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Identification and Characterization of Foliar Polyphenolic Composition in Sweetpotato (*Ipomoea batatas* L.) Genotypes

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Trials over two years were conducted using 1389 sweetpotato (*Ipomoea batatas* L.) genotypes collected from all over the world to characterize the polyphenolic composition in sweetpotato leaves. Wide variation was observed in relation to their total and individual leaf polyphenolic constituents. In all genotypes studied, the total polyphenol contents of sweetpotato leaf ranged from 1.42 to 17.1 g/100 g dry weight. The six different polyphenolic compounds were identified and quantified by NMR, FABMS, and RPHPLC analysis procedures. This is the first report of polyphenolic compositions in sweetpotato leaves. The relative levels of polyphenolic acids in sweetpotato leaves were as follows: 3,5-di-*O*-caffeoylquinic acid > 4,5-di-*O*-caffeoylquinic acid > chlorogenic acid (3-*O*-caffeoylquinic acid) > 3,4-di-*O*-caffeoylquinic acid > 3,4,5-tri-*O*-caffeoylquinic acid > caffeic acid. The highest 3,4,5-tri-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid occurred at 221 and 1183.30 mg/100 g dry weight, respectively.

KEYWORDS: Sweetpotato leaf; chlorogenic acid; caffeic acid; 3,5-di-*O*-caffeoylquinic acid; 4,5-di-*O*-caffeoylquinic acid; 3,4-di-*O*-caffeoylquinic acid; 3,4,5-tri-*O*-caffeoylquinic acid

INTRODUCTION

The consumption of sweetpotato (*Ipomoea batatas* (L.) Lam; Convolvulaceae) greens as a fresh vegetable in many parts of the world (1–3) indicates that they are acceptable as edible vegetables like other traditional leafy vegetables. Sweetpotato leaves have the advantage of being harvested several times in a year, and the annual yield of sweetpotato leaves is therefore ultimately much higher than that of other green vegetables. Furthermore, as one of the few vegetables that can be grown easily during the monsoon seasons of the tropics, sweetpotato leaves are usually the only greens available in some countries after a flood or a typhoon. They are rich in vitamin B, iron, calcium, zinc, and protein, and are more tolerant of diseases, pests, and high moisture than many other leafy vegetables grown in the tropics (4–7). Our previous experiment revealed that sweetpotato leaves were an excellent source of antioxidative polyphenolics compared to other commercial vegetables (8).

The plant phenols, because of their diversity and extensive distribution, are the most important group of natural antioxidants, and they contribute to the organoleptic and nutritional qualities of fruit and vegetables. Polyphenols and phenolic compounds have attracted special attention because they may protect the

human body from oxidative stress, which can cause many diseases including cancer, aging, and cardiovascular diseases (9–18). The antimutagenic activity in the storage root of sweetpotato cultivars was mainly associated with phenolic compounds (19). The aim of the present study is to characterize and categorize the sweetpotato genotypes in relation to their foliar polyphenolic compositions, to clarify the chemical characteristics of the above polyphenols, and to clarify the relationship between the polyphenolic quality and quantity. To the best of our knowledge this is the first report of polyphenolic compositions in sweetpotato leaves.

MATERIALS AND METHODS

Plant Material and Cultural Methods. A total of 1389 sweetpotato cultivars, lines, and genotypes, collected from Japan and other parts of the world, were preserved in the gene bank of the National Agricultural Research Center of Kyushu Okinawa Region, Japan (NARCKO). The above materials were grown at the Department of Upland Farming Research of NARCKO, Miyazaki, Japan. Trials were carried out over two years during growing seasons 2000 and 2001. Only 850 genotypes were cultivated in 2000, but in 2001 all 1389 genotypes were used, including the first year's genotypes. The storage roots were planted on March 15 in different greenhouses, and they were grown with standard production practices. The average temperatures during the experimental period of 2001 were as follows: March 12 °C, April 18 °C, May 22 °C, and June 26 °C. Each entry consisted of 10 plants, grown in rows 1 m apart with 30 cm between plants. Three months after planting, 30

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to 40 leaves per genotype were harvested, washed gently, transferred into pre-labeled individual vinyl bags and immediately frozen at -85°C . The following day all the frozen samples were freeze-dried for 48 h in a vacuum freeze-dryer (model TR-PK-3-80, Trio Sciences Co., Ltd., Tokyo, Japan) with a plate temperature of $27-30^{\circ}\text{C}$. The freeze-dried samples were powdered by a blender and used for laboratory analysis.

