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Variation of Pigments in the Bulbs of Shallot (*Allium cepa* var. *ascalonicum*) and *Allium*×*wakegi*

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Variation of the content of anthocyanins and flavonols in the accessions of shallot and *A.*× *wakegi* collected in Indonesia, Japan and Korea was investigated. Cyanidin was the dominant anthocyanins in both shallot and *A.*×*wakegi*. Peonidin was also detected in a small amount only in two shallot and one *A.*×*wakegi* accessions and in a trace amount in all other accessions. Only quercetin was detected as a flavonol and it distributed with wide variation in either species. *A.*×*wakegi* accessions showed significantly higher values of flavonol/anthocyanin than shallot accessions with the formulas of $Y=0.330+19.109X$ ($R=0.771$) and $Y=1.566+24.832X$ ($R=0.814$) for shallot and *A.*×*wakegi*, respectively, where Y is flavonol content and X is anthocyanin content.

INTRODUCTION

Shallot (*Allium cepa* var. *ascalonicum*; *A. cepa* Aggregatum group) is a very popular small–bulb onion cultivated widely in southeast Asian countries. *Allium*× *wakegi* is another small–bulb onion and it is an F_1 hybrid of Welsh onion (*Allium fistulosum*)×shallot (Tashiro, 1980). It is grown in Western Japan, Korea and some other Asian countries including Indonesia (Fujieda *et al.*, 1980; Inden and Asahira, 1990) although it is a minor crop in southeast Asian countries when compared with shallot. It is cultivated in some places being mixed with shallot without discrimination (Arifin and Okubo, 1996).

Like in common onion (*Allium cepa*) bulb colors in shallot vary from deep reddish purple to white through many shades of yellow and brown and they are very important to consumer preference. They can be also considered as a potential trait of classification in taxonomy (Clarke *et al.*, 1944; El–Shafie and Davis, 1967). The red and yellow colors in alliums are due to the presence of anthocyanins and flavonols, respectively. Fuleki (1971) has separated seven cyanidins and one peonidin from red onions, and Fenwick and Hanley (1990) have isolated quercetin from the outer skins of allium. Patil *et al.* (1995) found that the presence of quercetin was higher in the skins of colored onions than in white onion cultivars.

The role of pigments is suggested not only to control the bulb color but also to influence storability and disease resistance of the plants. Hurst *et al.* (1985) reported that white onions showed higher losses in the skin weight than yellow ones. Saxena *et al.* (1974) showed that red cultivars have a higher storage potential in comparison with

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yellow and white. Clarke *et al.* (1944) found that colored onion bulbs are highly resistant to the onion smudge organism, *Colletotrichum circinans* (Berk.) Vogl., whereas white bulbs tend to be more susceptible. In pungent cultivars, the whites suffered considerably more from neck-rot disease caused by *Botrytis allii* than the colored ones (Owen *et al.*, 1950).

One of the cultivation problems of small bulb onions in the high humidity of tropical regions is the high intensity of pest and disease attack. The main diseases caused by *Colletotrichum gloesporiedes, Alternaria porri* and *Fusarium oxysporum*, and pests of aphids and thrips resulted yield losses of shallot production in Indonesia as reported by Permadi (1994). Farmers use chemicals in high intensity to control pest and diseases, and it causes environmental pollution and unsafe products for consumption. Combination of breeding for resistance and cultural measures can reduce or even eliminate the problems and improve the profitability of crop production to a large extent. Variation in the skin colors of collected accessions can be manipulated for breeding materials.

This study was subjected to clarify the variation of flavonoids in the accessions of shallot and *A.*×*wakegi* collected in Indonesia, Japan and Korea.

MATERIALS AND METHODS

Flavonoid status was analyzed in 15 accessions of shallot collected in Indonesia and 22 accessions of *A.*×*wakegi* collected in Indonesia (12 accessions), Japan (9 accessions) and Korea (one accession) which had different isozyme patterns in the previous study (Arifin and Okubo, 1996). They were propagated in the field of Kyushu University.

Bulb samples were extracted with 1% HCl methanol and the absorbance of the extracts was measured at 530 and 360 nm wavelengths of a spectrophotometer for anthocyanin and flavonol, respectively. Acid hydrolysis of the extracts was done with 2N HCl at 90°C for 80 min and HPLC analysis was run for separating the aglycones using a column packed with Cosmosil $5C_{18}$-AR with solvents of 4% H_3PO_4/CH_3CN=82/18 (25 min) and 4% H_3PO_4/CH_3CN=65/35 (20 min) for anthocyanidins and flavonol analyses, respectively with a flow rate of 1.0 $ml min^{-1}$. Identification of peaks was based on the anthocyanidins extracted from purple snapdragon for cyanidin, peony for peonidin and on flavonols from purchased standards of quercetin and kaempferol.

