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Sensitivity of sex expression and sex variation in castor (*Ricinus communis* L.) to environmental changes

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Abstract

The development of pistillate lines triggered the commercial exploitation of heterosis in castor (*Ricinus communis* L.). Pistillate lines were maintained conventionally by retaining 20 per cent monoecious or sex revertants as pollen source resulting in high cost of rouging and low genetic purity in certified hybrid seed production plots. Pistillate lines when exposed to high temperatures (32°C) produce male flowers in between female flowers or capsules called interspersed staminate flowers (ISF). This environment sensitive character, ISF, has been used in refined method for the maintenance of pistillate lines in summer season resulting in high genetic purity and less cost of rouging in hybrid seed production plots. However, pistillate lines have to be maintained in summer season exposing the crop to seasonal difficulties *viz.*, high temperature, desiccating winds, poor irrigation resulting in low seed set. An attempt has been made to isolate isogenic lines of the pistillate line VP-1 to overcome such problems in summer season. The F_2 ratios of Pistillate x ISF indicated the role of modifying genes in the expression of ISF. Variants of ISF were classified as sparsely, moderately and highly interspersed. The progeny of the sex variants of ISF *viz.*, sparsely and moderately interspersed ISF tend to be pistillate while highly interspersed types produced 10-12 per cent plants with ISF.

Key words: Castor, pistillate and interspersed staminate flowers, VP-1.

Introduction

Castor (*Ricinus communis* L.) is a sexually polymorphic species. The most natural occurrence of annual and perennial castor is monoecious form. The spike has basal 1/3 rd to 1/2 male flowers while the top portion has female flowers. In between these few whorls have both male and female flowers in an interspersed fashion. Pistillate (P) spike occurs as a rare recessive mutant with the spike having female flowers through out the spike. A variant of pistillate form with male flowers interspersed, through out the female flowers on the spike is termed as interspersed staminate flower (ISF). Sex revertant is basically a female form that turns to monecious form or reverts at later stage [1].

India has a virtual monopoly in the world castor market and exported castor oil worth Rs. 1000 crores during 2000-01. The national productivity has increased from less than 300 kg/ha to 1027 kg/ha (1999-2000) with extensive cultivation of early and medium duration improved varieties like Aruna, Bhagya, Sowbhagya, VI-9, 48-1 etc., and hybrids like GCH 3, GAUCH 1, GCH 4, GCH 5, DCH 32 and DCH 177. Development of an indigenous stable pistillate VP-1 from an introduced pistillate line TSP 10 R triggered the development of castor hybrids.

Maintenance of pistillate line of hybrid castor is done either by conventional method or refined method. In conventional system of maintenance of pistillate line, 20 to 25% monoecists are retained in the pistillate population as a pollen source. This method has been modified or refined (1) to overcome the high cost of rouging, low genetic purity and high proportion of monoecists than expected. The refined or modified method utilizes environmentally sensitive genes for interspersed staminate flowers (ISF) in late revertant progeny of pistillate population. The method is advantageous as it recovers high proportion of pistillate plants (90-100%) in seed production plots with high genetic purity, less cost of rouging and less proportion of monoecists. Unlike the conventional method, seed production in the refined method should be taken up either in summer/*kharif* season, making it imperative to plan a year ahead of hybrid seed production. In addition, high temperature coupled with lack of irrigation facilities and desiccating winds reduce the seed yield of pistillate lines. Thus, simple manipulations either genetically or physiologically to induce ISF expression even in *kharif* season were initiated in this project. Similar efforts were made in erstwhile USSR at Vavilov Institute of Plant Industry (1987) resulting in 3 way hybrids using ISF lines termed as "dispersed type" [2].

Efforts initiated earlier (1990-1992) in this direction at the Directorate of Oilseeds Research resulted in the development of eleven ISF lines and few non-ISF lines.

