



METHOD OPTIMIZATION FOR PARENTAL LINE SYNCHRONIZATION IN HYBRID RICE SEED PRODUCTION

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Abstract

Parental lines synchronization is a very crucial fact and major determinant in successful hybrid seed production. In this study seeding schedule of parental lines of Pant Sankar Dhan 1 for multiplication of A line (A/B) and hybrid seed production (A/R) was determined using three conventional methods *i.e.* growth duration difference (GDD), Effective accumulated temperature (EAT) and Leaf number difference (LND). In growth duration difference (GDD) method, B and R lines are earlier on an average of three planting dates by 5 and 4 days, respectively, with respect to their seed parent (CMS). Effective accumulated temperature (EAT) for flowering of A, B and R lines were recorded as 1485.22, 1394.67 and 1422.96°C. Leaf number difference (LND) for flowering of A, B and R are 16.50, 14.96 and 15.30, respectively. Based on observations recorded, seeding of parental lines to achieve complete synchronization should be done accordingly. Seeding of B and R lines is suggested when the A line attains LND of 1.45 and 1.07; EAT of seed parent (A line) attains 90.55 and 62.26 and after 5 and 4 days of seeding of A line, respectively for A/B and A/R seed production. Study revealed that greater consistency in flowering days in CMS line was with LND method, as compared to other two methods. It suggests LND method to be more reliable across the planting dates followed by EAT and GDD to predict synchronization between parental lines of hybrids.

Key words: Parental lines synchronization, Hybrid rice seed production enhancement.

Introduction

Rice is a self pollinated crop, where the extent of natural out crossing is only 0.3-3.0% (Kumar, 1996). Hybrid rice seed production therefore, required specialized techniques to promote out crossing, which need to be thoroughly understood before embarking this endeavor. The success of hybrid seed production depends on factors like choice of field, seeding time, planting pattern and weather condition during flowering, synchronization in flowering of parental lines, GA₃ application, supplementary pollination techniques etc. For the economic feasibility and commercial viability of hybrid rice technology, development of an efficient and economic seed production package is pre-requisite. Over the years (1976-94), mean seed yield in China increased from 0.27 to 3.4 tons/ha, with a high of 7.39 tons/ha. At IRRI, hybrid rice seed yields increased from 0.15 tons/ha in 1989 to 1.09 tons/ha in 1994, with yield of 2.05 tons/ha. In India,

the average seed yield increased from 0.5 tons/ha in 1991 to 2.1 tons/ha now (Janaiah, 2010).

The synchronization in flowering between the parental lines assumes greater significance as the failure to obtain proper synchronization results in poor seed yields (Kader, 2017). This is because the seed set on the female parent depends on the pollen supplied from the male parent during the flowering period. To achieve better synchronization between parental lines, determination of seeding interval of these lines is an important component in hybrid seed production. Parental lines of the most hybrid combinations differ in their growth duration, so the seeding of parental lines has to be adjusted in such a way that they come to the flowering at same time.

Materials and methods

Geographically, experimental site is situated at 29°N latitude and 79.3°E longitude and an altitude of 243.8 m

above the mean sea level and in the foothill of *Shivalik* range of the Himalayas in narrow belt called *Terai*. Experiments were conducted at the N. E. Borlaug Crop Research Centre (CRC), Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (U.S. Nagar), Uttarakhand, India during 2008-09.

Experimental materials and approaches

Experiments comprised parental lines of Pant Sankar Dhan 1 (UPRI 95-17A, UPR1 95-17B and UPR1 92-133R), were conducted at the N. E. Borlaug Crop Research Centre (CRC), Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (U.S. Nagar), Uttarakhand, India during 2008-09. Three approaches namely growth duration difference (GDD), effective accumulated temperature (EAT) and leaf number difference (LND) were utilized to assess the seeding intervals of parental lines of hybrid Pant Sankar Dhan 1.

Growth Duration Difference (GDD)

The CMS (A), maintainer and restorer lines were staggered sown and transplanted in well puddled field. Each entry composed of four rows each of 3 m. length. Row to row distance was 15 cm. Single seedling per hill was transplanted. Standard cultural practices were applied to raise healthy normal crop. At flowering, days to 50% flowering was recorded. The seeding interval or growth duration was calculated as the differences in flowering between female (A line) and male parental (B and R) lines.

Effective Accumulated Temperature (EAT)

Seeding interval was determined on the basis of difference in effective accumulated temperature (EAT) of both parents from seeding to 50% flowering. Here, 12°C was taken as the temperature of lower limit and 27°C, the temperature of upper limit by the formulae as below:

$$EAT = \Sigma (T-H-L)$$

Where,

T = Mean daily temperature, H = Temperature over upper limit (27°C), L = Temperature of lower limit (12°C), Σ = Accumulated temperature from beginning to end of a certain growth stage.

