Comparative Physicochemical Properties and Structure of Rice Containing the *sck* + *cryIAc* Genes and Its Nontransgenic Counterpart

X. LI, K. HUANG, B. ZHU, Z. LIANG, L. WEI, AND Y. LUO

ABSTRACT: The physicochemical properties and structure of an insect-resistant rice, Liangyou Kefeng Nr. 6 (IRR), containing the *sck* and *cryIAc* genes were compared with those of its nontransgenic counterpart designated as Liangyou 2186 (control), considering their key role in determining commercial value. Basically the appearance of IRR was not affected in terms of size and shape after foreign gene transformation but improved with lower chalkiness. The milling yield of IRR with lower chalkiness was higher measured by head rice yield compared with its parental control. The differences in appearance and milling quality were confirmed by the structure of raw rice grain by scanning electron microscopy (SEM). Slight differences were observed in pasting properties and textural quality determined by rapid viscosity analyzer and texture analyzer which was in agreement with the result of the structure of cooked rice grain by SEM. The above differences might be as a result of a positional effect of T-DNA insertion. On the whole, the appearance, milling quality, and eating quality of IRR were not adversely affected by transgenes, which will facilitate its acceptance by the consumer after commercialization.

Keywords: physicochemical properties, structure, transgenic rice

Introduction

Rice (*Oryza sativa* L.) is the most important staple crop on which half of the world's population depends as the source of their calories (Coffman and Juliano 1987). With the expanding world population, the pressure on rice production is increasing rapidly, which is intensified by the decreasing arable land, water, and other resources. Recent advances in rice biotechnology have opened up new avenues for production of rice varieties with agronomically important traits, including pest and disease control, abiotic stress resistance (drought, heat, cold, salt), and increased nutritional value (fatty acids, vitamins, and other micronutrients) (Bajaj and Mohanty 2005). The application of biotechnology in rice breeding offers a promising and effective method for the addition of specific characteristics, such as insect resistance, which either would be impossible or require much more time using conventional breeding technologies (Giri and Laxmi 2000).

The yield loss caused by insect damage is quite serious in China, estimated at more than 10% of total rice production was damaged per year (Zhu 2001). Despite tremendous efforts, endogenous genes for sufficient levels of resistance against a variety of insects have not been available (Schuler and others 1998). Genetic engineering of rice for insect resistance provides a potent, cost-effective, and environment friendly option. Several varieties of transgenic rice with the insect-resistant trait have been successfully developed (Xiang and others 1999; Zhu and others 1999; Zhu 2001; Qi and others 2007). One variety of transgenic insect-resistant rice (IRR) developed by Chinese rice scientists contained *sck*, a modified cowpea trypsin inhibitor gene and *cryIAc* encoding insecticidal protein from *Bacillus thuringiensis* (Deng and others 2003). Field test indicated that the coexpression of, *sck* and *cryIAc* genes with different insecticidal mechanism renders the rice plant wider spectrum and prolonged phase of resistance (Li and others 2004; Jiang and others 2005; Liu and others 2005a, 2005b), which resulted in higher yields and reduced pesticide use compared with conventional counterpart (Zhu 2001; Huang and others 2005).

For its importance as a staple crop food of the world, heated debate over the safe release of the transgenic rice is on the rise. Of all the biosafety issues being debated, the main safety concerns related to rice are (a) gene flow to wild or weedy relatives, (b) health effect (toxicity and allerginicity), and (c) impact on nontarget organisms such as unintended toxicity of introduced insecticidal protein to beneficial insects. Harmonized regulations used for the safety assessment of GMOs have been developed by international agencies such as Organization for Economic Co-operation and Development (OECD 1993, 1996, 1997) and the United Nations World Health Organization/Food and Agricultural Organization (FAO/WHO 2000). Also, governmental regulations have been elaborated in all major developer and user countries to oversee the safe release of GM crops (Nap and others 2003; Jaffe 2004).

For the safety assessment of transgenic IRR containing *sck* and *cryIAc* genes, much work has been carried out on releasing this transgenic rice into the environment. Initial results showed that no adverse effects were observed (Liu and others 2006; Rong and others 2006). Careful investigations on food safety have also been made and demonstrated that the transgenic IRR was substantially equivalent to its nontransgenic counterpart in terms of chemical composition and nutrition (Li and others 2007).