The expression of ISF character varied from 0 (October 1991) to 46 per cent (April 92) reflecting the environmental sensitivity of the character [3]. The genetic nature of ISF, the variations in the expression of ISF per spike and its correlation with weather parameters has not been clearly understood until now. The present study was initiated with an objective to confirm the sex expression and breeding behavior of available sex variants and to work out the genetic basis of environment sensitive ISF character.

Materials and methods

Earlier work initiated at Directorate of Oilseeds Research involved crossing between different sex revertants in all possible combinations like pistillate, monoecious, early, late and non-revertants of VP-1. The resultant population was screened and selected for individual plants with ISF (ISF) and without ISF (NISF) and then further multiplied and maintained through selfing. All the available sex variants were screened for the expression of ISF, percentage of pistillate plants in both the seasons and order of reversion. Weekly observations on the number of pistillate plants, number of plants reverting to monoecism in different order of spikes, number of plants showing ISF character, expression of ISF in different order of spikes and weather data *viz.*, temperature, RH, rainfall, day length etc., were recorded.

Results and discussion

ISF lines : An initial observation trial was conducted in *Kharif* 1995 to confirm the sex expression of ISF lines. Among the ten ISF lines evaluated, five ISF lines ISF 4, 2, 8, 9, 10 were capable of producing interspersed staminate flowers through out the season starting from September reaching maximum in October and slightly decreased in November and December. However, the number of interspersed staminate flowers per spike were more in ISF 4 (Table 1). This line behaved as highly ISF line when sown in *rabi* (19.11.95) season and expressed high percentage of ISF expression in April. The production of ISF was low in primaries and increased with the next order of spikes up to quaternaries or pentanaries in all the ISF lines while ISF 4 produced maximum number of flowers (1168) per spike in hexenary in July. The number of spikes showing ISF was high up to April and later decreased in May due to high temperatures and increased in July due to the onset of monsoon. The line ISF 2 has also shown continuous production of ISF through out the year (Table 2). The lines ISF 2 and 4 were classified as Less Environmentally Sensitive (LES) while ISF 1, 3, 5, 6, 7, 8 & 10 were classified as Highly Environmentally Sensitive (HES) types. The selfed progeny of these LES type ISF lines when studied in *kharif* 1996 proved the superiority of ISF 4 with 25 per cent of the progeny showing pistillate with ISF expression. During the period of study maximum and minimum temperatures varied from 27.1°C to 43.7°C and 12.8°C to 28.4°C (Table 3). Earlier studies at Directorate of Oilseeds Research indicated that a monthly mean day temperature of 32°C to 33°C was desirable for the induction of ISF [3] for the maintenance of pistillate line.

Since the interspersed staminate flowers were induced on the primary spike when the female flowers were fertilized or dropped off, ISF lines did not set seed on selfing. To overcome this problem, ISF lines - ISF 2 and ISF 4 were maintained in isolation at ICRISAT farm in open pollination in *kharif* 1997. The seed collected from individual progeny lines were evaluated in *rabi* 1997-98. Forty per cent of the plants observed (90) showed ISF expression both in primary and secondary order of spikes. However, in later generation, the percentage of the plants showing ISF varied from 2.3 (1998-99 *rabi* selfed) to 13.7 (1998-99 *rabi* OP) in primary spikes and 21 in secondary spikes (Table 4). Among the individual ISF progeny of the open pollinated population, ISF 4-14 has shown some promise with 23 per cent ISF expression in secondary order of spikes (Table 5). This material has been multiplied in large scale to evaluate the feasibility of I SF 4 for the maintenance of VP-1 in *kharif* season.

