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Breeding CMS-based white seeded pigeonpea hybrids

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ABSTRACT

Most people in Gujarat prefer vegetarian diets. Their protein requirement is derived from pulses, vegetables, and various milk products. However, *tuar dal* (pigeonpea) is, by far, the most popular and cheap source of protein in their diets. The state produces 2.4 lakh tonnes of pigeonpea annually, but this quantity is insufficient to meet the ever increasing domestic needs. Since the scope of increasing pigeonpea area in the state is limited, the emphasis is now centered on increasing its productivity. In this context, the recently developed CMS-based hybrid pigeonpea technology has shown a promise and hybrids such as ICPH 4506, ICPH 4503, ICPH 4502 and ICPH 4429 have demonstrated >50% yield advantage over the popular cultivars. The commercial hybrid seed production technology was successfully tested for over two years and, on average, yield of about 1000 kg/ha has been recorded. This paper summarizes the successful joint efforts made byICRISAT and Biogene Agritech, Ahmedabad (a private seed company) in developing and testing the first ever white-seeded high-yielding, and wilt and sterility mosaic disease resistant pigeonpea hybrid for Gujarat.

Key words: Disease resistance, Hybrids, Male-sterility, Pigeonpea, Seed production

Gujarat is one of the important pigeonpea producing states of India with an area of 2.7 lakh ha and production of 2.4 lakh tonnes (DAC, 2011). Pigeonpea in this state is predominantly consumed as *dal,* but afair proportion of its produce is also used as fresh vegetable. The agricultural environment of the state is characterized by semi-arid growing conditions with two-thirds of its area experiencing frequent early season and/or terminal drought. Traditionally, medium maturing (180-200 days) pigeonpea cultivars are grown in Vertisols and seed colour of all the local cultivars and landraces is white. This colour preference is unique in the state as in rest of the country brown seeded pigeonpea is preferred. The *tuar dal* (pigeonpea), by far, is the most popular and cheap source of daily proteins but its production is not sufficient to meet the ever increasing local demand. Since the scope of increasing pigeonpea area in the state is limited, the emphasisis now centered on increasing its productivity. In this context, the most reliable and proven approach of breeding high yielding hybrids offers scope. In pigeonpea, this technology has recently been developed at ICRISAT; and the first ever commercial hybrid was released in India in 2010 (Saxena *et al.,* 2013). This was possible due to successful breeding of a stable cytoplasmic nuclear male-sterility (CMS) and using the natural partial out-crossing (Saxena, 2008) systems. Considering the potential of heterosis breeding in enhancing yield, sincere efforts have been initiated to breed high yielding hybrids for Gujarat state. To achieve this, it was found essential that the hybrids have white bold seeds and resistance to diseases, besides high yields. This endeavour began with the breeding of white seeded, *fusarium* wilt and sterility mosaic (SM) disease resistant hybrid parents. This paper describes the process of developing white seeded hybrid parents and hybrids, besides discussing their performance, seed production system, and overall prospects of hybrid technology for Gujarat state.

MATERIALS AND METHODS

In order to develop appropriatemale and female hybrid parents, 34 white-seeded advanced breeding and germplasm lines identified as potential testers. Since the resistance to diseases was important, the parents and segregating populations were screened for resistance in a sick nursery. For SM screening, three week old seedlings were inoculated by stapling the infected leaves (Nene *et al.,* 1981); while for wilt screening a mean inoculum load @ 5 x10^6 spores /m^2 was maintained in the soil of sick nursery (Mamta Sharma, unpublished). Two 4m long rows of each test material were sown along with susceptible checks after every six rows. At the end the season counts were made in each plot for diseased and healthy plants and the data were presented as percentage incidence. To initiate a breeding programme, all the testers were crossed to a brown seeded medium duration stable CMS line ICPA 2043. Each hybrid plant was examined for pollen sterility and fertility. The lines which exhibited 100% pollen-sterility in hybrid combination were backcrossed to their respective testersto develop new male-sterile (A-) lines. In each back cross population, selections were exercised for white seed coat colour, disease resistance, plant type, and productivity. Each year the recurrent parents were selfed to produce pure seed. The BC_4F_1 populations appeared phenotypically uniform with respect to important morphological traits such as maturity, plant height and plant growth. These male-sterile genotypes were crossed to 14 known white-seeded fertility restorers to produce the first set of experimental hybrids. Simultaneously, further backcrossing to their respective maintainers was also continued to stabilize the female parents. The first set of 14 experimental hybrids was evaluated in 2009 rainy season at Patancheru. During the same year, 22 new hybrids were also synthesized for evaluation in 2010 at Ahmedabad. Similarly in 2010, eight promising hybrid combinations were made for evaluation in 2011 at both Patancheru and Ahmedabad.

