蕎麥芸香苷之研究

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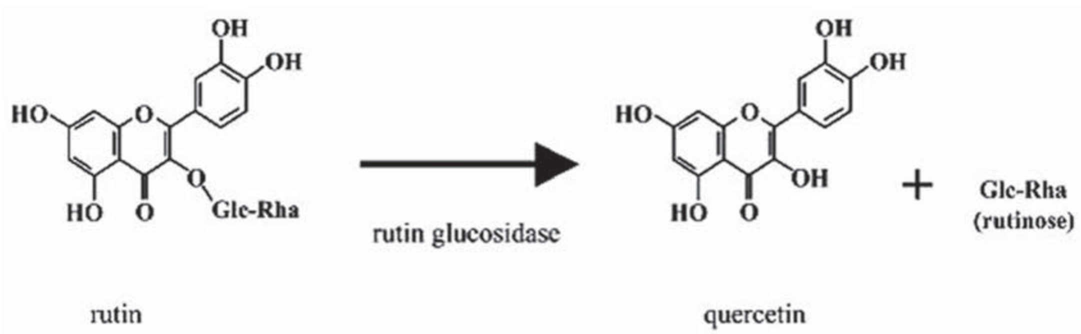
前言

芸香苷(*rutin*)是一種類黃酮(flavonoid)配醣體成分，最初是由Weiss(1842)從芸香(*rue*, *Ruta graveolens*)中所分離出來的成分。在普通蕎麥(common buckwheat, *Fagopyrum esculentum* Moench)以及韃靼蕎麥(tatary buckwheat, *Fagopyrum tataricum*)中發現大量的芸香苷的存在(Couch *et al*., 1946; Kitabayashi *et at*., 1995)。

蕎麥被認為是擷取芸香苷的主要飲食來源(Oomah *et al*., 1996)，芸香苷主要的研究是應用在對降低人類的高血壓、血管出血性等相關疾病及微血管之修補作用，其具有良好的保健功效(Matsubara *et al*., 1985)。

芸香苷為槲皮素(quercetin)的糖苷的其中一種，即芸香苷會透過芸香苷葡糖苷酶活性(rutin glucosidase activity)經過水解作用後形成槲皮素(Fig. 1)，所以，芸香苷含量在蕎麥的生長期間會有增減的變化(Suzuki *et al*., 2005)。

本文將探討芸香苷在蕎麥生長時其含量之表現，在不同蕎麥品種間的差異性，還有芸香苷含量與蕎麥農藝性狀間的相關性。



(Suzuki *et al*., 2005)

內容

一、芸香苷含量的差異性及相關性

蕎麥全株均含有芸香苷，包含其莖、葉、花、種子，Gupta *et al*.(2011)將蕎麥的生長期劃分成9個時期，並針對不同時期調查該時期之代表樣品，檢測其芸香苷含量之變化(Fig. 2)，在其研究中，蕎麥生長期中，第1時期之蕎麥芽的芸香苷含量稍高，到長出第一對葉子(第2時期)會下降，之後隨著蕎麥的生長，芸香苷的含量會增加，直到盛花期(第6時期)芸香苷含量達最高，之後在種子形成及充實期(第7、8時期)再下降，最後種子成熟時(第9時期)，芸香苷含量會再提升(Fig. 3)。

	Growth stage	Sample	Days after germination
Stage 1	Emergence	Whole plant	4
Stage 2	First pair of leaf formation	Whole plant	8
Stage 3	Bud show & leaf growth	Recently mature leaf (second)	15
Stage 4	Vegetative growth & leaf growth	Mature leaf	20
Stage 5	Flowering & no leaf growth	Mature leaf	30
Stage 6	Peak flowering	Inflorescence	40
Stage 7	Seed formation started	Immature seeds	52
Stage 8	Seeds are in the milk or dough stage	Immature seeds	62
Stage 9	filled seeds are brown (Leaves have a yellowish cast)	Mature brown seeds	75