Table 1. Mean no. of interspersed staminate flowers per plant in ISF lines (*kharif* 1995 - *rabi* 1996)

Month	ISF 2	ISF 4	ISF 5	ISF 6	ISF 8	ISF 10	Temp. Range (°C)	
							Max	Min
kharif 1995								
Aug	2	-	-	-	-	2	28.5-30.2	21.9-25.5
Sep	5	7	-	-	3	3	28.2-33.1	21.6-22.3
Oct	33	25	4	-	11	15	28.0-31.2	18.5-21.2
Nov	-	18	4	-	9	2	28.7-30.4	14.7-17.6
Dec	-	26	9	-	17	15	28.3-29.0	10.8-13.9
rabi 1995-96								
Jan	4	2	5	2	-	-	26.5-32.0	12.8-15.0
Feb	19	22	5	5	13	6	28.0-35.5	13.9-15.8
Mar	23	42	5	14	7	9	31.0-40.5	14.0-18.6
Apr	14	138	21	22	15	39	28.0-42.0	19.7-22.6
May	20	14	26	7	17	12	36.0-42.5	23.1-28.4

Table 2. Behavior of ISF lines in *rabi* season (1995-96)

Month	No. of interspersed staminate flowers in different orders														Total no. of ISF in all orders		No. of spikes with ISF	
	Pr		S		T		Q		Pen		H		Septenery					
	SF 2	ISF 4	ISF 2	ISF 4	ISF 2	ISF 4	ISF 2	ISF 4	ISF 2	ISF 4	ISF 2	ISF 4	ISF 2	ISF 4	ISF 2	ISF 4	ISF2	ISF4
Jan	2	2	-	-	-	-	-	-	-	-	-	-	-	-	15	2	4	1
Feb	61	2	141	-	97	-	-	-	-	-	-	-	-	-	101	299	8	21
Mar	39	103	167	7	630	-	197	-	-	-	-	-	-	-	117	1033	11	38
Apr	16	16	88	31	39	210	8	878	-	24	-	-	-	-	151	159	11	35
May	-	-	2	41	33	33	100	24	27	55	-	11	-	-	162	164	17	20
June	-	-	-	9	13	20	284	26	232	26	107	75	-	-	636	156	38	14
July	-	-	15	144	222	109	66	585	425	350	225	867	58	302	1011	2476	36	94
Aug	-	-	10	-	5	123	31	178	131	51	73	120	5	169	255	700	31	75
Sep	-	-	-	-	-	6	32	55	38	163	101	95	25	4	196	23	18	20
Total	135	118	222	533	319	1228	521	1943	853	669	506	1168	88	475	2646	6316	176	322

Table 3. Weather parameters in the year 1995-96

Months	Temperature (°C)		(Max – Min.) Temp. (°C)	Mean temp. (°C)	Day length	Relative humidity (%)	Rainfall (mm)
	Maximum	Minimum					
August	29.4	23.3	6.1	26.4	12.72	87	39.7
September	30.2	21.9	8.3	26.1	12.23	92	55.7
October	29.5	20.3	9.2	24.9	11.60	83	27.3
November	29.9	16.2	13.7	23.0	11.22	82	6.5
December	28.5	12.7	15.8	20.6	11.07	77	0.0
January	29.7	12.8	16.9	21.6	11.11	65	0.0
February	31.0	14.8	16.2	23.9	11.26	82	0.0
March	34.7	16.3	18.4	26.6	11.67	74	0.0
April	36.1	21.1	15.0	29.8	12.27	63	2.3
May	41.3	24.5	16.8	33.3	12.73	61	0.0

Table 4. Breeding behavior of ISF 4 over seasons and years

Year	Season	Source	No. of plants observed	No. of plants in Primary			No. of plants in Secondary		
				P	ISF	M	P	ISF	M
1997-98	*rabi*	Selfed	90	50	37	1	59	39	2
1998-99	*kharif*	Selfed	116	87	10	19			
1998-99	*kharif*	OP	227	186	18	23			
1998-99	*rabi*	OP	183	154	25	4			
1998-99	*rabi*	OP	171	20	4	0	122	36	0
1998-99	*rabi*	Selfed	183	154	25	4			
1998-99	*rabi*	Selfed	171	20	4	0	121	36	0

Table 5. Performance of individual progenies of ISF 4 in *rabi* season (1998)

