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- Note -

Increasing Kale Sulforaphane Contents by Combining Geraniol with Water Stress in Indoor Farm Aeroponics

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Sulforaphane is a sulfur-containing substance found in large amounts in cruciferous plants and has been reported in several studies to have anticancer effects. Kale is a representative cruciferous plant known as a superfood and is widely used as an ingredient in various dishes. In this study, in order to investigate a cultivation method for increasing kale's content of sulforaphane, kale was treated with geraniol or methyl jasmonate and water stressed during cultivation using a aeroponic culture system in a fully enclosed plant factory. Geraniol or methyl jasmonate were sprayed on the kale's leaf surface once a day for 2 days, and water deprivation stress was conducted for 3 days after 7 days from first treatment day. No difference in growth between control, geraniol, methyl jasmonate treated groups were observed during cultivation. The study results showed that the kale sulforaphane content increased by 60% in the group treated with geraniol compared to the control group and that the group treated with water deprivation stress in addition to geraniol showed a significant increase of 414%. These results show that kale with an increased content of sulforaphane can be grown and that geraniol can be a good research material for increasing the content of functional substances in plants.

Key words : Aeroponics, geraniol, kale, sulforaphane, water stress

Introduction

Interest in food with chemoprevention properties has been steadily increasing. Cruciferous vegetables in particular have attracted in rich isothiocyanates, for example, sulforaphane [5, 10]. Kale is a cruciferous vegetable, characterized by leaves along the stem, which have gained a great popularity as a 'superfood'. Kale leaves are usually consumed fresh in salads and as kale leaf juice, and cooked as diverse soup dishes, omelets, and stir-fry. Recently, dried kale or so-called 'kale chips' became very popular, although draying significantly decreases its nutritive and phytochemical content, sulforaphane [6].

Sulforaphane is an isothiocyanate that is present naturally in widely consumed cruciferous vegetables. Multiple characteristics of sulforaphane have been widely reported recently. Sulforaphane has various effects including antioxidant, antitumor formation and anti-inflammatory effect [7-9, 11, 12]. This compound has been shown to suppress or prevent tumor formation and development [8, 12].

Geraniol (3,7-dimethylocta-trans-2,6-dien-1-ol) is an acyclic monoterpene alcohol with the chemical formula $C_{10}H_{18}O$. Geraniol has characteristic rose-like odor and the taste (at 10 ppm) is described as sweet floral rose-like, citrus with fruity, waxy nuances [3]. This monoterpene alcohol is a widely used fragrance material. In addition, geraniol exhibits various biochemical and pharmacological properties. Researchers have shown geraniol to be an effective plant-based insect repellent[10] and its potential as an antimicrobial agent has been highlighted in several studies [1, 4].

The purpose of this study is to increase the sulforaphane contents of kale, famous for superfood, by use of MeJA, Geraniol and water stress in aeroponic system of indoor farm.

Materials and Methods

Chemicals and materials

Prethanol A (Duksan company, Korea), Geraniol (Dotter, Korea), MeJA (methyl jasmonic acid) (Zhuoer Chemical Co., Ltd., China), Potassium nitrate, Calcium nitreat, Monopotassium phosphate, Magnesium sulfate and Sodium molybdate (Now-chem. Co. Ltd, Korea), Ammonium nitrate and Nitric

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acid (Hyun science, Korea), EDTA-Fe (Shijiazhuang Jackchem co.,ltd, China), Boric acid, Copper sulfate (Officeahn, Korea), Manganese sulfate (Dof), Zinc sulfate (DAEJUNG), kale seed (*Brassica oleracea*) (Asia Seed Co. Ltd., Korea), Seedling sponge (Gafatech, Korea), Seedling tray (Yeong-nong-sa, Korea), Aeroponic system (Insungtec, Korea), Culture room (WOORI TECHNOLOGY INC., Korea) were used for this study.

Seedling

Put a seedling sponge on the seedling tray, pour water and let it soak well. Sow 1 kale seed on the seedling sponge soaked in water, cover with plastic, and in a growing room at 23±2℃ 80±10% without light supply for 2-3 germinate day. The germinated seedling tray is removed from the plastic and grown under LED light until planting. In the 3rd week after sowing, add the nutrient solution little by little. It is planted in a cultivation bed about 4 weeks after the roots are formed to some extent.