Although significant efforts have been made to address the safety concerns of transgenic rice, the factors influencing the profit of the

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processors and the acceptance of consumer were underestimated. For the foreign genes with agronomically important traits are often introduced into rice variety with good economic quality. The maintenance of the good quality of its parental line will facilitate the acceptance of the transgenic line after commercialization. Furthermore, understanding of physicochemical properties of newly developed rice variety can make the raw rice material better exploited in food industry, for the requirement of physicochemical properties for different rice product is quite diversified. Nevertheless, little research has been conducted on the effect of foreign gene insertion on the physicochemical properties of rice grain which are closely related to its economic value.

Most rice consumers essentially prefer uniform (size and shape) and translucent kernels. Chalkiness is undesirable because it detracts from the visual appearance. Moreover, higher chalkiness generally results in lower milling yield. Milling yield is measured by head rice yield, which is the mass percentage of rough rice kernels that remain as head rice, or rice that is three-fourths of a kernel length or longer after milling (Chinese National Standard GB/T 17891 1999). As the price of rough rice is determined largely by head rice yield, its economic concern for the rice processor is maximized. Because rice is consumed mostly in the steamed or boiled form of whole milled grain, the pasting and textural properties of rice grains determine the basic eating quality and palatability of the cooked rice product (Islam and others 2001; Zhou and others 2002; Suwannaporn and others 2007).

Therefore, the physicochemical properties of economic importance, including appearance (size, shape, chalkiness), head rice yield, texture profile, and pasting properties, were compared between the transgenic rice containing *sck* and *cryIAc* and its non-transgenic control. For their importance in determining the eating quality, comparison of amylose content and structure was also conducted to obtain a deeper insight into the variation in physicochemical properties.

Materials and Methods

Samples

Indica rice (*Oryza sativa* L., cv Liangyou 2186) was used as a non-transgenic parental control and as the host for the *cryIAc* and *sck* gene for the production of IRR (*Oryza sativa* L., cv Liangyou Kefeng Nr. 6). The paddy rice for study was obtained from the experimental field of Fujian Academy of Agricultural Sciences, Fujian Province, China in 2005. The transgenic line and its parental line were grown in adjoining fields under the same conditions. The samples were stored at low temperature (4 °C) before use.

Appearance, head rice yield, and amylose content

Duplicate samples of 200 g rough rice were dehulled with a Satake THU-35C dehuller (Satake, Tokyo, Japan) and the resulting brown rice grains were weighed and polished for 30 s in a Satake TM-05C grain mill (Satake). The broken milled rice was removed by a Satake TRG-05B rice grader (Satake) at 10 degrees for 1 min. The resulting whole milled rice was weighed. Head rice yields were calculated as percentage by weight of rough rice. Only head rice kernels were used in this study. The length and width of 10 whole grains were measured by enlarging the photo 10 times the original size. The chalkiness was calculated according to Chinese National Standard GB17891 (1999). Amylose content was determined by iodine colorimetry (Juliano and others 1981).

Sample preparation

Whole milled rice was ground in a cyclone mill with an 80-mesh sieve for amylose assay and rapid viscosity analysis. Cooked rice for scanning electron microscopy (SEM) and texture profile analysis was prepared with the procedures as follows: Thirty grams of milled rice was put into the aluminum cup of the rice cooker, 42 mL distilled water (rice: water ratio = 1:1.4) was added, and the rice was soaked for 30 min at room temperature. The sample was then steamed for 30 min and kept warm in the electric rice cooker (SR-W180, Toshiba Co. Ltd., Japan) for 10 min, then left at room temperature for 2 h before use (Tran and others 2001, 2004, with modifications).

RVA characteristics of rice flour

Paste viscosity properties of rice flour samples were determined with a rapid visco analyzer (RVA-super 3, Newport Scientific, Warriewood, Australia), which was controlled by computer software, the thermo cycle for Windows according to AACC Approved Method 61-02 (AACC 2000).

Scanning electron microscopy

Raw and cooked whole grain sections of milled rice from IRR and control were compared for morphological features using SEM. Raw grains were dry-fractured using a razor blade.