ISF lines	January			February				March				
	No. of plants	Primary		No. of plants	Secondary		Tertiary		No. of plants	Secondary		Tertiary	
		P	ISF		P	ISF	P	ISF		P	ISF	P	ISF
4-1	33	27	6	36	5	0	28	3	19	5	2	9	3
4-5	30	26	4	30	1	0	24	5	17	4	0	10	3
4-8	36	33	3	36	7	1	25	3	13	5	0	8	0
4-11	17	14	2	14	1	1	10	2	7	4	0	3	0
4-14	67	54	10	55	6	2	34	13	32	8	2	15	7
ISF 2	17	16	1	15	2	0	13	0	4	2	0	2	0
ISF 8	17	16	1	16	2	0	12	2	14	3	0	10	1

The ability of ISF lines as maintainer lines of VP-1 was also judged by crossing the pistillate lines produced by ISF lines with 48-1, the male parent of GCH 4. The resulting F_1s were compared with normal GCH 4 produced by VP-1 x 48-1 in a RBD with three replications in *kharif* 1996 (Table 6). The results indicated that the yields of VP-1 x ISF 4 x 48-1 were comparable with GCH 4. Similar results were indicated [4] when single hybrids with 85-97 per cent gynoecious plants were obtained using plants with dispersed inflorescence (male and female flowers interspersed on the inflorescence) as pollinators and pistillate lines as female lines. These single hybrids when crossed with recommended and promising varieties resulted in 3-way hybrids with 95-97 per cent gynoecious plants.

Table 6. Comparison of GCH 4 developed from VP-1 maintained by ISF with GCH 4*

Hybrids	Number of nodes to primary spike	Spike length (cm)	Seed yield (g/plant)
(P 17 x ISF 2) x 48-1	13.1	34.9	41.6
(P 23 x ISF 2) x 48-1	13.9	40.3	33.5
(P 24 x ISF 2) x 48-1	13.9	35.3	33.5
(P 25 x ISF 2) x 48-1	13.9	43.1	40.2
(P 34 x ISF 2) x 48-1	13.5	42.5	35.5
(P 5 x ISF 4) x 48-1	13.2	32.2	34.4
(P 13 x ISF 4) x 48-1	13.3	40.5	33.7
(P 23 x ISF 4) x 48-1	13.9	34.2	33.3
(P 24 x ISF 4) x 48-1	13.3	32.9	35.8
(P 8 x ISF 8) x 48-1	12.3	38.7	41.3
(P 23 x ISF 6) x 48-1	14.3	46.3	37.0
GCH 4 (VP-1 x 48-1)	12.9	27.4	36.3
Gen. Mean	13.5	37.4	36.4
SE (m) +/-	0.29	3.47	3.5
CV (p=0.05)	3.68	16.1	NS
CD	1.14	10.18	

*GCH 4 -VP-1 x 48-1 in which VP-1 is maintained by refined method

Penetrance and expression of ISF character : During the evaluation of ISF lines it was observed that there is a variation in the expression of interspersed staminate flowers like the number of male flowers per spike in a plant. Some pistillate plants produce one or two interspersed staminate flowers and remained pistillate through out the season. While some lines classified as ISF produced more number of male flowers on one or two spikes and remained pistillate in the later order of spikes. On this basis, the pistillate plants were classified as sparsely, moderately and highly interspersed. The classification is based on the number of male flowers per spike when the flowering period coincided with mean temperatures ranging from 30°C-33.5°C in April and May and 19.5°C to 23.8°C in November and December. Similar differences in penetrance and expressivity of ISFs were observed [5] in CNES pistillate lines when monthly average temperature is 32.78°C in July.

