

indirect control of CO₂ production and linkage to the carbon cycle, we would study the rapidly occurring silica depletion in nature.

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Temperature-sensitive male-sterility system in pigeonpea

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The present communication reports successful breeding of the first temperature-sensitive male-sterility system in pigeonpea (*Cajanus cajan* (L.) Millsp.). This material was derived from a cross involving *Cajanus sericeus* (Benth. ex Bak.), a wild relative of pigeonpea, and a cultivar (ICPA 85010). The selections demonstrated marked changes in the fertility status of the plants under field conditions in different temperature regimes. At $\geq 25^{\circ}\text{C}$ the plants were completely male-sterile, while at $\leq 24^{\circ}\text{C}$ the same plants became fully male-fertile. These genotypes can be used to develop a two-line hybrid breeding system in pigeonpea.

Keywords: Fertility status, pigeonpea, temperature-sensitive male-sterility, seed production.

WHEN pedigree and population breeding programmes failed to break the decades-old low-yield plateau in pigeonpea (*Cajanus cajan* (L.) Millsp.), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) breeders turned towards exploring hybrid technology. This vision was essentially based on the possibility of exploiting a small window of natural out-crossing for seed production. This endeavour began in 1974 with a massive germplasm search for a suitable male-sterility

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system that ended in the identification of a genetic male-sterility (GMS) system¹ and release of the world's first pigeonpea hybrid ICPH 8 in 1992 (ref. 2). However, the large-scale seed production problems led to the demise of this hybrid. In the second phase of hybrid breeding, a comprehensive research programme was launched to breed more efficient cytoplasmic–nuclear male-sterility (CMS) system. Among various wild species tried to develop a CMS system, success came from a cross between *Cajanus cajanifolius* and cv. ICP 28 (ref. 3), and with this technology the large-scale hybrid seed production became a reality. This CMS system was used extensively in hybrid pigeonpea breeding and a high-yielding hybrid, ICPH 2671 was released in 2010 (ref. 4). But it still involved expensive seed production of male-sterile (A-) line that was dependent on a corresponding maintainer (B-) line and insect pollinators, and hence required special pollinator-rich locations for seed production. Now, a new concept in hybrid breeding is being developed at ICRISAT and this involves a temperature-sensitive male-sterile line which would ease seed production of female parent and eliminate its dependency on insect pollinators and maintainer line. This system is already established in rice, but is unique for pigeonpea. The present communication, besides describing the results of field evaluation of the temperature-sensitive selections under different environments, discusses their prospects in developing a two-parent hybrid breeding system in pigeonpea.

In an attempt to breed a stable CMS system, *Cajanus sericeus* (Benth. ex Bak.), a wild relative of pigeonpea, was crossed as female parent with a cultivar; the materials and methods used to generate early generation breeding materials have been described earlier⁵. The materials generated in that study⁵ were further carried forward for the next seven generations and lines with temperature-sensitive gene(s) were selected⁶. The present study was conducted with four temperature-sensitive selections, namely Envs Sel 1, Envs Sel 2, Envs Sel 3 and Envs Sel 5, made on the basis of their effective male-sterility reversion. These selections were planted under insect-proof cages during 2007, 2008 and 2009 in the middle of June. Observations on each plant for pollen fertility were recorded at the onset of flowering in September using acetocarmine test. The second round of fertility evaluation was done in November, when the plants started developing young pods. The final observations were recorded in March, when the temperatures rose again.

In the second experiment conducted in 2008, the bulk of the four lines was grown on two planting dates to assess their behaviour with respect to fertility status and ability to produce self-pollinated seed. In this experiment, the first sowing was done on 15 June and the second on 30 September under irrigated conditions on ridges, 75 cm apart. In the first sowing data were recorded on pollen fertility and sterility in the months of August, November and March while in second sowing, the data on

male-fertility/sterility were recorded in November and March.

Inherently, pigeonpea has a number of traits that are present in a typical perennial plant. However, it does not survive for many years and hence is classified as a 'short-lived perennial'. The late maturing type with its strict short photo-period requirement flowers once in a year at the onset of short days. On the contrary, an early maturing genotype is photo-insensitive⁷ and can flower any time of the year; once flowered, it continues flowering throughout the year. In this study, only early maturing selections were made and it provided an excellent opportunity to study the effects of different temperature regimes on the fertility reversal of the same plants over a period of time.