Extraction and Measurement of Total Phenolics. Determinations of total polyphenols were made according to the Folin-Ciocalteu method with slight modification (20). The lyophilized sweetpotato leaf flour was vigorously mixed with 10 times its equivalent volume of 80% ethanol. The mixture was boiled for 5 min and centrifuged at $5000g$ for 10 min, and the supernatant was collected. The residue was mixed with additional 80% ethanol and boiled for 10 min to re-extract the phenolics, and centrifuged under the same conditions. The extracts were combined and made up to 10 mL and used for the measurement of total phenolics. The alcohol extract was diluted to obtain an absorbance reading within the range of the standards ($800-40\text{ }\mu\text{g}$ 3-*O*-caffeoylquinic acid/mL). The absorbance was measured at 600 nm with a dual-wavelength flying spot scanning densitometer (Shimadzu Co., Japan) with a microplate system. The results were expressed as g/100 g dry leaf powder.

Identification of Isolated Sweetpotato Leaves Phenolics. The dried leaves of sweetpotato (150 g) were extracted with MeOH ($2 \times 2\text{ L}$) at room temperature. The extract (17 g) was partitioned between benzene and water. The water layer (8 g) was chromatographed on MCI gel CHP20P (50 mm \times 35 mm i.d., $75-150\text{ }\mu$, styrene polymer, Mitsubishi Chemical Ind., Tokyo, Japan) eluted with water, 20% MeOH, 40% MeOH, 60% MeOH, 80% MeOH, and 100% MeOH successively. The 40% and 60% MeOH eluates were chromatographed on ODS (30–50 μ , Fuji Silisia Ltd., Nagoya, Japan; used gel vol. 25 mm \times 140 mm) (20–70% MeOH) to give caffeic acid (15 mg), 3-*O*-caffeoylquinic acid (400 mg), 3,4-di-*O*-caffeoylquinic acid (2 mg), 3,5-di-*O*-caffeoylquinic acid (60 mg), 4,5-di-*O*-caffeoylquinic acid (21 mg), and 3,4,5-tri-*O*-caffeoylquinic acid (2 mg). These compounds were identified by ^1H and FABMS spectra (21, 22). The NMR spectra were taken on a JEOL A-500 and FABMS was taken on a JEOL JMS-DX303HF spectrometer. The data were summarized as follows.

Caffeic Acid. ^1H NMR (CD_3OD) δ : 6.29 (1H, d, $J = 16\text{ Hz}$, caff C8–H), 6.85 (1H, d, $J = 8\text{ Hz}$, caff C5–H), 6.98 (1H, dd, $J = 2, 8\text{ Hz}$, caff C6–H), 7.10 (1H, d, $J = 2\text{ Hz}$, caff C2–H), 7.51 (1H, d, $J = 16\text{ Hz}$, caff C7–H).

3-*O*-Caffeoylquinic Acid. ^1H NMR (CD_3OD) δ : 2.03–2.13 (2H, m) and 2.16–2.28 (2H, m) [C2,6–H], 3.73 (1H, dd, $J = 3, 9\text{ Hz}$, C4–H), 4.17 (1H, d, $J = 4\text{ Hz}$, C5–H), 5.34 (1H, m, C3–H), 6.26 (1H, d, $J = 16\text{ Hz}$, caff C8–H), 6.78 (1H, d, $J = 8\text{ Hz}$, caff C5–H), 6.95 (1H, dd, $J = 2, 8\text{ Hz}$, caff C6–H), 7.05 (1H, d, $J = 2\text{ Hz}$, caff C2–H), 7.55 (1H, d, $J = 16\text{ Hz}$, caff C7–H).