Leaf Number Difference (LND)

Seeding interval was determined by the difference in heading date of parents of hybrid based on the difference in leaf number of main tiller. Procedure adopted was to tag ten seedlings in each parent. Observation was recorded as per the main tillers at every three day interval usual on three point scale used for calculation of leaf

number as in the following table.

Scale	Description
0.2	The leaf just emerged but not opened
0.5	The leaf opened but not fully
0.8	The leaf fully opened

Results and Discussion

Experiment was conducted with the objective to determine the growth duration of A, B and R lines of hybrid Pant Sankar Dhan 1 and effective methods under *Tarai* condition of Uttaranchal. The present study reports results from three synchronization methods commonly used viz., growth duration difference (GDD), leaf number differences (LND) and effective accumulated temperature (EAT).

Effect of synchronization methods on flowering

Analysis of variance of all three methods (table 1) for parental lines of Pant Sankar Dhan 1 (A, B and R lines) sown in three staggered date revealed that in all methods and planting dates, variances in 50% flowering were highly significant due to genotypes. Over year (pooled) variances in 50% flowering dates of parental lines were also highly significant due to genotypes in all methods and only in EAT were highly significant due to planting dates and genotype-planting dates (G×P) interactions (table 2). Synchronization of flowering between seed and pollen parent is the key determinant to an efficient and economic hybrid seed production programme aimed at higher seed yields (Xu and Li, 1998; Siddiq, 1995; Viraktamath, 1995a 1998b). The per cent seed set on the female parent depends largely on the pollen supplied by the male parent at the time of anthesis in production plot.

Determination of proper seeding interval is the first and foremost step in successful synchronization of the parental lines. However, the seasonal and weather fluctuations highly influence the flowering of female and male lines and therefore, seed setting. The seeding interval may not be the same for all the locations (Singh *et al.* 1998). The three parental lines differed significantly from each other for days to 50% flowering in individual plantings (table 1 and pooled analysis (table 2) under three synchronization methods but significant mean square due to sowing dates and its interaction with genotype was evident only in respect of EAT method, it suggest necessity to work out flowering days of parental lines at different planting time/seasons to get perfect synchronization during seed production at particular location.

Flowering synchronization between A and B lines

The data from different synchronization methods

Table 1: ANOVA of growth duration difference (GDD), leaf number difference (LND) and effective accumulated temperature (EAT) method of synchronization of CMS, maintainer and restorer lines.

Source of variance	D.F.	Mean square								
		GDD			LND			EAT		
		Sowing dates			Sowing dates			Sowing dates		
		13 June 2008	16 June 2008	19 June 2008	13 June 2008	16 June 2008	19 June 2008	13 June 2008	16 June 2008	19 June 2008
Replication	9	0.35	0.25	0.63	0.04	0.32	0.08	86.64	570.36**	188.42**
Genotype	2	9.74**	123.90**	24.65**	0.89**	1.54**	0.87**	3269.81**	3274.00**	4765.30**
Error	18	0.35	0.36	0.21	0.02	0.08	0.09	93.54	402.62	324.83
Total	29									

*, ** Significant at 5% and 1% level of significance, respectively

Table 2: ANOVA of growth duration difference (GDD), leaf number difference (LND) and effective accumulated temperature (EAT) method of synchronization of CMS, maintainer and restorer lines for pooled data over sowing time

Source of variance	D.F.	Mean square		
		GDD	LND	EAT
Replication	9	0.45	0.51	221.60**
Genotype	2	117.88**	113.55**	5609.44**
Sowing dates	2	19.71	23.06	968.43**
S x G	4	21.33	27.98	2806.45**
Error	54	0.54	0.56	2.143
Total	71			
CV (%)		0.95	0.65	3.19

*, ** Significant at 5% and 1% level of significance, respectively

revealed that some adjustment in sowing dates of parental lines need to be practiced before seeding. Under growth duration difference (GDD) method (table 3), CMS (UPRI 95-17A) flowered in 98.56 days, whereas, this period of maintainer line (UPRI 95-17B) was only 95.17 days. Hence, the difference in growth duration between them was 3.39 (says 4) days. The CMS line was late in flowering than its maintainer line. Therefore, the sowing of A line is to be preceded by same days than the first seeding of maintainer line. The second seeding of maintainer line should be done three days after the first seeding. This will ensure perfect synchronization between A and B lines.

