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Identification of new fertility restorers for development of early maturing pigeonpea hybrids

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ABSTRACT

The paper reports the identification of two early maturing fertility restorers *viz.*ICPR 2433 and ICPR 2438. The fertility restoration was found to be under the control of two duplicate dominant genes. Multi-location evaluation of the hybrids involving these restorers revealed that the fertility restoration of the hybrids was stable across seven environments. On average, the two hybrids (ICPH 2348 and ICPH 2433) out-yielded the control ICPL 88039 by a margin of over 100%. These results showed that with targeted breeding and selection of fertility restorers it would be possible to breed early maturing hybrids in the near future.

Key words: Cytoplasmic male sterility, Early maturity, Fertlity restoration, Hybrid.

INTRODUCTION

Commercial hybrids in legumes were not known until the world's first pigeonpea hybrid was released in India by Saxena *et al*. (2013). This technology is based on three hybrid parents, commonly identified as male sterile (A-), maintainer (B-), and restorer (R-) lines. The cytoplasmic nuclear male-sterile (CMS) line used in this endeavour was bred using cytoplasm of a wild relative of pigeonpea, representing secondary gene pool (Saxena *et al*., 2005). The availability of stable fertility restorers in the medium maturing pigeonpe group is well established (Saxena *et al*., 2014a) but it is a real issue among early maturing types. This paper, besides reporting the identification of early maturing fertility restorers and inheritance of genes governing this trait, also discusses strategies to breed new early maturing fertility restorers and high yielding pigeonpea hybrids.

MATERIALS AND METHODS

An early maturing and stable CMS line ICPA 2039 was selected for this study. Out of hundreds of early maturing testers evaluated at ICRISAT in the last few years, only two inbred lines ICPR 2438 and ICPR 2433 exhibited stable fertility restoration and these were used for inheritance study. Pure seeds of the three genotypes were obtained from ICRISAT's breeders seed production programme. In 2009 rainy season, two crosses ICPA 2039 x ICPR 2438 and ICPA 2039 x ICPR 2433 were made and their hybrids were grown in 2010. A few hybrid plants were bagged to produce pure F_2 seed; and, at the same time, backcrosses were also made using the hybrid plants as male parent and ICPA 2039 as female parent. In 2011 rainy season, seeds of F_1, F_2 and BC_1F_1 generations of both the crosses were sown in Alfisols at ICRISAT centre, Patancheru, under irrigated conditions. All the recommended cultural practices were adopted to grow a normal crop of pigeonpea. At flowering, each plant was assessed for its pollen fertility. For this, 5-6 fully grown but unopened floral buds were harvested from different branches between 9-11 a.m. and their anthers were crushed on a glass slide and drenched with 2% aceto-carmine solution. In each slide 3-5 microscopic fields were studied using 10X magnification for recording pollen viability data. Plants with <5% pollen viability were considered male sterile. The data were analysed using the standard chi-square test. For assessing the stability of yield and fertility restoration the two hybrids ICPH 2438 (restorer ICPR 2438) and ICPH 2433 (restorer ICPR 2433) were evaluated at seven locations in the trials replicated three times. Four rows of five meter length constituted a plot. Besides seed yield (t/ha), the data were recorded on the number of fetile and sterile plants. For recording fertility data, individual plants in each plot were examined visually for the presence/absence of pollen grains.

RESULTS AND DISCUSSION

The cytoplasmic nuclear (or genetic) male sterility (CGMS) in plants generally arises due to some deleterious interactions occur between nuclear and cytoplasmic genomes. The dominant nuclear genes of other genotypes can restore male fertility of the hybrid plants. The number and action of genes may vary from crop to crop and among crosses.