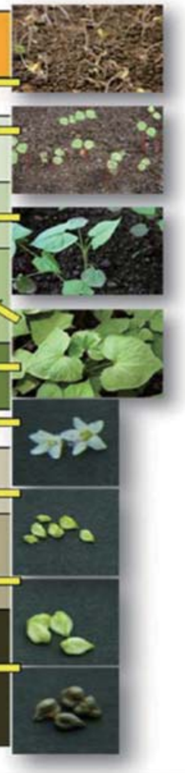


Fig. 2. The rutin glucosidase catalyzation of rutin to quercetin.

(Suzuki *et al*., 2005)

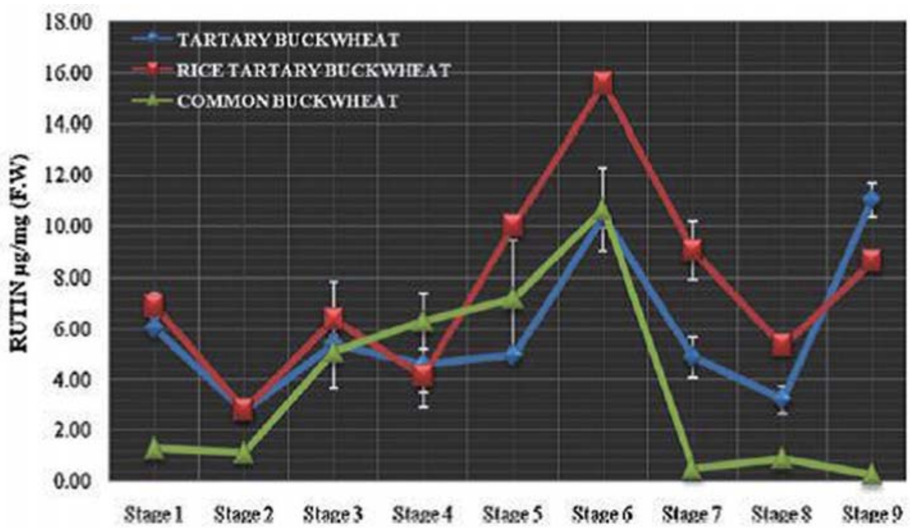


Fig. 3. Rutin content variation in different growth stages of Fagopyrum sp. Buckwheat accessions: Tartary Buckwheat (IC-14889), Rice Tartary Buckwheat (IC- 329457), Common Buckwheat (IC-5408858).

(Gupta *et al*., 2011)

除了蕎麥植株不同器官上的芸香苷含量會有差異性外，在蕎麥不同部位之葉片芸香苷含量也會顯現出差異性。Suzuki *et al*.(2005)針對不同部位的葉片進行芸香苷含量及芸香苷葡糖苷酶活性調查，發現在L7葉片的每葉所含之芸香苷含量最高，而每克乾重之芸香苷則在L8葉片上最高，顯示芸香苷含量在鮮葉上的含量最高，並隨著葉片的位置降低而下降(Fig. 4)。除了葉片位置不同其芸香苷含量會有差異外，在同一葉片上的不同部位的芸香苷含量亦不相同，葉片中上表皮部位的芸香苷含量最高，達53.1%，下表皮部位次之，葉肉部位之芸香苷含量最低(Table 1)，這是因為芸香苷含量與蕎麥遮蔽紫外線(ultraviolet, UV)照射有關。

Table 1. Relative distribution of flesh weight, rutin content and rutin glucosidase activity in different leaf discs cut out from tartary buckwheat leaf.

Type of leaf disc	Fresh weight (%)	Rutin content (%)	Rutin glucosidase activity (%)
Upper epidermis	27.1	53.1	13.6
Mesophyll	42.8	17.1	19.1
Lower epidermis	30.1	29.8	67.4

Data are means of two independent experiments.

