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IMPROVEMENT OF Bg 360 FOR SUBMERGENCE TOLERANCE BY MORPHOLOGICALLY AND MOLECULAR MARKER ASSISTED BACK CROSS BREEDING

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ABSTRACT

Submergence of rice is a major constraint specially in Matara, Galle, Kalutara, Ratnapura, Gampha and Colombo districts in Sri Lanka. Thus, at Rice Research and Development Institute, Batalagoda, a molecular marker assisted backcross breeding programme was initiated to incorporate *Sub 1A* allele to improve popular varieties for submergence tolerance. This paper reports the progress of improvement of Bg 360 for submergence tolerance using Swarna-*Sub 1* developed at International Rice Research Institute (IRRI). In this program Bg 360 the recurrent parent was corssed with Swarna-*Sub 1* the donor parent and F_1, BC_1F_1, BC_2F_1, BC_3F_1, BC_2F_2 genrations were raised and submergence tolerant plants similar to Bg 360 were selected by submergence screening and molecular screening. In submergence screening 10 day old seedlings were screened for submergence tolerance. The plants were scored for survival 10 day after submergence and elongation at desubmergence and for recovery at 14 days after desubmergence. Recovered plants were subjected to molecular screening. Rice SSR marker RM219 and RM 23869 were used to select heterozygotes in back crossed populations and heterozygotes/homozygotes in selfed populations for *Sub 1A* allele. By combining of phenotyping and genotyping precision of selection was improved and altogether 12 promising BC_3F_1 and BC_2F_2 submergence tolerant lines were identified for evaluation for other agronomic traits.

KEYWORDS: Rice, SSR markers, Swarna-*Sub 1A* allele, Submergence tolerance.

INTRODUCTION

Over 22 million ha of lowland rainfed rice (*Oryza sativa* L.) lands which contribute 18% of global supply of rice are vulnerable to submergence and flash floods worldwide. Submerged conditions are severe in Asian countries such as India, Bangladesh and Thailand. Most of the submergence prone fields were cultivated with submergence tolerant landraces such as FR13A and FR43B with a poor yield of about 2 mt/ha (Neeraja *et al.*, 2007). In Sri Lanka, 0.2 million ha were destroyed in *maha* 2010/2011 season due to floods leading to a yield loss of one third of total production. In 2013, approximately 75,000 ha of paddy lands had been affected due to flooding

prevailed throughout the *maha* season. Submergence is also one of the major environment constraints in Kalutara (41%), Matara (33%), Galle (29%), Ratnapura (22%), Colombo (22%) and Gampaha (17%) districts (Walisinghe *et al.*, 2013). To overcome this constraint the most promising approach is to develop high yielding varieties that are tolerant to submergence. Introduction of Swarna-*Sub 1*, the first example of a submergence-tolerant mega variety showed a 2-fold or higher yield advantage over Swarna after submergence for 10 days or more during the vegetative stage. Therefore rice breeders should select appropriate varieties with higher yields to improve flood resistance for those areas. The major issue of submergence is the carbohydrate starvation of affected plants (Xu and Mackill, 1996).

Submergence-tolerant cultivars can restart their growth during de-submergence by using preserved carbohydrates. Another strategy is an escape strategy where the low-oxygen escape syndrome which involves fast elongation of internodes to rise above the water level and is used by deep water rice cultivars. Both strategies depend on ethylene-responsive transcription factors (Nishiuchi *et al.*, 2012). Submergence-1 (*Sub 1*) is a major Quantitative Trait Locus (QTL) affecting tolerance to complete submergence in lowland rice (Xu and Mackill, 1996). Submergence tolerance in lowland rice is given by a specific allele variation of *Sub 1A* that dampens ethylene production and gibberellic acid responsiveness, causing stillness in growth that correlates with the capacity for re-growth upon de-submergence.