RESULTS AND DISCUSSION

Anthocyanins

Anthocyanidins of both shallot and *A.*×*wakegi* were very simple and they were dominated by cyanidin in all the samples (Tables 1 and 2) in agreement with previous studies in onion (Fuleki, 1971; Terahara and Yamaguchi, 1985). Peonidin was also detected in 6.2–11% of the total anthocyanidins only in two accessions of shallot 'Dien' and 'Medan Merah' and one *A.*×*wakegi* 'Ciledog -4', originated in Indonesia, whereas it was only a trace amount in all the other samples. It is well considerable that the red color of *A.*×*wakegi* was derived from the anthocyanins of shallot since there are no red pigmentation found in Welsh onion.

The mean anthocyanin content in shallot was 0.198 abs. with the range from 0.115 to

Table 1. Anthocyanidin and flavonol status in shallot.

Accession	Anthocyanin (Abs.)	Cyanidin (%)	Peonidin (%)	Flavonol (Abs.)	Flavonol / anthocyanin
Dien	0.286 a	86.66	11.08	3.176	19.835bc
Batu–2	0.269 ab	90.41	trace	6.485	24.339a
Siniang	0.254 ab	93.97	trace	5.840	18.870bc
Batu–1	0.249 ab	96.26	trace	6.106	19.364ab
Batu–3	0.246 ab	96.10	trace	6.160	25.172a
Batu–4	0.194 ab	94.03	trace	4.114	15.584bc
Kayutanduk–2	0.187 b	97.32	trace	3.956	20.754ab
Medan Merah	0.182 b	93.79	6.20	2.632	11.864c
Baraka–1	0.150 c	92.45	trace	3.240	21.599ab
Saruran–1	0.141 c	91.88	trace	2.427	16.978bc
Kepung	0.140 c	91.55	trace	2.757	19.633ab
Karang Tengah	0.135 c	93.18	trace	3.042	22.724ab
Manado	0.120 c	89.87	trace	2.958	25.011a
Majalengka–2	0.117 c	94.29	trace	2.952	24.787a
Cingkir	0.115 c	97.01	trace	2.322	20.103ab
Mean	0.186	93.25	–	3.878	20.441

Mean separation within columns by Duncan's multiple range test at 5% level.

0.286, while it was 0.102 abs. ranging from 0.049 to 0.239 in *A.×wakegi*. Seven accessions of shallot contained higher amount of anthocyanins than average among which six were from Java Island where those containing low amount of anthocyanins were also found as done in other places of the country (Table 1). Seven of nine Japanese accessions of *A.× wakegi* contained higher amount of anthocyanins than average although one accession from Indonesia 'Ciledog–4' exceptionally contained the highest amount of anthocyanins in all the accessions of *A.×wakegi* (Table 2). 'Dien' contained the highest amount of anthocyanins among shallot accessions. Shallot 'Dien' and *A.× wakegi* 'Ciledog–4' were the two of only three accessions that contained a detectable amount of peonidin, and they are grown geographically very closely to each other in Java Island. A Korean accession of *A.× wakegi*, 'Shunsen' contained lower amount of anthocyanins as other Indonesian accessions.

Significant difference of anthocyanin content between shallot and *A.×wakegi* was revealed by ANOVA (Table 3) although some accessions of shallot contained lower amount of anthocyanins than some *A.×wakegi* accessions.

Flavonol

Only quercetin was detected in either species in the present study although kaempferol was reported by Bilyk *et al.* (1984) to exist in red onion (Tables 1 and 2). Flavonol amount between shallot and *A.×wakegi* was not significantly different (Table 3), and there was a wide variation within species. Some shallots ('Batu–1', 'Batu–2' and 'Batu–3') and *A.×wakegi* ('Ciledog–4' and 'Yatsushiro') showed high content with more than 6 units abs., while other accessions had only around 2 units abs. Of seven accessions of shallot that contained higher amount of anthocyanins than average six accessions also

Table 2. Anthocyanidin and flavonol status in *A.* × *wakegi*.