In each year the selection of thermo-sensitive genotypes was made in mid-June sowings under netted field conditions. The field testing data recorded in the three years were in agreement with each other (Table 1); all the four selections possessed environment-sensitive gene(s). The changes recorded with respect to fertility status of the plants were distinct. In the progenies tested in 2007, out of 53 plants grown in the four selections, 46 (86.8%) were male-sterile, suggesting some degree of impurity with respect to thermo-sensitive gene(s); the removal of such fertile segregants during selection enhanced their genetic purity. In 2009, all the four lines were pure and no fertile plant was observed (Table 1).

In comparison to the length of photo-period (11.2–12.6 h) during experimentation, the variation for temperature (19.1°C to 30.3°C) was large and in different years it did not vary significantly (Table 2). Further, the temperatures during August, November and March were vastly different; this significantly affected the fertility status of the plants. Therefore, it can be inferred that the prevailing temperatures during these months played an important role in the manifestation of male-sterility/fertility of the selections. It was also observed that under the temperature regime of $\geq 25^{\circ}\text{C}$ that prevailed during the standard weeks of 34 and 35 (in August), the plants were completely male-sterile with absolutely no pollen produced (Table 2). In contrast, during the standard weeks of 47 and 48 (in November), when daily mean temperatures ranged between 19.1°C and 23.9°C, the male-sterile plants turned fully fertile and produced self-pollinated pods. In the next cycle of flowering during the standard weeks of 12 and 13 (in March), the same plants again transformed their sex and reverted to male-sterility with no trace of pollen grains.

In pigeonpea both temperature and photo-period have been reported to be important in determining flower initiation and their appearance⁸, but their role in determining male-fertility/sterility has not been established. Information available on different crops revealed that the reversion of male-sterility to fertility is genotype-specific and it may be controlled by either photo-period, temperature

Table 1. Field observations recorded in three months on male-sterility and fertility in four temperature-sensitive selections

Year	Selection	September		November		February	
		Sterile plants	Fertile plants	Sterile plants	Fertile plants	Sterile plants	Fertile plants
2007	Envs S-1	13	1	2	12	11	0
	Envs S-2	11	2	1	12	11	2
	Envs S-3	9	1	2	8	8	0
	Envs S-5	13	3	2	14	12	2
	Total	46	7 (13.2%)	7	46 (86.8%)	42	4 (8.7%)
2008	Envs S-1	22	0	1	21	22	0
	Envs S-2	8	0	1	7	8	0
	Envs S-3	10	0	0	10	7	0
	Envs S-5	18	0	3	15	16	0
	Total	58	0 (0.0%)	5	53 (91.4%)	53	0 (0.0%)
2009	Envs S-1	37	0	0	37	37	0
	Envs S-2	32	0	0	32	32	0
	Envs S-3	27	0	0	27	25	0
	Envs S-5	23	0	0	22	21	0
	Total	119	0 (0.0%)	0	118 (100.0%)	115	0 (0.0%)

Values in parenthesis are percentage of fertile plants.

Table 2. Mean temperatures and photo-periods during critical standard weeks recorded at Patancheru (17°N)

Standard week	Period	Day length (h)	Average air temperature (°C)		
			2007–08	2008–09	2009–10
34.0	20–26 August	12.6	26.0	26.0	25.8
35.0	27 August–2 September	12.5	26.0	26.7	25.2
	Mean	12.6	26.0	26.4	25.5
47.0	19–25 November	11.2	19.1	23.9	22.9
48.0	26 November–2 December	11.2	20.3	22.3	20.4
	Mean	11.2	19.7	23.1	21.7
12.0	19–25 March	12.1	25.8	27.0	29.0
13.0	26 March–1 April	12.2	26.5	28.3	30.3
	Mean	12.2	26.2	27.7	29.7

or both. In the present study, the mean photo-period (including twilight) ranged between 11.2 and 12.6 h. This variation may be critical under certain situations, but does not appear to be so in this material under field experiments at Patancheru (17°N). Hence, it is difficult to conclude if the photo-period played any important role in determining the sex of the plants. However, based on the available literature on this aspect in other crops, the role of photo-period in determining pollen fertility cannot be overlooked. Hence, elaborate experiments under controlled environment facility involving various day/night temperatures and photo-periods are required to determine threshold/reversion points under different environments.