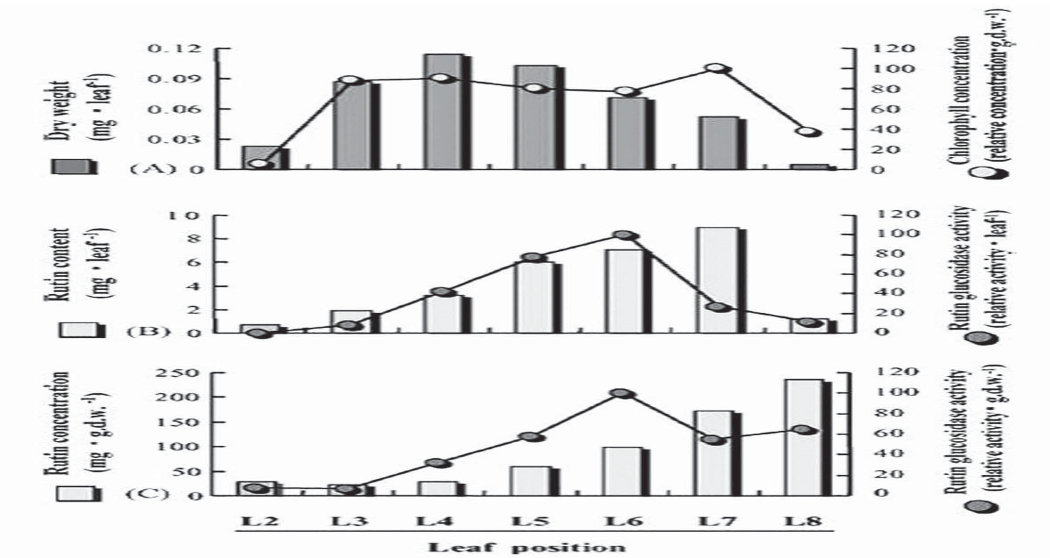


Fig. 4. Measurement of rutin concentration, rutin glucosidase activity, soluble protein concentration and chlorophyll concentration. Tartary buckwheat leaves in different leaf positions grown in the experimental field were harvested on 28 DAG (L2, senescent leaf; L3–L6, mature leaves; L7 and L8, young expanding leaves). Then, dry weight (A); rutin concentration (B and C); rutin glucosidase activity (L6 = 100) (B and C); and chlorophyll concentration (L7 = 100) (A) were measured (see Section 2). Data are means of two independent experiments. The two measurements did not differ by more than 16.9% (A and B (bar); C (bar)); and 29.2% (B (dot) and C (dot)).

(Suzuki *et al*., 2005)

除了在同一蕎麥植株上不同器官或不同部位之葉片，其所含芸香苷會有差異之外，在不同的蕎麥品種系上，其芸香苷含量也會有差異性，Kitabayshi *et al*.(1995)針對所收集之各地區蕎麥品種系進行芸香苷含量之分析，種子芸香苷含量在不同普通蕎麥品種(系)中有很大的變異性，從最高含量品系的35.9~12.6 (mg/100g)，種子芸香苷含量最高的尼泊爾品系Tatopani達35.9 (mg/100gDW)，要較歐洲栽培種Bogatyr的種子芸香苷含量12.6 (mg/100gDW)高3倍。除了種子芸香苷含量在不同品系中的變異性很大之外，葉子中芸香苷含量在不同的品種系中的變異性也很大，變異範圍從3600至1880 (mg/100gDW) (Table 2)。

Table 2. Mean values of rutin content in common buckwheat.

