Korean J. Crop Sci. 47(1): 42~47(2002)

Effect of Light Source on Organic Acid, Sugar, and Flavonoid Concentrations in Buckwheat

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ABSTRACT : The major free sugars of buckwheat plants were fructose, glucose, and maltose but their contents and compositions were influenced by the different wavelength of light. Free sugar contents of Clfa 39 (*Fagopyrum tataricum*) were higher than those of Yangjul-maemil (*Fagopyrum esculentum*) regardless of the light sources. As treated with red and blue light, the free sugar contents in the leaves of buckwheat plants were slightly increased, but their contents in the stems and flowers were lower than those of natural light condition. Under the natural light condition, maltose was detected in every tissues of buckwheat plants, but as treated with blue and red light, it was not detected in the flowers of buckwheat plants. Citric, malic and acetic acid were detected as major organic acids in buckwheat plants. Red and blue lights decreased the total organic acid contents in buckwheat plants as compared with natural light condition. It was considered that blue light are less active than red light for the accumulation of organic acids. Tataric acid was detected only in the leaves of buckwheat plants, however, as treated with red and blue light, it was not detected in the leaves of Clfa 39. Flowers of Yangjul-maemil contained a considerable amount of rutin and quercitrin. Only small amount of quercitrin was detected in leaves, but it was not detected in stems. On the other hand, Clfa 39 leaves contained a considerable amount of rutin, quercetin and small amount of quercitrin, but quercitrin and quercetin were detected only in the stems of Clfa 39. Red and blue lights significantly decreased the contents of rutin, quercitrin, and quercetin in buckwheat plants as comparing with natural light condition. Rutin content in the flowers of Clfa 39 was increased under the red and blue light conditions.

Keywords : Blue light, Buckwheat, Organic acids, Quercetin, Quercitrin, Red light, Rutin, Sugars

Light is one of the major environmental factors which affect the growth and development of crops. Many studies have demonstrated that the plant response and the light intensity are closely related each other.

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The spectral region of most significance to plant development consists of blue, red, and far-red radiation (Tomasko, 1993). In fact, the relative amounts of red and far-red radiation, termed the R:Fr ratio, have assumed particular importance because of the effect on the photoequilibrum and biological activity of phytochrome. The chemical nature of the blue light photoreceptors is not established, and many studies consider it likely that more than one type of blue light receptor exists. Certainly, the response to blue light vary greatly in type, and include effects on endogenous rhythms, organ orientation, stem extension, stomatal opening and cytoplasmic streaming (Mohr & Schopfer, 1995; Hart, 1988). Many of the response to blue light also show a strong dependence on the amount of light (Hart, 1988).

The organic acids of the Krebs cycle like citric, *cis*-aconitic, isocitric, isoketoglutaric, succinic, fumaric, malic and oxalacetic acids have a major importance in the aerobic respiration harvesting substantial quantities of energy stored in ATP molecules. These acids are parts of a cyclic pathway consisting of chemical reactions that can be characterized as transformations of these acids into each other. The organic acid of Krebs cycle has additional roles in plant metabolic processes. Also the intermediates of the Krebs cycle are believed to play a role in the cation transport xylem vessels (Mohr & Schopfer, 1995).

Phenolic compounds include the anthocyanins and flavonoids are known as substances which are a light sensitive and their synthesis is triggered by light, and their concentrations are related to various light intensity and quality (Amrhein, 1979; Harper *et al.*, 1970; Hart, 1988; Lewis *et al.*, 1998; Mohr & Schopfer, 1995). It was reported that short irradiations with red light lead to increase markedly in phenylalanine ammonialyase (PAL) levels, which are probably mediated through the phytochrome system (Smith & Attridge, 1970), and the incorporation of ^{14}C-phenylalanine into the flavonoids is markedly stimulated by red light in a phytochrome-type response pattern (Harper *et al.*, 1970). Lewis *et al.* (1998) reported that although some potato cultivars produced anthocyanin in the dark, light enhanced the production of anthocyanins, other flavonoids and phenolic acids in all cultivars of potato have been studied. However, a little information is available on organic acids, free sugars, flavonoids and their analogues synthesis in buckwheat plants.

In this study, we studied the qualitative and quantitative distribution of organic acids, free sugars, flavonoids and their analogues such as rutin, quercitrin and their aglycon quercetin in buckwheat plants as treated with different wavelength of lights.

