= 200; Water availability (%), A_1= 50, A_2= 75, A_3= 100. *Numbers followed by the same letter in the same row and column indicate no significant difference in the DMRT test at the 5% level

Table 11. The average dry weight of *B. nivea* with GA_3 treatment and variations in water availability 2 months after planting (g)

GA_3 Treatment	Water availability			Average
	A_1	A_2	A_3	
G_0	-	-	5.46	5.46[b]
G_1	2.22	2.50	1.86	2.19[a]
G_2	1.88	2.19	2.20	2.09[a]
Average	2.05	2.34	3.18	

Notes: Concentration of GA_3 (ppm), G_0= 0, G_1= 175, G_2= 200; Water availability (%), A_1= 50, A_2= 75, A_3= 100. *Numbers followed by the same letter in the same row and column indicate no significant difference in the DMRT test at the 5% level

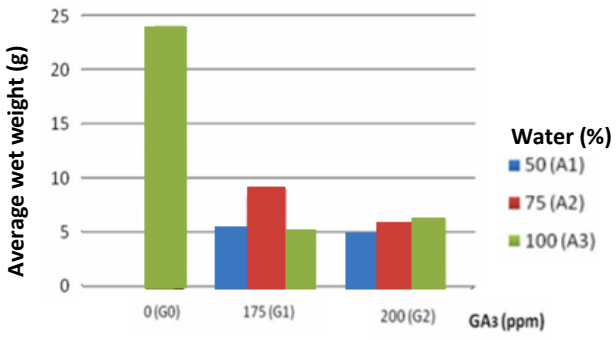

Figure 4. Fresh weight of *B. nivea* with GA_3 treatment and variations in water availability 2 months after planting. Notes: Concentration of GA_3 (ppm), G_0= 0, G_1= 175, G_2= 200; Water availability (%), A_1= 50, A_2= 75, A_3= 100

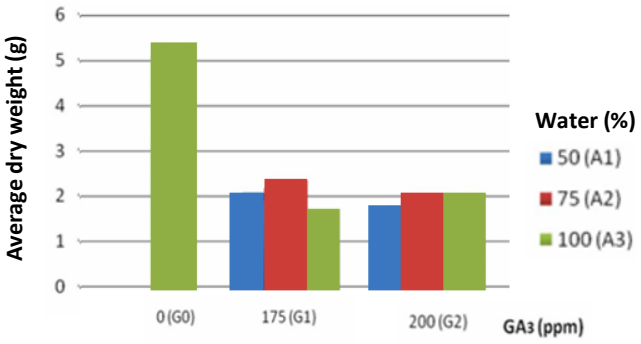

Figure 5. The dry weight of *B. nivea* with GA_3 treatment and variation of water availability 2 months after planting. Notes: Concentration of GA_3 (ppm), G_0= 0, G_1= 175, G_2= 200; Water availability (%), A_1= 50, A_2= 75, A_3= 100

Table 11 (Figure 5) shows that the highest dry weight of *B. nivea* was produced at a GA_3 concentration of 0 ppm, while the lowest dry weight of *B. nivea* was produced at a GA_3 concentration of 200 ppm. The highest dry weight of *B. nivea* was produced at 100 percent KL treatment, and the lowest dry weight was produced at 50 percent KL treatment for the water availability given in this study.

According to Delvin and Withan (1983) in Rahardjo et al. (1999) dry weight of plants can indicate how much plants respond to water stress because water is the main limiting factor for plant growth. Gardner et al. (1991) stated that severe water scarcity could cause stomatal closure, reducing carbon dioxide uptake and stunted growth and dry weight production.

According to Fitter and Hay (1998), water affects dry weight because of metabolism, specifically photosynthesis. The total dry weight of crop yields is the sum of net CO_2 assimilation yields accumulated over the growing season. Among others, utilizing the results of photosynthesis by plants is for forming body structures and food reserves. Photosynthesis fixes CO_2 for hexose production and respiration. Water stress can reduce the rate of photosynthesis, reducing the synthesis/formation of body structure and food reserves and, as a result, dry weight. Although water is a raw material in the photosynthesis process, reducing water in the leaves indirectly affects the rate of photosynthesis. The effect of soil water content will cause a reduction in photosynthesis rate because of: reduced diffusion capacity of the stomata due to stomatal closure, decreased hydration of chloroplasts and other parts of the protoplasm, thereby reducing the effectiveness of the photosynthesis mechanism, accumulation of sugars and thus inhibiting further photosynthesis (Haddy 1987). According to Fitter and Hay (1998), the closure of the stomata prevents CO_2 diffusion from the atmosphere to the leaves. As a result, photosynthesis cannot occur, and in the long run, it interferes with other physiological processes, inhibiting plant growth.

Fiber quality

Fiber tensile strength

The results of the average tensile strength of hemp fiber with GA_3 treatment and water availability are presented in Table 12.

Analysis of variance of the General Linear Model (GLM) showed that the GA_3 treatment had a significant effect on the tensile strength of the fiber, with a significance value of 0.008. The treatment of water availability, as well as the interaction between GA_3 and the provision of water availability, did not affect the fiber's tensile strength. A comparison of the tensile strength of *B. nivea* fiber with GA_3 treatment and variations in water availability are shown in Figure 6.

Figure 6 shows that the highest tensile strength of the fiber is 326 in the GA_3 treatment of 200 ppm and 50% water availability (G_2A_1), while the lowest tensile strength is 58 in the GA_3 treatment of 0 ppm and 100% water availability (G_0A_3/control). Figure 6 shows that the greater the concentration of GA_3 application, the greater the tensile strength of the fiber.

Cellulose and lignin contribute to fiber strength. The higher the cellulose and lignin content, the stronger the resulting fiber. However, cellulose, the main constituent of cell walls, contributes to the fiber's strength. One of the most important properties of cellulose is its flexibility, which allows it to withstand strain. The lignin increases the wall's resistance to stress and prevents cellulose microfibrils from folding. The orientation of the different microfibrils is an important factor in determining the wall's strength (Mudyantini et al. 2006).

According to Salisbury and Ross (1995), an increase in endogenous GA_3 can also cause an increase in the hydrolysis of starch, fructan, and sucrose into glucose and fructose molecules. Incorporating glucose units into macromolecular compounds insoluble in all commonly used solvents is known as cellulose (Fengel and Gerd 1995). Abidin (1990) claims that GA_3 can produce starch hydrolysis, which aids in the formation of α-amylase. The glucose concentration will rise as a result of this process.

A lack of water will cause disruptions in cell metabolism, including photosynthesis. Photosynthesis will be hampered if the main ingredient, water, is only available in limited quantities, resulting in lower photosynthesis results (Harjadi and Yahya 1988). According to Islami and Wani (1995), water stress causes changes in the type and amount of carbohydrate compounds in plants. Plants exposed to water stress produced less flour and more sugar.

