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Research Article

WET SEASON HYBRID RICE SEED PRODUCTION IN NEPAL

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ABSTRACT

Hybrid rice seed production trial was initiated first time in Nepal with an objective for producing F1 hybrid seed by private seed company- to supply hybrid rice seed in the local level. Restorer (R) and cytoplasmic male sterile (CMS). A parental lines of hybrid rice varieties (HJ G1, HJ G2, HJ G5 and HJ G10) were received by Karma Seed Company in collaboration with Hejia Seed Company, China, and hybrid rice seed production trials were carried out at Parsa, Chitwan in 2016 and at Pathariya, Jhapa in 2017 during wet season. R lines of HJ G2, HJ G5 and HJ G10 were seeded earlier on the same day on 26 June 2016 and their A lines were seeded latter on the basis of their flowering period while HJ G1 was seeded on four lots where R line of each lot was seeded three days earlier than A line in 2016. In 2017 only two varieties HJG5 and HJ G1 were seeded where R lines were seeded 15 days and three days earlier than their R lines, respectively. There was good synchronization in the first lot of HJ G1 in 2016 and in both varieties of HJ G1 and HJ G5 in 2017 after adjustment. However, the yield harvested was not so good due to cloudy, rainy and windy environment, particularly during pollination and fertilization period. Seed setting in HJ G5 (1761 kg ha^{-1}) was better than HJ G1 (1032 kg ha^{-1}) when good matching in flowering. Early June and very late seeding beyond July was not good for hybrid rice seed production due to rain likely to occur nearby shortening of growth duration and low temperature during heading, resulted in no synchronization in other varieties.

Key words: Restorer line, CMS line, pollination, synchronization

INTRODUCTION

Nepal is an agricultural country, the population is increasing and the cultivated area is decreasing hence the large quantity of rice is imported each year. Southern belt and some lower hilly areas where the climate is tropical and subtropical and soil is fertile are quite suitable for hybrid rice cultivation. Yield potentiality of hybrid rice is 15-25% higher than inbred rice varieties (MoAC, India, 2010). According to MoAD Nepal (2016) more than two third of rice growing area is irrigated and the year-round irrigated area is about 30% where hybrid rice could be produced successfully. The government of Nepal also envisaged program of hybrid rice in seed vision 2013-2025 (MoAD, Nepal, 2013). According to its target, 8 hybrid rice varieties are to be developed by 2025. Thirty-two hybrid rice varieties of different background introducing from other countries were registered after evaluation in different agricultural research stations under NARC for general cultivation for the farmers. The farmers are facing problems regarding seed as always depending on introduced seeds and there is no guarantee of varieties and quality of seed. Research on hybrid rice development has also been commenced in NRRP Hardinath, but so far, no any own hybrid variety was come out yet. So there is no seed production work in a commercial scale in the government sector and there is no any other seed company involved in hybrid rice seed production in Nepal. But now there is a need to work on hybrid rice seed as well. However, it is a risky business; there is no 100% guarantee for parental lines synchronization. Synchronization is a most important factor for higher grain yield in hybrid seed production. Two lines are used for hybrid seed production and they need to cross during flowering to get the seed. If no matching in flowering, no fertilization, and no seed setting. Planting of both male (R) line and female (A) line are arranged according to their growth duration, flag leaf number and effective accumulated temperature (EAT) but there are so many factors, like climate, biotic and abiotic affecting growth duration, arise problems in synchronization. Panicle initiation (PI) needs to check from the beginning of the PI stage for synchronization. Earlier the information about the difference in PI of the parents, better will be the result of an adjustment for synchronization. So we need to adjust heading time of parental lines as far as possible by applying fertilizers like Urea, TSP and MOP, providing stress by drying the fields and using chemicals for retardation and stimulation of growth. So this situation should be taken into consideration from the beginning and schedule of spraying of pesticides and other field operations should be followed timely and properly.

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Different varieties response differently with climatic factors and it is matters of past experience of particular varieties of a particular environment. Genetic factors are governed by the genetic makeup of plants. Thus, both climatic and genetic factors are adjusted on the basis of past study.