MATERIALS AND METHODS

Plant growth

Two buckwheat varieties, Yangjul-maemil (*Fagopyrum esculentum*) and Clfa 39 (*Fagopyrum tataricum*), were planted in the growth chambers that were specially fabricated to filter the natural light. Commercial colored acetate film was employed as a light filters in order to refine the spectral composition of light and served as the sources of blue (380 nm<λ<490) and red light (600 nm<λ<700), respectively. The spectra of natural, red and blue light were measured by a spectroradiometer (LI-1800, USA.).

Sample preparation for analysis

Leaves, stems, and flowers of Buckwheat plants were randomly collected at the flowering stage, and immediately frozen at -70°C deep-freezer, and there after freeze dried to prepare the samples. The freeze dried buckwheat plant tissues were milled about 100 mesh with disk mill for the analysis of organic acids, free sugars, and flavonoids.

Analysis of organic acids and sugars

Organic acid and free sugar contents were determined according to the previously described methods (Kim *et al.*, 2000) with Waters Ion-Exclusion column (7.8 × 300 mm) and Supelcosil LC-NH_2 column (4 × 250 mm), respectively.

20 ml of distilled water was added to the 0.5 g of freeze dried and milled buckwheat plants and shaken for 20 minutes at 35°C water-bath, centrifuged at 15,000 rpm and collected supernatant was filtered with Sep-Pak NH_2 cartridge, then 20 μl was injected on HPLC. Organic acids were analyzed by using Shimadzu SPD-7AV HPLC instrument (Japan) and free sugars were analyzed by using Waters 410 R.I. Detector (USA), respectively.

Analysis of rutin and its analogues

Rutin and its analogues were extracted with methanol and analyzed by reversed-phase HPLC (Waters 996 PAD and Waters 2690 Alliance system, USA.), as described before (Kim *et al*, 1998), using a H_2O/MeOH linear gradient from 20% to 80% MeOH in 35 min, a flow rate of 0.2 ml/min, and detected at 340 nm. Each solvent contained 0.1% H_3PO_4. Analysis were performed with a used XTerra MS C_{18}, 3.5 μm column (2.1 × 150 mm).

RESULTS AND DISCUSSION

Spectra of natural, red, and blue light

The spectra of natural, red, and blue light applied in this experiment were measured by a spectroradiometer. Different types of light filters have been frequently used in experimentation with radiation energy. These methods have been applied to plant research, particularly in PAR (photosynthetically active radiation, λ400-700 nm) investigation. As shown in the spectra of Fig. 1, each light filtering treatment showed their own specific photon spectral distributions. These results indicate the fact that commercial acetate films were effectively filtered the red (λ455-500 nm) and blue light (λ620-700 nm) regions.

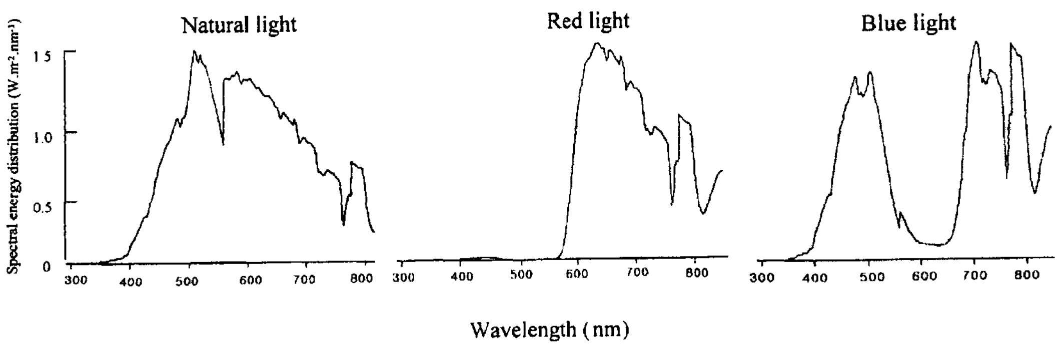

Fig. 1. The spectra of natural, red, and blue light measured by a spectroradiometer.

