

Growth and Total Flavonoids of Three Celery (*Apium graveolens* L.) Varieties in Shaded Environments in The Tropical Lowland

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Abstract

Celery is an introduced species of family Apiaceae from the subtropical areas. Celery leaves and stalks contain vitamins, phenolic compounds, essential oils, and other nutrients that have been used in the foods, cosmetics, and herbal industries. Shading can create a better environment for varieties adapted to the highlands to succeed to grow in the lowlands. However, the changes in the growing environment including light and temperatures might affect the flavonoid production. This study aimed to determine the growth and production of flavonoids of the highland celery varieties in the lowlands when treated with various level of shades, i.e. without shade/control, 50% shade, and 70% shade. The study used "Aroma", a celery variety adapted to the highland, and "Summer Green", a variety adapted to the lowland. Increases in the shade levels decrease air temperature, increases air humidity, and significantly improved the growth and production of the three celery varieties in terms of plant height, leaflets number except for the "Aroma" variety; increased leaf area, stem diameter, tiller number, crown diameter, total wet weight, total dry weight, chlorophyll a, chlorophyll b, total chlorophyll, anthocyanins, plant N (Nitrogen), P (Phosphor), K (Potassium), and total flavonoids. "Amigo" exposed to 50% shade had significantly higher flavonoids than "Aroma". Providing shades to "Summer Green" resulted in the lower levels of flavonoids compared to without shading.

Keywords: abiotic stress, growth, highland varieties, secondary metabolites, shading

Introduction

Celery (*Apium graveolens* L.) is a vegetable species from subtropical climates of Europe, Asia, Africa, and America (Rubatzky and Yamaguchi, 2012; Kooti et al., 2015). Celery leaves and stems contain vitamins, phenolic compounds, essential oils, and other nutrients that are useful in the food and cosmetic industries. Celery could also be used as a treatment for anti-inflammatory, anti-bacterial, anti-obesity, and lowering blood sugar (Li et al., 2017; Cho et al., 2019). Celery can be grouped into tuber celery (*Apium graveolens* var. *rapaceum*), leaf celery (*Apium graveolens* var. *secalinum*), and stem celery (*Apium graveolens* var. *sylvester*) (Fazal and Singla, 2012). Leaf celery is often found and cultivated in Indonesia. There are leaf celery varieties that are adapted to the highlands (700-1200 m above the sea level), and to the lowlands. Growing celery in the lowlands usually uses shade to reduce the light intensity and to maintain humidity around the plants (Bobihoe, 2010). Shading can affect the quality of light received by the plants. Photons from light activates photoreceptors that are linked to signaling pathways and lead to gene expression changes (Thoma et al., 2020). Therefore, differences in the light quality can cause variations in flavonoid production (Berquist et al., 2017).

Flavonoids are one of the secondary metabolites in celery, it is a phenolic group that has antioxidant activities (Ferreysa et al., 2012; Yao et al., 2010; Redha, 2010). Water availability, temperature, and soil composition (abiotic), as well as microorganisms, herbivores, and plant species (biotic) affect the biosynthesis and accumulation of secondary metabolites in plants (Pavarini et al., 2012). UV light optimizes the accumulation of phenolic compounds, particularly the concentration of anthocyanins (Jaakola and Hohtola, 2010, Bian et al., 2015). The

objective of this study is to determine the growth and production of flavonoids of the highland celery varieties grown in the lowlands with different levels of shading.

Material and Methods

Experimental Site and Research Design

The experiment was conducted at the Leuwikopo experimental field of the Department of Agronomy and Horticulture, IPB University, Indonesia, from December 2020 to June 2021. According to Balitsa (2007), optimal temperatures for celery is 16-21°C. December to June is the rainy season in Indonesia and the temperatures were cooler compared to those in the dry season, therefore this period would be more suitable to grow celery.

A nested design was used and consists of two factors, shading levels and celery varieties. Shading treatment consists of control/without shading, 50%, shade, and 70% shade, whereas celery varieties used are the highland varieties "Amigo" from Panah Merah and "Aroma" from Bintang Asia; and a lowland variety "Summer Green" which is set as a control variety. Shade level of 50% transmits 50% of the sunlight, and shade 70% transmitted 30% light to the plants.