Portions of cooked rice were dehydrated in 100% ethanol and dried in a critical-point dryer with CO_2 (Structure Probe Inc., West Chester, Pa., U.S.A.). The dried cooked rice grains were dry-fractured using a razor blade.

Specimens were mounted on aluminum studs with double sticky tape and coated with gold in a sputtering system (Eiko IB-5, Tokyo, Japan). The coated samples were observed with a Hitachi S570 SEM (Hitachi, Tokyo, Japan) at an accelerating voltage of 10 kV.

Texture analysis of cooked rice

Texture profile analysis was conducted with a texture analyzer (TAXT2i, Stable Micro Systems, London, U.K.) by placing a single kernel of cooked rice on the base plate of the analyzer at room temperature. A 2-cycle compression force compared with time program was used with a test speed of 2 mm/s and a rate of 80% strain using a cylindrical plunger with a diameter of 10 mm (An and others 2005). Textural parameters of hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness were determined using the software provided by the Stable Micro System Ltd. (1995 Godalming, Surrey, England).

Statistical analysis

The dimensions of rice kernel were measured on 10 whole grains. The amylose content and head rice yield were measured in duplicate. Physicochemical analyses including RVA were performed in triplicate, and the texture analysis of each variety of cooked rice was repeated on 20 replicated samples. The data were expressed as means ± standard deviation (SD). Statistical data were analyzed with the independent-sample *t*-test using Microsoft Excel 2003.

Results and Discussion

Appearance, head rice yield, and amylose content

There were no obvious differences in the appearances and weights between the control and IRR paddy rice as depicted in Figure 1. The paddy rice kernels used for the subsequent experiments were about 10.5 mm in length × 3.0 mm in width and about 29.5 mg in weight.

The size (length and width), shape (length/ width), and chalkiness of the milled rice kernels from the 2 cultivars are presented in Table 1. The milled rice sample of IRR was comparable to the control in size and shape. However, IRR had significantly less chalkiness than the control rice. The head rice yields of IRR were significantly higher than the control. This observation is in agreement with the previous work showing that higher chalkiness results in a reduced milling yield due to the breakage of chalky kernels (Kanda and others 1969; Khush and others 1979; Webb 1991).

The amylose content, which is related to the adhesiveness of cooked rice, was similar in IRR and control. According to the Intl. Rice Research Inst. (Juliano 1982), these 2 rice cultivars can be classified as low amylose varieties (12% to 19%). The amylose content was the same with the introduction in both the control and IRR, although the significant difference in chalkiness between IRR and control. The present results are in agreement with previous reports by Cheng and others (2005) suggesting that amylose content is not inherently related to chalk occurrence.

Therefore, IRR was substantially equivalent to the control in size, shape, and amylose content, while improved in appearance and milling quality, after foreign gene insertion.

Pasting properties

The pasting properties of rice flours as measured by RVA (rapid viscosity analysis) were generally used as one of the indirect indicators for eating quality (Reddy and others 1994; Champagne and others 1999). It can be seen from Table 2 that IRR had basically the same RVA profile as control although there was a slight but significant difference in the hot paste of RVA viscosity. Two key parameters for hardness and adhesiveness of the cooked IRR, respectively, breakdown viscosity and setback viscosity, were similar to cooked control. The initial temperature and peak and cool viscosity of the 2 cultivars were substantially the same for the equivalent amount of amylose content. This result suggested that the transgenic cooked rice basically maintained the soft and tender texture as its parental did, although there were significant reductions in the hot paste viscosity. The present results concur with a previous report by Cheng and others (2005) that chalky occurrence is not closely related to RVA properties.

Texture analysis of cooked rice

Texture analysis was employed to achieve a deep insight of sensory properties of rice (Ong and Blanshard 1995). Individual cooked

