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Changes in the Chlorophyll of Garlic Chives (*Allium tuberosum*) Resulting from Fertilizer and Drought Stress

Man Kyu Huh[1]* and Byeongryong Lee[2]

[1]*Food Science & Technology Major, Dong-eui University, Busan 47340, Korea*
[2]*Department of Biology Education, Seowon University, Cheongju City 28674, Korea*

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The garlic chive (*Allium tuberosum* Rotter) is a prominent herb species in Asia and other nations of the world. Garlic chives is a favorite vegetable and used to garnish noodles in Korea. The effects of various doses of N, P, and K fertilizers and drought stress on the chlorophyll content in the leaves of garlic chives were investigated. The evaluations showed that chlorophyll a content was 0.386 at 10 mg/l N fertilizer and 0.584 at 50 mg/l N fertilizer. The treatment group showed a significant difference with regard to the contents of chlorophylls a and b and total chlorophyll at the 5% level ($p<0.05$). Pearson's correlation coefficient (Pearson's r) for chlorophylls a and b and total chlorophyll were 0.940, 0.966, and 0.971, respectively. The highest content of chlorophylls a and b and total chlorophyll in the leaves was recorded at 40 mg/l P fertilizer, while the values corresponding to 50 mg/l P fertilizer were lower than those for 40 mg/l P fertilizer. The content of total chlorophyll evaluated at 10 mg/l K fertilizer was 0.312 and that at 50 mg/l was 0.589. The simple linear regression showed the relationship between chlorophyll efficiency aand moisture. The slope factors of the dark-level fluorescence yield (Fo), the maximum fluorescence yield (Fm), the quenched state (Fv), and the maximal PSII quantum yield (Fv/Fm) for chlorophyll-efficient indicators were -0.931, 0.972, 972, and 0.950, respectively. NPK fertilizers and drought stress affected the chlorophyll content and efficiency of *A. tuberosum*.

Key words : *Allium tuberosum,* chlorophyll content, chlorophyll efficiency, drought stress, garlic chives

Introduction

Allium tuberosum Rotter is originated in the Siberian-Mongolian-North Chinese steppes [13]. or the Chinese province of Shanxi [22] and cultivated and naturalized elsewhere in Asia and around the world. *A. tuberosum*, garlic chives (or Chinese leek) are perennial plants that use leaves for food, and are characterized by seed breeding and root breeding. There is a short root stem in the ground and many scales are made to form a cape. The leaves are attached to each scaly stem and are 15-20 cm long and 3-10 mm wide.

In Korea, garlic chives can be harvested throughout the year. However, frequent harvesting exhausts the nutrients of the soil, so fertilizer must be supplied artificially. In addition, in winter and early spring, it is damaged by drought. Soils have played pivotal roles over the long history ages. The capacity to produce crop biomass remains an essential productivity function of the soil. This function is closely associated with food security, energy, water supply, carbon balance, and climate change [14]. It can only be delivered by a fertile and/or a healthy soil. Three major agricultural mineral fertilizers mean any substances with nitrogen (N), available phosphoric acid (P_2O_5), and soluble potash (K_2O), singly or in combination.

Abiotic stresses are one of the major constraints to crop production and food security worldwide. Agricultural production is directly affected by climate variables such as temperature and precipitation [16]. Environmental stress conditions such as drought, heat, salinity, cold, or pathogen infection can have a devastating impact on plant growth and yield under field conditions [7]. Drought is a multifaceted environmental stress that effects crop productivity in the fields. Moreover, drought stress could lead to extensive agricultural productivity losses and cause permanent damage to a plant such as stunted growth, hampered metabolism, re-

***Corresponding author**
Tel : +82-51-890-1592, Fax : +82-51-890-1521
E-mail : mkhuh@deu.ac.kr
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duced yield [1].