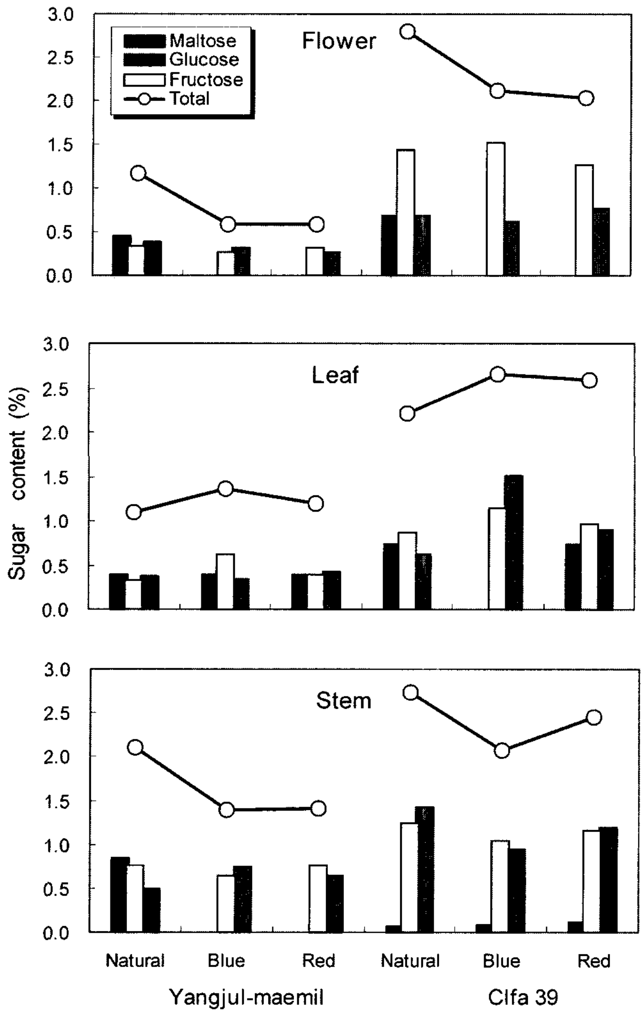

Fig. 2. Effect of natural, blue, and red light on the accumulation of free sugars in buckwheat plants at flowering.

Free sugars and organic acids

Fig. 2 shows the free sugar contents in the flower, leaf and stem of buckwheat plants. One of the interesting responses to the light treatments was the different pattern of free sugars in the buckwheat plants.

The major free sugars in buckwheat plants were fructose, glucose, and maltose, but, as treated with the different wave length of lights, their contents and compositions were changed in the buckwheat plants. The dominant soluble carbohydrates in the plants have been reported as sucrose, glucose, fructose, maltose, and polysaccharides (Drew, 1983). Kim *et al.* (1998) reported that buckwheat grains contained the sucrose and maltose as a major free sugar, but in this study sucrose was not detected in buckwheat plants.

Free sugar contents in the different tissues of Clfa 39 were higher than those of Yangjul-maemil regardless of light treatments. As treated with red and blue light, the free sugar contents in the leaves of buckwheat plants were slightly increased than those of natural light condition. But their contents in the stems and flowers of buckwheat plants were lower than those of natural light condition. Under the natural light condition, maltose was detected in the every tissues of buckwheat plants, however, as treated with blue and red light, it was not detected in the flowers of both buckwheat species and in the stems of Yangjul-maemil. In general, maltose appears in plants tissues at very low levels as a product of starch degradation. This process is intensified in photosynthetic tissues during dark periods, whereas when illuminated, maltose is almost undetectable in the plastids (Avigad, 1993). This experiment results were different from other

Table 1. Effect of natural, blue, and red light on the concentration of organic acids in buckwheat plants at flowering. (unit : mg/100 g)

Varieties	Organic acids	Leaf			Stem			Flower		
		NL†	BL	RL	NL	BL	RL	NL	BL	RL
Yangjul -maemil	Citric	30.5	26.3	28.5	0.9	0.5	0.8	5.9	4.2	5.0
	Tataric	5.7	6.6	6.7	-	-	-	-	-	-
	Malic	83.3	56.1	79.3	12.1	9.3	12.6	12.2	6.3	9.0
	Shikimic	1.0	1.4	1.5	0.1	0.1	0.1	0.4	0.2	0.3
	Glutaric	7.5	0.8	0.3	3.3	1.9	1.7	3.0	0.7	1.2
	Acetic	38.0	24.6	30.6	10.0	9.6	7.9	7.5	3.1	5.5
	Total	166.0	115.8	146.9	26.4	47.8	23.1	29.0	14.5	21.0
Clfa 39	Citric	38.2	6.5	16.9	1.5	4.2	6.4	16.6	5.2	9.3
	Tataric	3.5	-	-	-	-	-	-	-	-
	Malic	27.6	0.7	18.2	17.1	18.7	22.5	20.2	13.9	17.2
	Shikimic	1.0	3.3	0.3	0.1	0.1	1.0	0.6	0.2	0.3
	Glutaric	6.0	15.3	6.6	2.7	8.9	4.0	11.2	6.6	12.0
	Acetic	52.9	17.0	18.0	8.4	14.7	28.7	42.2	38.3	43.2
	Total	129.2	42.8	60.0	29.8	46.6	62.6	90.8	64.2	82.0

†NL : Natural light, BL : Blue light, RL : Red light.