Considering these all situations, Karma Seed Company Pvt. Ltd. test the hybrid rice varieties HJ G1, HJ G2, HJ G5, and HJ G10 were used for hybrid rice seed production program. Later three varieties HJ G1, HJ G5, and HJ G10 were got registered. There are two systems developed for hybrid rice development. Out of two one is three line systems where three lines CMS (A), maintainer (B) and Restorer (R) lines are required. Another system is two line systems where only two lines CMS line and R line are needed. On the basis of three line system R lines and A lines of all varieties were acquired from the above-mentioned company of China and B line was not made available to make always depending for parental lines on Company. R line is also called a male line and A line is called female/seed line. Work was done for two years in Chitwan, later shifted in Jhapa.

MATERIALS AND METHODS

Hybrid rice seed production trials were carried out in 2016 and 2017 wet season by Karma Seed Company Pvt. Ltd. in collaboration with Chinese Hejia Hybrid Seed Company. A lines and R lines of four varieties were received from China hybrid rice seed production was started.

Seeding and transplanting*:* Net seedbed area for all varieties was calculated on the basis of 40 g seed m^{-2}. Seed rate for A line was used 25 kg ha^{-1} for HJ G1, 35 kg for HJ G2 and HJ G10 and 37.5 kg for HJ G5 while 7.5 kg ha^{-1} for R line of each variety was used. HJ G1 was of coarse while others were of medium grain type varieties. The seed was sown in 1.5 m wide strip of required length with 30 cm gap between the two strips. Seedbeds were prepared by plowing two passes by cultivator followed by two passes by harrow after two to three days. Basal doses of N, P_2O_5, and K_2O were applied at a given rate of 67, 37 and 89 kg ha^{-1} respectively. Zinc sulfate was used as 15 kg ha^{-1}. In addition to that Multimix 15 kg ha^{-1} for micronutrients and Kartap hydrochloride 15 kg ha^{-1} for soil insects were also mixed in the soil before seeding in the seedbed. The seed was water soaked for 72 hours and incubated for 24 hours before seeding. While soaking, the seed was washed four times a day. R line and A line of HJ G1 are of the small difference in 50% flowering duration, so their seed beds were kept only at the three-day difference. A and R lines of HJ G5 are of different duration, so, that was not seeded together. The difference in seeding was maintained according to the difference in duration of A and R lines from seeding to 50% flowering. The required seed of R line was split into two replications for seeding at 7 days interval. Sprouted seeds were sown uniform ally and sparsely in well leveled wet seedbed. While seeding, the seed was kept uniformly and gently pressed with wet jute sack of seedbed width holding in both sides. Transplanting of HJ G1 was to be done in 6.5 ha in 2016. So seedbed of that variety was kept in four lots. Seeding of R line was done on 7th June, 22nd June, 24th June and 27th June 2016. The seedbed of A was kept three days later than R line in each lot as of 10th June, 25th June, 27th June and 30th June. The seedbed of R line of each HJ G2, HJ G5 and HJ G10 was kept on the same day on 26th June whereas their A lines were kept on 30th July, 27th July and 3rd August respectively. A and R lines of these varieties were of different maturity period and their A lines were earlier. So A lines of these varieties were sown late. The seedbeds of A and R lines of HJ G1 were kept on 10th July and 12th July in 2017. R2 was seeded 7 days after R1. R line of HJ G5 was seeded on 9th July and its A line was seeded at 10 and 15 days difference on 19 July and 24th July to evaluate synchronization between A and R lines.

Top dressing in the seedbed*:* Three top dressings were used in seed beds in the wet season. Before top dressing irrigation was given with about 2 cm water depth on the surface of the seedbed and dried after two days. First top dressing in seedbed was given at two complete leaf stages and thereafter every five day subsequent top dressings were done at a rate of 21, 28, and 35 kg N ha^{-1} respectively.