Chlorophylls take part in photosynthesis in plant cells that carries out the bulk of energy fixation. The absorbed energy of the light is transferred to an electron in a process called charge separation. Chlorophyll a is the most widely distributed form in terrestrial plants. Chlorophyll A absorbs light from the orange-red and violet-blue areas of the electromagnetic spectrum. Chlorophyll B absorbs blue light. The only difference between chlorophyll a and chlorophyll b is that the former has a methyl group where the latter has a formyl group. This difference causes a considerable difference in the absorption spectrum, allowing plants to absorb a greater portion of visible light. In diethyl ether, chlorophyll a has approximate absorbance maxima of 430 nm and 662 nm, while chlorophyll b has approximate maxima of 453 nm and 642 nm [5]. The absorption peaks of chlorophyll a are at 465 nm and 665 nm. Chlorophylls can be extracted from the protein into organic solvents [10]. In this way, the concentration of chlorophyll within a leaf can be estimated [5]. Methods also exist to separate chlorophyll a and chlorophyll b.

The aim of this study was assess the effect of various NPK fertilizers and drought stress on chlorophyll content in the leaves of garlic chives (*A. tuberosum*).

Materials and Methods

Measurement of chlorophylls

Seeds of garlic chives were sown on column-pots (water stress apparatus) with a baked particulated clay mixture and cultivated in a growth chamber (Dongweon Scientific Co., Korea). Temperature, humidity, light cycle, and three fertilizers inside the plant growth chamber were controlled. After germination plants are watered as needed to avoid water stress. We generally water plants by subirrigation for the first few weeks. Methods for applying soil water deficits applied to establish a gradual intensification of water deficit over at least several weeks [17]. The fertilizer was dissolved in a liquid state and sprayed in consideration of humidity at an appropriate concentration.

The content of the leaf chlorophyll was analyzed according with the Aron's method with some modification. The determination of chlorophyll a and b was performed with a spectrophotometer. Samplings were chosen in garlic chives from the upper complete open leaves with the length of 10-10.5 cm. Obtained samples were 1.5 g in weight. Leaves were cut promptly into small pieces and were ground to a fine powder with -70℃ liquid nitrogen. The 1.0 g of grinned powders was put in 80% acetone solution contained a small amount of $NaCO_3$. Then the samples were kept in the solutions for 24 hr in a dark place. Samples were centrifuged for 5 minutes at 10,000 rpm. The pellet was re-extracted with transferred to a second 1.0 ml aliquot of acetone, shaking for two minutes, centrifuging and removing the pellet. After this treatment, extract was properly diluted and measured by the intensity of the optical absorption. The optical density (OD) of the solution was read using the Microplate Reader (VersaMax, California, USA) at the wavelength 645 and 665 nm. The calculation of the contents of optical absorptions at wave lengths of 647 and 664.5 nm after Inskeep and Bloom's method [11] as fellow:

Chlorophyll a = 12.63 A664.5 – 2.54 A647

Chlorophyll b = 20.47 A647 – 4.73 A664.5

Total Chlorophyll = 17.95 A647 – 7.90 A664.5

Measurement of chlorophyll a fluorescence

Chlorophyll fluorescence analysis is used to measure photosynthetic parameters [3]. By exposing plants to a very bright flash of light, i.e. a saturating light pulse, the reactive centers of the plants' photosystems become saturated.

With the Imaging-Pulsed-amplitude modulation (PAM) Chlorophyll Fluorometer-2500 (Heinz Walz GmbH, Effeltrich, Germany), the current fluorescence yield (Ft) was continuously monitored. Plants were dark adapted for 20 min. On application of a saturation pulse, the dark-level fluorescence yield (Ft = F0) and the maximum fluorescence yield (Fm) were determined. The maximal PSII quantum yield, Fv/Fm, and the quantum yields of regulated and nonregulated energy dissipation in PSII, Y (NPQ) and Y (NO) were imaged. The variable fluorescence in the dark and in the quenched state (Fv′) is expressed by Fv = Fm – Fo, and Fv′ = Fm′ – Fo′, respectively. Fv/Fm was calculated according to the equation: Fv/Fm = (Fm − F0) Fm. Y (NPQ) was calculated according to Kramer et al. [12].

Statistical analysis

Experiments were performed in triplicate. Regression analysis was estimated the relationship of one variable (e.g., chlorophyll) with another (e.g., moisture) by expressing the one in terms of a linear function of the other [18].

Results

The content of chlorophylls a and b and total chlorophyll in garlic chives were obtained by setting up the equations.

