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Germination process increases phytochemicals in corn

[1]Chalorcharoenying, W., [2]Lomthaisong, K., [1,3]*Suriharn, B. and [1,3]Lertrat, K.

[1]*Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand*
[2]*Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand*
[3]*Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand*

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Abstract

Sprouts and seedlings increase nutritional value of seeds as they increase phytochemicals that are beneficial to health. Corn sprouts and seedlings can be consumed as germinated grains, vegetable and used as food additive. The objective of this study was to compare of phytochemical compounds from seeds, sprouts and seedlings of four small ear waxy corns, three waxy corns, three field corn, three sweet corns and three glutinous rice cultivars. sprouts and seedlings increased carotenoid content, gamma amino butyric acid (GABA) content, total phenolic content and total anthocyanin content, and the highest increases in these phytochemicals were found in seedlings. The levels of carotenoid content, GABA content, total phenolic content and total anthocyanin content among corn genotypes were low in seeds and sprouts but very high in seedlings. The correlation coefficients among Total anthocyanin content, carotenoid GABA and total phenolic content were high and significant ($P \leq 0.01$), ranging from $r=0.711$ to $r=0.871$. The results suggested that field corn is most suitable for germination at seedling stage in order to obtain the highest nutritional values. The utilization of sprouts and seedlings as vegetable and food additive is discussed.

Introduction

Germination process improves nutritional value of grains in many crops (Chavan and Kadam, 1989). Sprouted seeds and seedlings contain higher protein, vitamins, sugar, minerals and nutrient content than normal grains (Lorenz, 1980; Khalil *et al.*, 2007). In rice, phytochemical compounds such as gamma amino butyric acid (GABA), vitamin B1, total phenolic and total anthocyanin significantly increased during germination process (Khampang *et al.*, 2009; Chatsuwan and Areekul. 2010; Vongsudin et al., 2011). These phytochemicals have several health benefits such as reductions in the risk of cardiovascular disease, cancer, diabetes and aging, and they are also effective in controlling blood pressure, preventing the clogging and hardening of the arteries, reducing obesity and promoting better health for consumers (Hu *et al.*, 2003; Tsuda *et al.*, 2003; Jones, 2005).

Utilization of germinated seeds could be both at sprouted stage and seedling stage. In rice, hulled grains are germinated, dried and cooked alone or with normal rice to improve nutritional value. In other crop species such as beans, peanut and sunflower, the sprouted seeds are consumed as fresh or cooked vegetables. Another utilization is to make flour from both sprouted seeds and seedlings, and the flour provides both nutritional value and dietary fiber.

Ample information is available for the merit of germinated seeds that can improve nutritional value of the seeds in many crop species, and sprouted seeds have been extensively utilized. However, this information is not available for different types of corn. Which types of corn and germination stages are suitable for germinated seed production is still not clear.

Several distinct types of specialty corns are available (popcorn, sweet corn, high-protein quality corn, high waxy corn, high oil corn, etc.) (Hallauer, 2001.), and they are also different in kernel colors. The kernel colors would be white, brown, yellow, red, purple and black. Environmental conditions also affect the amount of this pigment, phytochemical compounds in corn kernels and seeds of other cereals (Adom and Liu, 2002). However, the information on the changes in phytochemicals during germination process in different types of corn is lacking. The

*Corresponding author.
Email: *bsuriharn@gmail.com*

Table 1. Corn and rice varieties/cultivars used in this study

Varieties/cultivars	Type of corn	Kernel color	Source
Tein Kaow	Small ear waxy corn	White	Khon Kaen University
Tein Luang	Small ear waxy corn	Yellow	Khon Kaen University
Tein Salubsee	Small ear waxy corn	Yellow and white	Khon Kaen University
Tein Line	Small ear waxy corn	Yellow, white and violet	Khon Kaen University
Samlee-Esaan	Waxy corn	White	Khon Kaen University
Khao Niew Khao kam	Waxy corn	Violet	Khon Kaen University
KKU-WX111031	Waxy corn	Violet	Khon Kaen University
Field corn check 1	Field corn	Yellow	Pacific Seeds Company Limited
Suwan 1	Field corn	Yellow	National Corn and Sorghum Research Center
Suwan 2	Field corn	Yellow	National Corn and Sorghum Research Center
Wandokkoon	Sweet corn	Yellow	Khon Kaen University
Sweet corn check 1	Sweet corn	Yellow and white	Syngenta Seeds Company Limited
Sweet corn check 2	Sweet corn	Yellow and white	Chia Tai Company Limited
Khao Maled Phai	Glutinous rice	Violet	Khon Kaen University
Khao Niew Dum Mong	Glutinous rice	Violet	Khon Kaen University
Khao Jao Hawm Nin	Rice	Violet	Kasetsart University