Cultivar or Strain		Rutin content	
		in seed (mg/100 gDW)	in leaf (mg/100 gDW)
Japanese cultivars (Diploid)			
Botan-Soba		16.4	2,730
Kitawase-Soba		18.9	2,160
Shinano-No.1		15.5	2,880
Hitachi-Akisoba		17.0	2,670
Kyushu-Akisoba		14.5	3,060
Japanese cultivars (Tetraploid)			
Hokkei-No.1		20.8	1,880
Shinshu-Ohsoba		20.0	3,300
Miyazaki-Ohtubu		22.1	2,750
Chinese strains			
Yunnan-1		19.4	2,700
Yunnan-2		21.3	1,950
Guizhou		16.9	2,240
Yulin		16.6	2,060
Taolin		15.5	2,170
Nepalese strains			
Matathati		34.9	2,820
Tatopani		35.9	3,190
Khinger		32.3	3,600
Jarkot-1		15.7	3,070
Jarkot-2		21.9	3,050
Chhenga		16.2	2,660
Muktinath		25.1	3,240
European cultivars			
Bogatyr'	(Russia)	12.6	2,880
Adja Tlacina	(Slovenija)	13.7	2,420
Sokurovskaja	(Slovenija)	14.5	2,320
LA HARPE	(France)	18.1	2,990
BRUSVILY	(France)	16.0	2,640
SAINT CONGARD	(France)	22.2	2,260
Local variety	(France)	18.8	2,270

(Kitabayashi *et al*., 1995)

同時，研究人員針對不同二倍體蕎麥品種系間的種子及葉子芸香苷含量及其主要農藝性狀進行變方分析，在葉子及種子的芸香苷含量中，不同的品種系之間達到極顯著差異，此外，芸香苷含量在兩個年度中也達顯著性差異，顯示出環境對芸香苷的含量具有顯著性的影響。在農藝性狀方面，第一朵開花期及千粒重在不同的品種系中也達極顯著差異，表示不同品種系中，其第一朵開花期及千粒重等兩個性狀的差異性很大。然而，在所有的性狀中(種子芸香苷含量、葉子芸香苷含量、第一朵開花期、千粒重及每株所含種子重)在不同的區域中均達極顯著差異，由此可發現不同的環境間的蕎麥品種系已適應其區域環境，進而產生不同區域性的差異(Table 3)。

Table 3. Analysis of varience for rutin content and main characters in common buckwheat.

Sources of variation		Degree of freedom	*F*-values					Formula of *F*-value
			Rutin content		Main characters			
			in seed	in leaf	Days to first flowering	1,000 seed weight	Seed weight /plant	
Year	(Y)	1	28.8*	45.8*	49.9*	246.3***	168.1***	MS_Y/MS_R
Block	(R)	2	0.4	3.2*	3.1	1.5	3.8*	MS_R/MS_E
Variety	(V)	23	5.7***	4.6***	21.6***	7.9***	1.4	$MS_V/MS_{Y\times V}$
Between areas		3	18.9***	19.4***	74.0***	39.4***	8.0***	
Within Japanese		4	0.4	2.8*	19.4***	3.4*	1.3	
Within Chinese		4	0.8	2.1	17.8***	3.3*	0.6	
Within Nepalese		6	10.0***	2.3	18.5***	1.0	0.1	
Within European		6	1.5	2.3	2.5	5.0**	0.1	
Y×V		23	2.3**	1.6	3.2***	3.4***	17.7***	$MS_{Y\times V}/MS_E$
Error	(E)	46						

*, **, *** : Significant at 5, 1 and 0.1 %, respectively.

$E[MS_Y]=\sigma^2_E+24\sigma^2_R+48\sigma^2_Y$, $E[MS_R]=\sigma^2_E+24\sigma^2_R$, $E[MS_V]=\sigma^2_E+4\sigma^2_V+2\sigma^2_{Y\times V}$, $E[MS_{Y\times V}]=\sigma^2_E+2\sigma^2_{Y\times V}$, $E[MS_E]=\sigma^2_E$

(Kitabayashi *et al*., 1995)