In the present study, both the crosses showed complete dominance of fertility restoration in F_1 generation

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(Table 1) and each hybrid plant had excellent pollen load. To select such genotypes Saxena *et al*. (2014a) suggested a simple field technique that is based on the hypothesis of positive relationship of the number of dominant genes with pollen load (> 90%). In F_2 generation, the crosses showed a good fit to 15 fertile : 1 sterile plants, suggesting presence of duplicate dominant gene action. This hypothesis was confirmed in the backcross generation, which segregated in a ratio of 3 fertile :1 sterile plants. Pooled data over the two crosses also confirmed the hypothesis of duplicate dominant gene action in controlling fertility restoration of A_4 CMS system. A perusal of published reports on the genetics of fertility restoration of A_4 CMS system in the medium maturing pigeonpea (Table 2) showed that either one or two dominant genes controlled the fertility restoration with variable gene action. Saxena *et al*. (2011a) reported that the fertility restoration in the early maturing pigeonpea was stable across environments only when both the dominant genes were present together in a single genotype; and the hybrids carrying either of the dominant genes were also fertile but not in all the environments. Dalvi *et al*.(2008) and Saxena *et al*.(2014a) examined over 2000 testers from pigeonpea germplasm across the maturities and reported that, in compaison to early maturing germplasm, the fertility restoring genes were more frequent in the medium maturing pigeonpea germplasm. These observations suggest that finding a stable and genetically diverse fertility restorers among early maturing germplasm will not be easy and breeding of new restorers is necessary to develop high yielding hybrids.

At present, breeding of early maturing hybrids is being persued at various ICAR centres such as Ludhiana, Jaipur, Kanpur and New Delhi; and, at each location, the availability of stable fertility restorers is the main bottleneck in breeding high yielding hybrids. This may be due to narrow genetic diversity for restoration in this group of materials, inadequate expression of restoration (due to a single restorer) under low-temperature environments. In the present study two early maturing restorers ICPR 2438 and ICPR 2433,each with two dominant genes have been identified; and their hybrids exhibited stable fertility restoration and good yield

Table 1: Segregation for male fertility and sterility in various generations of two crosses (early group) involving *C. cajanifoleus* cytoplasm

Cross	Generation	Total plants	Fertile plants	Sterile plants	Expected ratio	Chi-square Cal	Prob.
ICPA 2039 x ICPR 2038	F_1	78	78	0	01:00	-	-
	F_2	224	212	12	15:01	0.3	0.58
	BC_1F_1	219	172	47	03:01	1.46	0.23
ICPA 2039 x ICPR 2447	F_1	76	76	0	01:00	-	-
	F_2	113	110	3	15:01	2.49	0.11
	BC_1F_1	74	61	13	03:01	2.18	0.13
Pooled data	F_1	154	154	0	01:00	-	-
	F_2	337	322	15	15:01	2.45	0.11
	BC_1F_1	293	233	60	03:01	3.19	0.07

Table 2: Summary of reported gene action for fertility restoration in pigeonpea

Author	Female parent (A-line)	Male parent (R-line)	Maturity (R-line)	Gene action	Origin of male parent
Dalvi *et al*. (2008)					
	ICPA 2039	ICP 12320	Medium	Single dominant	Pakistan
	ICPA 2039	ICP 11376	Medium	Duplicate dominant	Malawi
	ICPA 2039	HPL 24-63	Medium	Duplicate dominant	India
Sawargaonkar *et al*. (2012)					
	ICPA 2043	ICP 2766	Medium	Duplicate dominant	A.P
	ICPA 2047	ICP 3513	Medium	Duplicate dominant	U.P
	ICPA 2048	ICP 3477	Medium	Single dominant	U.P
	ICPA 2092	ICP 2766	Medium	Single dominant	A.P
Kyu and Saxena (2011)					
	ICPA 2047	ICPL 20107	Medium	Duplicate interactions	ICRISAT
	ICPA 2052	MAL 9	Medium	Dominant Epistasis	U.P
	ICPA 2092	ICP 10928	Medium	Dominant Epistasis	
Saxena *et al*. (2011a)					
	ICPA 2043	Asha	Medium	Duplicate dominant	ICRISAT
	ICPA 2043	ICPR 3467	Medium	Duplicate dominant	U.P

in the multi-location trials (Table 3). This means that these two restorers are of value and can be used directly to develop early matuing hybrids; or alternatively serve as donor parents for transfering both the fertility restoring genes to non-restoring genotypes having high combining ability. Hence, in early maturing hybrid parent breeding programmes, care should be taken to select individual restorers with both the dominant genes together. This emperical execise involves physical examination of fully gown but unopened floral buds for pollen load on a bright sunny day. The hybrid plants with pollen load as good as a cultivar expectedly will carry both the dominant genes with high probability, while those with either of the dominant gene will produce sparse pollen. This approach is being used at ICRISAT and so far it has produced good results in selecting promising fetility restorers. Therefore to develop high yielding stable early maturing hybrids, selection of the testers should be based on their ability to produce large quantity of pollen grains on their hybrid plants. This will allow selection of hybrid male parents with both the dominant genes with high probability.

