Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(2): 2256-2263
Received: 22-01-2020
Accepted: 24-02-2020

Gouri Sharma
Department of Seed Science and Technology, CSKHPKV, Palampur, Distt. Kangra, Himachal Pradesh, India

JK Sharma
Department of Seed Science and Technology, CSKHPKV, Palampur, Distt. Kangra, Himachal Pradesh, India

Shailja Sharma
Department of Genetics & Plant Breeding, CSKHPKV, Palampur, Distt. Kangra, Himachal Pradesh, India

Corresponding Author:
Gouri Sharma
Department of Seed Science and Technology, CSKHPKV, Palampur, Distt. Kangra, Himachal Pradesh, India

Validation of hybrid seed production technology in rice under mid hill conditions and DNA fingerprinting of hybrids

Gouri Sharma, JK Sharma and Shailja Sharma

Abstract
The present investigation entitled, "Validation of hybrid seed production technology in rice under mid hill conditions and DNA fingerprinting of hybrids" was undertaken to study the extent of hybridity in some public bred rice hybrids under mid hill conditions. The experimental material comprising of 4 rice hybrids Sahyadri 1, Sahyadri 2, Sahyadri 3 & Sahyadri 4 each with two parental lines (male R and female A) were evaluated in Randomized Block Design with 4 replications during the *kharif* 2015 at experimental farm of Rice and Wheat Research Centre (RWRC), CSKHPKV, Malan. Staggered planting of male lines was done and dates were decided on the basis of standard planting protocol of respective lines of hybrids. One planting was done on the date of standard protocol and other two dates were 5 days early and 5 days later to protocol. Data were recorded on various morphological traits and were analyzed using standard procedures. The study revealed that maximum hybridity % and hybrid seed yield per ha (q) was observed for Sahyadri 4 hybrid and Sahyadri 2 hybrid and it was minimum for Sahyadri 1 hybrid and Sahyadri 3 hybrid. The significant differences for germination percentage first & final count, accelerated ageing test (24, 48 & 72 hrs for first & final count), speed of germination, seedling dry weight, seed vigour index 1 & 2 indicated that the seeds of 4 hybrids have different storability capabilities. Moderate PCV and GCV (15-30%) was observed for hybridity percentage, accelerated ageing test (24 hrs for first count), accelerated ageing test (48 hrs for first & final count), accelerated ageing test (72 hrs for final count), speed of germination and seedling dry weight. High heritability was found for florets per panicle, seeds per panicle, hybridity percentage, 1000 seed weight, germination percentage first count, accelerated ageing test (24 & 48 hrs for first & final count), accelerated ageing test (72 hrs for first count), speed of germination, seedling dry weight and seed vigour 2. Furthermore, the hybridity of F_1 crosses was confirmed at molecular level through SSR primers.

Keywords: Rice, hybridity, hybrid seed yield, germination, accelerated ageing, vigour index, DNA fingerprinting, SSR primers

Introduction
Rice (2n=24) belongs to the family Graminae, subfamily Orazoidea and is a staple food of more than 60% of world's population. It is a major cereal crop of high agronomic and nutritional importance. It is highly polymorphic with wide geographical and genetic differentiation. Hybrid rice is the genealogy of rice produced by cross-breeding different lines of rice. The first generation progeny (F_1) obtained by crossing two genetically different varieties/inbred (parents) of rice is called 'Hybrid rice'. Though there are two systems (2- line and 3-line) hybrid breeding and seed production, but presently three line method, using cytoplasmic male sterility system, is in practice. In this system three lines (parents) are involved in hybrid seed production, i.e. A line, B line and R line. The male-sterile parent (the one that cannot produce its own pollen) can then be pollinated by the other parent (which is fully fertile), creating the cross that results in a hybrid. The seed born from the male-sterile parent are the seed of the new hybrid rice variety. Presently, the rice hybrid seed production is not in practice in Himachal Pradesh as the hybridity protocols for the released hybrids of public sector has not been tested in the State. Thus, farmers are at the plight of private seed companies to meet out their requirement for hybrid seeds. Farmers can opt for rice hybrid seed production if the seed production technology is validated for suitable hybrids under mid hill conditions. Hybrid seed production of such hybrids will be popularized among farmers of the State, which can meet out their seed requirements as well as improve their economy by involving themselves in business.

Materials and Methods
The investigation involved field and laboratory experiments. The field experiment was conducted at Experimental Farm of Rice and Wheat Research Centre (RWRC) CSKHPKV,

Malan during *kharif* season of 2015 and the laboratory experiment was carried out in the Seed Technology Laboratory of Department of Seed Science and Technology, CSKHPKV, Palampur. The experimental material for the present research investigation consisting of four hybrids Sahyadri-1, Sahyadri-2, Sahyadri-3 & Sahyadri-4 along with its A & R lines was procured from Konkan Krishi Vishvavidyalaya, Dapoli. Since the climatic conditions of Konkan region with high humidity & temperature are almost similar to RWRC, Malan, hence the selection of these hybrids for seed production were selected as material for study. Out of these two hybrids Sahyadri-2 & Sahyadri-4 were early maturing i.e. 110-120 days and Sahyadri-1 &Sahyadri-3 were medium maturing i.e. 125-130 days. The four female parental (A) lines were planted in 9 rows each using three spacings of 15x15 cm, 15x20 cm and 20x20 cm, in four replications, respectively. The nursery sowing was conducted as per details in Table 1. The transplanting was done after 15 days of sowing. The female parental line (A) was grown along with R line in a standard row ratio i.e. 3:1. The planting of male parents was staggered at three stages of five days interval so as to ensure the continuous supply of pollen. Staggered transplanting of male and female lines for proper synchronization was done as shown in Table 1. The treatment T_2 was decided based on the suitable dates for synchronization of flowering as per standard protocol/requirement of the hybrid. Other two dates were decided 5 days early and 5 days later to meet out the probable differences in flowering due to climate change.