According to Hamid (2001) and Sjostrom (1995), cellulose biosynthesis begins with glucose. Therefore, by providing GA_3 and varying water availability, the glucose content in plants increases, which can then be used for cellulose photosynthesis, increasing cellulose content in plants.

Active glucosyl (UDP-glucose) is a precursor in cellulose synthesis. In the cytoplasm, UDP-glucose is produced from two sources: sucrose by sucrose synthase (i) (reversible reaction) and glucose by sequential reactions catalyzed by hexokinase (ii), phosphoglucomutase (iii), and UDP-glucopyrophosphosylase (iv). After passing through

the plasma membrane, UDP-glucose transfers the remaining glucosyl to the glucan growth chain (cellulose), releasing UDP. This incorporation is catalyzed by active sites on cellulose synthase complex subunits stored in the plasma membrane. The glucan chains that originate from one complex are thought to be linked by hydrogen bonds to form microfibrils, whose size varies between cell types. The orientation of the microfibrils can be determined as the synthesis progresses by complex motion in the fluid lipid bilayer. Microtubules on the inner surface of the plasma membrane can direct such movements (Sjostrom 1995).

Incrustation refers to the entry of additional materials into the cellulose framework of the cell wall. In higher plants, lignification is the most important incrustation process. Still, other materials such as suberin, tannins, cutin, quinine wax, and other organic and mineral materials can also coat the cell wall (Fahn 1991).

According to Neish (1968), Sarkanen (1971), Griseboch (1977), Gross (1977), and (1978) in Fengel and Gerd (1995), lignin biosynthesis starts from glucose. As a result of the addition of GA_3 and changes in water availability, the glucose content of plants increases, which can then be used for lignin photosynthesis, increasing the lignin content of plants.

Plants produce lignin macromolecules through complex biological, biochemical, and chemical systems. Many studies with radioactive carbon confirmed that p-hydroxy cinnamyl alcohol, p-coumaryl alcohol, p-coniferyl alcohol, and sinapyl alcohol are primary parent compounds (precursors) that serve as the building blocks for all lignin compounds (Fengel and Gerd 1995).

Lignin biosynthesis begins with glucose produced by photosynthesis. It is converted to shikimic acid, a byproduct of the shikimic pathway. As the pathway's final compounds, two aromatic amino acids, L-phenylalanine and L-tyrosine, are formed via reductive animation via prephenic acid. These are the starting materials (amino acid groups) for the enzymatic metabolism of phenyl propanoid (cinnamic acid pathway), which results in the formation of three cinnamyl alcohols via activated cinnamic acid derivatives. Amino acids are deaminated to cinnamic acid by deaminase (phenylalanine ammonia-lyase and tyrosine aminolyase). P-coumaric acid, caffeic acid, ferulic acid, 5-hydroxy-ferulic acid, and synaptic acid are produced from hydroxylation (by phenolase/hydroxylase). Cinnamyl alcohol (p-coumaryl alcohol, p-coniferyl alcohol, and sinapyl alcohol) is finally formed via the coenzyme-A trioster by enzymatic activation (CoA ligase) and reduction (NADP reductase, NADP hydrogenase) (p-coumaryl-alcohol). Aldehydes (p-coumaraldehyde, coniferal dehides, and synapyldehydes) (Fengel and Gerd 1995). The primary precursor compounds and building blocks of all lignin are p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (Fengel and Gerd 1995; Robinson 1995).

According to Biemelt et al. (2004), the administration of GA_3 increased lignin biosynthesis and stimulated xylem formation in transgenic tobacco. Li et al. (2003) stated that the administration of GA_3 during flowering and tiller induction increased the lignin content of *Myrica rubra* A.Chev. According to Mudyantini (2008), the administration of GA_3 increased the lignin content of *B. nivea*.

Fiber elasticity strength

The results of the average elasticity strength of hemp fiber with GA_3 treatment and water availability are presented in Table 13.

Table 12. Average *B. nivea* fiber tensile strength with GA_3 treatment and water availability 2 months after planting (gr)

GA_3 treatment	Water availability			Average
	A_1	A_2	A_3	
G_0	-	-	58	58[a]
G_1	102	148	82	110[a]
G_2	326	178	180	228[b]
Average	214	163	106	

Notes: Concentration of GA_3 (ppm), G_0=0, G_1=175, G_2=200 Water availability (%), A_1=50, A_2=75, A_3=100. *Numbers followed by the same letter in the same row and column indicate no significant difference in the DMRT test at the 5% level

Table 13. The average tensile strength of *B. nivea* fibers with GA_3 treatment and water availability 2 months after planting (%)

GA_3 treatment	Water availability			Average
	A_1	A_2	A_3	
G_0	-	-	1.88	1.88
G_1	0.94	2.09	1.26	1.43
G_2	1.74	1.66	2.23	1.88
Average	1.34[a]	1.87[b]	1.79[ab]	

Notes: Concentration of GA_3 (ppm), G_0= 0, G_1= 175, G_2= 200; Water availability (%), A_1= 50, A_2= 75, A_3= 100. *Numbers followed by the same letter in the same row and column indicate no significant difference in the DMRT test at the 5% level

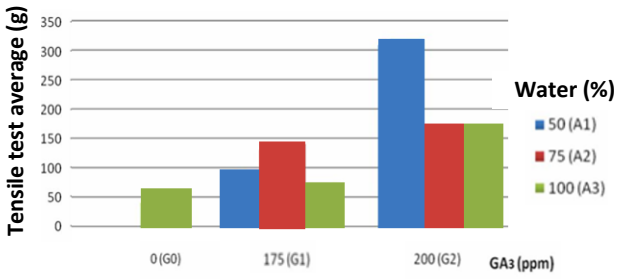

Figure 6. The *B. nivea* fiber tensile strength with GA_3 treatment and water availability treatment 2 months after planting. Notes: Concentration of GA_3 (ppm), G_0= 0, G_1= 175, G_2= 200; Water availability (%), A_1= 50, A_2= 75, A_3= 100

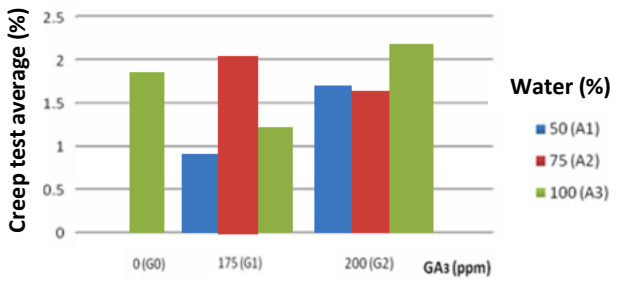

Figure 7. The elasticity strength of *B. nivea* fiber with GA_3 treatment and water availability treatment 2 months after planting. otes: Concentration of GA_3 (ppm), G_0= 0, G_1= 175, G_2= 200; Water availability (%), A_1= 50, A_2= 75, A_3= 100

According to the analysis of variance of the General Linear Model (GLM), the GA_3 treatment and water availability did not significantly affect the fiber elasticity strength. Still, the interaction between GA_3 and water availability did, with a significance value of 0.017. Figure 7 compares the elongation strength of *B. nivea* fibers with GA_3 treatment and variations in water availability.