Plant Cultivation

Celery seeds were sown in rockwool blocks and sprayed with 10 mL of AB mix fertilizer in 10 L water daily for 15 days after sowing the seeds. The emergence of 4 true leaves on the seedlings indicates that the seedlings are ready to be transferred to the field (about 300 m²); the plots are 1 m x 4 m with 20 cm x 20 cm space between plants. One planting hole contains one celery seedling. At 2 weeks after planting (WAP), seedlings were fertilized with NPK 16-16-16 every fortnight. Weeding and watering were conducted as necessary. Snails, which are pests investing the experimental plants, were controlled manually, whereas mealybugs were controlled chemically. Celery was harvested at 10 WAP.

Table 1. Light intensity, humidity and temperatures in the experimental field 1-10 weeks after planting

Variable	Shade level (%)		
	0	50	70
Light intensity (x1000 lux)	49.09	23.41	19.38
Humidity (%)	86.32	87.70	97.17
Temperature (°C)	25.89	24.62	23.77

Data Analysis

Quantitative data on "Amigo" and "Aroma" were analyzed with ANOVA at the 5% level. Means that were significantly different were further separated using the Tukey's Honestly Significant Difference (HSD) test, and using Student t-test with "Summer Green" as a control variety. All quantitative data were analyzed using R Studio application.

Result and Discussion

General Condition

The higher the level of shades, the less intensity of sunlight received by the plants, and the air temperature under the shade progressively decreases (Table 1). The impact of light intensity on air temperature in this study are highly significant, as also stated in Handriawan et al. (2016). High light intensity causes the air temperature in a location to increase, resulting the death of "Amigo" and "Aroma" in the control/ full light condition. The recapitulation data on the shade effects, varieties, and the interaction of the two factors are described in Table 2.

Growth and Production of Several Celery Varieties at Different Shading Treatments

The 50% shade resulted in a slight increase (0.5%) of leaflet number compared to the 70% shade. In a study by Sulistyowati et al. (2019) the plants adapted to shades by producing more leaves to promote photosynthesis. "Amigo" had a significantly (54.54%) more leaflets than "Summer Green", whereas "Summer Green" had 43.3% more leaflets than "Aroma" (Table 3).

"Amigo" had more stalks and wider leaf area than "Aroma" and "Summer Green", but their height and stem diameter were similar. "Summer Green" were the tallest, whereas "Aroma" had the largest stem diameter. Compared with the "Summer Green" in the treatment without shade, "Amigo", "Aroma", and "Summer Green" had wider leaf area, larger stem diameter, and taller plants (Table 3).

Table 2. Recapitulation of the ANOVA at 10 weeks after planting

Variable	F-test			
	Shade	Varieties	Shade*Varieties	CV (%)
Plant height	ns	ns	ns	14.11
Stalk number	ns	ns	ns	19.31
Leaflet number	**	**	ns	13.63
Tiller number	ns	ns	*	19.35
Leaf area	ns	ns	ns	46.89
Stem diameter	ns	ns	ns	18.20
Crown diameter	**	ns	**	10.46
Fresh weight	*	ns	ns	15.82
Dry weight	**	ns	ns	13.79
Chlorophyll a	ns	ns	ns	16.45
Chlorophyll b	ns	ns	ns	12.43
Total Chlorophyll	**	*	ns	11.06
Anthocyanin	ns	ns	ns	16.56
Carotenoids	ns	*	ns	11.60
Nitrogen	ns	**	ns	4.44
Phosphorus	ns	ns	ns	17.10
Potassium	ns	ns	ns	14.50
Total Flavonoids	ns	ns	**	6.64

Note: Plant height (cm), leaflet number, leaf area (cm^2), stem diameter (mm), fresh weight (g), dry weight (g), chlorophyll a ($\mu\text{mol/g}$), chlorophyll b ($\mu\text{mol/g}$), Nitrogen (%), Phosphorus (%), Potassium (%). CV= coefficient of variation; ns= not significant, *= significantly different at $\alpha= 0.05$, **= significantly different at $\alpha= 0.01$.