objective of this study was to evaluate phytochemicals in different types of corn at seed, sprouted and seedling stages. The information obtained from this study is useful for selection of suitable corn types and germination stages for production of sprouted corn.

Materials and Methods

Thirteen corn varieties including four small ear waxy corns, three waxy corns, three field corns and three sweet corns were used in this study, and three black rice cultivars were also used for comparison. The corn varieties used in this study were representatives of white, yellow and purple kernel types, and the details for these varieties are presented in (Table 1).

Sample preparation

Dry and well-filled seeds of corn and rice were used in the experiment. The seeds were soaked in water for 6 hours and germinated on moist filter paper in plastic trays at ambient temperature in the dark until shoot tips were about 0.5-1 mm in length or 24 hours. The seeds that germinated completely were cleaned and planted in sterilized sand for 7 days until the seedlings had green leaves. The sprouted seeds and the seedlings (Figure 1) were stored in liquid nitrogen to block the enzymatic activities at -20ºC until the samples were freeze-dried. Then the samples were ready for analysis.

Sample extraction

Sample extraction was carried out according to the modified method of Hu and Xu (2011). Briefly, 50 mg for each of the powder of seeds, sprouted seeds and seedlings was loaded in a flask of 250 ml containing 40 ml of methanol. Hydrochloric of 1% was added onto the flask and the sample was mixed thoroughly. The flask and the mixture was shaken stirrer (Diligent, Thailand) for two hours at

Figure 1. (A) dry kernels, (B) germinated kernels (24 hours), and (C) seedlings (7 days)

ambient temperature and in the absence of light. The homogenates were then centrifuged for 15 minutes in a centrifuge (Universal 30 RF) at 4,000 g and 4ºC. After centrifugation, the supernatants was filtered through Whatman #1 filter paper, evaporated under vacuum at 40ºC, and the resulting precipitates were re-suspended in 10 mL of 1% HCl/MeOH solvents (1 vol of 12.1 M HCl in 100 vol of methanol). The extracts of supernatant fluid were stored at -20ºC in the dark until further analysis for total anthocyanins and total phenolics.

Total anthocyanin content analysis

Total anthocyanin was determined using the spectrophotometric method as described by Hu and Xu (2011). Absorbance of appropriately diluted extracts at 535 nm was immediately measured to detect anthocyanins. Anthocyanin levels were expressed as milligrams of cyanidin 3-glucoside equivalents (CGE) per 100 g of dry weight (DW), using the reported molar extinction coefficient of 25 965 M^{-1} cm^{-1} and a molecular weight of 449.2 g/mol.

Total phenolics content analysis

The total phenolic content was determined based on the Folin-Ciocalteau colorimetric method as described by Hu and Xu (2011). Briefly, the extracted sample of 0.5 ml was mixed with 2.5 ml of distilled water and 0.5 ml of 1.0 M Folin – Ciocalteau phenol reagent. After incubation for 8 min, 1.5 ml of 7.5% sodium carbonate was added, mixed well and stored in the dark for two hours at room temperature. The samples were then vortexed, and the absorbance was measured at 765 nm using a UV spectrophotometer (Genesys 10S UV-VIS; Thermo scientific). Methanol was used as the blank, and gallic acid (GA) was used for calibration of standard curve (0-500mg/L). Phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per 100 g on dry matter basis (DM).

Carotenoid content analysis

Analysis of carotenoids was carried out according to the modified method of Schaub *et al.* (2004). The samples of seeds, sprouted seeds and seedlings were ground into very fine powder. The powder samples of 0.5 g each were loaded into 15ml blue cap plastic tubes (Falcon tube). EtOH:BHT of 6 ml was added into each tube, and the samples were mixed thoroughly by vortexing.