Each year the hybrids were evaluated in RCBD along with controls in three replications. Each experimental plot consisted of 4 m long four rows with row-to-row and plant-to-plant spacing of 75 and 30 cm, respectively. To study pollen fertility, five floral buds were harvested from different branches of each plant of each hybrid. The anthers were squashed and stained with 2% aceto-carmine solution and examined under light microscope using 10X magnification. The harvest from the middle two rows was used to estimateseed yield and their 100-seed weight was recorded on three random samples drawn from the net plot harvest.

RESULTS AND DISCUSSION

Breeding of Hybrid Parents

Pollen fertility data indicated that the F_1 hybrids, derived from line x tester crosses could easily be classified into three groups. The first group consisted of completely male-sterile hybrids. The male parents responsible for producing such hybrids were ICPL 211, ICPL 96053, SGBS 6, SIPS 10, ICPL 20175, and ICP 14085; and these testers were designated as 'maintainers' of the A_4 CMS (Saxena *et al.,* 2005) system. Such genotypes carry recessive ($fr_1 fr_1 fr_2 fr_2$) genes which interact with mitochondrial genome of the host plant and invalidate the normal process of microsporogenesis to produce a male-sterile phenotype (Kaul, 1988). Backcrosses were accomplished to substitute nuclear genome of the recurrent parents. In the second group, 15 hybrids, exhibiting full male fertility with pollen load as good as control cultivars, were included. The testers of these crosses were classified as 'fertility restorers'. According to Saxena *et al.* (2011a) high pollen load in the hybrids (similar to that of popular cultivars) is an indicator of the presence of two dominant genes ($Fr_1 Fr_1$ Fr_2 Fr_2), which conferred fertility restoration to the hybrid plants. Such *Fr* genes have the ability to overcome the ill-effects of recessive *fr* genes and allow the production of fertile pollen (Kaul, 1988). The third group of hybrids consisted of 13 combinations which segregated for male-sterility and fertility. This is an unwanted group of hybrids and indicated lack of genetic uniformity in the testers. This situation may arise either due to heterozygosity at fertility restoring loci or genetic impurity caused by some physical factors (e.g. mixtures etc.). In pigeonpea such possibilities are always high due to partial out-crossing nature of the species.

The inoculum levelof the two diseases in the sick nursery was excellent; and the susceptible checks recorded >95% wilt and 100% SM incidence. These observations suggested that screening under such high density inoculum for 2-3 consecutive generations will yield genotypes with high and stable resistances. Among diseases, SM is not severe in Gujarat but fusarium wilt is widespread in most pigeonpea growing areas;and it is one of the most threatening yield constraints and in the hybrid breeding programme high emphasis was given to incorporate resistance to this disease. The wilt disease is caused by a soil-borne fungus known as *Fusarium udum* Butler and it has inherent capacity to survive for over five years in the soil (Reddy *et al.,* 1990). Since chemical and cultural control measures are ineffective in reducing wilt losses; breeding resistant cultivars appears to be the best option. Information on the inheritance of wilt resistance is useful in developing appropriate hybridization and selection schemes. Pathak (1970) found two complementary genes for resistance; while Kotresh *et al.* (2006) reported a single dominant gene in controlling resistance to wilt. On the contrary, Jain and Reddy (1995) found a single recessive gene for resistance. Recently, Saxena *et al.* (2012) showed that resistance to wilt was due to the presence of one dominant and one recessive gene with epistatic inhibitor effect. Since, the published information on inheritance of wilt resistance is inconclusive; by and large, the plant breeders rely on the results of screening nursery for selecting desirable genotypes.