Table 1: Nursery sowing and transplanting dates of male and female lines

Hybrid	Nursery sowing dates		Transplanting dates of lines		Standard protocol
	Male	Female	Male	Female	
Sahyadri 1	10.06.2015	02.07.2015	25.06.2015	17.07.2015	Male lines to be planted 17 days earlier to female
	15.06.2015		30.06.2015		
	20.06.2015		05.07.2015		
Sahyadri 2	18.06.2015	08.06.2015	03.07.2015	23.06.2015	Male lines to be planted 15 days later to female
	23.06.2015		08.07.2015		
	28.06.2015		13.07.2015		
Sahyadri 3	10.06.2015	02.07.2015	25.06.2015	17.07.2015	Male lines to be planted 17 days earlier to female
	15.06.2015		30.06.2015		
	20.06.2015		05.07.2015		
Sahyadri 4	25.06.2015	08.06.2015	10.07.2015	23.06.2015	Male lines to be planted 21 days later to female
	01.07.2015		15.07.2015		
	05.07.2015		20.07.2015		

The experimental observations were recorded on days to panicle initiation, days to 50% panicle insertion, days to panicle completion, days to pollen shed, plant height (cm), florets per panicle, seeds per panicle, effective tillers per plant, % florets opened in A & R lines, hybridity %, 1000-seed weight (g), hybrid seed yield per plot (kg), hybrid seed yield(q/ha) and the laboratory observations were recorded on germination percentage, seedling length (cm), seedling dry weight (g), seedling vigour index, accelerated ageing test, speed of germination

Confirmation of hybridity using SSR markers

Molecular characterization

The parents as well as their F_1's were used to confirm hybridity through SSR markers. Twelve randomly chosen primers were screened for polymorphism. The experiment was carried out in DNA Fingerprinting Lab, Department of Seed Science & Technology and Molecular and Quality Control Lab, Department of Crop Improvement, CSK HPKV, Palampur.

Table 2: List of *Oryza sativa* L. microsatellite primer sequences used in the present study

Sr. No.	Primers	Forward sequence	Reverse sequence
1	Rm 7636	TCACTAACACAGGCAGAGCG	ACCAACTCAAGGCGAAGATG
2	Rm 2136	ATGTTTGAGAAAATGCAGAC	CACTAAGCTCGTTTTCAAAAG
3	Rm 5799	ATCGAACCATCCAGGATGAC	TTGCACAAGAGGCAACACTC
4	Rm 2229	AGCACCTAAGCATCTAGCAC	CATGTCACCCAAAACAATTA
5	Rm 3474	TTGGTTGCTTCCTCCCATAC	GGCATTGTACGACGGATCTC
6	Rm 2110	ATGTGGACAATGATATATGT	CTCCGTTTCATATTATAAGA
7	Rm 3468	TGCAGTCTCCATCATCGAAG	GTTAACGGGATCTTGTTCGC
8	Rm 2064	GCTACCTTAGCTAGGTGATC	ATGTAAAATTTGCATGTTTG
9	Rm 564	CATGGCCTTGTGTATGCATC	ATGCAGAGGATTGGCTTGAG
10	Rm 549	ACGAACTGATCATATCCGCC	CTGTGGTTGATCCCTGAACC
11	Rm 3501	TCCTAGTGCATCAGCACAGC	GTCCGTTTCAGCAAGCAAAC
12	Rm 8135	ATCTATATACATATGTGGCTAGATTCA	GGACCAAATTAAACAAACTGTG

DNA samples were extracted from pooled leaf samples of 14 days old seedlings of the hybrid and parental lines following CTAB method. The polymerase chain reactions were carried out using standard procedure. For amplification of genomic DNA, a reaction mixture of 25 µl volume was prepared using 12.3 µl of sterilized distilled water2 µl DNA, 5 µl 5X PCR buffer, 1.5 µl $MgCl_2$, 2 µldNTPmix, 1 µl of each primer pair and 5 Unit Taq Polymerase 0.2 µl (Promega). The amplifications were performed in a thermal cycler (TC-PRO BOECO, Germany) with an initial denaturation step of 5 minutes at 94 ˚C, followed by 39 cycles of denaturation, annealing and extension steps (55 ˚C for 30 seconds and 72 ˚C for 45 seconds, respectively) with a final extension of 5 minutes at 72 ˚C and a final hold at 10 ˚C. The amplifications were carried out in S1000^{TM} Thermal Cycler (BIO-RAD). The amplified PCR products were electrophoretically resolved on

3% (w/v) agarose (HIMEDIA) gel stained with ethidium bromide(0.5µg/ml). The agarose gel electrophoresis was carried out at 150 V for large horizontal gel system (Scie-Plas) and at 125 V for small horizontal gel system for three hours using 50X TAE buffer. After the completion of the electrophoretic run, the gel was visualized under UV light using a gel documentation system (BIO-RAD). The amplified DNA of parental lines and their F_1 hybrids generated SSR marker profiles; the presence of SSR bands was done manually. If both the band of two parents *viz.*, P_1 and P_2 were present in F_1 it showed that it is true hybrid.