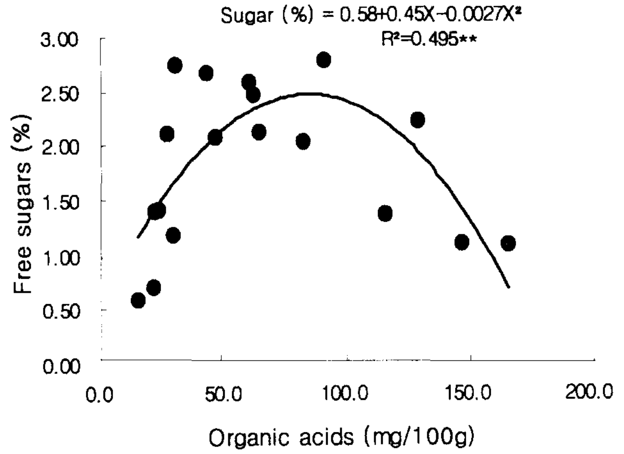

Fig. 3. Relationship between organic acids and free sugars in buckwheat plants at flowering.

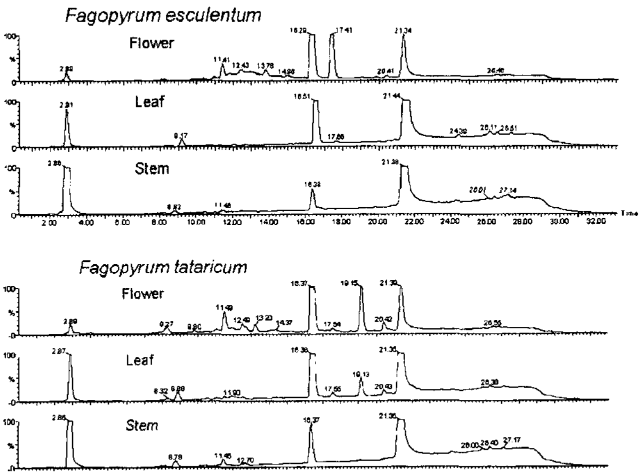

Fig. 5. Comparision on HPLC chromatograms of flavonoids between *Fagopyrum esculentum* (Yangjul-maemil) and *Fagopyrum tataricum* (Clfa 39).

plants, thus it was considered that the physiological responses of buckwheat plants are need to study further.

Table 1 shows the organic acid contents in the flower, leaf and stem of buckwheat plants at flowering stage. Citric, malic, and acetic acid were detected as the major organic acids in buckwheat plants. Their content and composition were various according to buckwheat species and plant tissues. Organic acid contents in the leaves of Yangjul-maemil were higher, but in stems and flowers were lower than those of Clfa 39. It was an interesting result that organic acid contents in the flower of Clfa 39 were approximately three times higher than those of Yangjul-maemil.

Organic acids are actively involved in the tricarboxylic acid (TCA) cycle and other metabolic mechanism in green plants. Red and blue light treatment decreased the total organic acid contents in buckwheat plants except the contents in the stems of Clfa 39 as comparing with natural light condition, and it was considered that blue light are less active than red light for the production of organic acids. Tataric acid was detected only in the leaves of buckwheat plants, however, as treated with red and blue light, it was not detected in the leaves of Clfa 39. Tataricum species are commonly cultivated in cold areas where common buckwheat species cannot be grown. For this reason, physiological response of Clfa 39, one of the tataricum species, was much different as comparing with Yangjul-maemil, one of the common buckwheat species.

As shown in Fig. 3, organic acids of buckwheat plants are significantly related with the free sugars, and their quadratic equation are sugar (%)=0.58+0.45X-0.0027X^2 (R^2=0.495**). Free sugar is a kind of soluble carbohydrate and carbohydrate metabolism has a close relationship with TCA cycle, sugars are exported from the chloroplast into the cytoplasm mainly *via* triose-phosphate. A phosphate translocator transports triose-phosphate, in exchange with inorganic phosphate, through the inner chloroplast envelope membrane to the outside, and eventually product the carbohydrates. On the other hand, carbohydrates are degraded into the pyruvate and acetate is formed from pyruvate by oxidative decarboxylation, and eventually introduced into the TCA cycle (Mohr & Schopfer, 1995). For these reason, it was considered that blue or red light are less efficient to