Table 1. The changes in chlorophyll (chl-) content when the different concentrations (0～50 mg/l) of three fertilizers were treated in garlic chives (unit: mg/g)

Concentration (mg/l)	Fertilizer	Chl-a	Chl-b	Total	Chl-a/Chl-b
0 (control)	0	0.351±0.027	0.156±0.001	0.411±0.026	2.244
10	N	0.386±0.023	0.164±0.002	0.418±0.030	2.363
	P	0.326±0.030	0.158±0.003	0.315±0.044	2.068
	K	0.317±0.018	0.152±0.001	0.312±0.048	2.093
20	N	0.451±0.018	0.191±0.014	0.485±0.021	2.366
	P	0.343±0.020	0.170±0.010	0.387±0.032	2.021
	K	0.338±0.024	0.166±0.011	0.374±0.041	2.037
30	N	0.556±0.024	0.259±0.017	0.672±0.022	2.147
	P	0.414±0.028	0.188±0.019	0.478±0.030	2.202
	K	0.399±0.202	0.186±0.021	0.465±0.040	2.148
40	N	0.572±0.010	0.291±0.012	0.778±0.087	1.964
	P	0.459±0.031	0.233±0.013	0.608±0.024	1.967
	K	0.436±0.016	0.225±0.019	0.535±0.016	1.938
50	N	0.584±0.005	0.298±0.006	0.801±0.090	1.959
	P	0.442±0.028	0.227±0.020	0.573±0.030	1.944
	K	0.479±0.038	0.244±0.014	0.569±0.015	1.959

Nitrogen (N) fertilizer with the various treatments contributed to an increase in chlorophylls a, b, and total in garlic chives (Table 1). Even for chlorophyll content of 10 mg/l N fertilizer was higher than control. The content of chlorophyll a was evaluated 0.386 at 10 mg/l N fertilizer and 0.584 at 50 mg/l N fertilizer. There was a significant difference with regard to the contents of chlorophylls a and b, and total chlorophyll at the 5% level (p<0.05) from the control group (t of chlorophyll a=4.989, t of chlorophyll b=2.759, and t of chlorophyll total = 4.083). A statistically significant liner correlation was found between chlorophylls and amount of N fertilizer in the leaves (Table 2). Pearson correlation coefficient (Pearson's r) for chlorophylls a and b and total chlorophyll were 0.940, 0.966, and 0.971, respectively. The r^2 for chlorophyll content (a, b, and total) were 0.883, 0.934, and 0.943, respectively.

The highest chlorophylls a, b, and total content of the leaves were recorded at 40 mg/l P fertilizer while those values of 50 mg/l P fertilizer were lower than those of 40 mg/l P fertilizer (Table 1). Pearson correlation coefficient (Pearson's r) for chlorophylls a and b and total chlorophyll by P fertilizer were 0.926, 0.946, and 0.947, respectively. The r^2 for chlorophyll content (a, b, and total) by P fertilizer were 0.857, 0.896, and 0.896, respectively (Table 2).

Table 1 was shown the effects for K fertilizer of chlorophylls a and b and total chlorophyll in garlic chives. It was observed that content of chlorophylls a and b and total chlorophyll go on increasing with enhancements in concentration of K fertilizer in garlic chives. The content of total chlorophyll evaluated at 10 mg/l K fertilizer was 0.312 and that of 50 mg/l was 0.589. There was a significant difference with regard to the contents of chlorophylls a and b, and total chlorophyll (p<0.05) (t of chlorophyll a = 2.599, t of chlorophyll b = 6.856, and t of chlorophyll total = 2.743). A statistically significant liner correlation was found between chlorophylls and amount of K fertilizer in the leaves (Table 2). Pearson

Table 2. The correlation of chlorophyll a and b content when three fertilizer concentrations are treated in garlic chives

Fertilizer	Chlorophyll	Liner relation	R^2
N	a	Y = 0.052x + 0.335	0.883
	b	Y = 0.004x + 0.127	0.934
	Total	Y = 0.011x + 0.312	0.943
P	a	Y = 0.004x + 0.291	0.857
	b	Y = 0.002x + 0.137	0.896
	Total	Y = 0.007x + 0.242	0.896
K	a	Y = 0.004x + 0.275	0.985
	b	Y = 0.002x + 0.127	0.973
	Total	Y = 0.007x + 0.241	0.994

correlation coefficient (Pearson's *r*) for chlorophylls a and b and total chlorophyll were 0.992, 0.987, and 0.997, respectively. The r^2 for chlorophyll content (a, b, and total) were 0.985, 0.973, and 0.994, respectively.