Results and Discussion

The effect of male and female treatment differences for hybrid Sahyadri 1. The results have been presented in Table 3

The number of days to panicle initiation were significantly influenced by the male and female treatment differences. Significantly lower number of days to panicle initiation was recorded in male lines i.e. T_1 - 90.67, T_2 - 91.67 and T_3 - 91.33 than female lines T_4 to T_6 (94.00, 93.67 & 93.33). Reddy and Ghosh (1989) [11] reported that delay in planting beyond 13 July resulted in decreased panicle length and grain yield. Days to 50% panicle insertion varied significantly among the treatments**.** Number of days to 50% panicle insertion were less in male lines as compared to female lines in general and were recorded significantly lower for T_1 - 99.00, T_2 - 101.33 and T_3 - 101.33 as compared to T_4 to T_6 (109.33,111.00 &111.33). These were at par for treatments T_2 and T_3 (101.33). Differences for T_1 were significant over T_2 & T_3 indicating taking less number of days to 50% panicle insertion. Singh *et al*. (2004) [12] carried out a field experiment on hybrid rice 'PRH 10' at New Delhi and reported that delayed transplanting showed significant reduction in yield and yield attributes.The number of days to complete panicle insertion were also significantly influenced by the male and female treatment differences. Significantly lower number of days to complete panicle insertion were recorded for male lines T_1 - 113.00, T_2 - 115.00 and T_3 - 115.67 as compared to female lines T_4 to T_6 (123.67, 123.67 & 124.00). Days to complete panicle insertion for treatments T_4 - 15x15 cm (123.67) and T_5 - 15x20 cm (123.67) were similar. Differences for T_1 were significant over T_2 & T_3 indicating taking less number of days to complete panicle insertion. Similar studies have been reported by various workers. Ferraris *et al*. (1973) [7] worked on hybrid rice at Thailand and found that plant spacing (25x25, 25x12.5 and 25x6.25 cm) did not influenced grain yield significantly. Days to pollen shed & stigma receptivity varied significantly for all treatments. Significantly lower number of days to pollen shed was recorded for males lines T_1 - 22.33, T_2 - 23.33 and T_3 - 24.33 as compared to stigma receptivity for female lines T_4 to T_6 (29.67, 30.00 & 30.67).Treatments T_2 and T_3 in male and T4, T5and T_6 in female lines were consistent and did not differ for all the traits. Differences for T_1 were significant over T_2 & T_3 indicating taking less number of days to pollen shed. Akram *et al*. (1985) [1] conducted a field experiment at Kashmir valley with rice cv. Basmati revealed that 8 June planting gave significant higher yield than that planting on 24 May, 24 June and 8 July.

The effect of male and female treatment differences for hybrid Sahyadri 2.

The results have been presented in Table 4

The number of days to panicle initiation recorded was significantly influenced by the male and female treatment differences. Significantly lower number of days to panicle initiation was recorded in male lines i.e. T_1 - 79.00, T_2 - 83.00 and T_3 - 83.67 than female lines T_4 to T_6 (91.00, 84.00 & 82.00). Differences for T_1 were significant over T_2 & T_3 indicating taking less number of days to panicle initiation. Dixit *et al*. (2004) [5] conducted an experiment on rice hybrid 'Sahyadri 1' at Maharashtra and observed that 25 June planting showed significantly highest grain yield (53.22 q/ha) than that planting on 5, 10 and 15 June. Days to 50% panicle insertion varied significantly among the treatments**.** Number of days to 50% panicle insertion were less in male lines as compared to female lines in general and were recorded significantly lower for T_1 - 86.00, T_2 - 90.00 and T_3 - 90.67 than female lines T_4 to T_6 (105.67, 106.00 &105.33). Differences for T_1 were significant over T_2 & T_3 indicating taking less number of days to 50% panicle insertion. Verma *et al*. (2004) [14] studied the response of hybrid rice 'PA 6201' to date of planting and found that early planting on 20 July produced significantly higher 11 grain yield than late planting on 5 and 20 August.The number of days to complete panicle insertion were also significantly influenced by the male and female treatment differences. Significantly lower number of days to complete panicle insertion was recorded for male lines i.e. T_1 - 100.00, T_2 -104.67 and T_3 - 104.33 as compared to female lines T_4 to T_6 (118.33, 122.33 & 121.00). Differences for T_1 were significant over T_2 & T_3 indicating taking less number of days to complete panicle insertion. Venugopal and Singh (1985) [13] observed that there was no significant differences in panicle length due to spacing of 15x15, 20x15 and 20x20 cm in rice cv. 'DR 92'.Days to pollen shed & stigma receptivity varied significantly for all treatments. Significantly lower number of days to pollen shed was recorded for male lines T_1 - 21.00, T_2 - 21.67 and T_3 - 20.67 as compared to stigma receptivity for female lines T_4 to T_6 (27.33, 38.33 & 39.00). Treatments T_2 and T_3 in male lines were consistent and did not differ for all traits. Similar studies were carried out by various workers. Asraf *et al*. (1989) [2] observed that the effect of transplanting dates on rice cv. Basmati 385. They were recorded highest yield 5.3 t/ha with transplanting on 15 June than the transplanting on 1 June, 16 July and 15 August.

The effect of male and female treatment differences for hybrid Sahyadri3.