Culture

For one week after planting, a nutrient solution with EC 0.5±0.1 mS/cm and pH 6.0±0.2 is supplied at 120 sec/30 min (2 min spray at 30 min intervals). The humidity of the cultivation room is set at 70±10% and the temperature at 23±2℃. The light supply cycle is 16 hr/8 hr (bright/dark), and the light source is 290±10 umol/m^2/s (250 mm distance) LED (Insungtec, Korea). After planting, adjust the EC to 0.7±0.1 mS/cm at the 2^{nd} week, 1.0±0.1 mS/cm at 3^{rd} week, and 1.5±0.1 mS/cm at the 4^{th} week after planting. Table 1 shows composition of the supplied nutrient solution.

Treatment

After planting, 500 ppm geraniol or 500 ppm MeJA (10%/EtOH diluted in water) was sprayed on the leaves of kale grown for 10 weeks after planting once daily for 2 days. The treatment concentration was determined by adjust the method of Mikkelsen et al. [13] in consideration of the growth rate. Samples are collected 7 days after the first treatment, and water stress treatment is performed for 3 days by supplying the nutrient solution at 60 sec/180 min (60 sec spray at 180 min intervals). After water stress treatment, the leaves are harvested and analyzed.

Analysis

Sulforaphane analysis by GBST Green Bio Research Facility Center (Pyeongchang-gun, Korea).

Statistics

Statistical analysis was performed using SPSS. Duncan was used to compare the data between groups.

Results

Seedling

Germination occurred 2-3 days after sowing, and the main leaves appeared after 2-3 weeks, and at this time, half of the seedling plate was filled with EC 1.5 nutrient solution. At 3-4 weeks, the root growth and the growth of the main leaves was enough for planting (Fig. 1).

Culture

Seedlings with good root growth and good leaf growth were planted in the cultivation bed. The cultivation environment is shown in Table 2. The temperature of the cultivation room was stably managed. After 7 weeks of vigorous growth and vigorous leaves, humidity increased due to transpiration. The temperature of the nutrient solution was maintained similar to the temperature of the cultivation room. As a result of comparing the growth of Control, MeJA, and Gera-

Table 1. Nutrient solution composition

A Tank		B Tank	
Potassium nitrate	105*	Potassium nitrate	105
Calcium nitrate, 10 H^2O	68	Monopotassium phosphate	65
Ammonium nitrate	34	Magnesium sulfate	55
EDTA-Fe	8	Boric acid	1.192
		Manganese sulfate	0.816
		Zinc sulfate	0.090
		Copper sulfate	0.019
		Sodium molybdate	0.012

* g/4L (100X solution)

Table 2. Environment condition during kale culture in aeroponic system

Time	Humidity (%)[a)]	Temperature (℃)[b)]	pH	EC (mS/cm)	Nutrition temperature (℃)
TPN	64~72	22.9~24.4	6.0	0.5	22.8
1st Week	65~71	22.4~24.8	6.0	0.6	23.5
2nd Week	67~72	22.6~24.5	5.9	0.7	23.1
3rd Week	67~73	22.4~24.4	6.0	1.0	23.0
4th Week	69~73	22.2~24.1	6.0	1.5	22.7
5th Week	71~76	22.1~24.0	6.0	1.5	22.9
6th Week	72~76	22.1~24.0	6.0	1.5	22.9
7th Week	75~83	21.7~23.4	6.1	1.5	22.8
8th Week	81~87	21.6~23.4	6.0	1.4	23.1
9th Week	83~85	21.4~23.1	6.0	1.5	22.7
10th Week	82~89	20.9~23.1	6.0	1.5	22.7
1st Treatment	85~89	20.9~22.4	5.4	1.4	22.5
2nd Treatment	85~88	20.9~22.2	6.0	1.5	22.6
Sampling+WST	79~84	21.0~22.4	6.0	1.4	22.5
Sampling	73~76	21.7~23.7	5.7	1.3	23.5

[a)]Mini~Max humidity during a day, [b)]Mini~Max temperature during a day

niol-treated groups, Control and Geraniol showed similar growth, and MeJA-treated group showed some scars on the leaf surface (Fig. 2).