The relationship between chlorophyll efficiency and moisture concentrations on garlic chives were observed. The current fluorescence yield (Fo) was increased to the decrease of water in soils (Fig. 1). The regression line slopes were shown downward from left to right. Fig. 2 was the maximum fluorescence yield (Fm) at different moisture (%). The maximal PSII quantum yield, Fv/Fm was the highest at 80% moisture among nine moisture percentage stages (Fig. 3). The simple type of regression showed the relation between chlorophyll efficiency as a function of moistures. The slope factors of Fo, Fm, Fv, and Fv/Fm for chlorophyll efficient indicators were -0.931 (Fig. 1), 0.972 (Fig. 2), 972 (Fig. 3), and 0.950 (Fig. 4), respectively. The regression line slopes of Fig. 2 - Fig. 4 were shown upward from left to right.

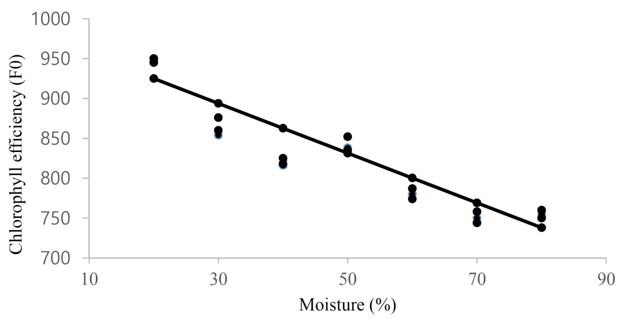

Fig. 1. The values of chlorophyll efficiency at different moisture (%). Fo: The current fluorescence yield. The values of figure listed their moisture (X-axis) in soil pots and dark-level fluorescence yield (Y-axis). Figure showed the scatter diagram and the regression line for the data on seven treated groups.

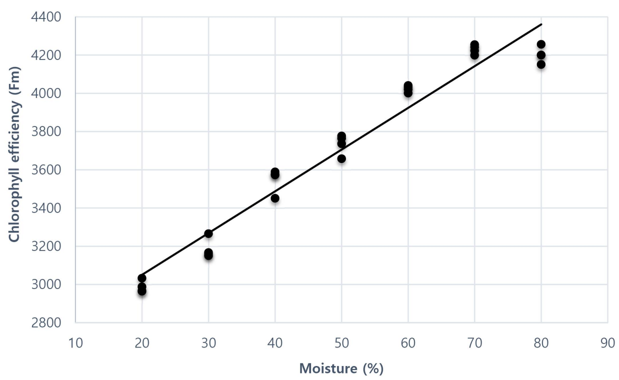

Fig. 2. The maximum fluorescence yield (Fm) at different moisture (%). The values of figure listed their moisture (X-axis) in soil pots and maximum fluorescence yield (Y-axis). The regression line slopes upward from left to right.

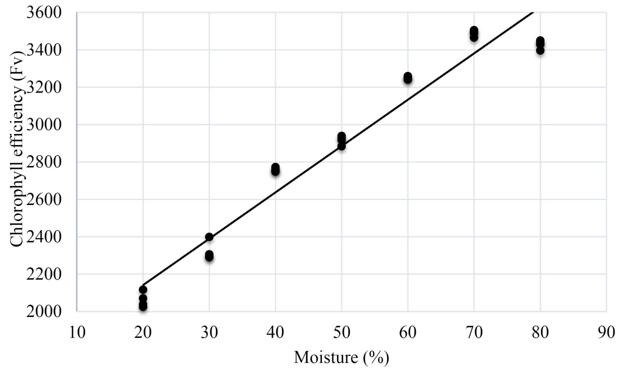

Fig. 3. The values of chlorophyll efficiency in the quenched state. Fv: The quenched state. The values of figure listed their moisture (X-axis) in soil pots and the quenched state (Y-axis).