The results have been presented in Table 5

The number of days to panicle initiation recorded was significantly influenced by the male and female treatment differences. Significantly higher number of days to panicle initiation was recorded in male lines i.e. T_1 - 93.67, T_2 - 94.33 & T_3 - 95.33 than female lines T_4 to T_6 (81.67, 84.00 & 83.67). Reddy and Ghosh (1989) [11] reported that delay in planting beyond 13 July resulted in decreased panicle length and grain yield.Days to 50% panicle insertion varied significantly among the treatments**.** Number of days to 50% panicle insertion were more in male lines as compared to female lines in general and were recorded significantly higher for T_1 - 101.67, T_2 - 102.33 & T_3 - 103.33as compared to T_4 to T_6 (86.67, 90.33 & 90.33). Differences among T_5 - 15x20 cm (90.33) and T_6 - 20x20 cm (90.33) were not observed. Singh *et al*. (2004) [12] carried out a field experiment on hybrid rice 'PRH 10' at New Delhi and reported that delayed transplanting showed significant reduction in yield and yield attributes, timely transplanting on 3 July led to 8.4 and 19.1 per cent higher grain yield than transplanting on 10 and 17 July, respectively. The number of days to complete panicle

insertion were also significantly influenced by the male and female treatment differences. Significantly higher number of days to complete panicle insertion were recorded for male lines T_1 - 115.67, T_2 - 116.33 & T_3 - 117.33 as compared to female lines T_4 to T_6 (98.00, 100.67 & 99.33). Similar studies have been reported by various workers. Ferraris *et al*. (1973) [7] worked on hybrid rice at Thailand and found that plant spacing (25x25, 25x12.5 and 25x6.25 cm) did not influenced grain yield significantly. Days to pollen shed & stigma receptivity varied significantly for all treatments. Significantly higher number of days to pollen shed were recorded at par for male lines T_1 - 22.00, T_2 - 22.00 & T_3 - 22.00 as compared to stigma receptivity for female lines T_4 to T_6 (16.33,16.67 & 15.67).

The effect of male and female treatment differences for hybrid Sahyadri 4.

The results have been presented in Table 6

The number of days to panicle initiation recorded were significantly influenced by the male and female treatment differences. Significantly lower number of days to panicle initiation were recorded in male lines i.e. T_1 - 68.00, T_2 - 72.00& T_3 - 72.00 than female lines T_4 to T_6 (93.00, 94.00 & 94.33). The performance among treatments T_2 to T_3 in male lines was similar. Differences for T_1 were significant over T_2 & T_3 indicating taking less number of days to panicle initiation. Verma *et al*. (2004) [14] studied the response of hybrid rice 'PA 6201' to date of planting and found that early planting on 20 July produced significantly higher 11 grain yield than late planting on 5 and 20 August. Days to 50% panicle insertion varied significantly among the treatments**.** Number of days to 50% panicle insertion were less in male lines as compared to female lines in general and were recorded significantly lower for T_1 - 75.00, T_2 - 80.00 & T_3 - 80.00 than female lines T_4 to T_6 (101.67, 104.00 & 102.33) . These were at par for T_2 and T_3 (80.00). Differences for T_1 were significant over T_2 & T_3 indicating taking less number of days to 50% panicle insertion. Dongarwar *et al*. (2005) [6] reported that early transplanting on 15 and 30 July resulted significantly higher grain yield 31.29 and 32.61 q/ha, respectively than late transplanting on 15 August (28.40 q/ha).The number of days to complete panicle insertion were also significantly influenced by the male and female treatment differences. Significantly lower number of days to complete panicle insertion were recorded for male lines T_1 - 89.67, T_2 - 96.00 & T_3 - 96.67 as compared to female lines T_4 to T_6 (119.67, 123.00 & 124.33). Days to complete panicle insertion for treatments T_2 and T_3 were at par. Differences for T_1 were significant over T_2 & T_3 indicating taking less number of days to complete panicle insertion. Similar studies have been reported by various workers. Venugopal and Singh (1985) [13] observed that there was no significant differences in panicle length due to spacing of 15x15, 20x15 and 20x20 cm in rice cv. 'DR 92'.Days to pollen shed & stigma receptivity varied significantly for all treatments. Significantly higher number of days to pollen shed was recorded for T_1 - 21.67, T_2 - 24.00& T_3 - 24.67 as compared to stigma receptivity for female lines T_4 to T_6 (26.67, 29.00 & 30.00). Treatments T_2 and T_3 in male lines were consistent and did not differ for all the traits.Asraf *et al*. (1989) [2] observed that the effect of transplanting dates on rice cv. Basmati 385. They were recorded highest yield 5.3 t/ha with transplanting on 15 June than the transplanting on 1 June, 16 July and 15 August.

Table 3: Effect of male and female treatment differences for hybrid Sahyadri 1

Treatment	Panicle initiation	Days to 50% panicle insertion	Days to complete panicle insertion	Days to pollen shed & stigma receptivity
Days after transplanting of male lines				
T_1 25-06-2015	90.67*	99.00*[a]	113.00*[a]	22.33*[a]
T_2 30-06-2015	91.67*	101.33*	115.00*	23.33*
T_3 05-07-2015	91.33*	101.33*	115.67*	24.33*
Spacing of female lines				
T_4 15x15 cm	94.00	109.33	123.67	29.67
T_5 15x20 cm	93.67	111.00	123.67	30.00
T_6 20x20 cm	93.33	111.33	124.00	30.67
SE (m±)	0.53	0.48	0.42	0.60
CD (5%)	1.60	1.45	1.28	1.81

Table 4: Effect of male and female treatment differences for hybrid Sahyadri 2

Treatment	Panicle initiation	Days to 50% panicle insertion	Days to complete panicle insertion	Days to pollen shed & stigma receptivity
Days after transplanting of male lines				
T_1 03-07-2015	79.00*[a]	86.00*[a]	100.00*[a]	21.00*
T_2 08-07-2015	83.00*	90.00*	104.67*	21.67*
T_3 13-07-2015	83.67*	90.67*	104.33*	20.67*
Spacing of female lines				
T_4 15x15 cm	91.00	105.67	118.33	27.33
T_5 15x20 cm	84.00	106.00	122.33	38.33
T_6 20x20 cm	82.00	105.33	121.00	39.00
SE (m±)	0.66	0.79	0.61	0.75
CD (5%)	2.00	2.39	1.85	2.26