在調查了蕎麥的芸香苷含量及主要農藝性狀後，可以知道在不同的品種系間，芸香苷含量與農藝性狀均有顯著性的差異性。一般偵測蕎麥植體上芸香苷的含量，必須採集植體並進行化學成分分析，目前最常用的分析方法為高效液相層析儀(high-performance liquid chromatography, HPLC) (Fabjan *et al*., 2003)，利用成分分析方法得知蕎麥芸香苷含量，必須針對蕎麥植體進行破壞性檢測，且無法在田間栽培時就可迅速獲得訊息。然而，為了得知蕎麥植株各部位芸香苷的芸香苷訊息，而不需利用成分分析方法調查芸香苷的含量，可利用蕎麥農藝性狀與蕎麥芸香苷含量的相關性，間接的獲得芸香苷含量的資訊。Kitabayashi *et al*.(1995)為利用芸香苷含量與農藝性狀進行相關分析，種子中芸香苷含量及葉子芸香苷含量均與第一朵開花期有著顯著正相關，顯示蕎麥第一朵開花期會影響到蕎麥植株種子及葉子上的芸香苷含量。種子芸香苷含量與葉子芸香苷含量也有正相關的趨勢，顯示出種子芸香苷含量多寡與葉子芸香苷含量會有影響。然而，無論是種子芸香苷

含量或葉子芸香苷含量，均與千粒重及每株粒種有著負相關的趨勢，顯示無論是種子或葉子上的芸香苷含量均隨著種子粒重的增加而減少，即在種子逐漸充實成熟時，無論是種子芸香苷或是葉子芸香苷的含量均會降低(Table 4)。

Table 4. Correlation matrix among rutin content and main characters in common buckwheat in 1992 and 1993.

	1 000 seed weight	Seed weight /plant	Rutin content in seed	Rutin content in leaf
(1992)				
Days to 1st. flow.	−0.58**	−0.68***	0.61**	0.46*
1,000 seed wt.	—	0.59**	−0.24	−0.32
Seed wt. /plant		—	−0.48*	−0.45*
Rutin cont. in seed			—	0.36
(1993)				
Days to 1st. flow.	−0.49*	−0.13	0.69***	0.54**
1,000 seed wt.	—	0.60**	−0.52**	−0.44*
Seed wt. /plant		—	−0.41*	−0.02
Rutin cont. in seed			—	0.15

*, **, *** : Significant at 5, 1 and 0.1 %, respectively.

(Kitabayashi *et al.*, 1995)

二、芸香苷與抗氧化能力之關係

蕎麥為保健食物中抗氧化能力的來源作物之一，抗氧化能力為人類生活中一種重要的特性，許多包括抗突變、抗癌、抗老化等作用的主要特性均與抗氧化能力有關(Cook and Samman, 1996)，蕎麥中具抗氧化能力的物質，被認為是酚類次級代謝物質，如類黃酮、芸香苷、酚酸等物質等及其衍生物(Watanabe *et al.*, 1997)。Holasova et al.(2002)利用燕麥(oats)、大麥(barley)及蕎麥進行抗氧化能力測定，在相同劑量的情況下(20% wt.)，蕎麥葉片的抗氧化能力(protection factor, PF=8.0)最大，接著依序為蕎麥種子(PF=4.0)、蕎麥去殼種子(PF=3.1)、大麥(PF=2.2)、燕麥(PF=1.8)、蕎麥種子殼(PF=1.8)及蕎麥莖稈(PF=1.3)，可明顯看出蕎麥的抗氧化能力遠大於大麥及燕麥(Table 5)。此外，其更進一步針對燕麥、大麥、蕎麥種子、蕎麥去殼種子及蕎麥葉片來檢測其總酚(total phenolics)含量、芸香苷含量、母生育酚(tocol)含量，並估計其相對的抗氧化能力(Table 6)。在其試驗中可發現，抗氧化能力的高低，與總酚含量及芸香苷含量有相關，蕎麥葉片的總酚含量(39,514mg/kg)及芸香苷含量(23,443 mg/kg)為遠高於其他材料，其抗氧化能力(PF=8)亦遠高於其他材料，可證明總酚含量、芸香苷含量及母生育酚含量均與抗氧化能力之高低有關。即總酚含量、芸香苷含量及母生育酚含量越高，其抗氧化能力就越強(Table 7)。

Table 5. Antioxidant activity of oats, barley and buckwheat seed and plant parts.