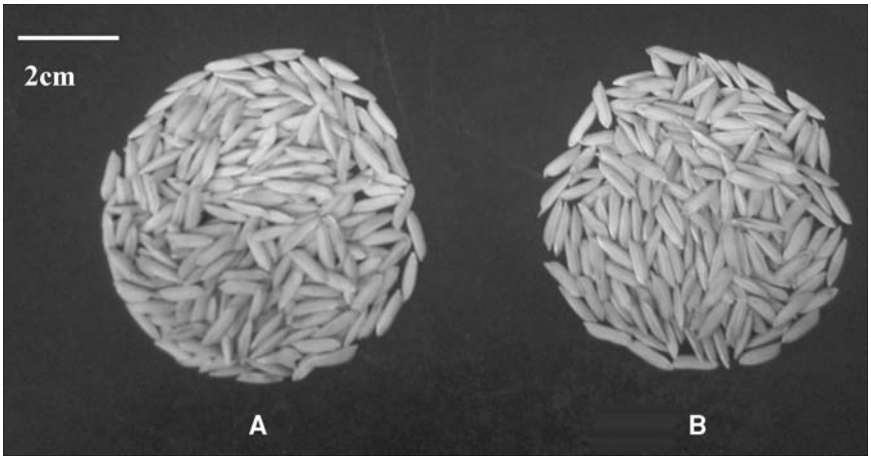

Figure 1 — Paddy rice samples used for comparison of structure and physicochemical properties. A, control; B, IRR. The bar indicates 2 cm in length.

Table 1 — Appearance, head rice yield, and amylose content of control and IRR.

Variety	Length (mm)	Width (mm)	Length/ width	Chalkiness	Head rice yield (%)	Amylose content (%)
Control[a]	6.32 ± 0.04	2.32 ± 0.02	2.73 ± 0.01	9.40 ± 0.14	56.3 ± 0.1	16.10 ± 0.06
IRR[b]	6.35 ± 0.03	2.33 ± 0.01	2.72 ± 0.01	6.60 ± 0.05	63.8 ± 0.4	16.20 ± 0.06
D[c]	0.03	−0.01	−0.01	−2.80 [d]	7.5 [d]	0.10

[a] Control, nontransgenic parental rice variety Liangyou 2186.
[b] IRR, the insect-resistant rice variety Liangyou Kefeng Nr. 6, containing the *sck* and *cryIAc* genes.
[c] *D*, difference between control and IRR.
[d] Significant at $P < 0.05$ by *t*-test.

Table 2 — Pasting properties of rice flours of control and IRR.

Variety	Initial pasting temperature (°C)	Viscosity (RVU)[d]				
		Peak	Hot paste	Cool paste	Breakdown[e]	Setback[f]
Control[a]	76.0 ± 0.01[g]	275.97 ± 0.46	116.83 ± 1.17	218.03 ± 9.27	159.14 ± 0.71	57.94 ± 9.00
IRR[b]	75.8 ± 0.56	280.97 ± 8.84	111.64 ± 1.81	211.22 ± 1.65	169.33 ± 7.05	69.75 ± 7.19
D[c]	−0.02	5.00	−5.19[h]	−6.81	10.19	11.81

[a] Control, nontransgenic parental rice variety Liangyou 2186.
[b] IRR, the insect-resistant rice variety Liangyou Kefeng Nr 6, containing the *sck* and *cryIAc* genes.
[c] *D*, difference between control and IRR.
[d] RVA (rapid viscosity analysis) units.
[e] Peak viscosity minus hot viscosity.
[f] Cool viscosity minus peak viscosity.
[g] Mean ± SD ($n = 3$).
[h] Significant at $P < 0.05$ by *t*-test.

Table 3—Texture profile analysis of cooked rice of control and IRR.

Variety	Hardness (g)	Adhesiveness (gcm)	Springiness (cm)	Chewiness[e] (gcm)	Gumminess[f] (g)	Cohesiveness
Control[a]	587.27 ± 57.04[d]	14.01 ± 2.27	0.84 ± 0.06	202.76 ± 27.91	239.57 ± 22.17	0.41 ± 0.03
IRR[b]	619.03 ± 48.38	12.95 ± 2.15	0.71 ± 0.06	174.09 ± 14.29	244.58 ± 17.60	0.40 ± 0.04
D[c]	31.76	−1.06	−0.13[g]	−28.67[g]	5.01	−0.01

[a] Control, nontransgenic parental rice variety Liangyou 2186.
[b] IRR, the insect-resistant rice variety Liangyou Kefeng Nr 6, containing the *sck* and *crylAc* genes.
[c] *D*, difference between control and IRR.
[d] Mean ± SD ($n = 20$).
[e] Chewiness (gcm) = gumminess (g) × springiness (cm).
[f] Gumminess (g) = hardness (g) × cohesiveness.
[g] Significant at $P < 0.05$ by *t*-test.

rice grains of IRR had higher hardness, springiness, gumminess, and chewiness values but lower adhesiveness values than control (Table 3), and only springiness and chewiness were statistically different. Meanwhile, cohesiveness was similar between the 2 groups. The equivalence in hardness for the equivalent amount of amylose was in agreement with a previous report by Ramesh and others (1999) and Reddy and others (1993), who indicated that the hardness of rice texture is positively correlated to amylose content. It may be suggested that chalky occurrence changed, to some extent, the textural properties of rice grain.