Figure 7 shows that the highest fiber elasticity strength is 2.23 percent in the GA_3 treatment of 200 ppm and 100 percent water availability (G_2A_3). In comparison, the lowest elasticity strength is 0.94 percent in the GA_3 treatment of 175 ppm and 50 percent water availability (G_1A_1). This demonstrates that each plant requires the proper concentration for growth.

One of the most important properties of cellulose is its flexibility, which allows it to withstand strain. The lignin increases the wall's resistance to stress and prevents cellulose microfibrils from folding. The orientation of microfibrils is an important factor in determining wall strength. The tensile strength of cellulose is its most notable mechanical property, whereas the cellulose fibrils bend under compressive stress. Cell walls' physical properties include strain, strength, resistance to pressure, swelling, and permeability, which are determined by differences in the composition and structure of the lamellae, which continue to increase during the wall formation process. Structure differences can result from differences in the direction and density of cellulose microfibrils, differences in lignin content, and other factors (Fahn 1991).

Giving GA_3 and varying water availability can increase the glucose content in plants, which increases the cellulose content. According to Abidin (1990), GA_3 can produce starch hydrolysis, aiding in the formation of α-amylase. The glucose concentration will rise as a result of this process. According to Mudyantini (2008), GA_3 treatment can increase the cellulose content of plants by increasing the glucose content. The best GA_3 treatment for increasing cellulose in *B. nivea* was at a concentration of 200 ppm and cellulose content of 26.33% b/b.

Lack of water can slow photosynthesis because the turgidity of stomata guard cells decreases (Haryati 2003). According to Harjadi and Yahya (1988), photosynthesis will be hampered if water, the main ingredient, is only available in small quantities, resulting in lower photosynthesis results. According to Islami and Wani (1995), water stress causes changes in the type and amount of carbohydrate compounds in plants. Plants exposed to water stress produced less flour and more sugar. According to Lee (1999) in Indrawan (2007), Moisture affects the fiber's tensile strength. The higher the humidity, the higher the tensile strength of the fiber, while the lower the humidity, the lower the tensile strength.

Based on the research findings, it is clear that: (i) GA_3 treatment increased *B. nivea* growth at shoot length with a concentration of 200 ppm but decreased fresh and dry weight. The presence of GA_3 did not affect the number of shoots and leaves. At the same time, water availability treatment and the interaction between GA_3 and water availability did not affect all growth parameters of *B. nivea*. (ii) At a concentration of 200 ppm, GA_3 treatment increased the tensile strength of the fiber but did not affect the fiber's elasticity. The treatment of water availability did not affect all fiber parameters, but the interaction between GA_3 and water availability had a 75% effect on the elasticity strength of *B. nivea* fiber.

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Variations in growth, proline content, and nitrate reductase activity of *Canna edulis* at different water availability

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Abstract. *Nugraheni W, Solichatun, Etikawati N. 2019. Variations in growth, proline content, and nitrate reductase activity of* Canna edulis *at different water availability. Cell Biol Dev 3: 30-39.* Information on the physiological characteristics of Indian shot (*Canna edulis* Ker Gawl.), especially regarding the effect of water availability in its cultivation, is still limited. This study aims to determine the effect of variations in water availability on growth, proline content, and nitrate reductase activity in two intraspecies variations of *C. edulis*. Information on the physiological characteristics of *C. edulis* can be used as a basis for plant breeding efforts to optimize their cultivation. The study was conducted using a completely randomized design (CRD) with one factor, particularly water availability (A1=100% FC, A2=75% FC, A3=50% FC), with 3 replications for each intraspecies variation. The treatment of variations in water availability was given by watering once a day for 3 months. The data obtained for each intraspecies variation were analyzed by analysis of variance (ANOVA). Suppose there was a significant difference between the treatments for variations in water availability. In that case, it is followed by Duncan's Multiple Range Test (DMRT) at a 5% level. In contrast, the data obtained on both intraspecies variations were analyzed by *t*-test to compare the response of the two intraspecies variations towards variations in water availability. The research results on each intraspecies variation of *C. edulis* showed that the treatment of variations in water availability affected growth, proline content, and nitrate reductase activity. In the variables of the number of leaves, leaf area, respiration rate, plant height, gross plant weight, and plant dry weight, the optimal growth is at 100% FC water availability. Proline content and nitrate reductase activity were influenced by variations in water availability, with the highest proline accumulation at 50% FC water availability, while the highest nitrate reductase activity was at 100% FC water availability. Both intraspecies variations of *C. edulis* plants have the same growth response, proline content, and nitrate reductase activity towards variations in water availability.

Keywords: *Canna edulis*, growth, nitrate reductase activity, proline content, water availability

INTRODUCTION

Indian shot (*Canna edulis* Ker Gawl.) is a tuber-producing plant that has the potential as a substitute for wheat flour. In addition, it can also be used as an alternative food source and a source of bioethanol raw materials (Plantus 2007; Grehenson 2009). Tubers of *C. edulis* Ker. contains 80% carbohydrates and 18% water content and has a brownish White color with a smooth texture. High carbohydrate content indicates that *C. edulis* tubers can be used as raw materials for glucose production and ethanol fermentation (Putri and Sukandar 2008).

The *C. edulis* is one of the non-rice food ingredients with high nutritional value, especially the content of calcium, phosphorus, and carbohydrates. The nutritional content of *C. edulis* per 100 g completely consists of calories= 95.00 cal; protein= 1.00 g; fat= 0.11 g; carbohydrates= 22.60 g; calcium= 21.00 g; phosphorus= 70.00 g; iron= 1.90 mg; vitamin B1= 0.10 mg; vitamin C= 10.00 mg; water= 75.00 g; edible part= 65.00% (Directorate of Nutrition, Ministry of Health of RI 2007).

The *C. edulis* has not been widely cultivated and has a high potential to be cultivated (Grehenson 2009). The *C. edulis* can grow easily, either cultivated or wild (Putri and Sukandar 2008), and has tolerance to shade (Grehenson 2009). Efforts for plant cultivation include plant breeding efforts. One of the basics in plant breeding efforts is to know the physiological characteristics of *C. edulis*. Given the important value of *C. edulis*, which has potential as a substitute for wheat flour, alternative food sources, and bioethanol raw materials, a scientific study of this plant needs to be carried out so that *C. edulis* can be cultivated optimally.

The *C. edulis* cultivation can be done intensively by knowing the physiological characteristics of the plant. One of the most important aspects of plant cultivation is water because it functions as a solvent for plant nutrients in the soil and plays a role in the translocation of nutrients and photosynthesis in the plant body. The available water in the soil ranges from very low (drought) to waterlogged conditions (Gardner et al. 1991).