Tested material	Addition (% wt.)	Protection factor[a]
Oats	20	1.8
Barley	20	2.2
Buckwheat seeds	10	2.0
Buckwheat seeds	20	2.6
Buckwheat seeds	40	4.0
Buckwheat seeds dehulled	20	3.1
Buckwheat straws	20	1.3
Buckwheat hulls	20	1.8
Buckwheat leaves	1	1.6
Buckwheat leaves	5	3.6
Buckwheat leaves	10	4.5
Buckwheat leaves	15	5.3
Buckwheat leaves	20	8.0

[a] Each value is based on triplicate independent model samples.

(Holasova *et al.*, 2002)

Table 6. Relationship between antioxidant activites an total phenolics, rutin and tocol contens.

Tested material	Total phenolics[a] (mg/kg DM)	Rutin[a] (mg/kg DM)	Tocols[a] (mg/kg DM)	Protection factor
Oats	1138	<0.1	19.0	1.8
Barley	2168	<0.1	45.4	2.2
Buckwheat seeds	3303	178	36.2	2.6
Buckwheat seeds dehulled	3903	184	20.3	3.0
Buckwheat leaves	39514	23443	104.7	8.0

[a] Each value is based on two independent analysis:
Protection factor = 1.5291×10^{-4}[total phenolics] + 1.990, $R^2=0.987$, $P<0.002$;
Protection factor = $2.4012.10^{-4}$ [rutin] + 2.377, $R^2=0.972$, $P<0.002$;
Protection factor = $6.749.10^{-2}$ [tocols] + 0.4747, $R^2=0.867$, $P>0.61$.

(Holasova *et al.*, 2002)

此外，Morishita et al.(2007)針對普通蕎麥及韃靼蕎麥進行抗氧化能力之分析，Table 8為針對兩個普通蕎麥品種及兩個韃靼蕎麥品種進行抗氧化能力檢測之結果，其中韃靼蕎麥的抗氧化能力遠大於普通蕎麥，除抗氧化能力外，韃靼蕎麥的芸香苷含量亦遠大於普通蕎麥(Table 9)，這顯示抗氧化能力可能與芸香苷含量有明

顯的關係存在。因此，再進一步進行檢測四個品種中總酚含量、芸香苷含量等成分之抗氧化能力，顯示2個普通蕎麥品種的抗氧化能力大部分由未知成分所貢獻，而2個韃靼蕎麥的抗氧化能力則由芸香苷所貢獻，亦即芸香苷含量越高，其抗氧化能力越高(Fig. 9.)，這與Holasova et al.(2002)所進行的試驗結果(Table 7)是一致的。

Table 7. Antioxidative activities in common buckwheat and tatary buckwheat varieties.

[Common buckwheat]	
Hitachi akisoba	16.4 ± 0.6
Kanto No.1	15.3 ± 0.7
[Tartary buckwheat]	
Rotundatum	52.9 ± 0.8
Pontivy	57.4 ± 1.6

Unit : μmol-Trolox g^{-1} DW.
3 rep. AVG ± SD.

(Morishita *et al.*, 2007)

Table 8. Various polyphenol contents in common buckwheat and Tatary buckwheat grains.