The samples were then incubated at 85°C in a water bath for 6 minutes, and, after incubation for 3 minutes, the samples were vortexed for 10 seconds. Care must be taken to make sure that the caps were not closed tightly during heating, and the caps were closed tightly during vortexing. KOH of 120 µl (1g/ml H_2O, prepared fresh daily) was added into the tubes and the samples were vortexed thoroughly for 20 seconds.

For saponification, the samples were later Incubated for 5 minutes at 85ºC, vortexed for 10 seconds and further incubated for 5 minutes at 85ºC. After incubation, the samples were allowed to cool in ice bath. Distilled water (H_2O) of 4ml and PE:DE (2+1,v,v of 3 ml as added into the tubes. The samples were shaken or vortexed and centrifuged for 10 minutes at 1400g. Phase separation of the samples was visible.

The upper phase of each tube was transfer to a new 15 ml tube. Care must be taken not to transfer all portion of upper phase, and it is better to leave a little portion for recovering in the next steps. The recovering of upper phase was repeated two times. The organic epiphases from three extraction times were mixed together in the new tubes. This should produce approximately a total of 8-9 ml of the extract. The supernatants of each sample were added with 10 ml of PE:DE (2+1,v,v) and mixed well. The optical density (O.D.) was then measured at 450nm in a spectrophotometer with the appropriate blank (PE:DE (2+1,v,v) and Lambert-Beer's equation was used for calculation of carotenoids.

GABA content analysis

GABA content was determined using method described previously (Kitaoka and Nakano, 1969) with a minor modification. The powder samples of 1.5 g of corn and rice (seeds, sprouted seeds and seedlings) were extracted in 30 ml of 80% ethanol. The tubes were vortexed to mix the powder with ethanol. The samples were then filtered through a number 1 filter paper and dried in vacuum at 40ºC. The resulting precipitates were resuspended in 3 ml of water.

In order to determine GABA content, the sample extracts of 0.2 ml were added with 0.2 ml of borate buffer (0.2 mol boric acid 50 ml with 0.2 mol sodium borate 59 ml); 1ml of 6% phenol. The mixtures were allowed to cool at room temperature. After cooling, the samples were then added with 0.4 ml of 7.5% NaOCl and mixed well. The samples were heated in a hot water bath for 10 minutes, and they were cooled immediately in an ice bath for 5 minutes. The absorbance was measured at 630 nm using a UV spectrophotometer. GABA content was then compared with the graphs of the standard solution. The standard curve was constructed by GABA at concentrations of 0, 50, 100, 150, 200, 250, 300,350, 400, 450 500 mg/I) and the concentrations of standard samples were calculated using the standard curve equation;

$$y=0.0009x-0.0091,$$

where x is the concentration of GABA, y for the 630 nm visible light absorbance, R^2=0.983 derived GABA content.

Statistical analysis

The data of three replications were analyzed statistically according to a completely randomized design. Where main effect was significant, least significant differences (LSD) at 0.05 probability level was used to compare means. Correlation coefficients among carotenoid content, GABA content, total phenolic content and total anthocyanin content were computed to determine the relationships among these phytochemicals.

Results

Significant differences ($P \leq 0.01$) among germination stages were observed, and germination stage was the largest source of variations for carotenoid content, GABA content, total phenolic content and total anthocyanin content (Table 2). Significant differences ($P \leq 0.01$) among varieties were observed

Table 2. Mean squares for carotenoid content, GABA content, total phenolic content and total anthocyanin content of different corn types and rice evaluated at three germination stages (seed, sprout and seedling)

Source of variation	df	Carotenoid content	GABA content	Total phenolic content	Total anthocyanin content
Germination stage (S)	2	11013.2**	0.2518**	142.568**	42.276**
Variety (V)	15	132.7**	0.00262**	0.858**	0.9501**
S×V	30	122.6**	0.00545**	0.707**	0.5573**
Error	64	2.2	0.00001	0.174	0.0489