In the present study six male-sterility maintainers (ICPL 211, ICPL 96053, SGBS 6, SIPS 10, ICPL 20175, and ICP 14085) were identified and the derived male-sterile lines were designated as ICPA 2101, ICPA 2166, ICPA 2188, ICPA 2189, ICPA 2198 and ICPA 2199, respectively. These male sterile lines represented a useful genetic variation (Table 1) for starting a fairly good hybrid breeding programme. All the A-lines had attractive white seed coat colour and expressed 98-100% male-sterility in BC_6 generation. Among the A-lines, ICPA 2101 was earliest (106 days) to flower while ICPA 2166 (148 days) was the latest. The plant height in this group ranged from 138 to 215 cm. All the A-lines were resistant to SM but only two linesICPA 2198 and ICPA 2199 exhibited resistance to fusarium wilt. All the 15 fertility restorers exhibited resistance to both wilt and SM diseases. The 50% flowering among the restorers varied from 110 to 149 days. ICPL 20098 (14.3 g/100 seeds) and ICPL20137 (14.8 g/100 seeds) were most promising with regard to seed size (Table 1).

Evaluation of experimental hybrids

In 2009, 14 white seeded experimental hybrids were evaluated in two trials at Patancheru. Three hybrids in trial 1 and two hybrids in trial 2 were found segregating for fertility restoration. Hybrids ICPH 4171 (35% superiority), ICPH 4331 (33% superiority), ICPH 4330 (28% superiority), and ICPH 4182 (34% superiority) performed well (Table 2). All these hybrids had acceptable seed size. These hybrids matured between

Table 1. Important plant and grain characteristics of white seeded B-and R-lines

Genotype	Days to		Plant height (cm)	Seeds/ pod	100-seed mass (g)	Disease %		% pollensterility (BC_6F_1)
	flower	mature				wilt	SM	
B-lines								
ICPB 2101	106	150	138	4.4	14.5	67	0	98
ICPB 2166	148	172	213	4.1	15.1	78	11	100
ICPB 2188	123	172	179	3.7	10.5	97	0	100
ICPB 2189	117	169	170	4.4	10.7	100	0	100
ICPB 2198	122	165	212	3.9	10.1	0	0	100
ICPB 2199	122	170	215	4.0	11.4	0	0	100
R-lines								
ICPL 20098	130	174	235	4.2	14.3	0	0	-
ICPL 20105	131	187	285	4.2	13.6	1	0	-
ICPL 20106	125	170	230	4.2	13.6	8	0	-
ICPL 20108	122	165	235	4.3	11.4	0	0	-
ICPL 20110	126	175	245	4.1	9.8	2	0	-
ICPL 20112	123	165	218	3.7	9.1	12	0	-
ICPL 20115	126	182	250	3.9	9.4	0	0	-
ICPL 20135	126	179	237	3.8	9.8	15	0	-
ICPL 20137	130	187	262	3.8	14.8	8	1	-
ICPL 20138	128	170	230	3.9	13.8	0	0	-
ICPL 94062	110	163	217	3.9	10.9	2	0	-
ICPL 96053	122	170	215	3.9	11.4	0	0	-
ICPL 99044	124	168	220	3.9	10.2	0	0	-
ICP 8094	129	189	210	4.0	9.1	15	10	-
ICP 13092	149	185	215	3.7	14.7	0	0	
Checks								
ICP 8863 (C)	114	162	198	4.0	9.3	-	100	
ICP 2376 (C)	116	168	205	3.9	9.8	100	-	-

Table 2. Performance of promising white- seeded experimental hybrids at Patancheru and Ahmedabad.