The sensitivity of any male-sterility system to the environment may arise primarily due to mutations that could be spontaneous or induced in origin. In pigeonpea seven

CMS inducing cytoplasm have been reported⁹, but the thermo-sensitivity has been recognized only in *C. sericeus* (A₁) cytoplasm. This situation is more or less similar to maize, where only S-cytoplasm is thermo-sensitive. According to Pring *et al.*¹⁰, in the S-mitochondria some auto-replicating linear plasmid-like DNA are present which are responsible for male-sterility and these structures disappear in the reverted fertile plants. This situation may also be influenced by genetic background of genotypes and in certain situations the relationship between plasmid and sterility reversion may not exist¹¹. In general the fertility genes (*fr*) are prone to environmental changes, particularly abiotic factors such as temperature, photo-period, light intensity, soil nutrition, pH, etc. In early generations of breeding this material, Saxena *et al.*⁶ observed that some male-sterile pigeonpea plants

RESEARCH COMMUNICATIONS

Table 3. Segregation for male-sterility and fertility as affected by date of planting at Patancheru during 2008

Date	15 June sowing				30 September sowing			
	Sterile plants	Fertile plants	% Fertile plants	Yield/plant (g)	Sterile plants	Fertile plants	% Fertile plants	Yield/plant (g)
28 and 29 August	164	13	7.9	–	–	–	–	–
25–30 November	3	148	–	68–113	8	204	–	12–53
21–24 March	113	8	7.1	–	168	13	7.7	–

converted to male fertility much earlier than the rest, and these were classified as ‘early’ and ‘late’ converters. This suggested the presence of more than one *fr* gene with different temperature thresholds to produce fertile plants. According to Sun *et al.*¹² and Maruyama *et al.*¹³, the genes responsible for this trait were simply inherited. In other reports the male-sterility gene was found tightly linked to temperature-sensitive nuclear gene^{14,15}. The anther lobes of the male-sterile plants in the present case were rudimentary and dark in colour. This situation occurs when microsporogenesis is aborted at pre-meiotic stage¹⁶. Kaul¹⁷ while reviewing the subject concluded that the pre-meiotic stage is highly sensitive to thermal changes because during this stage DNA synthesis takes place and any disruption during its repair or synthesis can cause male-sterility.

The first true thermo-sensitive male-sterility was identified in rice¹⁸ in China and it was a spontaneous mutant. This mutant expressed male-sterility under high temperature and male-fertility under low temperature; it was similar to that reported here in pigeonpea. Another thermo-sensitive rice mutant was isolated from a population that was derived through irradiation using 20 kr gamma rays¹³. This mutant was completely male-sterile at 31°/24°C; partial male-fertile at 28°/15°C; and complete male-fertile at 25°/15°C. On the contrary, Zhang *et al.*¹⁴ reported a temperature-sensitive mutant with a reverse response to variations in temperature; it was male-sterile at 24°C and male-fertile at 27°C. Besides rice, the temperature-sensitive male-sterility system has also been reported in crops such as faba bean, sorghum, rye, pearl millet, carrot and capsicum¹⁷. The threshold for sex reversal may be different for different species and their expression may also be influenced by genetic background of the genotype.

To assess the performance of the study material under field conditions, bulk seed of the four elections was sown in June and September. To avoid any incidence of cross-pollination, these populations were grown in insect-proof cages. The results showed that the two crops behaved differently with respect to their pollen fertility (Table 3). The September-sown crop appeared like a normal pure line variety and out of 212 plants grown, 204 (98.1%) were male-fertile; by November end, a full crop was harvested by pod-picking. In this sowing, the plants produced less biomass and yield/plant varied from 12 to

53 g. In contrast, in the June-sown crop, the plants had relatively more biomass and out of 177 plants grown 164 (92.6%) were male-sterile; these did not set any pod in the main season. Subsequently, with the onset of low temperature regime in November, this population started converting to male-fertility and by November end, 98% of the plants had abundant pollen grains and produced self-pollinated pods. In this sowing the seed yield ranged from 68 to 113 g/plant. In these plants the reversion of male-sterility to male-fertility was attributed to significant temperature differences during August (when plants were male-sterile) and November (when plants were male-fertile).