** Significant at 0.01 probability level

Table 3. Carotenoid content, GABA content, total phenolic content and anthocyanin content of different corn types and rice evaluated at seed stage, sprout stage and seedling stage

Variety	Carotenoid (mg/g)			GABA (mg/100g)			Total phenolic (mg/100g)			Anthocyanin (mg/g)		
	Seeds	Sprouts	Seedlings	Seed	Sprouts	Seedlings	Seeds	Sprouts	Seedlings	Seeds	Sprouts	Seedlings
Corn												
Tein Kaow	0.008	0.018	15.093	0.030	0.028	0.231	0.126	0.186	3.568	0.049	0.091	2.302
Tein Luang	0.087	0.156	10.253	0.016	0.022	0.145	0.130	0.205	3.396	0.045	0.166	2.024
Tein Salubsee	0.065	0.097	10.347	0.023	0.020	0.199	0.123	0.200	3.358	0.052	0.113	2.129
Tein Line	0.065	0.086	19.160	0.028	0.021	0.202	0.081	0.213	4.034	0.094	0.190	2.053
Samlee-Esaan	0.025	0.032	23.653	0.026	0.040	0.212	0.162	0.247	3.537	0.052	0.051	1.704
Khao Niew Khao kam	0.025	0.033	29.693	0.021	0.033	0.224	0.234	0.221	3.445	0.151	0.682	2.165
KKU-WX111031	0.491	0.658	33.707	0.038	0.053	0.209	0.004	0.006	3.400	1.471	1.051	2.070
Field corn check 1	0.531	0.708	30.373	0.038	0.036	0.232	0.129	0.147	2.842	0.059	0.045	1.917
Suwan 1	0.390	0.602	30.053	0.066	0.038	0.173	0.145	0.151	3.697	0.113	0.115	1.858
Suwan 2	0.384	0.675	34.760	0.047	0.031	0.150	0.105	0.148	1.359	0.098	0.130	1.097
Wandokkoon	0.241	0.398	37.627	0.035	0.090	0.092	0.003	0.006	3.527	0.068	0.069	1.841
Sweet corn check 1	0.341	0.522	51.560	0.028	0.041	0.184	0.004	0.008	3.725	0.082	0.127	2.219
Sweet corn check 2	0.160	0.286	37.013	0.033	0.027	0.164	0.005	0.007	3.287	0.068	0.059	1.879
Mean	0.216	0.329	27.946	0.033	0.037	0.186	0.096	0.134	3.321	0.185	0.222	1.943
Rice												
Khao Maled Phai	0.029	0.048	23.133	0.026	0.068	0.110	0.002	0.006	3.096	0.669	1.048	1.834
Khao Niew Dum Mong	0.024	0.028	20.880	0.021	0.053	0.034	0.003	0.007	1.106	1.089	1.231	0.826
Khao Jao Hawm Nin	0.050	0.044	16.120	0.064	0.085	0.056	0.003	0.005	1.894	0.944	0.447	3.443
Mean	0.034	0.040	20.044	0.037	0.069	0.067	0.003	0.006	2.032	0.901	0.909	2.034
Overall mean	0.182	0.274	26.464	0.034	0.043	0.164	0.079	0.110	3.079	0.319	0.351	1.960
$LSD_{(0.05)}$	0.112	0.012	4.277	0.002	0.003	0.011	0.040	0.038	1.196	0.278	0.141	0.653
C.V.(%)	37.13	2.55	9.72	3.27	3.79	3.99	31.23	42.71	23.35	52.47	24.15	20.01

** Significant at 0.01 probability level

for carotenoid content, GABA content, total phenolic content and total anthocyanin content. The significant interactions (P≤0.01) between germination stage and varieties were observed for carotenoid content, GABA content, total phenolic content and total anthocyanin content. Although differences among varieties and the interactions between variety and germination stage were significant, these variations contributed much smaller portion to total variations in carotenoid content, GABA content, total phenolic content and total anthocyanin content compared to germination stage. As differences in germination stages for these phytochemicals were much higher than differences in genotypes, germination stages should be considered for use of colored corn and rice for functional food products.