Entry No	Days to		Plant height (cm)	Seeds /pod	100-seed mass (g)	Yield (kg/ha)	
	flower	mature				Hybrid	% Gain
2009 Trial 1 (n=7) (Patancheru)							
ICPH 4171	124	178	275	3.8	13.6	1769	35
ICPH 4331	122	165	242	3.9	10.8	1743	33
ICPH 4330	110	160	235	4.0	12.0	1685	28
Maruti (C)	112	162	220	4.1	9.8	1313	-
SEm±	1.7	3.3	3.7	0.07	0.36	203.4	-
CV(%)	2.0	3.0	2.2	2.34	4.57	20.5	-
2009 Trial 2 (n=7) (Patancheru)							
ICPH 4182	116	163	260	4.0	11.4	2518	34
Maruti (C)	112	160	190	3.8	9.7	1881	-
SEm±	1.7	1.9	3.3	0.04	0.31	260.7	-
CV(%)	1.9	1.6	2.0	1.41	3.62	21.7	-
2010 Trial 1 (n=11) (Ahmedabad)							
ICPH 4434	124	186	285	4.2	9.1	1585	43
ICPH 4436	124	184	290	4.1	10.3	1559	41
ICPH 4229	117	180	265	3.9	9.4	1537	39
ICPH 4431	118	180	292	4.1	9.7	1415	28
Maruti (C)	116	178	265	3.9	9.6	1107	-
SEm±	1.1	1.1	5.9	0.11	0.86	120.2	-
CV (%)	1.3	0.9	2.9	3.76	13.14	13.2	-
2010 Trial 2 (n=11) (Ahmedabad)							
ICPH 4184	119	181	318	3.9	9.2	1970	55
ICPH 4429	120	182	295	4.2	10.7	1918	51
ICPH 4421	124	184	320	3.9	9.2	1867	47
ICPH 4426	126	186	312	4.1	9.0	1792	41
Maruti (C)	116	177	262	3.9	9.3	1270	-
SEm±	1.0	1.1	4.3	0.18	0.62	164.8	-
CV (%)	1.2	0.8	2.0	6.016	10.35	16.8	-

160-178 days. In general the hybrids were taller than the cultivars. Highest yield of 2518 kg/ha was recorded by ICPH 4182 with 34% superiority over control cultivar Maruti. This hybrid matched well with the control in flowering and maturity. In 2010, 22 white seeded hybrids were evaluatedin two trials along with control Maruti at Ahmedabad. The promising hybrids (Table2) were ICPH 4434, ICPH 4436, ICPH 4229, ICPH 4184, ICPH 4429, ICPH 4421 and ICPH 4426. The yield advantage in this group of hybrids ranged between 28 and 55%. Among these, hybrid ICPH 4184 (1970 kg/ha) was the best with 55% more yield than control Maruti and resistance to both wilt and SM diseases.

In 2011, a set of eight promising hybrids was evaluated at Patancheru and Ahmadabad. The standard heterosis in these trials ranged from -55% to 83%. Based on the two location data, ICPH 4506 (83% superiority), ICPH 4503 (73% superiority), ICPH 4502 (52% superiority), and ICPH 4500 (37% superiority) were found promising (Table 3). Hybrid ICPH 4506 recorded highest yield both at Patancheru (2306 kg/ha) and Ahmedabad (1875 kg/ha). All the hybrids had white seeds with acceptable seed size. Hybrid ICPH 4506 involved parents of diverse origin; the male-sterile line was of Indian origin and the restorer was from Africa. It is too early to conclude that heterosis for yield in this cross was a consequence of genetic diversity of the parents.