3,4-di-*O*-Caffeoylquinic Acid. neg. FABMS m/z 515 $[\text{M}]^-$. ^1H NMR (CD_3OD) δ : 2.11 (1H, m) and 2.29 (3H, m) [C2,6–H], 4.36 (1H, br s, C5–H), 5.12 (1H, br d, $J = 3\text{ Hz}$, C4–H), 5.63 (1H, m, C3–H), 6.18 and 6.27 (each 1H, d, $J = 16\text{ Hz}$, caff C8–H \times 2), 6.73 and 6.74 (each 1H, d, $J = 8\text{ Hz}$, caff C5–H \times 2), 6.88 and 6.90 (each 1H, dd, $J = 2, 8\text{ Hz}$, caff C6–H \times 2), 7.00 and 7.03 (each 1H, d, $J = 2\text{ Hz}$, caff C2–H \times 2), 7.51 and 7.60 (each 1H, d, $J = 16\text{ Hz}$, caff C7–H \times 2).

3,5-di-*O*-Caffeoylquinic Acid. neg. FABMS m/z 515 $[\text{M}]^-$. ^1H NMR (CD_3OD) δ : 2.20 (3H, m) and 2.35 (1H, br d, $J = 7\text{ Hz}$) [C2,6–H], 3.97 (1H, dd, $J = 3, 9\text{ Hz}$, C4–H), 5.38 (1H, m, C3–H), 5.43 (1H, br s, C5–H), 6.26 and 6.36 (each 1H, d, $J = 16\text{ Hz}$, caff C8–H \times 2), 6.78 (2H, d, $J = 8\text{ Hz}$, caff C5–H \times 2), 6.96 and 6.97 (each 1H, dd, $J = 2, 8\text{ Hz}$, caff C6–H \times 2), 7.07 (2H, br s, caff C2–H \times 2), 7.51 and 7.60 (each 1H, d, $J = 16\text{ Hz}$, caff C7–H \times 2).

4,5-di-*O*-Caffeoylquinic Acid. neg. FABMS m/z 515 $[\text{M}]^-$. ^1H NMR (CD_3OD) δ : 2.12 (2H, m) and 2.21 (1H, br d, $J = 6\text{ Hz}$), 2.37 (1H, br d, $J = 7\text{ Hz}$) [C2,6–H], 4.35 (1H, m, C3–H), 5.00 (1H, dd, $J = 3, 9\text{ Hz}$, C4–H), 5.63 (1H, br s, C5–H), 6.25 and 6.28 (each 1H, d, $J = 16\text{ Hz}$, caff C8–H \times 2), 6.72 and 6.77 (each 1H, d, $J = 8\text{ Hz}$, caff C5–H \times 2), 6.86 and 6.91 (each 1H, dd, $J = 2, 8\text{ Hz}$, caff C6–H \times 2), 7.01 and 7.03 (each 1H, br s, caff C2–H \times 2), 7.54 and 7.57 (each 1H, d, $J = 16\text{ Hz}$, caff C7–H \times 2).

3,4,5-tri-*O*-Caffeoylquinic Acid. neg. FABMS m/z 677 $[\text{M}]^-$. ^1H NMR (CD_3OD) δ : 2.06–2.40 (3H, m), 2.47 (1H, br d, $J = 6\text{ Hz}$) [C2,6–H], 5.34 (1H, dd, $J = 3, 9\text{ Hz}$, C4–H), 5.69 (2H, m, C3,5–H), 6.22, 6.23 and 6.35 (each 1H, d, $J = 16\text{ Hz}$, caff C8–H \times 3), 6.72–6.81 (4H, m, caff C5–H \times 3, C6–H \times 1), 6.93 (2H, m, caff C6–H \times 2), 7.01, 7.05 and 7.09 (each 1H, br s, caff C2–H \times 3), 7.52, 7.55 and 7.62 (each 1H, d, $J = 16\text{ Hz}$, caff C7–H \times 3).