Scanning electron microscopy results

Raw and cooked whole grain sections of milled rice from transgenic rice and its nontransgenic counterpart were compared for morphological features using SEM.

Figure 2 shows the transversely fractured surfaces of the midregion of raw rice. The endosperms of the IRR and control had similar morphology when viewed at low magnification (Figure 2A and 2D). The shape of the starch granules of IRR was distinctive to that of control when observed at high magnification (Figure 2C and 2F). The starch granules in control are loosely packed with no sharp angles or edges, whereas in IRR are polygonal with sharp angles and edges. The cracks (pointed by arrows in Figure 2C and 2F) between individual starch granules with compound starch granules was larger for control than for IRR, which corresponded to the higher occurrence of chalkiness and easier breakage resulting in lower head rice yield.

Figure 3 shows the interior and peripheral area of cooked rice grains from control and IRR rice. From the low magnification view of cross-section of cooked kernels, the endosperms of IRR and control were loose and porous (Figure 3A and 3D) with cell wall disrupted contrary to solid and compact as raw (Figure 2A and 2D). From the view of entire cross section of cooked rice kernel, it can be observed that water penetrates unequally into the grain during cooking: the core region of rice was in higher density with low water penetration, and large voids in the peripheral area with high water penetration (Figure 3B and 3E). Smaller voids of cooked rice grain of IRR were observed compared with that of control both in interior area (Figure 3B and 3D) and peripheral area (Figure 3C and 3F), which corresponded to the finer cracks as raw (Figure 2C and 2F). This observation concurred with the result that air space between individual starch granules serves as channels for water migration into the grain during cooking (Ogawa and others 2003). The more compact ultrastracture of cooked IRR rice shed lights on the lower springiness and chewiness of its textural quality compared with control.

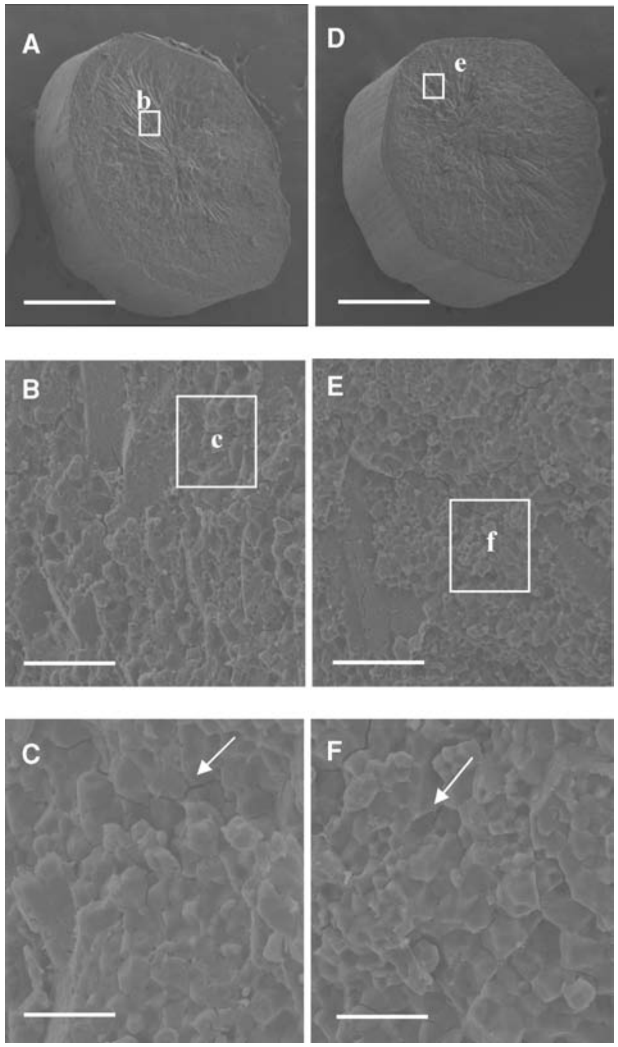

Figure 2—Structure of the transversely fractured midregion of whole raw grains from IRR rice (D, E, F) and control (A, B, C) by scanning electron microscopy. The rectangles b, e, c and f indicate areas shown in higher magnification in the subsequent micrographs. Scale bars: 0.75 mm (A, D); 50 μm (B, E); and 20 μm (C, F).