Sulforaphane contents

After 10 weeks of planting, MeJA and Geraniol were treated, and 7 days later, Control, MeJA and Geraniol treated kale leaves were harvested and analyzed for sulforaphane, and sulforaphane of 3 treatments leaves was analyzed by treatment with WST (water stress) for 3 days from the 7th day. Kale leaves withered during water stress like Fig. 3.

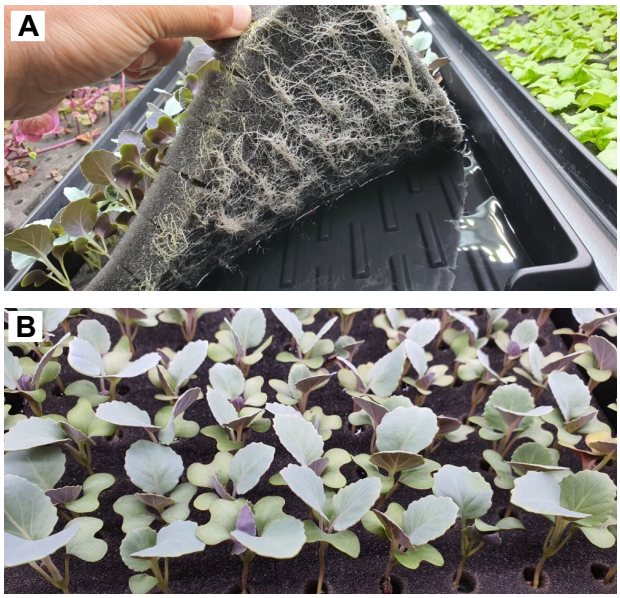

Fig. 1. Picture of seedlings at the time to TPN (transplantation). A: Roots, B: Leaves.

As shown in Fig. 4, 7 days after treatment, MeJA increased the sulforaphane content by 28% and Geraniol by 46% com-

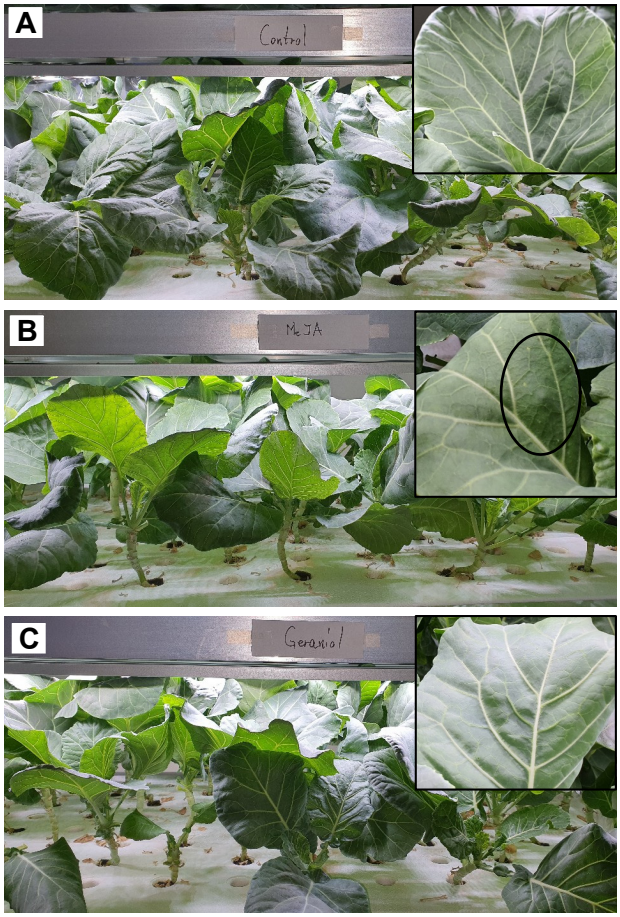

Fig. 2. Growth comparison of Control, MeJA, and Geraniol treatment groups. A: Control, B: MeJA, C: Geraniol.

Fig. 3. Kale leaves withered during water stress.

pared to the control. After WST was treated for 3 days, the MeJA-treated group increased the sulforaphane content by 120% compared to the control, and the Geraniol-treated group increased it by 414%. The values between each treatment group were all found to be significant difference.