Transplanting: The total transplanted area under hybrid rice seed was 6.65 ha and 0.83 ha by HJ G1 and 0.085 ha and 0.097 ha by HJ G5 in 2016 and 2017 respectively. The R1 line was transplanted at the distance of 50 cm plant to plant and 30 cm row to row. Then 3 days after, R2 was transplanted in between two R1 plants maintaining a distance of 25 cm from R1 to R2. After completed R2 transplanting, A line was transplanted at the distance of 20 cm from R line maintaining 17 cm line to line and 20 cm plant to plant in G1 and 13 cm line to line and 15 cm plant to plant distance in HJ G2, HJ G5, and HJ G10. A line was started to transplant

at 5.5 leaf stage and completed as early as possible on the basis of labor availability. The fields of HJ G2, HJ G5, and HJ G10 were not allowed to dry and their fields were always maintained with the thin layer of water until transplanting of A line was completed. While transplanting, seedling hill^{-1} was kept 3-4 for R line and 2-3 for A line. Male-female line ratios were maintained 2:13 for HJ G1 in both years whereas 2:9 for HJ G2, HJ G5, and HJ G10 in 2016 and 2:11 in 2017.

Fertilization for the main field and top dressing*:* Fertilizer was used as 157, 95, and 126 kg N, P_2O_5 and K_2O ha^{-1} for HJ G1 and 144, 71, and 126 N, P_2O_5, and K_2O kg ha^{-1} for HJ G2, HJ G5 and HJ G10 as basal dose and Zinc sulfate was used at the rate of 15 kg ha^{-1} in all during land preparation. For top dressing N at a rate of 35 kg ha^{-1} and K2O @ 21 kg ha^{-1} were used 5-7 days after transplanting only in R line and there after 7 days A line transplanting N @ 41 kg ha^{-1} and K_2O @ 45 kg ha^{-1} were applied in both A+R lines for 2nd top dressing.

Water management*:* After transplanting, 2-3-cm water depth was maintained for a week to establish transplanted hills and to check germination of weed plants. When plant settled, the water level was raised for up to 5 cm for increasing tillers. After attaining 10-12 tillers per hill, the fields were dried for 5 -7 days to control ineffective tillers. Then fields were irrigated again maintaining 5-6 cm of water at 5-7 days interval. However, the field should not be allowed for moisture deficit (cracking). PI, heading, and grain filling are critical stages for water requirement, so there should be a thin layer of water from PI to grain filling period. Surface irrigation was available in Chitwan and underground water was used in Jhapa.

Rouging and Isolation: Rouging was done at late tillering, heading and maturity stages to harvest pure and quality seeds. Isolation was maintained by keeping the barrier of plastic sheets in between two varieties during flowering with the support of bamboo sticks.

PI checking*:* PI checking is necessary to examine the status of PI stages of male and female parental lines to know about the difference between them for adjustment of synchronization. Male and female parents of HJ G1 are of the almost same duration of 135 to 140 days. Its PI was started to check after 40 days of transplanting. In HJ G2, HJ G5 and HJ G10, A line was earlier in maturity and its PI was started to check 20 days after transplanting and R line was checked 40 days after transplanting. Thumb rule of checking the PI is 30-35 days prior to 50% flowering day of that parental line of a particular variety.

Synchronization adjustment*:* When PI was checked in 2016 there was a big variation in PI initiation between A and R lines in all varieties. The differentiation was more than 10 days in HJ G2 and more than 15 days in HJ G5 and HJ G10 where no adjustment was tried. Variation varied from 2-15 days in different lots of HJ G1. Minimum 2 days difference was observed in the first lot, no any adjustment necessary there; >5 days in 4th (last) lot, and > 10 days difference in the 2nd and 3rd lots was observed where A line was late in comparison to R line. MOP @ 0.6% in A line and Urea @ 0.3% in R line of HJ G1 were applied and repeated in 2nd and the 3rd lot after a week and N was used as top dressing @ 17.25-23 kg ha^{-1} in R line, and somewhere K_2O @ 30 kg ha^{-1} was also repeated. After PI checking in the beginning, A line of HJ G1 was found about 5-7 days earlier whereas in HJ G5 A line was about 10 days in the 1st lot and 3-4 days earlier in the 2nd lot in 2017. For adjustment Paclobutrazol @ 500 g ha^{-1} was sprayed in A line and MOP @ 0.6% in R line of HJ G1and no adjustment was done for HJ G5.