A major QTL on chromosome 9, *Sub 1* has provided the opportunity to apply marker assisted back crossing (MABC) to develop submergence tolerant versions of rice cultivars (Neeraja *et al.*, 2006). More recently *Sub 1* gene has been successfully introduced through MABC into a popular high-yielding variety 'Swarna' from India. Swarna-*Sub 1*, the first example of a submergence-tolerant mega variety, had being evaluated in submergence-prone areas of India and Bangladesh (Neeraja *et al.*, 2007). Under non-submerged control conditions, no significant differences in agronomic performance, grain yield and grain quality between Swarna and Swarna-*Sub 1* were observed indicating complete restoration of the Swarna background in Swarna-*Sub 1* (Neeraja *et al.*, 2007). The major benefit of using the MABC approach is the resultant *Sub 1* varieties retain all the desirable features of the recurrent parent, especially the yield and grain quality characteristics. Initially the *Sub 1* locus was monitored by markers shown to be closely linked with the gene (Xu *et al.*, 2004). Using tightly linked (RM 464 A) and flanking (RM 219, RM 316) markers, as suggested by Hospital and Charcosset (1997), ensured efficient foreground and recombinant selection. At Rice Research and Development Institute, Batalagoda, a MABC was initiated to incorporate *Sub 1A* allele to improve popular varieties for submergence tolerance. This paper reports the improvement of popular Bg 360 which is a highly submergence sensitive variety for submergence

tolerance using *Sub 1* donor Swarna-*Sub 1* developed at International Rice Research Institute (IRRI).

MATERIAL AND METHODS

In 2011, a recurrent parent Bg 360 (Keeri Samba) and a *Sub 1* donor Swarna-*Sub 1* received from IRRI were crossed to obtain F_1. Subsequently, BC_1F_1, BC_2F_1, BC_3F_1 and BC_2F_2 genrations were raised. Crosses were done using hot water method (García-Yzaguirre and Carreres, 2008). Submergence tolerent plants were selected by screening in a cement tank filled with irrigation water. Subsequently survived plants were screened by molecular markers. The methodology is given in a schematic diagram shown in the Figure 1.

Morphological screening

Seeds were soaked for 24 hours in water and were sown on a petry dish for germination. Emerged seedlings were transferred to planting trays on nursery beds and kept 10 days for growing (Figure 2A). In a cement tank, 10 days old seedlings were screened by submerging 1 m deep in irrigation water for 10 days along with the parents (Bg 360 and Swarna-*Sub 1*; Figure 2B). The plants were scored for survival (Figure 2C) and elongation at desubmergence and for recovery at 14 days after desubmergence (Figure 2D). Recovered plants were subjected to molecular screening. Height of the plants before and after submergence, number of the plants survived before and after submergence and number of plants fully recovered at 14 days after desubmergence were recorded (Table 1).

Molecular Screening

Leaf samples of 3 cm length pieces were taken and DNA was extracted using a simple non-liquid N_2 method (Anushka *et al.*, 2008) at RRDI, Batalagoda. Presence of DNA of the samples were confirmed by using agarose gel (1%) electrophoresis. PCR was carried out using 3 µl of template DNA, 10x PCR buffer, 0.3 µl of 10 mM dNTPs, 1 µl of 10 µM each forward and reverse primers, 0.15 µl of 5 U/µl Taq polymerase and sterile water in 15 µl reaction volume. *Sub 1* linked SSR primers of RM 219, RM23869, RM 285, RM 105, RM 464A and RM 316 were tested for polymorphism between Bg 360 and Swarna-*Sub 1* and RM 219 and RM23869 were selected for marker assisted selection (MAS). In PCR amplification 35 cycles with a single preheat 94 ^{O}C, 5 minutes followed by 94 ^{O}C for 1minute for denaturation, 59.1 for 1 minute for primer annealing, 72 ^{O}C for extension and 72 ^{O}C for final extension was carried out in Biorad My cycler. For RM219 marker, selection of plants was done using PAGE and 2% agarose gel electrophoresis.