In comparison among seeds, sprouts and seedlings for overall means of corn and rice, sprouts were slightly higher than seeds for carotenoid content (0.274 and 0.182 mg/g), GABA content (0.043 and 0.034 mg/100g), total phenolic content (0.110 and 0.079 mg/100g) and anthocyanin content (0.351 and 0.319 mg/g), but they were significantly much lower than seedlings (26.464 mg/g, 0.164 mg/100g, 3.079 mg/100g and 1.960 mg/100g for carotenoid content, GABA content, total phenolic content and anthocyanin content, respectively) (Table 3). The results indicated that increases in carotenoid content, GABA content, total phenolic content and anthocyanin content were low at sprout stage but exceptionally high and significant at seedling stage.

Significant differences among corn genotypes and rice genotypes were found for caroteniod content, GABA content, total phenolic content and anthocyanin content in seeds, sprouts and seedlings. Carotenoid content values ranged from 0.008 to 0.531 mg/g in seeds, 0.018 to 0.708 mg/g in sprouts and 10.253 to 51.560 mg/g in seedlings. In general, corn genotypes with yellow kernels had the highest carotenoid contents in seeds, sprouts and seedlings with the ranges of 0.160 to 0.531, 0.296 to 0.708 and 30.053 to 51.560 mg/g, respectively, compared to corn genotypes and rice genotypes with white endosperm. These corn genotypes with high carotenoid content included KKU-WX111031, field corn check 1, Suwan 1, Suwan 2, Wandokkoon, Sweet corn check 1 and

Table 4. Correlation coefficients among carotenoid, GABA, total phenolic content (TPC) and total anthocyanin content (TAC)

	TAC	Carotenoid	GABA
Carotenoid	0.711**		
GABA	0.741**	0.769**	
TPC	0.771**	0.837**	0.871**

** significant at 0.01 probability level

Sweet corn check 2. It is interesting to note here that KKU-WX111031 with waxy endosperm and purple kernels was also had high carotenoid content.

GABA contents ranging from 0.016 to 0.066, 0.020 to 0.090 and 0.034 to 0.232 mg/100g in seeds, sprouts and seedlings, respectively, were observed among corn genotypes and rice genotypes. Suwan 1 had the highest GABA content (0.066 mg/100g) at seed stage. Wandokkoon had the highest GABA content (0.090 mg/100g) at sprout stage, and Field corn check 1 had the highest GABA content (0.232 mg/100g) at seedling stage. It is important to note here that, although GABA content increased with sprouts and seedlings, GABA contents at seed stage, sprout stage and seedling stage were not related.

Total phenolic content values ranging from 0.002 to 0.234, 0.005 to 0.247 and 1.106 to 4.034 mg/100g in seeds, sprouts and seedlings, respectively, were observed among corn genotypes and rice genotypes. Khao Niew Khao Kam had the highest total phenolic content (0.234 mg/100g) at seed stage. Samlee-Esaan had the highest total phenolic content (0.247 mg/100g) at sprout stage, and Tien Line had the highest phenolic content (4.034 mg/100g) at seedling stage.

Anthocyanin content values ranging from 0.045 to 1.471, 0.059 to 1.231 and 0.526 to 3.443 mg/100g in seeds, sprouts and seedlings, respectively, were observed among corn genotypes and rice genotypes. KKU-WX111031 had the highest anthocyanin content (1.471 mg/100g) at seed stage. Rice variety Khao Niew Dum Mong had the highest anthocyanin content (1.231 mg/100g) at sprout stage followed by KKU-WX111031 (1.051 mg/g). Khao Jaw Hawm Nin had the highest anthocyanin content (3.443 mg/100g) at seedling stage followed by sweet corn check 1 (2.219 mg/100g). Three rice varieties had high anthocyanin content at all stages compared to corn varieties.

Correlation coefficients among total anthocyanin content, carotenoid content, GABA content and total phenolic content were significant ($P \leq 0.01$), ranging from r=0.71 for total anthocyanin content and carotenoid content to r=0.871 for GABA content and total phenolic content (Table 4). Total anthocyanin was significantly correlated with GABA (r=0.741) and total phenolic content (r=0.771), whereas carotenoid was also significantly correlated with GABA (0.769) and total phenolic content (r=0.871).