GA_3 application: GA_3 is plant growth hormone and it is applied for complete panicle exertion of A line and to make R line comparatively taller than A line for proper pollination. Before application in the plant, it was first dissolved in ethyl alcohol making the concentration of 100 g lit^{-1}. GA_3 has been recommended on the basis of the volume of water required for one ha. 400 lit and 160 lit water were required for one ha A+R/A and R line spraying respectively. Simultaneously field should be watched every following day after spraying GA_3 for plant height in male and panicle exertion for female. During GA_3 application in 2016, there was a labor shortage. So GA_3 could not be applied in time. It was applied on the basis of heading percent monitored before application. First dose was varied from 30-120 g ha^{-1} according to variation from 10-50 percent heading. Subsequent 2nd and 3rd doses were also given on the basis of the requirement in A+R in each variety. In 2017, A line was earlier in HJ G1 and the first dose was started with 90 g ha^{-1} at 30% heading followed by 120 g ha^{-1}, 150 g ha^{-1} and lastly 30 g ha^{-1}. While in HJ G5, A line was late in heading and GA_3 was used @ 30 g ha^{-1} followed by 60 g ha^{-1}, 90 g ha^{-1}, and 120 g ha^{-1}. R lines of HJ G1 first lot, HJ G2 and HJ G10 in 2016 were late in heading by 1-4 days after an adjustment. Thus two special doses of GA_3 were given

at the rate of 60 g ha^{-1} and 90 g ha^{-1} whereas only one special dose 60 g ha^{-1} in HJ G1 and 90 g ha^{-1} in HJ G5 was enough in 2017.

Pollination*:* Pollination was started after GA3 application in between 9-10 AM to 12-13 PM when pollens come out from the spikelet in the sun. It was done for 8-10 days regularly till pollens observed in panicles. Ropes were used for pollination and it was done 4-6 times in a day till there is pollen exertion period. Two ends of the rope held by two people keeping down to the height of A line in opposite sides of rice lines. The rope should behold neither loosely nor tightly and moved fast opposite to lines.

Harvesting, threshing, drying and storage*:* Harvesting was done at 85% maturity, R lines were harvested by manuals, in the beginning, and then A line was harvested manually in 2017 and by a combine harvester in 2016. Thereafter threshing was done by tractor operated thresher. Cemented floor or plastic trials were used for drying for 6-7 days to bring down moisture level 12% for proper storage and stored in jute sacks for time being and later in IRRI bags for safe storage.

RESULTS AND DISCUSSION

Seeding and heading: Seeding on 7th and 22nd June in 2016, there was the 5-day difference in 50% flowering in R line of HJ G1 and that was reduced to three days from 22nd – 27th June seeding however no much difference was found in A line for flowering. However, there was not any difference on the basis of temperature recorded during that period (Figure 1). When flowering of 2016 was compared with flowering of 2017 where seeding was done on 12th July almost 20 days late, 11 days difference was observed between two years in both A and R lines in 50% heading duration. Lower duration was rated in 2017 seeded on 12th July perhaps due to a higher temperature during reproductive phase. A line of HJ G1 was soaked and seeded 3 days later than R line in 2016 whereas in 2017 A line was soaked 3 days earlier and seeded 2 days earlier than R line i.e. because A line was late by 2-8 days late (Table 2) in heading even after adjustment in 2016 and there was no synchronization.