A. *At higher temperature regime (when flowering coincided with 30 °C to 33.5 °C in April to May)*

1. Sparsely interspersed - SI (<10 male flowers per spike)
2. Moderately interspersed - MI (10-20 male flowers per spike)
3. Highly interspersed - HI (>20 male flowers per spike)

B. *At lower temperature regime (when flowering coincided with 19.5 °C to 23.8 °C in November and December)*

1. Sparsely interspersed - SISF (<10 male flowers per spike)
2. Moderately interspersed - MISF (10-20 male flowers per spike)
3. Highly interspersed - HISF (>20 male flowers per spike)

The breeding behavior of these SI, MI, HI and SISF, MISF, HISF was studied in the *rabi* 1996-97. Majority of the progeny were pistillate (90-95%) in all the orders of spike except for the progeny of Highly interspersed types which have shown 10-12 per cent plants with ISF expression (Table 7). All these variants of ISF were used as female, by removing male flowers and fertilized by the interspersed male flowers of LES type line ISF 4 and evaluated in *rabi* season. The progeny of variants of ISF *viz.*, Sparsely, Moderately (at both higher and lower temperature regimes) crossed with ISF 4 were pistillate up to 4th order while the progeny of Highly interspersed and LES type ISF had some proportion of ISF plants in all the orders (Table 8). The proportion of plants with ISF increased from 19 per cent in primary stage to 39 per cent in tertiary stage in the above progeny.

Table 7. Breeding behavior of ISF 4 over seasons and years

Year	Season	Source	No. of plants observed	No. of plants in primary			No. of plants in secondary		
				P	ISF	M	P	ISF	M
1997-98	*rabi*	Selfed	90	50	37	1	59	39	2
1998-99	*kharif*	Selfed	116	87	10	19	-*	-	-
	kharif	OP	227	186	18	23	-	-	-
1998-99	*rabi*	OP	183	154	25	4	-	-	-
	rabi	OP	171	20	4	0	122	36	0
1998-99	*rabi*	Selfed	183	154	25	4	-	-	-
	rabi	Selfed	171	20	4	0	121	36	0

*Not flowering

Table 8. Breeding behavior of sex variations of ISF (1996-97)

Sex variants of ISF	No. of pl. obsd.	Number of pistillate plants in different order of spikes			Number of ISF plants in different orders of spikes		
		Pr*	S	T	Pr	S	T
SI	96	83	72	10	9	5	-
MI	65	60	32	35	3	5	3
HI	67	55	47	45	13	4	2
SISF	6	5	5	-	1	-	-
MISF	15	15	14	3	-	-	-
SI x LES	155	155	136	92	8	21	13
MI x LES	181	167	163	107	14	10	6
HI x LES	**215**	**165**	**178**	**107**	**42**	**33**	**84**
SISFxLES	23	15	16	14	8	7	9
Pistillate (check)	46	46	46	28	-	1	2

This indicated that the frequency of ISF plants in the progeny is higher by choosing highly interspersed types as female plants. The Sparsely and moderately interspersed types have a tendency towards pistillateness irrespective of the temperature regimes that induced ISF expression. Thus, there may be a maximum number of interspersed staminate flowers below which the breeding behavior of plants tend to be pistillate and above as interspersed. The genetic nature of highly interspersed type was able to express even through pistillate cytoplasmic background of the female plant.

Inheritance of ISF : Twenty-seven F_1s of Pistillate x ISF were evaluated initially in *kharif* 1995. Among the F_1s 37 per cent of the lines had 90 per cent pistillate progeny, 41 per cent had 80 per cent pistillate and 22 per cent had 70 per cent pistillate progeny. Thus, in the majority of the F_1s of Pistillate x ISF, pistillateness is maintained by ISF lines or pistillateness is dominant to ISF. However, the expression of ISF in those 10-15 per cent ISF plants is higher in male promoting (March - April) than in the female promoting environment (October-January). Based on the initial results, five F_1s of Pistillate x ISF were evaluated in a randomized block design with three replications in *kharif* and *rabi* seasons for two years (Table 9). The results indicated that the pistillateness is dominant to ISF expression in F_1s of Pistillate x ISF crosses. However, the segregating pattern of F_2s of Pistillate x ISF did not fit into Mendelian ratio of 3:1 or any epistatic ratios of 9:3:4 or 12:3:1 or 15:1 (Table 10). This indicated that the ISF expression may be due to modifying genes that are activated with the environmental conditions like temperatures, rainfall and day length. These genes may be similar to unstable nuclear factors, which are transformations with reversible changes at regular times during development, as