Tabel 3. Celery vegetative growth at ten weeks after planting

Treatment	Plant height (cm)	Stalk number	Leaflet number	Leaf area (cm^2)	Stalk diameter (mm)
Shade (%)					
50	36.82	3.7	53.10a	90.66	9.48
70	32.78	3.3	35.00b	76.41	9.54
Varieties					
"Amigo"	31.93*	3.7	55.50a*	93.15*	9.13*
"Aroma"	33.31*	3.2	32.30c	83.15*	10.40*
"Summer Green"	39.16*	3.6	44.30b	74.30*	10.31*
"Summer Green" without shade	24.30	10.2	34.2	172.03	8.50

Note: followed by different letters within the same column are significantly different according to the Honestly Significant Difference test at $\alpha= 0.05$. Values followed by * within the same column are significantly different compared to "Summer Green" without shade according to t-test at $\alpha= 0.05$.

"Aroma" with 70% shading had the greatest tiller number but it was similar to "Amigo" in the same shading level (70%), or "Aroma" and "Summer Green" with 50% shading. The largest plant crown diameter was in "Aroma" with 50% shade, but it was

not significantly different from "Amigo". Higher shade level decreases the canopy diameter of "Amigo" and "Aroma" (Table 4), and significantly increased the tiller number of "Amigo", "Aroma", and "Summer Green" compared to "Summer Green" without shading. The

largest plant crown diameter was in "Aroma" with 50% shade, but it was not significantly different from "Amigo". Higher shade level decreases the canopy diameter of "Amigo" and "Aroma" (Table 4), whereas "Amigo", "Aroma", and "Summer Green" under shade had significantly larger diameters compared to the control (Table 5).

Nutrient and Secondary Metabolite Content of Several Celery Varieties at Different Shading Treatments

Fifty percent shade resulted in a 5.6% more fresh weight, 1.23% more dry weight, and 0.01% more total chlorophyll compared to 70% shading (Table 6). Chlorophyll b and carotenoids at 50% shade were similar to those at 70% shade. The celery varieties differ in the total chlorophyll and carotenoids but had similar fresh weight or total dry weight. Chlorophyll in the "Amigo" was significantly higher (0.01%) than "Aroma", but "Aroma" had similar chlorophyll level to "Summer Green". Similarly, carotenoid levels in "Amigo" was considerably higher (0.08%) than "Summer Green", but the levels in "Summer Green" was not significantly different from "Aroma" (Table 6).

The three celery varieties in 50 and 70% shade had higher chlorophyll a and b, carotenoids, and anthocyanins when compared to "Summer Green" without shade (Table 6). The presence of anthocyanins is in the cell vacuoles protect chloroplasts from high light intensity and reduce free radicals (Susanti et al., 2014; Pebranti et al., 2015). For example, in waterleaf

(*Talinum triangulare*), an increase in anthocyanin resulted in an increase in antioxidants which then acts as a protection against biotic or abiotic stress (Susanti et al., 2014).

Shading did not affect the plants' N, P, and K, but their levels were slightly more than without shades. "Aroma" had the highest N, but it was not significantly different from that in "Amigo". The t-student test showed that when compared to the "Summer Green" without shade, "Aroma", "Amigo", and "Summer Green" with shades, had significantly higher N, P, and K levels (Table 7). The study of Coble and Cavalieri (2015) reported that shades could affect the nitrogen distribution in the *Acer saccharum* leaves. The low light produced by the shade causes the nitrogen mass of *Acer saccharum* leaves to increase.

"Amigo" at 50% shading had in a significantly higher total flavonoids than "Aroma". Shading at 70% did not affect flavonoid content of "Aroma", but reduced it in "Amigo". Shaded "Summer Green" had lower levels of total flavonoids than in without shade. Although "Summer Green" is a lowland variety, it could benefit from shade treatment indicated by a higher flavonoid content with shading. The total flavonoids in "Summer Green" without shade indicates that "Summer Green" experienced abiotic stresses (Table 8). Our results agree with Chikmawati's (2013) in that plants produce more secondary metabolites, including flavonoids, when exposed to high temperatures stresses.