Table 5: Effect of male and female treatment differences for hybrid Sahyadri 3

Treatment	Panicle initiation	Days to 50% panicle insertion	Days to complete panicle insertion	Days to pollen shed & stigma receptivity
Days after transplanting of male lines				
T_1 25-06-2015	93.67*	101.67*	115.67*	22.00*
T_2 30-06-2015	94.33*	102.33*	116.33*	22.00*
T_3 05-07-2015	95.33*	103.33*	117.33*	22.00*
Spacing of female lines				
T_4 15x15 cm	81.67	86.67	98.00	16.33
T_5 15x20 cm	84.00	90.33	100.67	16.67
T_6 20x20 cm	83.67	90.33	99.33	15.67
SE (m±)	0.72	0.90	0.64	0.82
CD (5%)	2.17	2.72	1.94	2.48

Table 6: Effect of male and female treatment differences for hybrid Sahyadri 4

Treatment	Panicle initiation	Days to 50% panicle insertion	Days to complete panicle insertion	Days to pollen shed & stigma receptivity
Days after transplanting of male lines				
T_1 10-07-2015	68.00*[a]	75.00*[a]	89.67*[a]	21.67*
T_2 15-07-2015	72.00*	80.00*	96.00*	24.00*
T_3 20-07-2015	72.00*	80.00*	96.67*	24.67*
Spacing of female lines				
T_4 15x15 cm	93.00	101.67	119.67	26.67
T_5 15x20 cm	94.00	104.00	123.00	29.00
T_6 20x20 cm	94.33	102.33	124.33	30.00
SE (m±)	0.61	0.66	0.90	1.04
CD (5%)	1.85	2.00	2.71	3.12

The effect of male and female treatment differences in 4 hybrids for per cent florets opened and plant height. The results have been presented in Table 7 (fig 1.)

The number of per cent florets opened were significantly influenced by the male and female treatment differences. Number of per cent florets opened were observed less in female lines as compared to male lines in general and were recorded significantly lower for the treatments T_1 (36.1), T_2 (36.8), T_3 (36.0) and T_4 (37.4) as compared to T_5 (45.7), T_6 (45.8), T_7 (45.7) and T_8 (46.9). Treatments T_1, T_2 and T_3 in female and T_5, T_6 and T_7 in male lines were at par. Vijayalakshmi *et al*. (2006) [15] elucidated the maximization of seed set in hybrid rice (ADTRH1) through GA3 alternates.Plant height of A & R lines varied significantly for all the treatments. Plant height was observed to be less in female lines as compared to male lines in general and were recorded significantly lower for the treatments T_1 (113.4), T_2 (110.3), T_3 (118.1) and T_4(115.0) as compared to T_5 (141.6), T_6 (139.5), T_7 (144.5) and T_8 (143.4). Plant height of T_2i.e. Sahyadri 2 female line was significantly less than other three female lines.Similar studies have been reported by various workers. Paliwal *et al*. (1996) [10] found that early transplanting on 25 July produced significantly higher plant height (107.4 cm) than delayed transplanting on 10 and 25 August.

Table 7: Effect of male and female treatment differences in 4 hybrids for per cent florets opened and plant height

Character	% florets opened	Plant height
T_1	36.1*	113.4*
T_2	36.8*	110.3*[a]
T_3	36.0*	118.1*
T_4	37.4*	115.0*
T_5	45.7	141.6
T_6	45.8	139.5
T_7	45.7	144.5
T_8	46.9	143.4
SE (m±)	0.6	1.0
CD (5%)	1.8	3.0

T_1 – Sahyadri 1 female parental line	T_5 – Sahyadri 1 male parental line
T_2 – Sahyadri 2 female parental line	T_6 – Sahyadri 2 male parental line
T_3 – Sahyadri 3 female parental line	T_7 – Sahyadri 3 male parental line
T_4 – Sahyadri 4 female parental line	T_8 – Sahyadri 4 male parental line

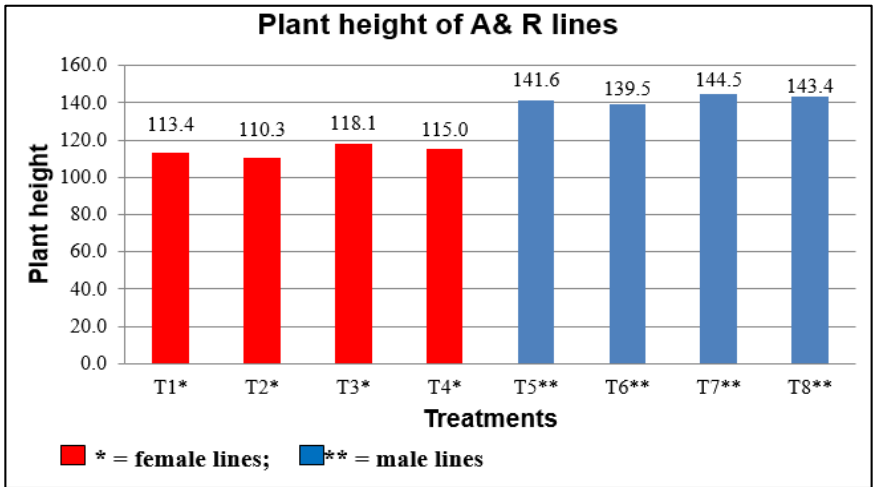

Fig 1: Effect of male and female treatment differences in 4 hybrids on plant height