Quantification of Phenolic Acids by RPHPLC. The lyophilized sweetpotato leaf flour (50 mg) was vigorously mixed with 4 mL of ethanol in a centrifuge tube with cap. The mixture was boiled for 5 min and centrifuged at $3000g$ for 10 min. The supernatant was filtered through a cellulose acetate membrane filter (0.20 μm , Advantec, Japan) and used for analysis. A 5- μL portion of the filtrate was injected into the HPLC system and eluted as described below. The HPLC system consisted of two model LC-10AT pumps, a model SIL-10AXL autoinjector, a model CTO-10AC column oven, and a model SPD-M10AVP UV–Vis photodiode array (Shimadzu, Kyoto, Japan). The column was a 150 mm \times 4.6 mm i.d., 5 μm YMC-Pack ODS-AM AM-302 (YMC, Kyoto, Japan). The column oven temperature was set at 40°C . The mobile phase consisted of water containing 0.2% (v/v) formic acid (A) and methanol (B). Elution was performed with the linear gradient as follows: 2% B from 0 to 15 min, 2% to 45% B from 15 to 50 min, and 45% B from 50 to 65 min. The flow rate was 1 mL/min. The polyphenols were detected at 326 nm. The retention times of the polyphenolic compounds were compared with those of authentic reagents.

Chemicals. Chlorogenic acid (3-*O*-caffeoylquinic acid) was purchased from Sigma Chemical (St. Louis, MO). Caffeic acid, dimethyl sulfoxide, and other chemicals used were the highest grade available supplied by Wako Pure Chemicals Industries Ltd., Osaka, Japan. The purified ($>97\%$) 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, and 3,4,5-tri-*O*-caffeoylquinic acid were used as standards for HPLC analysis.

RESULTS AND DISCUSSION

Total Phenolics of the Sweetpotato Genotypes. Our recent studies revealed that sweetpotato leaves contained much higher content of total polyphenols than any other commercial vegetables including sweetpotato storage root and potato tubers (7, 8, 23, 24). Therefore, we analyzed and characterized the total leaf polyphenol content of all the 1389 genotypes in the year 2001, but in the year 2000 we analyzed 850 genotypes. The above genotypes were collected from all over the world, and are being retained in the gene bank of the National Agricultural Research Center for Kyushu Okinawa region, Japan. A highly significant ($P > 0.001$) liner correlation was found between the polyphenol contents of the genotypes from 2000 and the genotypes from 2001 ($r = 0.812$; $n = 700$). Furthermore, our preliminary data ($n = 10$) indicated that the highest polyphenol concentration was in sweetpotato leaves ($6.19 \pm 0.41\text{ g/100 g}$ dry powder), followed by petioles ($2.97 \pm 0.26\text{ g/100 g}$ dry powder), stems ($1.88 \pm 0.19\text{ g/100 g}$ dry powder), and finally the tubers ($<1.00\text{ g/100 g}$ dry powder), indicating that polyphenolic concentrations are organ dependent. The frequency distribution of total polyphenolics of the sweetpotato genotypes studied is shown in **Figure 1**. The highest mode of total polyphenol of all the genotypes was 6.00 with 5.56 mean and 5.50 median. The genotypes differed widely in their polyphenolic contents. The highest found was 17.1 g/100 g and the lowest was 1.42 g/100 g . Most of the genotypes (75%) contained $>6.00\text{ g/100 g}$ dry powder total polyphenolics, which was a very high concentration compared to that of any other commercial vegetables (7, 23, 24). The genotypes were classified into three categories according to their polyphenolic content, namely: high polyphenol accumulator (78 samples) ($>9\text{ g/100 g}$ dry powder); medium polyphenol accumulator (778) ($5-9$

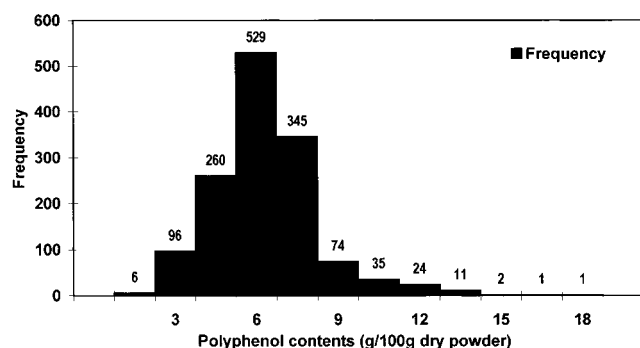


Figure 1. Frequency distribution of total polyphenol content (g/100 g dry powder) of 1389 sweetpotato genotypes.