Water requirements for plants are influenced by several factors, including the type of plant, its type and development, soil moisture content, and weather conditions (Fitter and Hay 1998). Lack of water in plants occurs due to insufficient water availability in the media and excessive transpiration or a combination of these two factors. In the field, even though there is enough water in the country, plants can experience stress

(lack of water). this happens if the absorption rate cannot compensate for water loss through transpiration (Sasli 2004).

Low water availability will affect all plant metabolic processes so that it can reduce its growth. The mechanism of plant adaptation to overcome low water availability is by regulating cell osmosis. In that mechanism, the synthesis and accumulation of organic compounds can reduce the osmotic potential of the cell. Therefore, the level of osmoprotectant compounds (such as proline) in plants can be used as a differentiator of the tolerance level of plants to water stress (Mathius et al. 2004).

Low water availability also interferes with nutrient uptake of nitrogen, thus reducing nitrate reductase activity (Foyer et al. 1998). Nitrate reductase activity can be used as a selection criterion for high-yielding plants in plant breeding programs because it positively correlates with plant growth and production (Delita et al. 2008). The nitrate reductase enzyme is useful for converting nitrate to nitrite, which then, after going through a series of other enzymes works. This nitrite will be converted into amino acids (Loveless 1991; Alnopri 2004; Alnopri et al. 2004; Komariah et al. 2004).

Based on the explanation, it is necessary to conduct a study as scientific evidence to determine the physiological character of *C. edulis*, especially related to the effect of water availability on growth, proline content, and nitrate reductase activity in this plant, so that it can be used as Information in optimizing its cultivation.

This study aims to determine: (i) Variations in plant growth of *C. edulis* at different water availability. (ii) The proline content of *C. edulis* at different water availability. (iii) Nitrate reductase activity of *C. edulis* at different water availability.

MATERIALS AND METHODS

Materials

The main ingredients needed are *C. edulis* tubers obtained from Boyolali, Central Java, Indonesia. A Plant Assimilation Analyzer (PAA) and a UV-VIS spectrophotometer are the main tools needed.

Experimental design

This study used a completely randomized design (CRD) with one factor, particularly the level of water availability (50%, 75%, and 100% the field capacity), with 3 replications for each intraspecies variation.

Procedure

Plant preparation and care

Preparation of growing media: (i) Soil and compost that have been dried are mixed with a ratio of soil: compost = 2:1. (ii) Three (3) kg of soil-compost mixture was taken and then put in 5 L polybags.

Determination of field capacity: (i) Each prepared media was weighed (initial weight). (ii) The media is doused with water until it is saturated, then left until the water from the media stops dripping. (iii) The weight of each medium after water administration was weighed (final weight). (iv) 100% field capacity was determined by subtracting the final weight of each medium from the initial weight of each medium. (v) Field capacities of 50% and 75% were determined based on the average field capacity of 100% obtained.

Seed preparation and treatment: (i) Tubers of *C. edulis* measuring ± 20 g with one shoot were prepared, then planted on the media provided. (ii) Treatment of water availability was given after the seedlings were one week old. Treatment of water availability was given by watering using water once a day (according to the level of water availability being tested). (iii) The addition of water given to the growing media was calculated based on the amount of evapotranspiration that occurred and was carried out by weighing.

HarvestPlants were harvested after being treated for 3 months. Harvested plants were removed from polybags and then cleaned for soil debris.

Growth analysis

The number of leaves: The number of leaves was calculated at the end of the treatment by counting the total number of leaves on each plant other than this still budding.

Leaf area: Leaf area was calculated at the end of treatment by gravimetric method. The leaf whose area was to be measured was made a replica on a piece of paper with known area and weight.

The formula calculated the leaf area:

$$LD = \frac{Wr}{Wt} \times LK$$

Notes:
Wr: Leaf replica weight (g)
Wt: Total paper weight (g)
LK: Total paper area (cm^2)
LD: Leaf area (cm^2) (Sitompul and Guritno 1995).

Respiration rate: Measurement of respiration rate was carried out by calculating the amount of CO_2 produced by plants using the Plant Assimilation Analyzer (PAA) according to the procedure of the tool (Horiba Plant Assimilation Analyzer ASSA-1610) as follows:

(i) The PAA appliance was turned on for 1 hour before use. (ii) Five polybags were put into the sample holder in the growth chamber, 1 container containing 1 polybag, and 1 container was left for measurement control. (iii) The volume of gas entering each sample was set to 2 L/min. (iv) Calibration was carried out to measure N_2 + CO_2 gas levels using the zero, span, and means buttons. (v) The zero button was used to measure the volume of gas that came out every 0.5 L/min on a

scale of 0 to measure the gas content of N_2. (vi) The span button measured the gas content of $N_2 + CO_2$. (vii) The means button read CO_2 levels directly ($CO_2 + N_2$) - $N_2 = CO_2$. (viii) The volume of gas escaping at 0.5 L/min was adjusted for each sample. Finally, (ix) CO_2 levels were measured by reading on a scale of ppm CO_2/L/minute.

Respiration rate = sample CO_2 - control CO_2
Respiration rate = CO_2/L/min

Plant height: Plant height was measured from the tip of the highest leaf of the plant to the understock at ground level (Hendriyani and Setiari 2009).

The gross weight of plants: Gross weight of plants was calculated by weighing the harvested plants that had been cleaned from the soil (Hendriyani and Setiari 2009).

Plant dry weight: Harvested crops cleaned of soil residue were put in paper bags ready to be oven (temperature 60ºC) for 4-5 days until a constant weight was reached. The constant weight achieved after the oven was the dry weight of the plant (Hendriyani and Setiari 2009).

Measurement of proline content

Proline accumulation was measured using the Ninhydrin method (Bates et al. in Umebese et al. 2009). (i) Materials in the form of fresh leaves (2nd leaf from the tip of the plant) as much as 0.5 g were ground in a mortar with 10 mL of 3% sulfosalicylic solution. (ii) The results of the leaf collision were then filtered with Whatman filter paper no.1. A total of 2 mL of the filtrate was reacted with 2 mL of ninhydrin acid and 2 mL of glacial acetic acid in a test tube at 100ºC for 1 hour. The reaction was ended by inserting the test tube into a beaker containing ice. (iii) An acid solution of ninhydrin prepared by heating 1.25 g of ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid until dissolved. (iv) The mixture was extracted with 4 mL of toluene, then shaken with a vortex for 15-20 seconds to form two separate liquid layers. The red toluene containing proline was located at the top. (v) The upper solution was aspirated using a pipette to measure the proline content with a spectrophotometer, and the absorbance was read at a wavelength of 520 nm. (vi) The proline content was determined based on reading the pure proline standard solution.