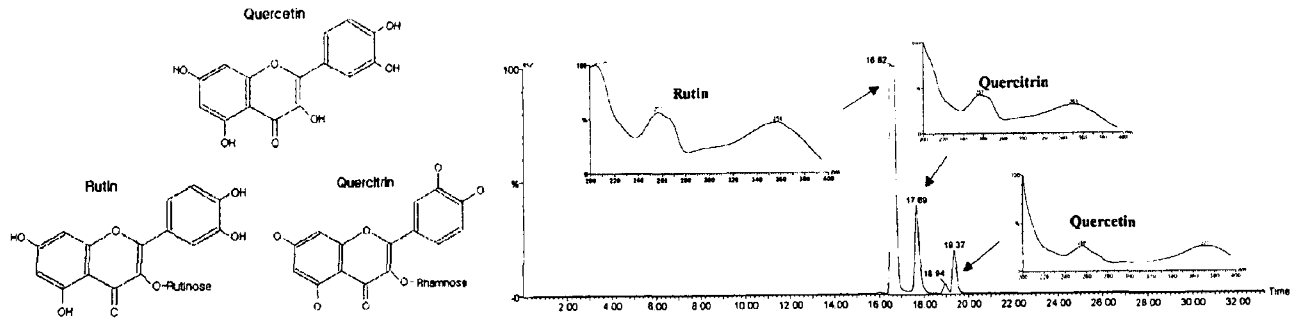

Fig. 4. Chemical structures and HPLC chromatograms of rutin, quercitrin, and quercetin.

Table 2. Effect of natural, blue, and red light on the accumulation of rutin and its analogues in buckwheat plants at flowering. (unit : %)

Varieties	Flavonoids	Leaf			Stem			Flower		
		NL†	BL	RL	NL	BL	RL	NL	BL	RL
Yangjul-maemil	Rutin	1.16	0.90	0.55	0.18	0.28	0.13	3.80	2.43	2.12
	Quercitrin	0.03	0.02	0.02	-	-	-	1.33	1.15	1.27
	Quercetin	-	-	-	-	-	-	-	-	-
Clfa 39	Rutin	0.58	0.19	0.23	0.22	0.19	0.23	1.12	2.22	1.20
	Quercitrin	0.06	0.03	0.02	-	tr	-	0.02	tr	0.01
	Quercetin	0.72	0.06	0.56	tr	tr	-	1.23	0.63	1.17

†NL : Natural light, BL : Blue light, RL : Red light, tr : Traced.

product the carbohydrate in buckwheat plants, and thus organic acids which are producted from the respiratory breakdown of carbohydrate were much lower than natural light condition.

Rutin and its analogues in buckwheat plants

Fig. 4 shows the chemical structures and standard chromatogram of rutin, quercitrin and quercetin. Fig. 5 shows the HPLC chromatogram of flavonoids in Yangjul-maemil (*Fagopyrum esculentum*) and Clfa 39 (*Fagopyrum tataricum*), respectively.

Buckwheat is known as an origin plant of rutin that is a kind of flavonol glycoside compounds used as hypertensive drug, vasculoprotector, and against circulatory disorders because it controls blood vessel and stabilizing high blood pressure (Couch *et al.*, 1946; Havsteen, 1983; Quettier-Deleu *et al.*, 2000).

As shown in Fig. 5, the flowers of Yangjul-maemil contain a considerable amount of rutin and quercitrin. However only small amount of quercitrin was detected in leaves, but not detected in stems. On the other hand, Claf 39 contained a considerable amount of rutin, quercetin and small amount of quercitrin. But, quercitrin and quercetin were detected only in the stem of Clfa 39. The rutin content in buckwheat plants depends on the stage of development, the plant organs and buckwheat species. Rutin is more concentrated in the young leaves than in the old ones. Hagels *et al.* (1995) reported that high amounts of rutin were found during the blossoming phase and the level of rutin was influenced by sowing time, sowing density and nitrogen fertilization. Flavonoid is known as a substance that is a light sensitive and its synthesis is triggered by light. Smith & Attridge (1970) reported that red light irradiation on *Pisum sativum* lead to increases the activity of phenylalanine ammonialyase (PAL) that is closely incorporated with a phytochrome response. However, as shown in Table 2, red and blue light significantly decreased the contents of rutin, quercitrin and quercetin in the whole buckwheat plant tissues as comparing with those of natural light condition. Smith & Harper (1970) reported that continuous illumination with red, blue and white light on *Pisum sativum* affected the contents of flavonoids such as kaempferol-3-triglucoside, kaempferol-3-*p*-coumaroyltriglucoside and quercetin-3-*p*-coumaroyltriglucoside, and these flavonoids were more increased under white light treatment than blue and red light. In contrast to the previous report (Smith & Harper, 1970), rutin content in the flower of Clfa 39 was increased as treated with red and blue light. These results explain the fact that physiological responses of tataricum species are much different as comparing with those of common buckwheat species when they have cultivated at mild temperature and different light condition.

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