Table 9. Breeding behavior of the progeny of ISF variants as females in *kharif* and *rabi* seasons

Cross	No. of plants obser-ved	No. of pistillate plants in different orders of spikes				Number of pistillate plants with ISF in different orders of spikes			
		Pr	S	T	Q	Pr	S	T	Q
MI x LES									
P8 x ISF 4	16	16	16	15	15	-	-	1	1
P16 x ISF 4	16	7	15	15	4	9	1	1	-
P17 x ISF 4	20	20	18	14	-	-	-	-	-
P18 x ISF 4	14	13	9	2	-	1	4	-	-
P28 x ISF 4	16	16	16	3	1	-	-	-	-
HI x LES									
P8 x ISF 2	15	11	14	8	-	4	1	7	-
P17 x ISF 2	33	28	30	19	11	5	2	9	4
P3 x ISF 4	32	30	20	10	9	2	11	21	2
P5 x ISF 4	16	15	15	5	7	1	1	11	-
P7 x ISF 4	24	3	20	12	8	13	11	7	8
P15x ISF 4	16	8	15	4	-	8	-	4	-

Pr-Primary, S-Secondary, T-Tertiary and Q-Quaternary order of spike

Table 10. Sex expression of Pistillate x ISF crosses in *kharif* and *rabi* seasons

Cross	No. of plants observed	Number of plants		Number of plants with ISF in different order of spikes			
		Pistillate	Pistillate with ISF	Pr*	S	T	Q
***kharif* 1995 & 96**							
P17 x ISF 2	68	49	3	-	1	2	-
P23 x ISF 2	70	51	3	-	-	3	-
P17 x ISF 4	69	53	3	-	-	-	2
P23 x ISF 4	55	49	1	-	-	-	1
P8 x ISF 8	71	50	3	-	-	-	2
***rabi* 1997**							
P17 x ISF 2	22	21	1	1	-	-	-
P23 x ISF 2	21	19	2	2	-	-	-
P23 x ISF 4	21	21	0	-	-	-	-
P8 x ISF 8	19	19	0	-	-	-	-
***rabi* 1997-98**							
P8 x ISF 2	74	70	0	2	1	-	1
P13 x ISF 4	36	34	0	1	-	-	1
P12 x ISF 8	36	35	0	1	-	-	-
P8 x ISF8	11	11	0	-	-	-	-

Table 11. Segregating pattern of ISF character in F_2 based on 3:1 ratio

Class	Number of plants		(Observed - Expected)	$(O-E)^2/E$
	Observed (O)	Expected (E)		
Pistillate	906	740	166	37
ISF	81	247	-166	111
Total	987	987		$X^2 = 149$

proposed by Shifriss [6]. This is evident by the increase in proportion of interspersed staminate flowers in male promoting environment (M), in late orders of spikes and immediately after rainfall. Similar results were obtained when pistillate lines and dispersed (ISF) lines observed among Russian and American varieties and hybrids were selfed, sibbed and crossed with monoecious varieties [7]. In the resulting F_1s, gene Ff for monoecious nature was conditioned by unstable nuclear factors (influenced by environment) confirming the Shifriss Hypothesis.

However, the results from the present study differ from the observation that pistillate flower distribution and expression of staminate flowers were governed by two pairs of genes - id_1, id_2 for differentiation of staminate flowers and Id_1 and Id_2 for their absence which were partially dominant over id_1 and id_2 [8] when the parents involved varied in the proportion of ISF. The highest level of interspersed staminate flowers was associated with id_1, id_1, id_2, id_2 and plants having this genetic make up were less susceptible to environmental effects. A collaborative work with molecular biology may give proper insight to the mechanism of expression of interspersed staminate flowers.

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