Measurement of nitrate reductase activity

(i) Fresh leaves (2nd leaf from the tip of the plant) were washed with distilled water until clean; then, the leaf bones were cleaned to obtain leaf blades. (ii) Leaf blades weighing 500 mg were cut into thin strips of about 1 mm using a sharp knife. The leaf pieces were put into a 5 mL phosphate buffer solution in a dark tube. After soaking for 24 hours, the buffer solution was replaced with a new one. (iii) Phosphate buffer was made from a mixture of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 (Manan 2008). (iv) A 0.1 mL of $NaNO_3$ was added with a micropipette, and the time was recorded as the start of incubation for 2 hours. (v) A dye reagent consisting of 0.2 mL of a 0.02% N-naphthylenediamine solution and 0.2 mL of 1% sulphanilamide in 3 N HCl was prepared. After being incubated for 2 hours, 0.1 mL of incubation liquid was taken from a dark tube and put into a test tube containing a dye reagent, then waited for a pink color to occur as a sign that nitrate was reduced to nitrite by the enzyme nitrate reductase. One test tube was not given the filtrate and was used as a blank. (vi) After the color change occurred, 2.5 mL of distilled water was added, then transferred to a cuvette to measure the absorbance in a spectrophotometer at 540 nm.

Nitrate reductase activity was expressed in micromoles nitrate/g tissue material per hour using the following formula (Indradewa et al. 2004):

$$\text{ANR} = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times 50 \times \frac{100}{\text{BB}} \times \frac{1}{\text{W}} \times \frac{1}{1000}$$

Where:
Standard absorbance: 0.0142
BB: Plant gross weight
W: Incubation time (hours)

Data analysis

The data obtained for each intraspecies variation in the form of quantitative data, including the number of leaves, leaf area, respiration rate, plant height, gross plant weight, plant dry weight, proline content, and nitrate reductase activity, were analyzed by analysis of variance (ANOVA). If there was a significant difference in water availability, the treatment was continued with DMRT (Duncan's Multiple Range Test) at a 5% level. The data obtained on both intraspecies variations were analyzed by t-test to compare the responses of the two intraspecies variations to variations in water availability (Santoso 2001).

RESULTS AND DISCUSSION

Water is necessary for plants to grow and develop (Noggle and Fritz 1983). Because water is important for plant growth and development, the availability of water in the soil must meet the needs of plants. If water availability is reduced, it will impact productivity (Gardner et al. 1991).

Plants that suffer from water stress are generally smaller than plants that grow normally. Therefore, water stress affects all aspects of plant growth. In this case, water stress affects plants' physiological and biochemical processes and causes anatomical and morphological modifications to plants (Islami and Utomo 1995).

Growth

Growth is expressed as an irreversible and restricted increase in size in living cells accompanied by metabolic processes that include the synthesis of macromolecules such as nucleic acids, proteins, lipids, and polysaccharides. Measurement of growth can be carried out in various ways, for example, by measuring plant height, leaf size (length, width, and surface area), gross weight, and dry

weight of plants or separate parts such as roots, stems, leaves and fruit, the number of cells in the plant tissues and organs, as well as the concentration of specific compounds (e.g., nucleic acids, dissolved nitrogen, etc.) in tissues and organs (Noggle and Fritz 1983).

The growth variables in this study included the number of leaves, leaf area, respiration rate, plant height, gross plant weight, and plant dry weight.

Number of leaves

Leaves are organs that are often observed in plants as growth parameters. The number of leaves will affect the results of photosynthesis which will be circulated to all parts of the plant because it is related to the light received by the leaves (Islami and Utomo 1995). Gardner et al. (1991) stated that genetic and environmental factors influence the number and size of leaves.

Leaf development is highly sensitive to environmental changes such as water availability. In addition, leaves are generally a place of carbohydrate synthesis for plants, so leaf observation is necessary as an indicator of growth and as supporting data to explain the growth process (Sitompul and Guritno 1995).

The results of the study on the number of leaves of *C. edulis* can be seen in Table 1.

The analysis of variance above shows significant differences in the number of leaves in the treatment of variations in water availability, both the Red and White varieties of *C. edulis*. Variations in water availability in this study included 100%, 75%, and 50% FC. Plants achieved the largest number of leaves with a water treatment level of 100% FC and the smaller at a water treatment level of 75% and 50% FC in both varieties of *C. edulis*.

In the 100% FC water treatment, plants can use the more available water, thus allowing photosynthesis to exceed respiration. In addition, the availability of sufficient water can maintain cell turgor so that cell enlargement can occur. Cell turgor pressure pushes the plant cell wall and causes the cell to enlarge (Salisbury and Ross 1995).

According to Fitter and Hay (1998), water affects cell growth; the lower the water availability, the lower the turgor pressure. Those results in a decrease in the growth rate; the number of leaves produced is low. Lack of water or drought causes stomata to close and inhibits CO_2 absorption, thus reducing the rate of photosynthesis.

The lower number of leaves in the water treatment of 75% and 50% FC was thought to be due to the lack of available water in the soil, which would reduce the turgor pressure of the plant so that it interfered with the plant's metabolism, including photosynthesis. Photosynthesis will be hampered if water, the main ingredient, is unavailable, so the photosynthesis results will also decrease. Those decrease in photosynthesis is caused by the closing of stomata so that CO_2 fixation is inhibited (Lawlor 2002). Photosynthate produced will also be hampered in its circulation to all parts of the plant.

Based on the results of the t-test, it can be seen that there is no significant difference in the number of leaves between the Red and White varieties of *C. edulis*. Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

The presence of many photosynthates, one of which is used to increase meristematic activity in leaf primordial formation. Hidayat (1995) stated that the increase in the number of leaves was thought to be due to increased division of primordial leaf cells and stem tip cell differentiation. Leaves as a means of photosynthesis can play an optimal role if it is supported by the availability of water, light, and sufficient nutrients.

Leaf area

The photosynthesis process can occur in other parts of the plant, but the leaf is generally seen as the main photosynthetic producing organ. Therefore, observation of leaves is highly necessary for addition to supporting data to explain the growth process that occurs in the formation of plant biomass and an indicator of growth (Sitompul and Guritno 1995).

Observation of leaves can be based on their function as light receivers and photosynthetic tools. On this basis, leaf area is used as the main parameter because the rate of photosynthesis per unit plant, in most cases, is determined largely by leaf area (Sitompul and Guritno 1995).

The results on the leaf area of *C. edulis* can be seen in Table 2. Based on the results of the analysis of variance above, it can be seen that there are significant differences in leaf area in variations in water availability, both Red and White varieties of *C. edulis*. Variations in water availability in this study included 100%, 75%, and 50% FC. Plants achieved the largest leaf area with a water treatment level of 100% FC and the smaller at a water treatment level of 75% and 50% FC in both varieties of *C. edulis*.

Table 1. The average number of leaves of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	4.6^{b}	4^{b}	2.3^{a}
White	5.6^{c}	4.3^{b}	2.6^{a}

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

Table 2. The average leaf area (cm^2) of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	217^{c}	195^{b}	175^{a}
White	238^{c}	216^{b}	191^{a}

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

The greatest effect of lack of moisture in early vegetative development is a reduction in leaf area. Even the slightest lack of water during vegetative development can reduce the leaf dilation rate and leaf area index at the next stage of development (Gardner et al. 1991).