In view of huge losses caused by *fusarium* wilt in pigeonpea, it is essential that all the recommended cultivars have high levels of disease resistance. Of the six white seeded A-lines, only two ICPA 2198 and ICPA 2199 had resistance to wilt disease (Table 1). On the other hand, the resistance for wilt was common among the fertility restorers. To select high yielding hybrid combinations, all the available A- lines were crossed to all the restorers. In some of the hybrids the female parent was susceptible to wilt disease. The evaluation of hybrids for disease reaction revealed that the hybrids such as ICPH 4184 and ICPH 4182, which involved wilt susceptible female and resistant male parents were also resistant, indicating dominance of wilt resistance over susceptibility, confirming the observations of Saxena *et al.* (2012). The availability of hybrid parents which carry a dominant gene for wilt resistance will enhance the probability of breeding high yielding hybrids with stable performance. This is because both the resistant x resistant and resistant x susceptible combinations will produce wilt resistant hybrids.

Based on three years of experimentation, a total of 12 experimental hybrids (Table 4) were selected. These hybrids were based on four A-lines (ICPA 2101, ICPA 2188, ICPA 2189,

Table 3. Performance of white-seeded hybrids at Patancheru and Ahmedabad in 2011.

Entry No.	Days to		Plant height (cm)	Seeds / pod	100-seed mass (g)	Yield (kg/ha)		Mean	% Gain
	flower	mature				Patancheru	Ahmedabad		
ICPH 4502	136	195	232	3.6	13.2	1611	1555	1583	52
ICPH 4503	129	196	245	3.9	12.4	1733	1875	1804	73
ICPH 4500	132	185	230	3.6	11.9	1828	1028	1428	37
ICPH 4506	134	197	235	3.4	11.3	2306	1493	1900	83
ICPH 4439	135	191	250	3.7	11.5	1072	1493	1278	23
ICPH 4504	124	190	230	3.7	12.5	939	1312	1126	8
ICPH 4501	130	197	232	3.3	13.3	1506	569	1038	-.1
ICPH 4505	137	198	220	3.4	12.2	350	587	469	-55
Maruti (C)	111	161	212	3.4	10.1	1194	885	1040	-
SEm±	2.1	2.5	7.2	0.14	0.26	294.4	59.3	-	-
CV (%)	2.3	1.8	4.4	5.81	3.03	29.6	7.4	-	-

Table 4. List of promising white-seeded hybrids, their standard heterosis, and wilt and sterility mosaic diseasereaction at Patancheru

ICPH No.	Parentage	Yield (kg/ha)		wilt%			SM%		
		Hybrid	% Gain	Female	Male	Hybrid	Female	Male	Hybrid
4434	ICPA 2101 x ICPL 20098	1382(2)	22	67	0	13	0	0	0
4431	ICPA 2101 x ICPL 20108	1375(2)	22	67	0	13	0	0	0
4438	ICPA 2101 x ICPL 20138	1344(1)	21	67	NA	0	0	NA	0
4184	ICPA 2188 x ICPL 20108	1483(2)	11	97	0	0	0	0	0
4421	ICPA 2188 x ICPL 20110	1867(1)	47	97	2	13	0	0	0
4182	ICPA 2189 x ICPL 20108	2117(2)	38	100	0	0	0	0	0
4426	ICPA 2189 x ICPL 20110	1792(1)	41	100	2	17	0	0	0
4429	ICPA 2189 x ICPL 20137	1918(1)	51	100	0	17	0	0	0
4500	ICPA 2199 x ICPL 20108	1428(1)	37	0	0	0	0	0	0
4502	ICPA 2199 x ICPL 20106	1583(1)	52	0	8	0	0	0	0
4503	ICPA 2199 x ICPL 20137	1804(1)	73	0	0	5	0	1	0
4506	ICPA 2199 x ICP 8094	1900(1)	83	0	15	10	0	0	0

( ) number of years

and ICPA 2199) and seven restorer lines (ICP 8094, ICPL 20108, ICPL 20106, ICPL 20138, ICPL 20110, ICPL 20098, and ICPL 20137). Yield and heterosis data represented either a single (8 hybrids) or two (4 hybrids) environments. Hybrids ICPH 4506, ICPH 4503, ICPH 4502 and ICPH 4429 recorded >50% yield advantage. The wilt disease in Gujarat can cause huge yield losses, and hence yield potential of the hybrids cannot be realized unless they carry high levels of genetic resistance to this disease. In the present set of materials all the 12 selected hybrids exhibited resistance to both wilt and sterility mosaic diseases. Hence, hybrid breeding technology involving disease resistant materials can be exploited for enhancing pigeonpea production and productivity in Gujarat state. In a dynamic hybrid pigeonpea breeding programme diversification of hybrid parents and breeding of high yielding hybrids for specific agro-ecological regions need attention. Since *Fusarium* wilt is an important constraint, the breeding programme should be geared to develop high yielding disease resistance parents with high combining ability. In addition, certain market preferred traits such as large and white seed, large pods, and earliness should also be given due importance.