The hybrids produced by crossing the two selected fertility restorers have demonstrated high stability with respect to their fertility restoring ability in hybrid combinations (Table 3). In both the hybrids > 95% of the plants were fully fertile with pollen load as good as the control cultivar ICPL 88039. These observations confirmed the conclusions that both the fertility restoration genes together in the restorer line will produce stable hybrids. The performance of both the hybrids over seven locations was excellent. On average hybrids ICPH 2438 and ICPH 2433 produced yield advantage of 119% and 123 %, respectively, over the most popular control cultivar ICPL 88039. The present results clearly demonstrated that, in pigeonpea, high yielding early maturing hybrids can be produced through breeding of elite fertility restorers with both the fertility restoring genes, high combining ability and market-preferred traits.

It is true that the famous Indian green revolution saved the country from hunger but its also true that in meeting the targets of cereal production, the pulses were put on the back burners. This resulted in the stagnation of their productivity and reduction in per capita availability from 52 to 31 g/day. Among the pulses, pigeonpea is the most important rainy season crop but its annual production of 2.93 m tonnes (FAO, 2013) falls short of domestic demand by a margin of about 0.5m tonnes. This challenge is quite serious as there is stagnation in the productivity and a little or no scope of expanding the traditional pigeonpea growing areas. Hence, to increase the national pigeonpea production, serious efforts are being made to i) increase the productivity of the crop through exploitation of hybrid vigour and ii) explore new pigeonpea production niches with early maturing cultivars (Saxena *et al.,* 2014b). The recent success in introducing early maturing pigeonpea in the northern hills (Saxena *et al.,* 2011b), dry areas of central India (Patel and Sharma, 1989), Rajasthan (S.J. Singh, personal communicatin), and in Indo-gangetic plains (Pande *et al*., 2006) has demonstrated the potential of early maturing cultivars in enhancing the gross production of this commodity. In this context, the early maturing hybrids can also play an important role in enhancing the productivty by a margin of 25-30% (Saxena *et al*., 2014b). Besides high yield, the pigeonpea hybrids are also known for greater drought toleance and stability. Unfortunately, so far efforts to breed early maturing stable high yielding hybrids have not been successful due to non-availability of good fertility restorers. The present study has opened up a channel to start breeding high yielding early maturing hybrids in pigeonpea.

Table 3: Stability of yield (t/ha) and fertility restoration expressed as fertile plants (%) in the hybrids based on the selected restorers ICPR 2438 (ICPH 2438) and ICPR 2433 (ICPH 2433)

Location (n =7)	**ICPH 2438I**		**ICPH 2433**		**Control (ICPL 88039)**		**SEm(±)**	**CV (%)**
	Yield (t/ha)	**Fertility (%)**	**Yield (t/ha)**	**Fertility (%)**	**Yield (t/ha)**	**Fertility (%)**		
Aurangabad	4.53	98	4.05	100	1.31	100	0.26	11
Jalna	0.62	100	1.83	100	0.56	100	0.1	15
Hyderabad	3.47	100	3.04	100	1.43	100	0.32	16
Phaltan	2.34	100	2.83	96	1.16	100	0.19	12
Jalna	1.37	98	0.87	100	0.98	100	0.07	9
Aurangabad (September planting)	2.83	100	2.67	98	1.09	100	0.16	10
Akola	1.48	98	1.7	100	1.07	100	0.1	10
Mean	2.42	-	2.38	-	1.09	-	-	-
% Superiority over control	119	-	123	-	-	-	-	-

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