In conclusion, it is possible to increase the content of sulforaphane, a functional component of kale, by treating MeJA, Geraniol with treating moisture stress when cultivating kale with the spray culture system in a plant factory. By treating the water stress with Geraniol, it was confirmed that the increase effect was more than 4 times compare to control. Also, this study will be the first report showing the effect of Geraniol to increase the functional components of plants. Therefore, future studies to elucidate the mechanism by which Geraniol increases the content of sulforaphane should be continued.

Discussion

Cruciferous vegetables in particular have attracted in rich isothiocyanates, for example, sulforaphane [5, 10]. Study on how the content of functional substances such as sulforaphane changes depending on the type or stress is also being conducted [14]. Wu et al. [15] reported that the sulforaphane content of kale shoots was about 150 mg/kg and that MeJA treatment increased it by about 60% to about 250 mg/kg. It is known that the sulforaphane content of the sprouts is relatively high, and the increase rate was relatively low at this test, but after water stress treatment, the increase effect was twice as high as that of Wu et al. [15]. Geraniol showed a higher sulforaphane-increasing effect than MeJA.

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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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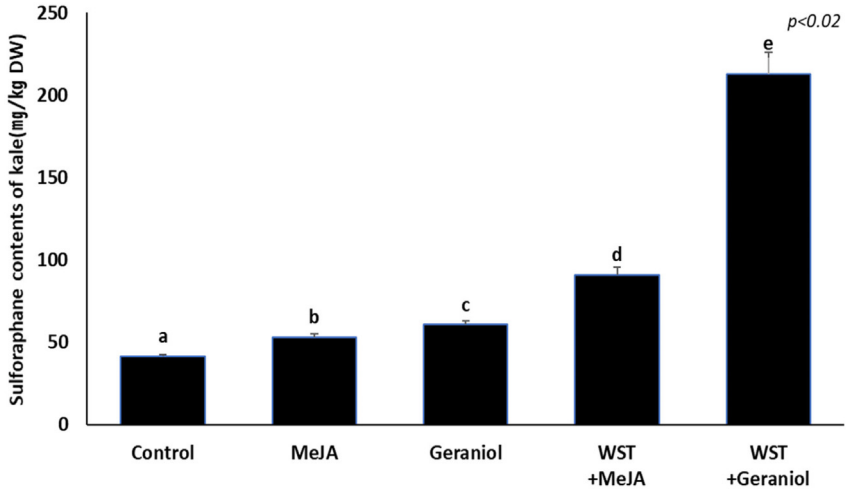

Fig. 4. Sulforaphane contents of Control, MeJA, Geraniol, WST+MeJA, WST+ Geraniol. The differences between treatment groups were shown with a, b, c, d, e (p<0.02).

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초록 : 분무경 식물공장에서 수분스트레스와 geraniol 스프레이에 의한 케일의 설포라판 함량 증가

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설포라판은 십자화과 식물에 다량 함유되어 있는 황을 함유하는 물질이다. 이 물질은 여러 연구에서 항암효과가 있다고 알려져 있다. 케일은 슈퍼푸드로 알려진 대표적인 십자화과 식물로 다양한 요리 재료로 널리 이용된다. 본 연구에서는 케일에서 이 설포라판의 함량을 증가시키는 재배방법을 연구하기 위해, 완전 밀폐식 식물공장에서 분무경재배시스템을 이용한 재배 중에 제라니올을 처리하고 수분스트레스를 처리하였다. 재배 중에 제라니올의 처리는 2일간 하루에 1회 엽면에 분무처리 하였고, 7일 후에 3일 동안 수분결핍 스트레스를 처리하였다. 재배 중에 처리구 간의 큰 생육 차이는 나타나지 않았다. 그 결과 제라니올을 처리한 처리구에서 대조구에 비해 설포라판 함량이 60% 증가하는 것을 확인하였고, 제라니올 처리와 함께 수분결핍 스트레스를 처리한 처리구에서는 414%에 상당한 증가를 보였다. 이 결과를 통해 설포라판의 함량이 증가된 케일을 재배할 수 있고, 제라니올이 식물의 기능성 물질의 함량을 증가시키기 위한 좋은 연구재료가 될 수 있다는 것을 보여주었다.