Conclusions

A comprehensive study of the physicochemical properties of economic importance, including size, shape, chalkiness, head rice yield, amylose content, structure, texture profile, and pasting properties, is of great importance to be made with a transgenic rice containing *sck* and *cryIAc* genes and its nontransgenic counterpart, although these properties may have no relevance in terms of food safety. In summary, most of the physicochemical properties of the IRR maintained after the introduction of foreign genes except for improved appearance and milling quality (lower chalkiness and higher head rice yield) and slight difference in texture (lower springiness and chewiness), which might be a result of a positional effect of T-DNA insertion. However, it should be noted that

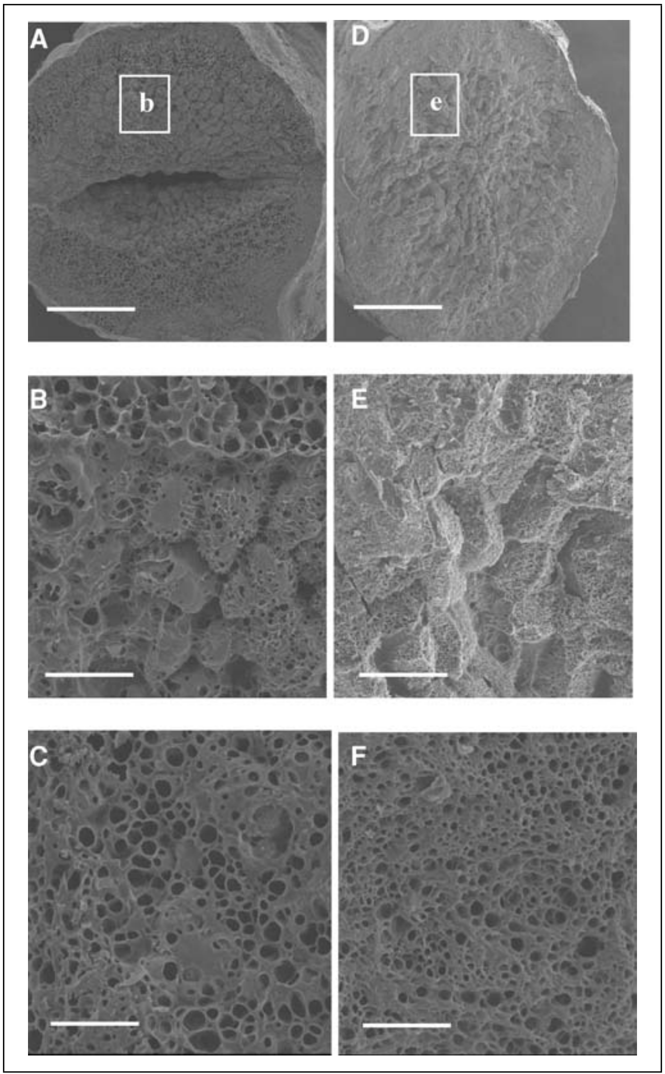

Figure 3—Scanning electron micrographs of interior (A, B, D, E) and peripheral area (C, F) of cooked kernels from control (A, B, C) and IRR rice (D, E, F). The rectangles b and e indicate areas shown in higher magnification in the subsequent micrographs. Scale bars: 0.60 mm (A, D); 100 μm (B, E); and 50 μm (C, F).

the occurrence of unintended effects is not a phenomenon specific to genetic engineering: it is also quite common in conventional breeding.

As for the uniqueness of rice in cereal grains, the physicochemical properties related to the commercial value of rice grain should be carefully investigated for each transgenic line developed, selecting favorable lines and discarding those exhibiting unwanted properties, for the convenience of its acceptability of the market after commercialization.

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