Large-scale Hybrid Seed Production

In pigeonpea the success in the seed production of hybrids and male-sterile parents is primarily determined by the population of pollinating insects (Saxena *et al.,* 1990). Since the pollinating insects can fly considerable distances, 500 m isolation distance has been recommended for maintaining genetic purity of pigeonpea. During 2010 and 2011 seasons, six seed production plots were grown using 3male-sterile and 1fertility restorer lines. At all the six locations the results were encouraging (Table 5). In 2010, the highest hybrid yield (1510 kg/ha) with a mean of 928 kg/ha was recorded. Similarly in 2011 also, mean hybrid seed yield of 1295 kg/ha was recorded. These results suggested that seed production of pigeonpea hybrids will not pose any problem in the promotion of hybrids in Gujarat.

Purity test of hybrids

Traditionally, genetic purity of hybrid seed is assessed through standard ‘Grow-out Test’. In pigeonpea, however, such tests will require one full season due to its long duration and photo-sensitivity. Therefore, simple, rapid, and cost effective seed quality tests based on molecular markers are being developed (Saxena *et al.,* 2010; Bohra *et al.,* 2011). This assay can now be used for reliable assessment of seed purity within the commercial seed lots of the hybrids to ensure the supply of high quality seeds. In addition, a morphological marker ‘obcordate leaf’ that is controlled by single recessive gene, is being incorporated in A- and B- lines to ensure the purity of female parents. It is a cost effective technology and takes only 4-5 weeks from harvest to identify off-types in the female line. This technology is easy and can be adopted at on-farm scale (Saxena *et al.,* 2011b).

Table 5. Seed production of CMS-based pigeonpea hybrid in Gujarat during 2010 and 2011

Year	Location	Area sown (m^2)	Row ratio (female: male)	Hybrid yield (kg/ha)
2010				
	Vadali	400	3:1	1510
	Daramali	400	3:1	525
	Idar	400	3:1	750
	Mean			**928**
2011				
	Dhagandhra	400	3:1	1669
	Halvad	400	3:1	1465
	Kolal	400	3:1	750
	Mean			**1295**

In order to make hybrid seed available to small holder farmers, it is essential that the hybrid seed is available at affordable prices. According to the studies conducted by Saxena *et al.* (2011c) at Indore (Madhya Pradesh), the cost of producing one hectare of hybrid pigeonpea seed, excluding land cost, was Rs. 26,395 and one kilogram of hybrid seed was produces @ Rs. 18.32. Soon we plan to generate similar information for the white seeded hybrids. Due to vigorous canopy of hybrid plants the recommended seed rate for the hybrids is 50% less than pure line cultivar; this means that the adoption of hybrids by even small farmers is possible because it will not increase the seed input cost.

Besides high yield, the cultivation of hybrid pigeonpea generally offers some additional benefits to the farmers (Saxena *et al.,* 1992). This include (i) rapid establishment of crop that makes the hybrid plants competitive against weeds and inter-crops, (ii) enhanced ability of hybrids to with stand intermittent droughts due to their greater root mass and depth, (iii) greater resilience against diseases attack, (iv) crop uniformity with respect to growth and maturity, and (v) about 50% less seeding rate without sacrificing productivity. All these factors together offer a greatscope for the adoption pigeonpea hybrids as a commercial crop. We consider that the CMS-based hybrid pigeonpea technology is ready to be delivered to the users and soon farmers of Gujarat will reap the benefits of this technology.

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