The EAT of A line is 1485.22°C, while the B line had it 1394.67°C (table 3). Hence, there is a difference of 90.55°C between the EAT of A and B lines. The A line required higher EAT for flowering hence, the A line is to be sown first. The corresponding B line should be sown when the EAT of A line has reached 90.55°C.

The Leaf number of A line is 16.54 while that of B line is 15.09 (table 3). Hence, there is a difference of

1.45 leaf numbers between the A and B line. The A line requires higher number of leaf for flowering hence, this parent is sown first. The B line should be sown when the leaf number of a line has reached 1.45.

Comparison of three methods of synchronization viz; growth duration difference (GDD), effective accumulated temperature (EAT) and leaf number difference (LND) methods over two years periods (table 3 and 4) indicate consistency in flowering dates in UPRI 95-17A in LND while significant difference in flowering due to sowing dates was evident under GDD and EAT methods. Response of male parents (B and R) of hybrid to sowing dates under three synchronization methods varied significantly. It was further confirmed by the amount of PCV for days to flowering under three methods. Explicitly revealed that LND method gives least variation in flowering across planting date followed by EAT and GDD method. The study suggests LND method to be most reliable in predicting the seeding sequence for multiplication of CMS line of Pant Sankar Dhan 1. EAT is the next best method of synchronization method followed by GDD. Results from the literature also suggest similar observations (Li, 1993).

Flowering of CMS, maintainer and restorer lines was delayed by 2-3 days by spraying 25ppm 2-4-D or 2% urea solution at panicle initiation stage. The flowering was advanced by 1.8 to 2.8 days by spraying 200ppm KNO₃ or 1% phosphatic solution. However, long gaps between the flowering time of seed and pollen parent cannot be adjusted by spraying of hormones. The synchronization can be adjusted only by differential seeding of the parental lines. At location where fluctuation in temperature are very frequent, the difference in days to flowering in not effective to determine the seeding interval (Viraktamath and Ramesha, 2000). Under such condition circumstances, the effective accumulated temperature (EAT) can be used.

Table 3: Mean of growth duration difference (GDD), leaf number difference (LND) and effective accumulated temperature (EAT) of CMS, maintainer and restorer lines for three dates of sowing.

Parental lines	GDD				LND				EAT			
	Sowing dates				Sowing dates				Sowing dates			
	13 June 2008	16 June 2008	19 June 2008	Pooled	13 June 2008	16 June 2008	19 June 2008	Pooled	13 June 2008	16 June 2008	19 June 2008	Pooled
UPRI 95-17A	97.75	100.65	97.15	98.56	16.65	16.45	16.57	16.54	1467.62	1513.56	1475.40	1485.52
UPRI 95-17B	95.25	94.00	96.10	95.17	15.23	14.97	15.08	15.09	1398.35	1378.25	1407.50	1394.67
UPRI 92-133R	98.00	97.35	98.65	98.00	15.63	15.32	15.46	15.47	1418.09	1412.55	1438.26	1422.96
Mean	97.00	97.33	97.30	97.24	15.84	15.57	15.70	15.70	1428.03	1434.78	1440.38	1434.38
S.Em.	0.24	0.21	0.24	0.16	0.08	0.06	0.09	0.53	2.98	4.48	5.33	4.31
CD at 5 %	0.57	0.85	0.68	0.40	0.23	0.47	0.55	0.37	4.05	3.31	4.78	7.33
CD at 1 %	0.90	1.03	1.00	0.53	0.37	0.61	0.56	0.45	4.78	4.30	5.02	8.92
CV%	1.23	1.65	1.81	0.95	0.39	0.59	0.62	0.65	0.81	1.23	1.24	3.19

Table 4: ANOVA for planting dates in three methods of synchronization.

Source of variance	D.F.	GDD			LND			EAT		
		Sowing dates			Sowing dates			Sowing dates		
		UPRI 95-17A	UPRI 95-17B	UPRI 92-133	UPRI 95-17A	UPRI 95-17B	UPRI 92-133	UPRI 95-17A	UPRI 95-17B	UPRI 92-133
		Replication	9	0.80	0.76	0.51	0.05	0.40	0.06	510.22
Planting dates	2	37.48**	5.82**	5.21**	0.11	0.22*	0.40**	6498.00**	2115.80**	1600.00**
Error	18	0.70	0.69	0.96	0.08	0.06	0.03	204.97	44.65	227.86
Total	29									
CV (%)		0.85	0.89	0.98	1.45	1.68	0.99	0.96	0.48	1.13

Table 5: Genotypic (GCV) and phenotypic coefficient of variance (PCV) for three methods of synchronization .