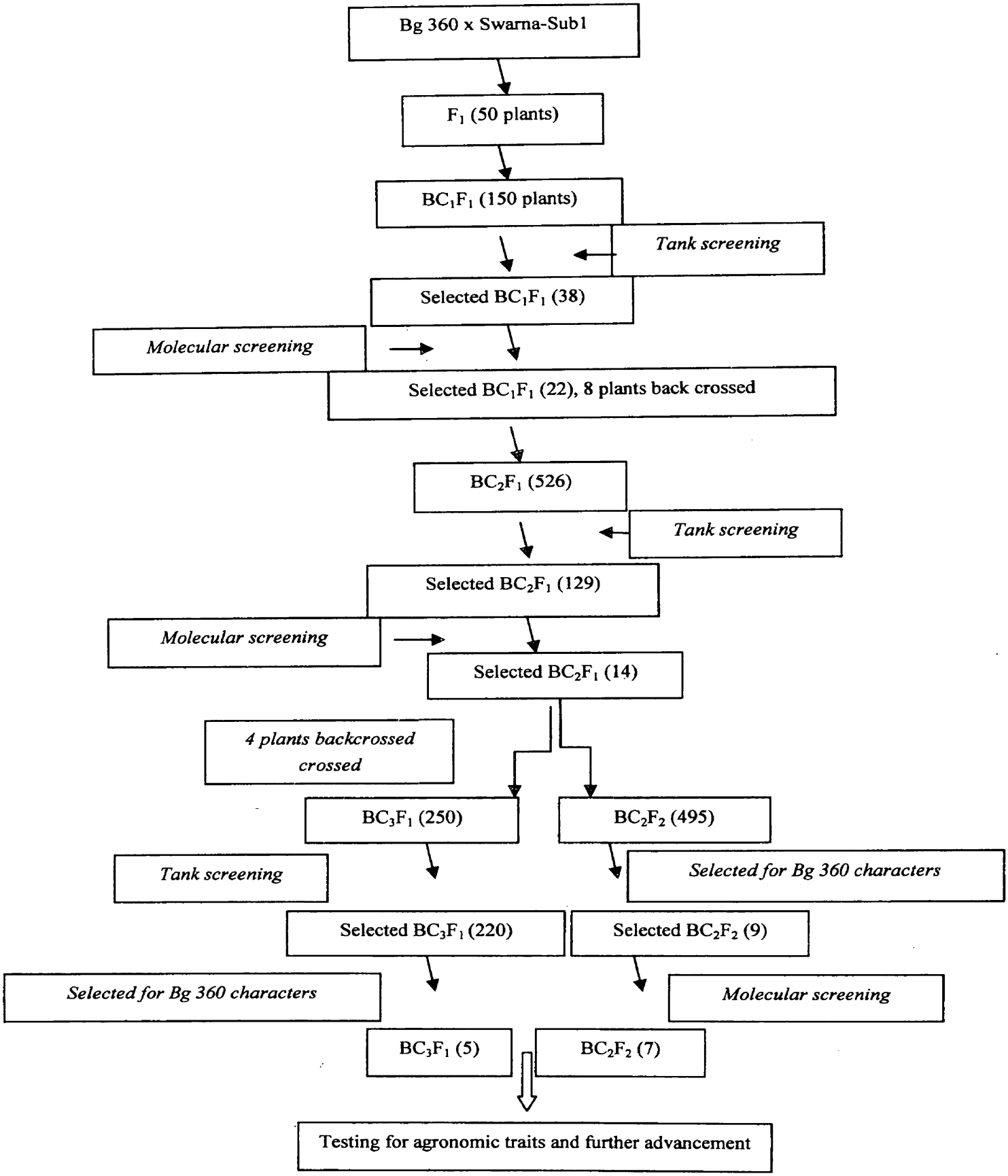

Figure.1: Schematic representation of Molecular marker assisted back cross program for Bg 360 x Swarna-*Sub 1*.

Figure 2. **Field level morphological screening of backcross progenies of Bg 360.**
Notes: A=10day old seedling raised in nursery trays; B=Submergence in 1 m of irrigation water; C=Just after de-submergence; D=Recovering plants.

RESULTS AND DISCUSSION

Morphological and molecular screening of BC_1F_1,BC_2F_1 and BC_3F_1populations

It was observed that after 10 days of submergence none of Bg 360 plants did not

Table 1. **Results of changing in plant height due to submergence and genotyping of Bg 360 /Swarna-*Sub 1*/ Bg 360 (BC_1F_1).**