Variety	(-)-Epicatechin	(-)-Epicatecingallate	Rutin	Quercitrin	Quercetin
Hitachi akisoba	20.2 ± 0.7	2.4 ± 0.1	13.6 ± 1.2	—	—
Kanto No.1	15.6 ± 2.3	1.3 ± 0.1	12.2 ± 0.8	—	—
	ns	**	ns		
Rotundatum	—	—	1808.7 ± 34.2	95.4 ± 4.5	2.0 ± 0.4
Pontivy	—	—	1853.8 ± 26.3	81.2 ± 3.8	2.4 ± 0.5
			ns	*	ns

3 rep. Avg ± SD.(mg $100g^{-1}$ DW).
* and ** indicate significant difference between varieties at 0.05 and 0.01 by t-test, respectively.
ns indicates not significant.

(Morishita *et al.*, 2007)

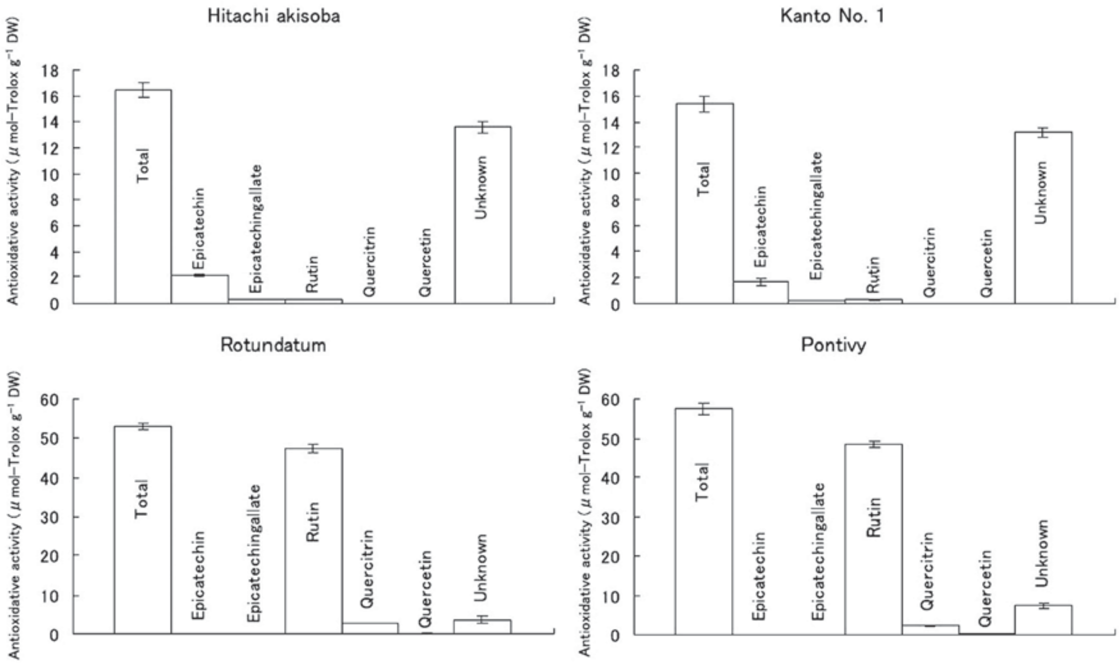


Fig. 5. Total antioxidative activity and contributions of each polyphenol in common buckwheat and Tatary buckwheat varieties.

(Morishita *et al*., 2007)

結論

韃靼種蕎麥的芸香苷含量遠高於普通種蕎麥，同植株上不同器官或不同位置葉片之芸香苷會有差異，且不同品種的蕎麥其芸香苷含量也會有差異性之存在，而蕎麥的芸香苷含量均與蕎麥之部分農藝性狀有相關性，芸香苷含量隨著第一朵花開花期增長而增加，但卻隨著種子重量的增加而降低。芸香苷含量的多寡，會影響到蕎麥的抗氧化能力，芸香苷的含量越多，其抗氧化能力越高。蕎麥所含芸香苷的含量，有助於人體的保健作用，藉由了解影響芸香苷多寡原因及其應用，可有助於提高蕎麥芸香苷育種作業。

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