The effect of female performance among 4 hybrids. The results have been presented in table 8

Table 8: Mean performance of female lines (4 hybrids combined)

Character	Sahyadri 1	Sahyadri 2	Sahyadri 3	Sahyadri 4	Mean	C.V.	F ratio	SE (m±)	CD (5%)
Florets/panicle	140.5	153.3	140.8	153.0	146.86	1.64	0.21	1.21	3.86
Seeds/panicle	22.5	38.1	23.1	40.2	30.99	7.09	0.34	1.10	3.51
Hybridity (%)	15.9	25.5	16.4	26.2	21.03	6.38	0.43	0.67	2.15
Effective tillers/plant	14.1	14.2	13.5	14.6	14.06	7.18	0.68	0.50	1.61
Hybrid seed yield/plot(kg)	0.05	0.11	0.05	0.14	0.09	24.00	0.33	0.01	0.03
Hybrid seed yield/ha(q)	4.9	10.7	5.2	13.5	8.57	24.00	0.33	1.03	3.29
1000 seed weight	17.1	21.5	18.4	21.3	19.56	2.16	2.37	0.21	0.68
Germination % 1st count	24.3	62.3	28.3	63.0	44.44	15.39	2.15	3.42	10.94
Germination % final count	72.5	91.3	83.8	91.8	84.81	5.32	2.17	2.25	7.21
AAT (24 hrs 1st count)	51.3	83.5	74.0	83.0	72.94	6.77	0.30	2.47	7.90
AAT(24hrs final count)	69.0	95.3	83.3	90.5	84.50	3.79	2.21	1.60	5.13
AAT(48 hrs 1st count)	39.5	72.3	61.8	72.5	61.50	9.76	0.18	3.00	9.60
AAT(48 hrs final count)	56.5	82.3	73.0	80.0	72.94	5.79	0.47	2.11	6.76
AAT(72 hrs 1st count)	29.8	60.3	53.3	60.8	51.00	11.61	0.28	2.96	9.47
AAT(72 hrs final count)	41.0	61.0	54.5	60.3	54.19	10.20	0.86	2.76	8.84
Speed of germination	6.0	8.9	6.9	9.0	7.70	8.83	1.08	0.34	1.09
Seedling length (cm)	12.0	11.4	11.8	11.6	11.70	2.98	4.52	0.17	0.56
Seedling dry weight	0.03	0.05	0.03	0.05	0.04	11.88	2.61	0.00	0.01
Seed vigour 1	8.7	10.4	9.9	10.5	9.87	4.63	5.65	0.23	0.73
Seed vigour 2	0.02	0.04	0.03	0.04	0.04	12.21	2.96	0.00	0.01

The range for the number of florets per panicle in female lines varied from 140.50 to 153.25 florets per panicle. Maximum number of florets per panicle was recorded in female line of Sahyadri 2 hybrid (153.3) and minimum florets per panicle were observed in female line of Sahyadri 1 hybrid (140.5). None of the female lines were significantly superior to the Sahyadri 2 female line whereas Sahyadri 4 female line (153.0) was statistically at par with Sahyadri 2 line. Chopra and Chopra (2004) [3] studied the effect of row spacing on rice at Karnal and noticed that wider spacing of 20x15, 30x15 and paired row 20:40:20 cm recorded significantly higher number of panicles per plant than the closer spacing of 15x15 cm. However, the seed yield was not affected due to different spacing. The range for the number of seeds per panicle varied from 22.48 to 40.22 seeds per panicle among different lines. Maximum number of seeds per panicle was recorded in Sahyadri 4 hybrid line (40.2) and minimum seeds per panicle were observed in Sahyadri 1 hybrid line (22.5). No hybrid was significantly superior to Sahyadri 4 line whereas Sahyadri 2 hybrid line (38.1) was statistically at par with Sahyadri 4 line. Mazid and Ahmad (1975) [9] observed that higher number of panicle per hill, number of grain per panicle and grain yield were found with transplanting of crop on 16 June. Significantly higher yield (8.07 t/ha) were found when crop planted on 30 June whereas delay in planting the yield decreased linearly to the lowest level of 3 t/ha. Sahyadri 4 hybrid line exhibited maximum hybridity (26.2%) therefore considered as best among all the four hybrid lines whereas Sahyadri 1 hybrid line showed the minimum hybridity (15.9%). The range for hybridity varied from 15.95% to 26.25% (Fig.2). None of the other line was significantly superior to Sahyadri 4 line whereas Sahyadri 2 hybrid line (25.5%) was statistically at par with Sahyadri 4.Effective tillers per plant were not significantly influenced by male and female treatment differences. The range for Effective tillers per plant varied from 13.45 to 14.55.Sahyadri 4 line resulted in maximum hybrid seed yield per plot (0.14 kg) therefore considered as best among all the four hybrid lines whereas Sahyadri 1 and Sahyadri 3 lines resulted in minimum hybrid seed yield per plot and were at par with each other (0.05 kg). The range for hybrid seed yield per plot varied from 0.05 to 0.14 kg. None of the hybrid line was significantly superior to Sahyadri 4 line whereas Sahyadri 2 line (0.11 kg) was statistically at par with Sahyadri 4. Mahal *et al*. (1999) [8] from a field study at Ludhiana reported that planting on 19 July gave significantly higher grain yield as compared to planting on 5 July and 2 August. Sahyadri 4 hybrid line resulted in maximum hybrid seed yield per plot (13.5q) therefore considered as best among all the four hybrids whereas Sahyadri 1 line produced minimum hybrid seed yield per plot (4.9q). The range for hybrid seed yield per plot varied from 4.88 to 13.45q. None of the hybrid line was significantly superior to Sahyadri 4 line whereas Sahyadri 2 line (10.7q) was statistically at par with Sahyadri 4. Dixit *et al*. (2004) [5] conducted an experiment on rice hybrid 'Sahyadri 1' at Maharashtra and observed that 25 June planting showed significantly highest grain yield (53.22 q/ha) than that planting on 5, 10 and 15 June. The range for 1000 seed weight varied from 17.13 to 21.45g. Maximum test weight was recorded in Sahyadri 2 hybrid line (21.5g) and minimum test weight was observed in Sahyadri 1 hybrid line (17.1g). No hybrid was significantly superior to Sahyadri 2 line whereas Sahyadri 4 hybrid line (21.3g) was statistically at par with Sahyadri 2 line. The mean range for the first and final count of germination under lab conditions varied from 24.25 to 63.00 per cent & 72.50 to 91.75 per cent respectively. Maximum first count and final count for germination was found in Sahyadri 4 hybrid line whereas minimum in Sahyadri 1 hybrid. The mean range for Accelerated ageing test (24, 48 & 72 hrs first count) under laboratory conditions varied from 51.25 to 83.50 per cent, 39.50 to 72.50 per cent & 29.75 to 60.75 per cent respectively (Fig. 3). The mean range for Accelerated ageing test (24, 48 & 72hrs final count) under laboratory conditions varied from 69.00 to 95.25 per cent, 56.50 to 82.25 per cent & 41.00 to 61.00 per cent respectively (Fig. 4). It is evident from accelerated ageing test for 24, 48 & 72 hrs that during first and final count, hybrid seed of Sahyadri 2 &Sahyadri 4 has the capacity of longer shelf life in comparison to Sahyadri 1 and Sahyadri 4. Delouche and Bhaskin (1973) [4] developed accelerated ageing test procedure to measure seed storablility and evaluate vigour. Based on speed of germination Sahyadri 4 hybrid line showed