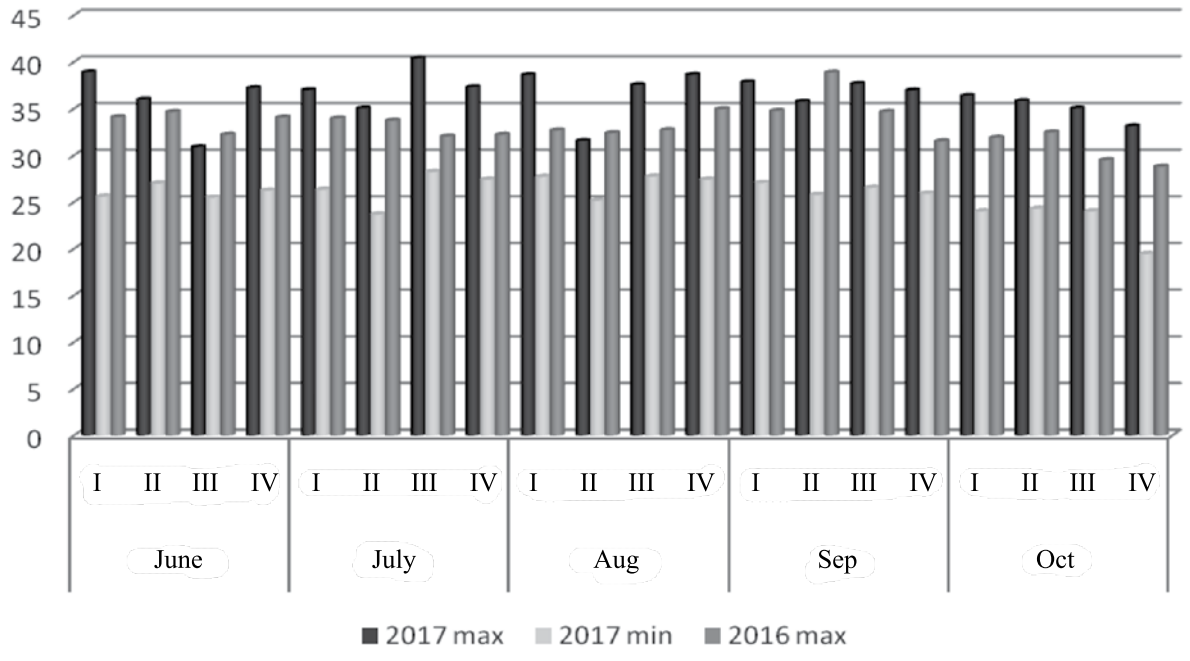

Figure 1. Weekly temperature (^{0}C) of rice growth period

Synchronization adjustment and seed yield: Different varieties response differently with climatic factors and it is matters of past experience of particular varieties of a particular environment. Genetic factors are governed by the genetic makeup of plants. So both climatic and genetic factors were adjusted on the basis of past studies. Even after doing everything in time, some day's difference might occur. 3-5 days differences could be adjusted if identified in the beginning. 5-7 days differences could be adjusted partially. More than 10 days differences could not be adjusted properly. Early identification of difference better will be an adjustment.

Table 1. Seed and grain production in the wet season of 2016-2017

Varieties	2016 wet season			2017 wet season		
	Seed (kg)	Seed (kg ha^{-1})	Fill grain (%)	Seed (kg)	Seed (kg ha^{-1})	Fill grain (%)
HJ G1	1950	694*	50	1000	1181	42
HJ G5 first lot				44	1032	39
HJ G5 second lot				100	1761	52
Total						

*Affected by plant hopper at reproductive stage

Seeding interval was adjusted on the basis of spring season study however in wet season due to the higher temperature, 50% flowering duration was no longer like spring season. As a result, there was no synchronization properly in any varieties. HJ G2, HJ G5, and HJ G10 were a complete failure in synchronization. Seeding interval in HJ G5 was 31 days in 2016, however, the difference in 50% flowering was recorded only 16 days (Table 2). Data for leaf counting and 50% flowering of 2017 also supported the same result (Table 3) of about 15 days. The seeding of A line was done 2 days earlier than R line in HJ G1in 2017 at Pathariya. Even that A line was found 3-4 days earlier than R line in PI checking. Adjustments were made by applying Paclobutrazol @ 500 g ha^{-1} (15%) in A line and MOP@ 0.6% in R line resulting in no difference in heading.

Table 2. Fifty percent flowering from seeding in wet season 2016

Growth stages	**Line**	**Varieties**						
		G2	**G5**	**G10**	**G1 1st**	**G12nd**	**G13rd**	**G14th**
Seeding	R line	26-Jun	26-Jun	26-Jun	7-Jun	22-Jun	24-Jun	27-Jun
	A line	30-Jul	27-Jul	3-Aug	10-Jun	25-Jun	27-Jun	30-Jun
Transplanting	Rline	21-Jul	21-Jul	21-Jul	4-Jul	18-Jul	20-Jul	21-Jul
	A line	17-Aug	15-Aug	22-Aug	10-Jul	23-Jul	26-Jul	29-Jul
Flowering	Rline	13-Sep	14-Sep	17-Sep	1-Sep	11-Sep	12-Sep	16-Sep
	A line	23-Sep	30-Sep	13-Oct	3-Sep	17-Sep	20-Sep	21-Sep
Total flowering period (day)	Rline	78	79	82	85	80	79	77
	A line	55	65	71	87	86	87	82
The difference in 50% flowering (day)		10	16	26	2	6	8	5
Synchronization (R line earlier)		10 days	16 days	26 days	2 days	6 days	8 days	5 days
Seeding Interval between R and A-lines		34	31	38	3	3	3	3
Total flowering period difference between R and A-lines		23	14	11	2	6	8	5