The reduction in leaf area at the water treatment level of 75% and 50% FC is considered to be closely related to plant adaptation to reduce transpiration and prevent evaporation of too much water from the plant body, a mechanism to reduce water use and prevent further damage due to water stress. Gardner et al. (1991) stated that the reduction in total leaf area is a strongly effective mechanism of plant adaptation to environmental stresses to prevent water evaporation.

Inhibition of cell division and enlargement due to water stress also affected the small leaf area produced by plants with 75% and 50% FC water treatment. Inhibition of cell enlargement occurs due to a decrease in cell turgor pressure. The cell turgor pressure pushes the plant cell walls and causes the cells to enlarge so that the decrease in turgor pressure causes the plant parts that are formed to be smaller than normal. The growth rate of plant cells and the efficiency of physiological processes reach the highest level when the cells are at maximum turgor.

The largest leaf area achieved in plants with 100% water treatment indicates the optimal level of water availability to support metabolic processes in plants so that sufficient energy is available for cell growth. In addition, the availability of sufficient water can maintain cell turgor so that cell enlargement can occur. Cell turgor pressure pushes the plant cell wall and causes the cell to enlarge. Harwati's study (2007) results show that tobacco plants with sufficient water availability have higher leaf area values. Based on the results of the *t*-test, it can be seen that there is no significant difference in leaf area between the Red and White varieties of *C. edulis*. Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

The leaf area is the measure of crown development and is very sensitive to water stress. Water stress results in decreased leaf formation and expansion, increased leaf senescence and shedding, or both. Leaf expansion is more sensitive to water stress than stomata closure (Nugraha 2008).

Cell growth is highly sensitive to water stress. Inhibition of cell enlargement occurs due to decreased cell turgor, forming small plant parts. The effect of lack of water during the vegetative development stage is the development of smaller leaves. During vegetative development, the slightest lack of water can reduce the rate of leaf dilation and leaf area at the next stage of development (Islami and Utomo 1995).

Respiration rate

Besides the process of photosynthesis, plants also carry out the process of respiration. Respiration is a process of disassembling energy from stored chemical energy to carry out life processes such as the formation of organic substances, activities in absorption (osmosis), accumulation of salts, protoplasm flow, and cell division, and other activities. There are two types of respiration, including aerobic respiration and anaerobic respiration. Aerobic respiration is a combustion reaction of carbon organic matter with O_2, which produces CO_2 and H_2O as well as the energy needed for growth. The respiration rate can be determined by measuring the volume of CO_2 released (Dwijoseputro 1994).

The results of the study on the respiration rate of *C. edulis* can be seen in Table 3. Based on the results of the analysis of variance above, it can be seen that there are significant differences in the rate of respiration in variations in water availability, both for the Red and White varieties of *C. edulis*. Variations in water availability in this study included 100%, 75%, and 50% FC. Plants achieved the highest respiration rate with a water treatment level of 100% FC and the smaller at a water treatment level of 75% and 50% FC in both varieties of *C. edulis*.

In the 100% FC water treatment, the plants were in a condition with more water, allowing a higher respiration rate. The availability of sufficient water can maintain cell turgor so that the stomata are always open. Those are related to the diffusion of O_2 from the atmosphere.

Anggarwulan and Solichatun (2001) stated that the availability of O_2 will affect respiration, considering its role as the final electron acceptor. In a thick tissue with a low surface-to-volume ratio, oxygen diffusion from the atmosphere decreases so that the respiration rate is low. In conditions of more available water, plants can increase their respiration rate so that the growth process also increases. In the respiration process, energy and carbon skeletons are obtained from the oxidation of photoassimilate, which is required for growth.

The lower respiration rate in the water treatment of 75% and 50% FC is considered to be due to the lack of available water in the soil, which will reduce the turgor pressure of the plant, causing the stomata to close and decreasing the diffusion of O_2 from the atmosphere. Based on the results of the t-test shows there is no significant difference in the respiration rate between the Red and White varieties of *C. edulis*. Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

Table 3. Average respiration rate (ppm/L/min) of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	36.3^{b}	26^{a}	20^{a}
While	39^{b}	30^{ab}	25^{a}

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

Plant height

The increase in plant height is a change or increase in the volume (size) of the stem because of cell enlargement to one dimension (vertical), particularly in the longitudinal direction so that the plant grows taller (Salisbury and Ross 1995).

Plant height is the most frequently observed plant size indicator of growth and is a parameter used to measure environmental influences or treatments applied. That treatment is done because plant height is the most easily seen growth measure. Therefore, plant height is sensitive to environmental influences as a parameter measuring environmental influences (Sitompul and Guritno 1995).

The results of research on plant height in *C. edulis* can be seen in Table 4. The analysis of the variance above shows significant differences in plant height in variations of water availability in both Red and White varieties of *C. edulis*. Variations in water availability in this study included 100%, 75%, and 50% FC. Plants achieved the largest plant height with a water treatment level of 100% FC and the smaller at a water treatment level of 75% and 50% FC in both varieties of *C. edulis*.

In the 100% FC water treatment, the plants were in conditions of sufficient water availability, so that plant growth was optimal. The availability of sufficient water can maintain cell turgor so that cell enlargement can occur. Cell turgor pressure pushes the plant cell wall and causes cell enlargement.

Gardner et al. (1991) stated that cell growth is a process in the plant body that is sensitive to water shortages. The value of the meristem tissue water potential during the day often causes a decrease in turgor pressure below that required for cell development. Those reduce protein synthesis, cell wall, and cell development, resulting in smaller growth. Inhibition of cell enlargement due to decreased cell turgor results in smaller plant parts.

The plant height achieved with 75% and 50% FC treatments was lower than 100% FC. Those were presumably related to the inhibition of cell enlargement due to decreased cell turgor pressure. The cell turgor pressure pushes the plant cell walls and causes the cells to enlarge so that the decrease in turgor pressure causes the plant parts that are formed to be smaller than normal. Besides, turgor pressure also affects the opening and closing of stomata, thus reducing the supply of CO_2. The stomata opening usually decreases when the leaf water potential decreases. Those decrease in the opening is due to an increase in the abscisic acid content produced by the leaf mesophyll (Goldsworthy and Fisher 1992). Umebese et al. (2009) stated that treatment of low water availability in spinach and tomato plants decreased plant height.

The research of Peng and Weyers (1994) on the leaves of the *Commelina commenis* L. stated that ABA levels in stomata guard cells affected the opening and closing of stomata. Water stress also reduces the translocation of nutrients and photosynthate in the plant body. Based on the results of the t-test shows there is no significant difference in plant height between the Red and White varieties of *C. edulis*. Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

Plant gross weight

Plant biomass is the most commonly used parameter to describe and study plant growth. Those are based on plant weight which is relatively easy to measure and is an integration of all events experienced by plants, so that parameter is the most representative growth indicator if the main goal is to obtain an overview of the overall appearance of a plant or a particular organ (Sitompul and Guritno 1995).