maximum speed of germination (9.0%) whereas the minimum speed of germination was recorded in Sahyadri 1 (6.0%). The range for this character varied from 6.03 to 9.00%. Seedling length was not significantly influenced by male and female treatment differences. The range for seedling length varied from 11.36 to 12.04 cm. Sahyadri 2 and Sahyadri 4 showed the maximum seedling dry weight (0.05g) whereas Sahyadri 1 and Sahyadri 3 showed the minimum seedling dry weight (0.03g). The range for this trait varied from 0.03 to 0.05g. Sahyadri 4 showed the maximum seed vigour index 1 (10.5) among all four hybrids whereas Sahyadri 1 showed the minimum seed vigour index 1 (8.7). The range of seed vigour varied from 8.73 to 10.50. None of the hybrid was significantly superior to Sahyadri 4 whereas Sahyadri 2 (10.4) was statistically at par with Sahyadri 4. Sahyadri 2 and Sahyadri 4 showed the maximum seed vigour index 2 (0.04) therefore considered as best among all four hybrids whereas Sahyadri 1 showed the minimum seed vigour index 1 (0.02). The range varied from 0.02 to 0.05. The performance of all the seed and seedling traits was high for the hybrid seeds of Sahyadri 2 and Sahyadri 4 in comparison to Sahyadri 1 and Sahyadri 3.

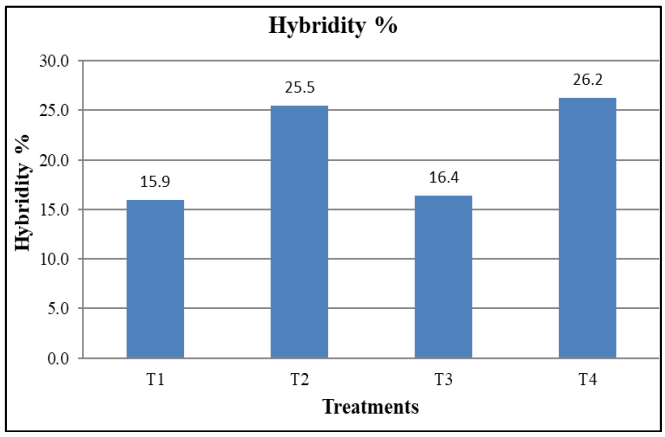

Fig 2: Effect of female performance among 4 hybrids on hybridity %

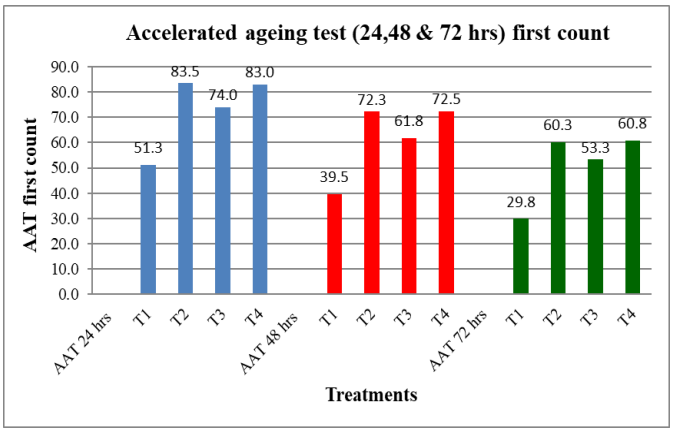

Fig 3: Effect of female performance among 4 hybrids on Accelerated ageing test (24, 48 & 72 hrs) first count