To make practical use of this genetic material in hybrid breeding programme, the seed production sites with strict temperature regimes and least fluctuations need to be identified. This would allow complete expression of the gene(s) responsible for this unique behaviour of the genotypes. This research may also help commercial seeds producers in producing quality seeds with minimum resources. The seed system strategy, involving environment-sensitive pigeonpea genotypes, would require two distinct sites, each with characteristically different temperature regime. For multiplication of female parent, the maximum safe mean temperature during crop growth, particularly reproductive phase, should not exceed 20°C. This temperature bar will maintain pollen fertility status of the plants and allow production of fertile flowers and normal pod-set. In case the temperature at such sites shoots up for a short period due to some sudden changes in weather conditions, it will also not affect seed quality of the female parent. This is because if some flowers revert to male-sterility and get pollinated by neighbouring fertile flowers, then seeds harvested from such pods will also produce male-sterile plants in the subsequent generation under warmer rainy season for hybrid seed production. The seed thus produced from such isolated plots will remain genetically pure in spite of minor temperature fluctuations. The hybrid seed production involving temperature-sensitive pigeonpea male-sterile lines should generally be done during rainy season when the temperatures are well over 26°C and to avoid low temperatures, the high-altitude locations should not be selected. Roguing of the female parent would be essential to eliminate any fertile plant arising due to short spells of temperature alterations.

The planting date experiment at Patancheru (Table 3) clearly showed that in early-maturity pigeonpea group, seed production of female parent and hybrid is possible at a single location. In this system the hybrid seed production plot must be sown in early rainy season (June); it will flower in about 60 days and all the flowers will be male-sterile. The cross-pollinated pods that would set on these plants can be harvested in another 40–45 days. However, for optimizing yields suitable agronomy packages need to be developed. The multiplication of female parent can be taken up in another isolation in September; the flowers produced on this crop will be fertile and a good harvest of female parent can be taken without the use of pollinating insects.

In crops where environment-sensitive gene(s) controlling male-sterility/fertility have been identified, the role of photo-period, temperature and their interaction is not well-defined. Future research on pigeonpea should now be concentrated on the issues such as identification of threshold temperature and photo-period at a given location that would control the function of fertility restoring system. Attempt should also be made to understand the molecular basis of sex reversion under different environments. Finally, it will be important to identify genes/quantitative trait loci responsible for controlling this trait that will facilitate quick transfer of these genes into heterotic hybrid parents.

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Monitoring of glacier changes and response time in Chorabari Glacier, Central Himalaya, Garhwal, India

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Chorabari Glacier (6.6 sq. km) in the Mandakini River basin, a tributary of the River Alaknanda, Central Himalaya, Garhwal (India) has been monitored in terms of its length and frontal area (snout) changes for the period between 1962 and 2012. Global Positioning System, Survey of India toposheet (1 : 50,000) and ground-based measurements were used to obtain the changes in morphology and size of the glacier. The result shows that the frontal area of the glacier has

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shrunk by 1% and 344 ± 24 m length loss, with an average rate of 6.8 ± 0.5 m a⁻¹ from 1962 to 2012. The observed terminus records of Chorabari Glacier indicate that the positive mass balance can cause terminus advance in about a 17-year timescale. The lag time of glacier signal transferred from accumulation area to the snout by glacier flow is about 562 years. These observations as well as other studies carried out in the region show a significant reduction in glacier area. The increased retreat rate of the glacier snout is probably a direct consequence of global warming.

Keywords: Frontal areas, glacier change, mass balance, response time, snout retreat.

MOST widely reported mountain glaciers are currently experiencing a period of recession, a trend that began in the mid-19th century with the end of the Little Ice Age^{1,2}. The rate of recession continued through the 20th century and has accelerated over the past three decades²⁻⁴. There is clear evidence that the retreat of glaciers in many locations of the world has accelerated in recent decades^{4,5}. However, glacier systems of the Himalaya have not responded uniformly to recent climate warming; some glaciers of western Karakoram Himalaya are advancing^{6,7}, while some glaciers of central and eastern Himalayas are retreating^{2,8}.