Plant no.	Change in plant height due to submergence			Genotyping		Response
	Plant height before submergence (cm)	Plant height after Submergence (10 days) (cm)	Height increase	RM 23869 marker		
				T Allele	S Allele	
61	13	16	3	T	S	Se
62	12.5	12.5	0	T	S	Se
63	15	18	3	T		R
64	14.5	8	-6.5	S		R
65	13	5	-8	NA		R
66	13	16	3	S		R
67	12.5	16.5	4	NA		R
68	13.5	10	-3.5	T	S	Se
69	12	17.5	5.5	T	S	Se
70	11	8	-3	NA		R
71	9.5	15	5.5	T	S	Se
72	13.5	17.5	4	T	S	Se
73	7.5	12	4.5	T	S	Se
74	9.5	15	5.5	NA		R
75	12	11	-1	T	S	Se
76	10	15	5	T	S	Se
77	10	4	-6	T	S	Se
78	10	10	0	T	S	Se
79	10	12	2	T	S	Se
80	11.5	9	-2.5	T	S	Se
81	12	12	0	NA		R
82	10.5	9	-1.5	T	S	Se
83	12	13	1	T	S	Se
84	10	11	1	T	S	Se
85	12	12.5	0.5	T	S	Se
86	12	12	0	T	S	Se
87	14	16	2	NA		R
88	11	13	2	T	S	Se
89	11	11	0	NA		R
90	10.5	14.5	4.5	NA		R
91	11	15	4	NA		R
92	13	13	0	T	S	Se
93	11.5	13	2.5	NA		R
94	7.5	7.5	0	NA		R
95	15.5	14	-1.5	NA		R
96	12	15	3	T	S	Se
97	12	14	2	T	S	Se
98	13	13.5	0.5	T	S	Se

Notes: T = Tolerant allele; S = Sensitive allele; R = Rejected plants; Se = Selected plants; NA-Not amplified

In BC_2F_1 also the donor gene should be in the heterozygous state. However there is 11.6% chance of recombination when using the marker RM219 (Neeraja *et al.*, 2007). Then, BC_2F_1 population may not show heterozygosity for RM 219. Plant numbers that showed heterozygous marker alleles for the *Sub 1* gene with double bands were 72-3, 72-11,72-18, 72-20, 73-2 and 77-48 (Figure 3). Some plants show homozygous state by presenting single band similar to the recurrent parent but those plants cannot be rejected as there is 11.6% chance of recombination. For acquiring higher accuracy, those plants were again checked by using a fine marker RM 23869 and appropriate plants were selected. By tank screening as well as molecular screening 5 submergence tolerant heterozygous plants were selected in BC_3F_1 for further advancement.