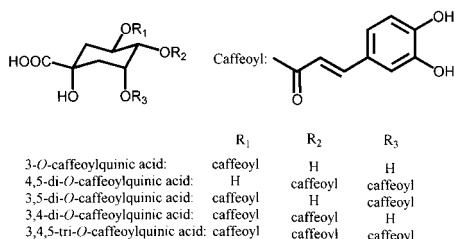


Figure 2. Chemical structures of polyphenolic compounds in sweetpotato leaves.

g/100 g dry powder); and low polyphenol accumulator (533) (<5 g/100 g dry powder). This result may be useful for various chemical breeding programs to improve desirable organoleptic and nutritional quality characteristics of crop plants.

Identification and Characterization of Individual Phenolic Acids. We selected sixty genotypes from the above three groups (twenty genotypes from each category) for the analysis of polyphenolic compositions. To the best of our knowledge, the present results are the first to show the composition of the phenolic acids in sweetpotato leaves. NMR, FABMS, and RPHPLC analysis procedures identified six different polyphenolic compounds in sweetpotato leaves. The chemical structures of these phenolic acids are shown in Figure 2. A typical HPLC chromatographic pattern of the phenolic acids of sweetpotato leaves is shown in Figure 3. All the HPLC profiles of the genotypes tested showed peaks at the same retention time, there being no qualitative difference among genotypes. However, the peak areas differed with genotypes. All the genotypes studied showed similar chromatographic patterns for the above phenolic acids. The results of this study revealed that the sweetpotato leaf phenolic profiles do vary according to genotypes, but proportions of the phenolic acids were relatively similar in all the genotypes studied. The phenolic acids are ubiquitous bioactive compounds found in plant foods and beverages (25–28). These phenolic compounds have received increased attention because of their potential antioxidant activities that may exert cardioprotective effects in humans (29), and it has been shown that intake of these compounds was inversely related to coronary heart disease mortality (30–32). Because polyphenol compounds show various physiological functions, sweetpotato leaves might also be expected to have physiologically active properties because they contain higher contents of polyphenolic compounds. The distribution of phenolic compounds of the 20 sweetpotato genotypes is presented in Table 1. Wide variation was observed in relation to individual phenolic compounds in sweetpotato leaves. In all genotypes studied, the caffeic acid, 3-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid, and 3,4,5-

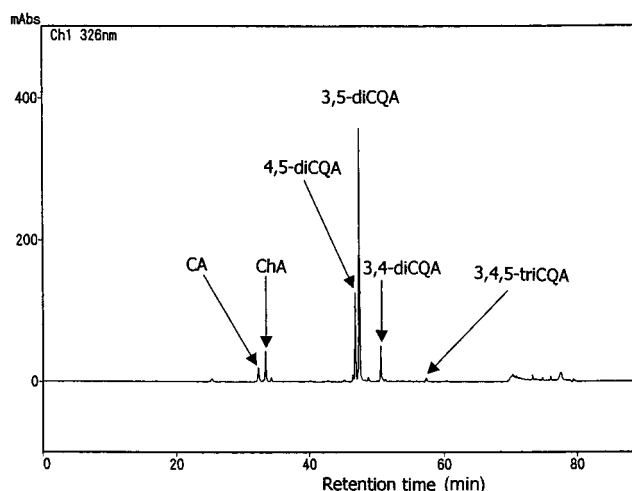


Figure 3. Reverse-phase HPLC (C₁₈) separation chromatograms for phenolic acids in sweetpotato leaf extracts: CA = Caffeic acid (32.27 min); ChA = Chlorogenic acid (33.42 min); 4,5-diCQA = 4,5-di-*O*-caffeoylquinic acid (46.72 min); 3,5-diCQA = 3,5-di-*O*-caffeoylquinic acid (47.34 min); 3,4-diCQA = 3,4-di-*O*-caffeoylquinic acid (50.55 min); 3,4,5-triCQA = 3,4,5-tri-*O*-caffeoylquinic acid (57.25 min).