The Himalayan Mountain Range contains thousands of glaciers of widely varying properties, which are spread over nearly 37,000 sq. km and over a 2400 km east-west range⁹. This large geographical extent and complex topography along with the variable climatic conditions across the Himalayas has resulted in various sets of glacier properties. The primary climatic factor from west to east is that of a decreasing influence by the mid-latitude westerlies and an increasing influence by the Indian summer monsoon (ISM)¹⁰. Thus, the distribution of glaciers in the Himalaya is uneven, with a higher concentration of glaciers in the NW than in the NE of the mountain range. Glaciers located in the Central Himalaya are influenced both by the westerlies as well as ISM¹¹. In general, ~70% of the ablation zone of the glaciers is covered by debris with thickness ranging from millimetres to tens of centimetres^{1,9,12}. Due to the thick debris cover and with a low gradient of the termini, many glaciers typically have a stable front position. As a consequence of this complex climate system, glacial geometry and properties, and the geology, the recession rates of the glaciers are variable¹².

During the last few decades, the long-term monitoring of snout position and mass balance measurements have been carried out in several glaciers in different parts of the Indian Himalaya^{2,13-15}. The studies suggest that most of the glaciers are retreating between 5 and 20 m a⁻¹, with negative mass balance varying from 0.2 to 1.2 m a⁻¹ water equivalent^{13,14,16}. With such considerations and realizing of the potential significance of the glaciers and their recession process, *in situ* measurements of snout positions,

surface area changes and meteorological observations were carried out to quantify the total changes in the Chorabari Glacier, Central Himalaya, Garhwal, during the period 1962–2012.

The Chorabari Glacier (30°46'20.58"N, 79°2'59.381"E) is a medium-sized compound valley-type glacier covering an area of ~6.6 sq. km. It is located in the Mandakini River basin of the Alaknanda catchment (a tributary of the Ganga), Central Himalaya, Garhwal, India (Figure 1). The Chorabari Glacier has its accumulation area below Bhart Khunta peak (6578 m amsl) and Kedarnath peak (6940 m amsl) and flows from north to south between 6400 and 3895 m amsl, with an average surface slope of 20° (Figure 1). The accumulation area is comparatively small, steep and formed by three tributary glaciers, whereas the ablation area is broad with gentle slope and covered by thick debris. A number of longitudinal and transverse crevasses are prominent in the upper ablation zone and several small, supra-glacial lakes (ponds) are present in the glacier surface in the lower ablation zone. Debris thickness increases along the glacier, and is greater than >50 cm at the terminus (Figure 2). A second glacier (Companion) flows parallel to Chorabari. The traces of medial moraines (Figure 2) suggest that recession has separated the two glaciers. The extension of these lateral moraines is observed 6 km downstream at Rambara town (2800 m amsl). There appear to have been four stages of recession where traces of the lateral moraines are well preserved throughout the valley¹⁷. These glacier stages are Rambara Glacial Stage (RGS; 13 ± 2 ka), Ghindurpani Glacial Stage (GhGS; 9 ± 1 ka), Garuriya Glacial Stage (GGS; 7 ± 1 ka) and Kedarnath Glacial Stage (KGS; 5 ± 1 ka)¹⁷. During the 16 and 17 June 2013, very heavy rains together with breaching of moraine dammed Chorabari lake triggered flash floods that washed away all the glacial deposits in the down valley¹⁸. In summary, the characteristic features of this glacier are a south-facing, wide and broad terminus with thick debris covers. Some of the salient features of the glacier are given in Table 1.

The glaciers of the Himalayan region are not well documented because of inaccessibility of the area and poor meteorological and hydrological data. The monitoring of the Chorabari Glacier began in 2003. A manned meteorological observatory (3820 m amsl) was installed to monitor air temperature, wind speed and precipitation during the study period. The automatic weather station (AWS) (Campbell Instrument) was installed in 2007, near the snout of the glacier at an altitude of 3820 m amsl.

The general climate of the study area is humid-temperate in summer and dry-cold in winter¹⁹. The daily mean air temperature was observed near the snout of Chorabari Glacier at the height of 3820 m amsl for different seasons of 2007–2012. The average daily air temperature ranged from -13.46°C to 11.56°C , whereas the maximum and minimum air temperatures ranged from