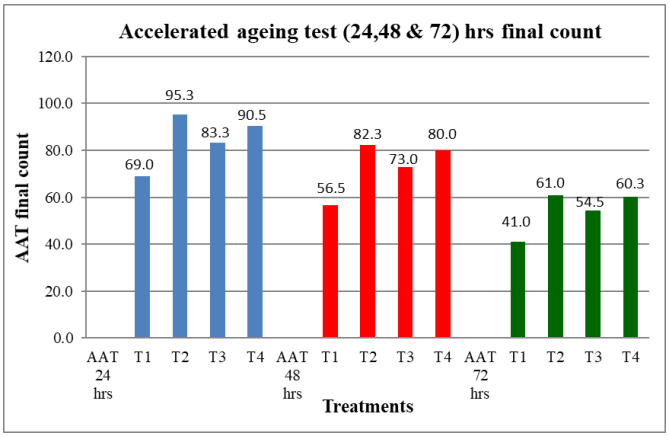

Fig 4: Effect of female performance among 4 hybrids on Accelerated ageing test (24, 48 & 72 hrs) final count

Conclusions

The hybrid seed production of Sahyadri 1 hybrid rice under Malan conditions is expected to be successful if its male lines are planted 10 days later than its female counter part line. Likewise hybrid seed production technology for Sahyadri 2 remains same as per standard protocol i.e. planting male line 15 days later than female line. For the hybrid seed production of Sahyadri 3, male parent can be planted 12 days earlier than female line as per standard protocol which is 17 days. Hybrid seeds of rice hybrid Sahyadri 4 can be produced by planting male lines 19 days earlier than female line i.e. 2 days earlier than standard protocol. Hybrid seed production of Sahyadri 4 was most successful among all 4 hybrids. Adjusting the best transplanting dates of male and female parental lines for the best synchronization are required to be studied extensively thereby fostering the hybrid seed production in rice under all suitable conditions. These are the preliminary studies in which it was found that Sahyadri 4 hybrid was the best among all and its hybrid seed production can be taken up under mid hill conditions but confirmatory studies are still required so that these can be made authentic. The hybridity of F_1 crosses was confirmed at molecular level through SSR primers. A total of twelve SSR primers were screened to confirm the hybridity of F_1's. Out of these, only two markers viz., *Rm3468* and *Rm2136* were found polymorphic between parents and hybrids and showed robust and reproducible bands.

References

1. Akram M, Cheema AA, Awn MA, Maqabool A. Effect of planting date and fertilizer level on grain yield and protein content of rice. Pakistan Journal of Agricultural Research. 1985; 6:165-167.
2. Asraf M, Mahmood S, Musif M, Yousif M. Relationship between transplanting time and grain of Basmati-385. International Journal of Rice Research. 1989; 14:8.
3. Chopra NK, Chopra N. Seed yield and quality of 'Pusa44' rice as influenced by nitrogen fertilizer and row

spacing. Indian Journal of Agricultural Sciences 2004; 74:144-146.
4. Delouche JC, Baskin CC. Accelerated aging techniques for predicting the relative storability of seed lots. Seed Science Technology. 1973; 1:427-452.
5. Dixit AJ, Thorat ST, Gaikwad VV, Jadhav MG. Yield attributes and yield of parental lines of sahyadri hybrid rice as influenced by sowing dates. Journal of Agrometreology. 2004; 6:95-97.
6. Dongarwar UR, Shinde VS, Monhde CB. Performance of scented rice varieties under different transplanting date in eastern vidarbh zone. Pakistan Journal of Agricultural Research. 2005; 29:239-241.
7. Ferraris R, Tromjainut S, Firth PM, Chauviroj M. Effect of nitrogen and spacing on photoperiod non-sensitive hybrid rice grown in the central plain of Thailand. Thailand Journal of Agricultural Sciences. 1973; 6:145-158.
8. Mahal SS, Uppal HS, Mankotia BS. Influence of planting date and irrigation management in scented rice varieties in Trans Gangetic Plain region. Environment Ecology. 1999; 17:557-559.
9. Mazid A, Ahmad S. Effect of transplanting date on paddy yield and other plant characters in different rice varieties. Journal of Agricultural Research. 1975; 13:447-454.
10. Paliwal AK, Khandalker VS, Paraye PM, Tomar NS. Effect of transplanting time on grain yield and its attributes in dwarf scented rice. Indian Journal of Agricultural Sciences. 1996; 66:48-50.
11. Reddy BB, Ghosh BC. Effect of seedling and hill density on the performance of rice under intermediate deep water conditions. Oryza. 1989; 21:233-337.
12. Singh T, Shivay YS, Singh S. Effect of date of transplanting and nitrogen on productivity and nitrogen use indices in hybrid and non-hybrid aromatic rice. Acta Agronomica Hungarica. 2004; 52:245-252.
13. Venugopal K, Singh RD. Effect of plant density and age of seedling on the yield of DR-92 rice in Sikkim. Oryza. 1985; 22:162-165.
14. Verma AK, Pandey N, Tripathi RS. Leaf growth, chlorophyll, nitrogen content and grain yield of hybrid rice as influenced by planting times and nitrogen levels. Annals of Agricultural Research. 2004; 25:456-458.
15. Vijaylakshami C, Ravichandran V, Thiyayaraja K. Maximization of seed setting in hybrid rice (ADTRH1) through GA_3 alternates. Plant Archives. 2006; 6:209-211.