Accession	Anthocyanin (Abs.)	Cyanidin (%)	Peonidin (%)	Flavonol (Abs.)	Flavonol / anthocyanin
Ciledog-4 (I)[z]	0.239a	91.40	6.79	7.599	32.035ab
Zairai (J)	0.180ab	93.89	trace	4.747	26.576b
Shirodane (J)	0.173ab	92.87	trace	5.769	26.203b
Kihara (J)	0.154bc	96.40	trace	5.295	26.626b
Teruma-2 (J)	0.147bcd	95.67	trace	4.564	30.504b
Yatsushiro (J)	0.124bcd	91.99	trace	6.686	54.270a
Uehara (J)	0.116bcde	95.82	trace	4.843	42.466ab
Karatsu (J)	0.107bcde	94.78	trace	4.571	65.141a
Amami (J)	0.101bcde	91.35	trace	4.772	42.819ab
Tonsewer (I)	0.100bcde	92.70	trace	3.924	39.154ab
Mojo (I)	0.093cde	93.45	trace	5.387	61.272ab
Alam Indah (I)	0.084de	95.51	trace	4.221	43.104ab
Saruran-7 (I)	0.084de	96.11	trace	2.926	34.368ab
Tongging-1 (I)	0.080de	94.32	trace	2.580	31.935ab
Wataes-3 (I)	0.073de	92.72	trace	3.715	46.425ab
Shunsen (K)	0.071de	97.12	trace	2.546	29.270b
Sinawungan (I)	0.071de	93.67	trace	2.788	39.822ab
Wonokrio-2 (I)	0.069e	95.48	trace	3.742	50.354ab
Panundaan (I)	0.068e	93.33	trace	1.916	27.897b
Wonokrio-1 (I)	0.066e	96.28	trace	3.950	59.342ab
Ginoza (J)	0.055f	94.77	trace	2.772	43.523ab
Saruran-5 (I)	0.049f	94.52	trace	2.342	48.409ab
Mean	0.105	94.55	–	4.166	40.978

[z] (I); collected in Indonesia, (J); collected in Japan and (K); collected in Korea.
Mean separation within columns by Duncan's multiple range test at 5% level.

Table 3. ANOVA of flavonoid content in shallot and *A.* × *wakegi*.

		df	SS	MS	F
Anthocyanin	Species	1	0.058	0.058	20.644**
	Error	35	0.099	0.00283	
	Total	36	0.157		
Flavonol	Species	1	0.741	0.741	0.342
	Error	35	75.874	2.168	
	Total	36	76.615		
Flavonol/anthocyanin	Species	1	3761.652	3761.652	41.708**
	Error	35	3156.622	90.189	
	Total	36	6918.274		

Significant difference P=0.01.

had higher amount of flavonols than average. Similarly to the tendency of anthocyanin content, Japanese accessions of *A.×wakegi* contained higher amount of flavonols than Indonesian accessions. Eight of nine Japanese *A.×wakegi* contained higher flavonols than average. The amount of flavonols in shallot and *A.×wakegi* is apparently correlated with that of anthocyanins as Patil *et al.* (1995) have already reported that the presence of quercetin was higher in the skins of colored onions than in white onion cultivars.

Flavonol / anthocyanin ratio

A.×wakegi accessions showed significantly higher values of flavonol/anthocyanin than shallot accessions, and the lowest value in *A.×wakegi* was still higher than the highest value in shallot (Tables 1–3).

The regression analysis revealed that there was strongly enough relation between these two compounds with the following formulas:

$Y=0.330+19.109\ X\ (R=0.771)$ and $Y=1.566+24.832\ X\ (R=0.814)$

for shallot and *A.×wakegi*, respectively, where Y is flavonol content and X is anthocyanin content (Fig. 1). These results may support the suggestion that *A.×wakegi* and shallot could be chemically separated, and also that they are still closely standing from one to another.

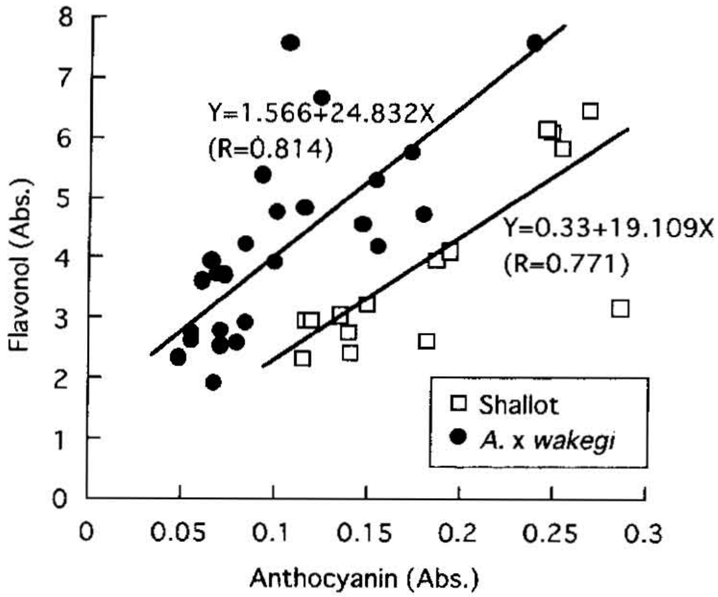

Fig. 1. Regression plots between anthocyanin and flavonol in shallot and *A.×wakegi*.

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