Genotype	GDD			LND			EAT		
	GCV	PCV	CD 5%	GCV	PCV	CD 5%	GCV	PCV	CD 5%
UPRI 95-17A	1.88	2.09	0.79	0.34	1.61	0.23	1.68	1.94	12.81
UPRI 95-17B	0.74	1.16	0.78	0.87	1.08	0.22	1.02	1.13	5.99
UPRI 92-133R	1.23	1.05	0.86	0.37	1.05	0.14	0.82	1.35	13.39

Based on observation (table 3), study suggest seeding sequence for parental line (A/R) and hybrid seed production. For A line multiplication using GDD method, A line should be seeded two days earlier than the first seeding of male (B) line. The second seeding of male line is done three days after the first seeding to get perfect synchronization between both two parents for higher yield.

Synchronization of parents for hybrid seed production (A and R)

Data of GDD for flowering of A and R lines were revealed that female parent (CMS) is taken 98.56 days to 50% be flowered whereas male (restorer) flowered in

98.00 days. Their days to flowering (50%) differed by 0.56 (says 1) days and the male parent is earlier in flowering by 1 days. Therefore, the sowing of A line has to be done one day before than the first seeding of restore line. The second seeding of R line is done after three days to first seeding of R line. This will give perfect synchronization between A and R lines.

The EAT for A and R lines of hybrid *i.e.*, UPRI 95-17A and UPRI 92-133R is 1485.22°C and 1422.96°C, respectively. The difference in EAT of both parents is 62.26°C. Therefore, for achieving perfect synchronization, the late parent (A) has to be sown first followed by the

sowing of R line, when the EAT of A line has reached 62.56°C.

The leaf number at the time of flowering of the A and R lines of hybrid are 16.54 and 15.47, respectively. The difference (LND) in them is 1.07. For achieving perfect synchronization, the seed parent should be sown first followed by the sowing of pollen parent, when the leaf number of seed parent has already reached up to 1.07.

In respect of hybrid (A/R) seed production, A line should be sown one day earlier than the first seeding of male (R) line. The second seeding of R line is done three days after its first seeding.

Temperature variation at Pantnagar during *Kharif* season (June) are not much, however, EAT for A, B and R lines was 1485.5°C, 1394.64°C and 1422.96°C. It revealed difference of 90.84°C and 66.54°C between the A and B, A and R, lines respectively. The A line required higher EAT for flowering as compared to B and R lines. Hence, A line is sown first to male parent in parental (A/B) or hybrid seed (A/R) production. For A/B multiplication, B line is sown when the EAT of A line has received 90.84°C temperature (Plate no. 4.2). Similarly, for hybrid (A/R) seed production using GDD method, A line should be seeded one day earlier than the first seeding of male (R) line. The second seeding of male line is done three days after the first seeding to get perfect synchronization between both two parents for higher yield. (Plate no. 4.3).

In respect of hybrid (A/R) seed production, A line should be sown one day earlier than the first seeding of male (R) line. The second seeding of R line is done three days after its first seeding (Plate no. 4.2). These results are in agreement with Dayal (2000) who reported hybrids EAT of A line (1431.1°C) as compared to R line (1332°C) of Pant Sankar Dhan 1.

Effects of planting dates on flowering lines

Mean squares (variances) due to planting dates of UPRI 95-17A (A), UPRI 95-17B (B) and UPRI 92-133R (R) lines of hybrid studied under three synchronization methods is given in Table 4. The variances due to planting dates for 50% flowering for each of the three parental lines in two of the three synchronization methods were highly significant *i.e.* GDD and EAT. However, in LND, the variance for R line was highly significant, while it was significant at 5% level of significance for B line and non-significant for A line.

Coefficient of variance (GCV and PCV) of flowering days in synchronization methods

Data from the table 5 revealed that the PCV was higher than GCV in all cases except R line in GDD. The GCV for A and R lines is lowest in LND (0.34 and 0.37) followed by EAT (1.68 and 0.82) and GDD (1.88 and 1.23) methods. However, for B line, it was minimum in the case of GDD (0.74) followed by LND (0.82) and EAT (1.02). Among the A, B and R lines, the PCV was higher (2.09, 1.61 and 1.94) for A line in all methods of synchronization as compare to B line (1.16, 1.08 and 1.13). Similar pattern also revealed between A and R lines.

Conclusion

The study suggests the most reliable method to determine the seeding dates of parental lines by the leaf number difference (LND) method. Accordingly, for A/B multiplication, the A line requiring higher number of leaf for flowering is sown first followed by seeding of the male parent (B line) on date at which the leaf number of female parent (A line) has reached 1.45. Similarly, for A/R multiplication, the A line requiring higher number of leaf for flowering is sown first followed by seeding of the male parent (R line) on date at which the leaf number of female parent (A line) has reached 0.93.

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