Table 4. Celery tiller number and crown diameter at ten weeks after planting

Shade (%)	Tiller number			Crown diameter (cm)		
	"Amigo"	"Aroma"	"Summer Green"	"Amigo"	"Aroma"	"Summer Green"
50	0.7b	0.8ab	1.0ab	6.23a	6.49a	4.38b
70	0.9ab	1.2a	0.7b	3.86b	3.60b	4.52b

Note: Values followed by different letters within the same column are significantly different according to the Honestly Significant Difference test at $\alpha = 0.05$.

Table 5. Tiller number and crown diameter of three varieties of celery compared to "Summer Green" without shade at ten weeks after planting

Varieties	Tiller number	Crown diameter (cm)
"Amigo"	0.81*	5.04*
"Aroma"	1.01*	5.05*
"Summer Green"	0.85*	4.45*
"Summer Green" without shade	0.5	3.34

Note: Values with * are significantly different according to t-test $\alpha = 0.05$.

Table 6. Celery fresh weight, chlorophyll, anthocyanin and carotene levels at ten weeks after planting

Shades (%)	Fresh weight (g)	Dry weight (g)	Chlorophyll a ($\mu\text{mol.g}^{-1}$)	Chlorophyll b ($\mu\text{mol.g}^{-1}$)	Total chlorophyll (mg.g^{-1})	Anthocyanin (mg.g^{-1})	Carotene (mg.g^{-1})
50	6.64a	2.23a	0.09	0.53	0.04a	0.09	0.39
70	5.45b	1.69b	0.09	0.54	0.03b	0.09	0.40
Varieties							
"Amigo"	5.99	2.02	0.10	0.60*	0.04a	0.10*	0.44a
"Aroma"	5.70	1.90	0.08*	0.52*	0.03b	0.09*	0.39ab*
"Summer Green"	6.44	1.97	0.09*	0.50*	0.03ab	0.09*	0.36b*
"Summer Green" without shade	5.65	2.08	0.06	0.37	0.03	0.06	0.30

Note: Values followed by different letters within the same column are significantly different according to the Honestly Significant Difference test at $\alpha= 0.05$. Values followed by * within the same column are significantly different to "Summer Green" without shade according to the Student's t-test at $\alpha= 0.05$

Table 7. Celery nutrient content at ten weeks after planting

Shades (%)	N (%)	P (%)	K (%)
50	2.89	0.34	3.74
70	3.02	0.30	3.78
Varieties			
"Amigo"	3.04a*	0.30	3.82*
"Aroma"	3.10a*	0.34*	3.90*
S. Green	2.72b*	0.32*	3.55*
"Summer Green" without shade	2.42	0.27	2.65

Note: N=nitrogen, P= phosphorus, K= potassium. Values followed by different letters within the same column are significantly different according to the Honestly Significant Difference test at $\alpha= 0.05$. Values followed by * within the same column are significantly different to "Summer Green" without shade according to the Student's t-test at $\alpha= 0.05$

Table 8. Celery total flavonoid at ten weeks after planting

Shades (%)	Total flavonoids (mg QE.g^{-1} of dry weight)		
	"Amigo"	"Aroma"	"Summer Green"
50	1358.01a	1078.22b	1230.56ab e
70	1159.79ab	1383.14a	1021.89b
0			1255.99

Note: Values followed by different letters in the same column are significantly different according to the Honestly Significant Difference test at $\alpha= 0.05$. Values followed by * within the same column are significantly different to "Summer Green" without shade according to the Student's t-test at $\alpha= 0.05$

Conclusion

Providing shades are important to grow highland celery varieties in the lowlands. "Amigo", "Aroma", and "Summer Green" have different growth, production, and flavonoid levels. "Amigo" grew best with 70% shade whereas "Aroma" with 50% shade. A lowland variety "Summer Green" benefits from shading for the better growth and production. The highest total flavonoid was in "Amigo" with 50% shade, "Aroma" with 70% shade, and "Summer Green" without

shading. For fresh consumption, providing 50% shades to "Summer Green" resulted in the higher fresh weight in the lowland. High shade (70%) should be provided for a highland variety "Aroma" to grow in the lowlands to produce higher levels of total flavonoids. Future studies should include an organoleptic test to determine if the shade treatment affects the taste and texture of the celery leaves. It is also important to determine the levels of bioactive compounds in celery.

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