Discussion

The problem of food security is increasingly important worldwide. Improvement of food quality and diversification of food utilization is an important means to increase food security when food is in shortage caused by natural disasters such as flooding and storms. In this situation, corn can be an important item in disaster rescue bags, and the victims can consume corn kernels immediately as grains and germinate corn kernels for sprouts or seedlings.

In this study, very low increases in carotenoid content, GABA content, total phenolic content and total anthocyanin content were found in sprouts of corn and rice. However, very high increases in these phytochemicals were observed in seedlings. Germination of corn at seedling stage is therefore very interesting for utilization than germination at sprout stage. Higher carotenoid content in seedlings than in seeds and sprouts indicated that carotenoid was newly synthesized in seedlings, and corn seedlings is the good source for this phytochemical.

In previous investigation in rice, the germination process significantly ($p<0.05$) increased GABA content, total phenolic content and antioxidant capacity, and there was little change in the early stage of germination and then a marked increase after 36 hours. For the GABA content after soaking, it gradually increased from 80 to 220 mg/100 g embryo fresh weight from 12 to 60 hours (Maisont and Narkrugsa, 2010). In another study, the highest GABA content was obtained when the rice was germinated for 36 hours and 48 hours, depending on rice varieties, and the GABA content in germinated brown rice increased 9.43-16.74 times compared to non=germinated brown rice (Banchuen *et al*., 2010). Similar increase in GABA content from germination process in rice were also reported in other studies (Saikusa *et al.*, 1994a, 1994b; Varanyanond, 2005; Sasagawa *et al*., 2006; Watchraparpaiboon, 2007). Appropriate germination is the germination stages that provide the highest GABA content and temperature, moisture and oxygen are involved in the germination process (Duangpatra, 1986).

Khumkah *et al*. (2009) reported that GABA content at seedling stage was significantly lower than that at sprout stage. The results were similar to our results for Khao Niew Dum Mong and Khao Jao Hawm Nin, which slightly decreased GABA content, but different from Khao Maled Phai, which increased in GABA content. For corn, however, most genotypes increased GABA content at seedling stage. GABA with antioxidant activity can be increased in corn and rice seeds during germination process and many enzymes are involved in synthesis of GABA (Lopez-Amoros *et al.*, 2006; Ngyuen and Ooraikul, 2008; Watchararparpaiboon *et al*., 2010).

For phenolic content, Khumkah *et al.* (2009) found in rice that germinated seeds had higher total phenolic content than non-germinated seeds, and the similar results were also reported in edible seeds of 13 species (Cevallos-Casals and Cisneros-Zevallos, 2009). Our results were in agreement with previous results and also added new information that corn seedlings had higher phenolic content than sprouts.

Anthocyanin content was similar to total phenolic content which increased remarkably at seedling stage. The anthocyanin content in grains is found in the area tissue and an outer layer of tissue in (Abdel-Aal *et al.*, 2006). However, the increase in anthocyanin content at seedling stage compared to seed stage and sprout stage indicated that anthocyanin was newly-synthesized in seedlings.

Differences among genotypes were low, but germination stage contributed to large variations for these phytochemicals. However, corn genotypes with yellow endosperm had the highest carotenoid content, and corn genotypes with purple kernels had the highest anthocyanin content, which were similar to black glutinous rice cultivars. When corn is consumed as vegetable, sweet corn and small ear waxy corn are very useful.

Although seedlings had the highest phytochemicals, whole corn seedling powder can be used as food additive to increase nutrition values in food, and grain maize is most suitable for these purposes. In this study, corn seedlings are a good source of bioactive compounds in our diet. The utilization of corn seedlings for food ingredient with health benefit is a means to increase value-added price in corn as production of corn seedlings is simple and not expensive. Corn seedling is a good source of diet fiber.

Conclusion

This research compared different types of corn and purple glutinous rice varieties at seed stage, sprout stage and seedling stage for carotenoid content, GABA content, total phenolic content and total anthocyanin content. Germination at sprout stage slightly increased these phytochemicals. However, very high increases in these phytochemicals were found at seedling stage in corn and rice. Therefore, corn and rice seedlings can be used as food additive to improve nutritional values in food. Grain maize is the most suitable for this purpose.

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