There was synchronization only in the first lot of HJ G1 in 2016 where there was only two days difference between A and R lines in flowering however in other lots 5-8 days difference was observed after adjustment. So the seed was harvested only from the first lot where there was matching. There was also damage from the stem and planthopper in the reproductive stage. In addition to that, there was rain during heading that created a problem in pollination and seed setting. Whatever seeds formed, after ripening harvesting could not be done in time due to rain and the most of seed got germinated in the panicles and lower seed yield 694 kg ha^{-1} was obtained in the first lot (Table 1).

There was single lot HJ G1 in 2017, however, some uprooted seedling were left for transplanting

and that was transplanted 3 days late due to continuous heavy rain and that was named as a 2nd lot. In this case, 2 days difference was observed in 50% flowering. 2 days difference in the heading was no problem in synchronization. There was a difference of 4-5 days in the 1st lot of HJ G1 during the early stage of PI checking and there was complete matching in heading after adjustment by using Paclobutrazol (15%) as a growth retarder. Even after complete matching seed yield obtained from HJ G1 was only 1181 kg ha^{-1} due to windy, cloudy and rainy weather during fertilization. There were two lots of HJ G5, one was of 10 days difference and 2nd lot was of 15 days difference in seeding between R and A lines. A line was of short duration seeded late two times for evaluation and 15 days interval seeding had good matching in heading only two days difference in flowering and A line was earlier (Table 3). Thus 17 days was needed for seeding interval instead of 15 days. The seed yield harvested here was comparatively higher 1761 kg ha^{-1} incomplete matching and 1032 kg ha^{-1} in case of partial matching. Higher yield in HJ G5 was because of parental matching and uniform heading trait.

Table 3. Fifty percent flowering from seeding in wet season 2017

Growth stages	Line	Varieties			
		G11st lot	G1 2nd lot	G5 1st lot	G5 2nd lot
Seeding	R line	12-Jul	12-Jul	9-Jul	9-Jul
	A line	10-Jul	10-Jul	19-Jul	24-Jul
Transplanting	Rline	4-Aug	12-Aug	5-Aug	5-Aug
	A line	8-Aug	15-Aug	9-Aug	11-Aug
Flowering	Rline	24-Sep	30-Sep	2-Oct	2-Oct
	A line	24-Sep	28-Sep	26-Sep	30-Sep
Total flowering period (days)	Rline	74	80	85	85
	A line	76	80	69	68
Difference in 50% flowering (days)		2	4	14	17
Synchronization (difference)		0	2	6	2
Seeding Interval between R and A-lines		2	2	10	15

In wet season 2016 according to seeding date of R line and A line, June first fortnight would be proper for seeding R and A lines together because when seeded R line on 7th June and A line on 10th June, A line was found two days late in 50% flowering i.e. on 3rd September and they were got ready for harvest on September 2nd fortnight. 15 June onward seeding increased the gap between R and A lines in 50% flowering from 2 to 8 days by 27th June thereafter decreased to 5 days difference when seeded on 30th June (Table 2). Early seeding, got disturbed from rain, cloudy and windy weather duration pollination and fertilization. On the basis of data obtained in 2016 when seeding was done late in the last June or after that then A line was found earlier than R line in heading. So to get synchronization, the seed of A line was soaked three days earlier and seeded two days earlier than R line and there was synchronization after adjustment. So for July seeding, A line should be seeded 4 days earlier than R line.

CONCLUSION

There was proper synchronization in the first lot of HJ G1 in 2016 and in both varieties HJ G1 and HJ G5 in 2017; however, the yield harvested was not so good due to cloudy, rainy and windy environment particularly during pollination and fertilization period. Early June and very late seeding beyond July was not favorable for hybrid rice seed production mainly due to low temperature during flowering, thus when possible effort should be made to transplant uprooted seedling on the same days, or next following day. A line of HJ G1 was about 4 days longer than R line. So A line should be shown 4 days earlier than R line.

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