The gross weight of the plant is obtained by harvesting and weighing it immediately before too much water has evaporated from the material. In species with rapid and abundant leaf development, the rate of photosynthesis will increase, which will then increase the overall plant (Salisbury and Ross 1995).

The study's gross weight of plants in *C. edulis* can be seen in Table 5. The analysis of the variance above shows significant differences in plants' gross weight in water availability variations, both for the Red and White varieties of *C. edulis*. Variations in water availability in this study included 100%, 75%, and 50% FC. The largest gross weight was achieved on plants with a water treatment level of 100% FC and the smaller at a water treatment level of 75% and 50% FC in both varieties of *C. edulis*.

The high value of plant gross weight in 100% FC water treatment is considered to be due to sufficient water for photosynthesis associated with turgor pressure in the tissue. The availability of water will increase photosynthesis. The photosynthesis results will be translocated throughout the plant body tissue through the phloem. The energy from photosynthesis will activate the growth of shoots so that the number of branches increases. Increasing the number of branches will increase the number of leaves. The increasing number of leaves causes the gross weight of the plant also to increase (Lakitan 1995).

Table 4. Average plant height (cm) of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	42.7[c]	33[b]	26.7[a]
White	51.7[c]	38.3[b]	28.7[a]

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

Table 5. Average plant gross weight (g) of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	95.7[c]	74.3[b]	55.3[a]
White	116.7[c]	84[b]	65[a]

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

The lower value of plant gross weight at 75% and 50% FC water treatment was considered to be due to a lack of water availability for plants, causing inhibition of plant vegetative growth due to a decrease in turgor pressure in plant tissues. Gardner et al. (1991) stated that cell growth is a process in the plant body that is sensitive to water shortages. Therefore, lack of water availability for plants causes a decrease in turgor pressure below that required for cell development. Those cause a reduction in protein synthesis, cell wall, and cell development, resulting in smaller growth so that the gross weight of the resulting plant is low. Jumin (2002) also added that the inhibition of plant vegetative growth in stressed conditions was caused by inhibition of cell division and protein synthesis used for plant growth.

Based on the results of the t-test, it can be seen that there is no significant difference in plant gross weight between the Red and White varieties of *C. edulis*. Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

The plant's gross weight shows the plant's metabolic activity and is influenced by the water content of the tissue, nutrients, and metabolic products. Therefore, the value of a plant's gross weight also shows the amount of water in plant tissues or organs other than organic matter (Salisbury and Ross 1995).

Plant dry weight

The plant dry weight results from drying the gross weight to remove the plant's water content. Drying is intended to remove all the water content of the material and stop metabolic activity (Lakitan 1995).

Gardner et al. (1991) stated that the dry weight yield of plants is a balance between CO_2 uptake (photosynthesis) and CO_2 expenditure (respiration). Photosynthesis increases plant dry weight due to CO_2 uptake, while respiration catabolism causes CO_2 release and reduces plant dry weight.

The main components of plant dry weight are polysaccharides and lignin in the cell wall, plus cytoplasmic components such as proteins, lipids, amino acids, and organic acids (Salisbury and Ross 1995). The plant's dry weight is about 25% of the gross weight. Plant carbon comes from CO_2 gas in the atmosphere, which is bound in the form of carbon through photosynthesis. These compounds are then used to form other compounds needed to form plant cell structures and support other metabolic activities or are accumulated by certain organ cells (Sitompul and Guritno 1995).

The study's results on plant dry weight in *C. edulis* are shown in Table 6. Based on the analysis of variance above, it shows there are significant differences in plant dry weight in variations in water availability, both Red and White varieties of *C. edulis*. Variations in water availability in this study included 100%, 75%, and 50% FC. Plants achieved the largest plant dry weight with a water treatment level of 100% FC and the smaller at a water treatment level of 75% and 50% FC in both varieties of *C. edulis*.

Table 6. Average plant dry weight (g) of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	13^{c}	11.3^{b}	8.6^{a}
White	16^{c}	12.7^{b}	10^{a}

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

The increase in dry weight in 100% FC water treatment occurred due to the availability of sufficient water to increase the rate of photosynthesis, which will produce photosynthate, the end product of metabolism. The total dry weight of crop yields results from the accumulation of net CO_2 assimilation yields throughout the growing season. Plant photosynthesis utilization, among others, is for forming body structure and food reserves. Photosynthesis fixes CO_2 for use in the production of hexose, and then the hexose is utilized in plant respiration.

The end product of the photosynthesis process is carbohydrates. Carbohydrates are the basic building blocks of organic matter in plant cells, such as structural, metabolic, and important food reserves. Plant cell parts such as cytoplasm, cell nucleus, and cell wall are composed of these organic materials. Those process results in the accumulation of dry weight (Salisbury and Ross 1995).

Water stress can reduce the rate of photosynthesis which will gradually reduce the formation of body structure and food reserves, thereby reducing dry weight; Those are shown in the water treatment of 75% and 50% FC. The reduced rate of photosynthesis due to water stress also occurs because the leaves formed in those conditions experience inhibition of cell enlargement, resulting in leaves that are formed having a smaller size when compared to plants that grow normally. this means that light absorption decreases so that the photosynthetic ability also decreases (Gardner et al. 1991). Research by Hamim et al. (1996) and Hanum et al. (2007) showed a decrease in plant dry weight in several soybean varieties with low water availability.

Water is one of the raw materials in the photosynthesis process, and the effect of reducing water in the leaves on the rate of photosynthesis generally occurs indirectly. The effect of water content in the soil will cause a reduction in the rate of photosynthesis; Those could be explained as follow:

Reduced diffusion capacity of the stomata due to closing of the stomata. The closing of the stomata causes the diffusion of CO_2 from the atmosphere to the leaves to stop. As a result, (i) photosynthesis cannot occur and, in the long term, will interfere with other physiological processes, so plant growth is inhibited (Fitter and Hay 1998). (ii) Decreased hydration of the chloroplasts and other parts of the protoplasm, thereby reducing the effectiveness of the photosynthesis mechanism. (iii) There is an accumulation of sugar that inhibits the process of further photosynthesis (Heddy 1987).

Based on the results of the *t*-test, it can be seen that there is no significant difference in plant dry weight between the Red and White varieties of *C. edulis.* Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

Dry weight accumulation is generally used to characterize growth because it usually has the greatest economic importance. However, the gross weight of the plant is less useful because the number fluctuates depending on the moisture state of the plant (Gardner et al. 1991).

Proline content

One of the organisms' most common responses to water-deprivation treatments is the accumulation of compatible osmolytes. Osmolites compatible are neutral organic compounds with an active osmotic ability, which protects plants during stressful conditions (Chutipaijit et al. 2009). In addition, the accumulation of compatible osmolytes can reduce the water potential in the cells (Taylor 1996; Mathius et al. 2001).

The amino acid proline is the most widely distributed osmolyte compatible. Proline synthesized during water shortage could provide organic nitrogen, which is useful in cell recovery. Proline degradation in mitochondria is directly related to the electron transport system in the respiratory system and ATP production (Elthon and Stewart 1981), besides improving the energy status of cells recovering from water shortage conditions (Lawlor 2002).