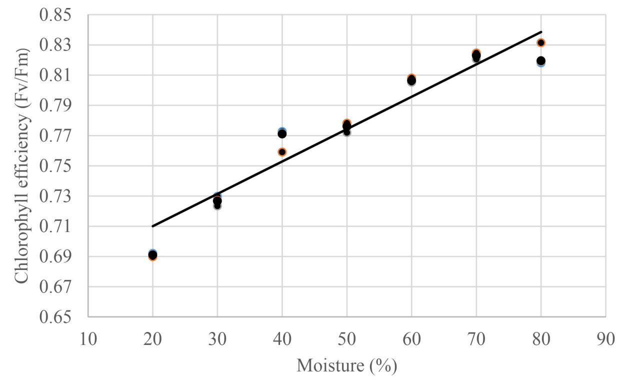

Fig. 4. The maximal PSII quantum yield of garlic chives. Fv/Fm: The maximal PSII quantum yield. The values of figure listed their moisture (X-axis) in soil pots and the maximal PSII quantum yield (Y-axis).

Discussion

Nitrogen (N), phosphorus (P), and potassium (K) are primary nutrients required by the plant for proper growth and development. Interaction of nutrients in soils and plants is an important factor in determining the yield of crops. Since nitrogen is required for the formation of chlorophyll and phosphorus for cell division and elongation [4]. Many fertilizer studies have investigated the effect on crops in a mixed form rather than a single ingredient. For example, the effect of the N-P-K mixing ratio on crops in the form of 15-15-15 or 20-20-20 was investigated [2, 15]. In that case, it is difficult to show the chlorophyll content effect of a single fertilizer. In this study, nitrogen fertilizers had a higher chlorophyll production effect than phosphoric acid or potassium fertilizers (Table 1). Previous literature reported that N application increased the yield of different crops by reducing the loading rate and enhancing photosynthetic performance [6, 19]. N fertilizer and high water inputs are commonly seen to achieve high yields [21]. The total crop water requirement is the maximum amount of water that a crop can use productively when the soil water content is not limiting [20]. Crops mainly need water for cooling purposes; most of the root's water uptake is released back to the atmosphere through transpiration, and only a negligible fraction is retained for crop growth [9]. Drought, created by withholding water, led to significant reductions in the absolute (-3.6%) and relative (-24.8%) water content of chives (*Allium schoenoprasum*) leaves, a significant rise in the osmolality of the leaf sap (+18.9%) and a loss of leaf transpiration (leaf diffusion resistance >20 s/cm) [8].

Garlic chive has long been a source of valuable nutrients and medicinal ingredients for humans. However the species is less known in many countries and therefore underrated. This species is grown on a large scale in Korea and has widely used as noodle garnish. These days, garlic chives are popular for chlorophyll intake. This study can be used as a basis for chlorophyll studies on garlic chives. In addition, if excessive chemical fertilizer is given to garlic chive, it will be lost from agricultural land to rivers, so research on appropriate fertilizer stocks will also be needed.

The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 비료와 가뭄 스트레스에 의한 부추의 엽록소 변화

허만규[1]* · 이병룡[2]
([1]동의대학교 식품공학과, [2]서원대학교 생물교육과)

부추는 아시아를 포함한 전세계적으로 분포하는 초본이다. 한국에서 부추는 양념용 채소와 국수용 고명에 사용된다. 질소비료, 인산비료, 칼리비료와 수분 스트레스가 부추(*Allium tuberosum* Rotter) 잎에 미치는 영향에 대해 조사하였다. 엽록소 a 함량은 10 mg/l 질소 비료에서 0.386였고, 50 mg/l에서는 0.584였다. 엽록소 a, b, 전체 엽록소량은 대조군에 비해 유의한 차이를 나타내었다(p<0.05). 엽록소 a, b, 전체 엽록소에 대한 피어슨 상관계수(Pearson's r)는 각각 0.940, 0.966, 0.971였다. 잎의 엽록소 a, b, 전체 엽록소는 인산비료인 경우 40 mg/l가 50 mg/l 시비보다 높았다. 인산 비료인 경우 10 mg/l에서 전체 엽록소량은 0.312였고 50 mg/l 일 때 0.589였다. 엽록소 효율과 습도와의 관계를 산출하였다. 광합성 효율의 척도로 기저 수준의 형광산율(Fo), 최대 형광산율(Fm), 전자소멸 상태(Fv), 최대 PSII 광계비율(Fv/Fm)의 기울기는 각각 -0.931, 0.972, 972, 0.950였다. 질소, 인산, 칼륨 비료와 가뭄스트레스는 부추의 엽록소함량과 효율성에 영향을 주었다.