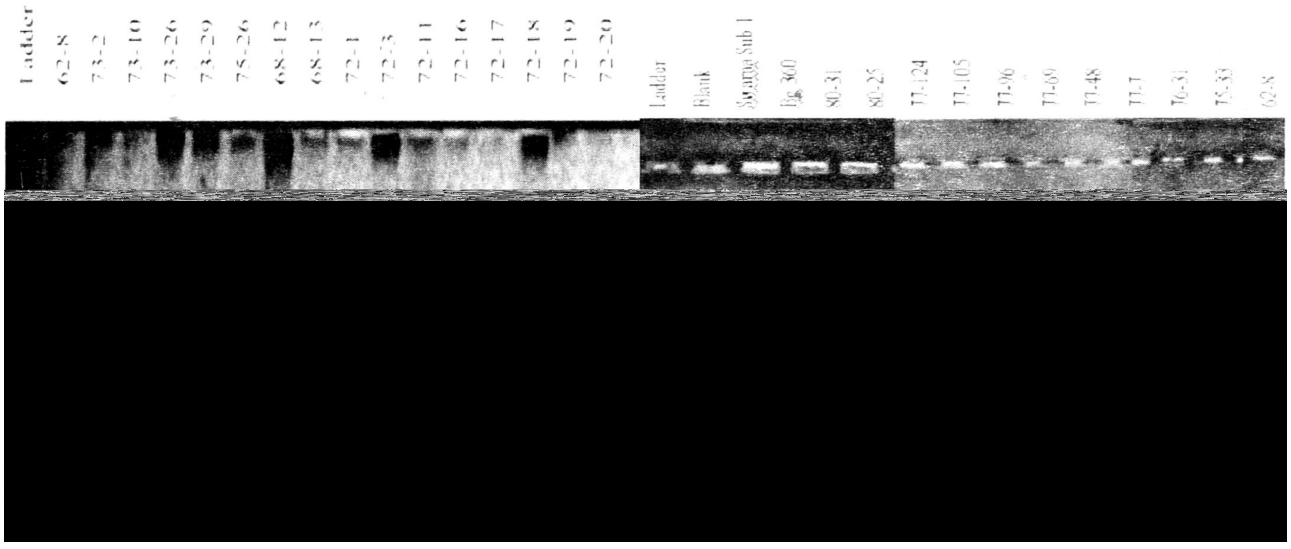

Figure 3. Visualization of PCR products of BC_2F_1 plants performed on 3% agarose gel.
Note: Buffer 0.5X TBE, duration 4 hours. Marker utilized RM 219 [Tightly linked molecular marker].

Morphological and molecular screening of BC_2F_2 population

By allowing to self after 2 backcrosses there is a possibility of identifying recombinants better than the recurrent parent. Therefore, we selected plants with better agronomic characters similar to Bg 360 from the phenotyped population and advanced in parallel to back crossing. Then genotyping was done for the selected plants as shown in Figure 4 and Table 2. In this case the plants having similar morphology to Bg 360 and homozygous /heterozygous for marker RM 23869 allele *Sub 1* were selected for advancing further to have higher number of BC_3F_3 lines.

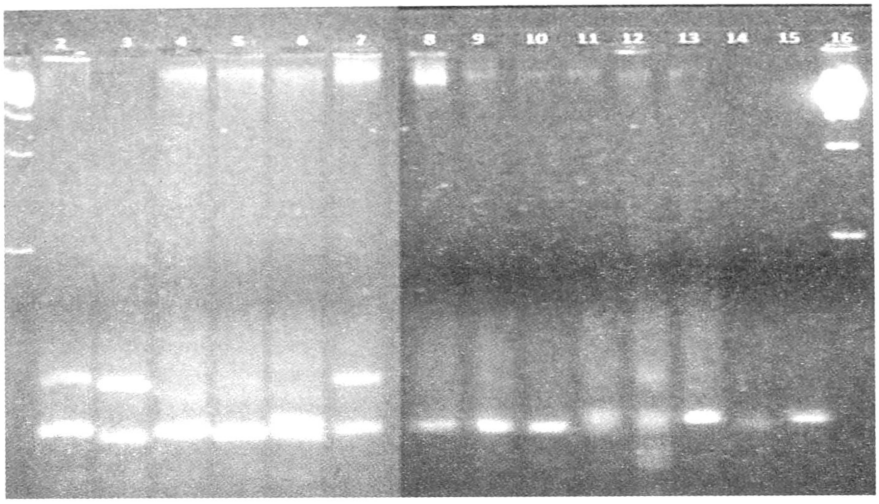

Figure 4. **Visualization of PCR products of BC_2F_2 plants performed on 3% agarose gel.**
Notes: Buffer 0.5X TBE, duration 4 hours. Marker utilized RM 23869 [Tightly linked molecular marker]. Lanes: 1=1 kb ladder, 2=Bg 360, 3=Swarna-*Sub 1*, 4=73-10-1, 5=73-10-2, 6=80-4 -3, 7=77-10-1, 8=80 -4-1, 9=73-52-4, 10=73-52-2, 11=73-39-1, 12=73 -39-2, 13=77-10-1, 14=Suwarna *Sub 1*, 15=Bg 360, 16=1 kb ladder.

Table 2. **Results of molecular marker analysis of BC_2F_2 plants.**

Serial no.	Plant number	RM 23869 marker		Response
		T allele	S allele	
1	73-10-1		S	R
2	73-10-2	T		SE
3	80-4-3	T	S	SE
4	77-10-1		S	R
5	73-39-1	T	S	SE
6	73-39-2	T	S	SE
7	73-52-2	T		SE
8	73-52-4	T		SE
9	80-1-1	T		SE

Notes: T = Tolerance allele, S = Sensitive allele, R = Rejected plants and Se = Selected plants.

CONCLUSIONS

By phenotyping complemented with genotyping the precision of selection was increased and we identified altogether 12 promising introgressed lines in BC_3F_1 and BC_2F_2 with submergence tolerance for evaluation for other agronomic traits.

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