The results of research on proline content in *C. edulis* can be seen in Table 7. The analysis of the variance above shows significant differences in proline content in variations in water availability, both Red and White varieties of *C. edulis.* Variations in water availability in this study included 100%, 75%, and 50% FC. Plants achieved the highest proline content with a water treatment level of 50% FC and the smaller at a water treatment level of 75% and 100% FC in both varieties of *C. edulis*.

The high accumulation of proline in 50% FC water treatment is considered to be because proline in plants with low water availability is synthesized as a consequence of cell osmotic regulation by increasing levels of dissolved compounds in cells so that the intracellular osmotic potential is lower or at least comparable to the osmotic potential of the medium around the cells. Some research showed an increase in proline content under conditions of low water availability, including spinach and tomato plants (Umebese et al. 2009) and corn plants (Heidari and Moaveni 2009).

Mathius et al. (2001) stated a positive correlation between proline accumulation and plant adaptation to drought stress. The accumulation of compatible osmolytes can reduce the water potential in the cell, thus allowing additional water uptake from the environment and protecting the mechanism from the effects of water deprivation. According to Rodriguez et al. (1997), an osmotic adjustment in plants can help deal with water stress.

Proline accumulation is a common response of plants to water stress (Darusman et al. 1991; Hamim et al. 1996; Mathius et al. 2001; Hamim et al. 2008; Ganesh et al. 2009; Umebese et al. 2009). Proline can act as an osmolyte compatible, a membrane and enzyme protective agent, a temporary transit site for organic nitrogen, and a free radical scavenging agent (Hare et al. 1999). Widyatmoko (2005) stated that proline accumulation is a plant's effort to maintain cell turgidity.

Proline content that was not too high in plants treated with 75% and 100% FC water was because there was still sufficient water available for plants so that plants did not have to accumulate osmolyte-compatible compounds that could reduce the water potential in the cells, which could allow additional water uptake from the environment. For example, Mathius et al. (2001) showed low proline levels in oil palm (*Elaeis guineensis* Jacq.) with sufficient water availability.

The t-test result shows no significant difference in proline content between the Red and White varieties of *C. edulis.* Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

Proline in plants is synthesized due to cell osmotic regulation caused by low water availability. That condition will spur some plants to increase their respiration rate to produce ATP, which is used to activate cells under stress as well as dissolved osmotic substances that can reduce the osmotic potential of cells, thus increasing cell water uptake which will simultaneously increase turgidity and activity (Hare et al. 1999).

Nitrate reductase activity

The enzyme nitrate reductase is useful for converting nitrate to nitrite. After going through a series of other enzymes, that nitrite will be converted into amino acids and proteins involved in metabolism. The activity of the nitrate reductase enzyme in mature plant leaves is related to the yield of the plant so that the level of nitrate reductase enzyme activity can be used as a selection criterion to select the genotype of a plant with high yields (Loveless 1991; Alnopri 2004; Alnopri et al. 2004; Komariah et al. 2004). Therefore, the positive correlation of nitrate reductase in the growth phase will impact high yields (Delita et al. 2008).

Nitrate ions absorbed from the soil must be reduced back to ammonium ions before their nitrogen components can be recombined into amino acids and other organic nitrogen compounds. The reduction of nitrate to ammonium in plants occurs in 2 stages (Noogle and Fritz 1983):

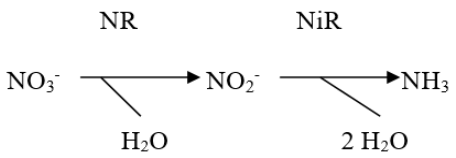

Table 7. The average proline content (mol/gram fresh leaf weight) of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	0.72[a]	0.86[a]	1.51[b]
White	0.73[a]	0.91[a]	1.93[b]

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

Table 8. Average levels of ANR (mole nitrate/gram tissue material per hour) of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	0.68[b]	0.60[a]	0.55[a]
White	0.66[b]	0.59[a]	0.54[a]

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

The results of the study on ANR levels in *C. edulis* can be seen in Table 8. Based on the results of the analysis of variance above, it can be seen that there are significant differences in ANR levels in variations in water availability, both Red and White varieties of *C. edulis.* Water treatment variations in this study included 100%, 75%, and 50% FC. Plants achieved the highest ANR levels with a water treatment level of 100% FC and the smaller at a water treatment level of 75% and 50% FC in both varieties of *C. edulis*.

The high ANR in plants with 100% FC water treatment is considered to be due to the availability of abundant water in the soil so that nitrogen transport from the soil to the plant body runs smoothly. Alnopri (2004) and Komariah et al. (2004) stated that the increased availability of nitrate would accelerate nitrogen synthesis so that the activity of nitrate reductase increases. According to Indradewa et al. (2004), plants that obtain stagnant water will increase ANR because plants absorb nitrate from the soil, so ANR in leaf shoots will increase when nitrate is available.

The low levels of ANR at water availability levels of 50% and 75% FC may be inhibited nutrient transport in the soil due to reduced transpiration, causing low nitrate reductase activity in plants. For example, Brandao and Sodek (2009) stated that reduced absorption of nitrogen nutrients from the soil caused a decrease in nitrate movement to the leaves, resulting in low nitrate reductase activity.

Chen and Sung's (1983) study on soybean nodules showed inhibition of nitrate reductase activity in nodules due to water stress. Likewise, the study by Umebese et al. (2009) showed decreased nitrate reductase activity in spinach and tomato plants under water shortage conditions. Foyer et al. (1998) stated that lack of water resulted in disruption of nutrient absorption. It reduced nitrate supply to the leaves, disrupting nitrate reductase activity. Based on the results of the *t*-test, it can be seen that there is no significant difference in ANR levels between the Red and White varieties of *C. edulis*. Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

Nitrate reductase is one of the most sensitive plant enzymes studied (Alnopri et al. 2004). Nitrate reductase has been studied intensively because its activity often affects the rate of protein synthesis in plants that absorb nitrate as the main nitrogen source. The activity of nitrate reductase is influenced by several factors, including the rate of synthesis and the rate of an overhaul by protein-destroying enzymes. In addition, inhibitors and activators also influence it in cells (Salisbury and Ross 1995).

The research carried out above shows that: (i) Variations in water availability affect the growth of *C. edulis*. In the variables of the number of leaves, leaf area, respiration rate, plant height, gross plant weight, and plant dry weight, optimal growth was achieved by giving 100% FC water treatment and decreased at 75% and 50% FC water availability. (ii) Variations in water availability affected the proline content of *C. edulis*. The highest proline accumulation was produced by plants with 50% FC water treatment and decreased at 75% and 100% FC water availability. (iii) Variations in water availability affected the nitrate reductase activity of *C. edulis*. The highest ANR accumulation was produced by plants with 100% FC water treatment and decreased at 75% and 50% FC water availability.

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