Table 1. Individual Phenolic Compounds in the Leaves of Different Sweetpotato Genotypes (mg/100 g Dry Powder)

accession numbers	CA ^a	ChA	4,5-diCQA	3,5-diCQA	3,4-diCQA	3,4,5-triCQA
45000918	16.15	411.63	965.18	1062.34	143.00	79.25
45001029	11.36	427.19	916.91	2328.77	495.47	79.41
45003139	7.33	643.09	1037.18	2101.59	524.35	149.31
45001258	88.40	232.38	533.57	1677.50	744.94	15.84
45001284	80.50	365.00	1153.99	1814.80	286.51	142.05
45001311	179.65	265.32	735.68	3503.61	750.59	59.04
45001236	29.08	327.34	881.67	1652.37	316.61	110.45
45003192	23.05	392.49	670.09	1040.81	467.84	182.57
45006585	9.85	602.24	1046.48	2381.62	207.65	56.27
45006596	60.64	350.18	1047.38	1412.51	222.17	86.92
45006600	67.05	276.40	741.58	1876.42	112.73	23.71
45001419	17.80	510.73	666.92	1002.44	235.94	60.15
45001551	11.87	637.57	914.76	1497.19	359.11	65.90
45008741	16.76	701.47	1011.47	1280.73	369.68	50.00
45001622	21.51	334.06	1183.30	1514.05	230.71	220.95
45001933	23.36	657.12	315.38	2278.05	238.74	63.43
NARCKO-1	23.58	410.79	551.86	952.92	332.92	139.11
NARCKO-2	10.74	485.39	775.64	1260.83	364.96	60.69
NARCKO-3	26.28	454.58	813.48	1349.07	366.75	142.98
NARCKO-4	17.49	437.35	883.83	1619.28	247.28	64.85

^a CA = Caffeic acid; ChA = 3-*O*-caffeoyl quinic acid; 4,5-diCQA = 4,5-di-*O*-caffeoyl quinic acid; 3,5-diCQA = 3,5-di-*O*-caffeoyl quinic acid; 3,4-diCQA = 3,4-di-*O*-caffeoyl quinic acid; 3,4,5-triCQA = 3,4,5-tri-*O*-caffeoyl quinic acid.

tri-*O*-caffeoylquinic acid contents are in the following ranges, respectively: 7.33–179.65, 232.38–701.47, 315.38–1183.30, 952.92–3503.61, 112.73–750.59, and 15.84–220.95 mg/100 g dry powder. The results suggest that the main phenolic compound in sweetpotato leaves is 3,5-di-*O*-caffeoylquinic acid followed by 4,5-di-*O*-caffeoylquinic acid. The average level of polyphenolic compounds in leaf of 60 genotypes were in the following order: 3,5-di-*O*-caffeoylquinic acid (1528 mg/100 g dry powder) > 4,5-di-*O*-caffeoylquinic acid (706 mg/100 g dry powder) > 3-*O*-caffeoylquinic acid (332 mg/100 g dry powder) > 3,4-di-*O*-caffeoylquinic acid (277 mg/100 g dry powder) > 3,4,5-tri-*O*-caffeoylquinic acid (71 mg/100 g dry powder) > caffeic acid (63 mg/100 g dry powder). 3-*O*-Caffeoylquinic acid of sweetpotato leaves was much higher than that of sweetpotato storage root (23), potato tubers (24), and apple fruit (33), and

it has various physiological functions (33–36). Among other polyphenolic compounds, caffeic acid was the most effective inhibitor of tumor promotion in mice skin, and the 3-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid, which were extracted from steamed sweetpotato root, suppressed the melanogenesis equally (13, 15). Mahmood et al. (37) reported that the 3,4,5-tri-*O*-caffeoylquinic acid exhibited a greater selective inhibition of HIV replication than 4,5-di-*O*-caffeoylquinic acid and caffeic acid had only slight anti-HIV activity.

The present experimental results showed that sweetpotato leaf contains a very high concentration of biologically active polyphenolic compounds, which may have significant medicinal values for certain human diseases. These results may help in future breeding for specific constituents and enhance the content of antioxidant components available for consumption, which plays an important dietary role in human health.

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