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Considerations for Breeding, Maintenance, and Utilization of TGMS Lines for a Two-Parent Hybrid System in Pigeonpea

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ABSTRACT

Thermo-sensitive male sterility (TGMS) system has already proven its worth in rice. After China, it is now finding its roots in other rice growing countries. In pigeonpea, the first thermo-sensitive male sterility has now been reported, and this provides a good option to pigeonpea breeders for breeding high yielding hybrids. This will ease the process of seed production of male sterile (A-line) parent, because it will eliminate the need of maintainer (B-line) and more importantly, the pollinating insects. Besides this, for hybrid seed production it will not require the fertility restorer (R-line) also. To assist the pigeonpea breeders, this paper outlines salient feature of TGMS-based hybrid breeding technology (two parent hybrid breeding) such as breeding new hybrid parents, seed production, and identifying critical sterility and fertility points.

Introduction

For the transformation of vegetative buds to reproductive phase, the plants always get some critical signals through external factors such as temperature, photoperiod, nutrition etc. These in turn influence normal physiology of different plant systems, and some of them induce the reproductive phase. Any deviation in these delicate biochemical growth and differentiation processes, caused by either genetic or environmental factors, may lead to the appearance of male sterility. The credit of reporting the first ever case of male sterility goes to Kolreuter (1763). Darwin (1890) considered this phenomenon a positive natural event which helps in evolution. The male sterility in plants may be genetic in origin (GMS) or may arise due to interaction between nuclear and cytoplasmic (CMS) genomes. In the recent times, the male sterility caused by some specific environmental factors is also getting recognition. The history of the environment-induced male sterility is recent and it originated in China, when Professor Shi Ming Song identified the first photo-period induced male sterility (PGMS) in rice at the University of Hubei in 1973. It was a spontaneous mutant recovered from a rice variety 'Nongken 58', and later the mutant was established as 'Nongken 58S'. This mutant was found to be male sterile under longer (>14 h) photo-periods and male fertile in shorter (<13 h 45 min) photo-periods. It is also true that this was not the first record of any environmental factor influencing the reproductive biology of plants. In various laboratory and field experiments, this type of effect was recorded in a number of plants of different genera and species (Kaul, 1988); but unlike Shi Mong Song, none of the scientists tried to establish its pure line or thought of using it in commercial hybrid breeding programmes.

Soon the rice mutants wherein the transformation of male sterility to fertility and vice versa was controlled by temperature was also discovered by Zhou et al. (1988) and Sun et al. (1989). Subsequently, these thermo-sensitive (TGMS) lines were also used in breeding hybrid rice. In this endeavour, China is the clear champion with environment-induced (PGMS and TGMS) male sterility based hybrids being commercialized in rice, maize, sorghum and brassica. This breeding approach is popularly called 'two parent hybrid technology'. According to Chinese scientists, the two-parent hybrid technology has clear advantages over the traditional three-parent hybrids in cost, quality, and heterotic advantage.

In pigeonpea, both the genetic (GMS) as well as cytoplasmic-nuclear (CMS) male sterility systems have been reported (Saxena, 2008) and used in hybrid breeding programmes. The temperature-sensitive male sterility (TGMS) system in pigeonpea is unique and it has a very recent record (Saxena, 2014). In this case, the expression of male sterility/fertility is conditioned by genes whose action is controlled/modified by the temperature variations. In this paper, the authors have not attempted to review the subject of sensitivity of male sterility due to variations in various environmental factors; but the effort is to provide guide lines to pave the way for breeding of TGMS based hybrids. Hence, the schemes related to breeding of TGMS lines, their maintenance, and their use in hybrid breeding programmes are outlined. Since pigeonpea is a new crop with respect hybrids, the authors believe that this synthesis of thoughts will stimulate and attract some breeders towards this novel hybrid breeding approach.

Report of the First TGMS in Pigeonpea

Pigeonpea is a crop of many unexplored research possibilities and since it is a major pulse crop of India, there is a need to develop plans for using this new TGMS based hybrid technology to break the decades old yield plateau. Now opportunities are there to take a step forward for breeding TGMS-based pigeonpea hybrids. In this context, a breakthrough has already been achieved by identifying the first set of TGMS lines derived from an interspecific cross involving Cajanus sericeus and C. cajan (Saxena, 2014). These TGMS selections show marked changes in the pollen fertility under different temperature regimes. At >25°C mean temperature (high mean temperature), the plants are completely male sterile, while the same plants become fully male fertile at <24°C (low mean temperature). Field evaluation of this material at Patancheru (17° N) showed that in the June sown crop, the plants were male sterile (Table 1) and did not produce any pollen when examined at the first flowering in the month of September (high temperature). These plants, however, turned male fertile in the month of November (low temperature); and when the temperatures rose in the month of February, the same plants again reverted back to male sterility and thus confirmed their thermo-sensitivity. These genotypes can be used either directly in a hybrid breeding programme or used as donor source for TGMS gene to diversify the genetic base of A-lines.

However, there may be threshold point for mean low temperature as well. In some thermo-sensitive genotypes of pigeonpea (IPA 209 and IPA 06-1), filaments of stamens fail to enlarge if mean temperature falls below 18°C, affecting opening of flower buds. Pollen dehiscence does not occur too, although pollens are

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fully fertile. As a consequence, unfertilized flowers wither and fall down, resulting in no pod formation in these genotypes under such low temperature (IIPR Annual Report, 2008-09; Choudhary et al., 2014). Further, these TGMS lines should also be assessed for photosensitivity before their utilization in two-parent hybrid breeding programme.

3. Advantages of TGMS over CMS

The thermo-sensitive genetic male sterility (TGMS) systems were discovered in rice, first by Sun et al (1989) in China and later in Japan by Maruyama et al. (1991) and by Virmani and Voc (1991) at International Rice Research Institute (IIRI), Philippines. They considered the TGMS-based hybrid system superior to CMS system and hence, it can be adopted easily in tropical and sub-tropical areas where the sites with desired temperatures requirements can be identified for seed production using different seasons, altitudes, and latitudes. Yuan (1987) proposed the use of TGMS system in rice hybrid breeding programmes in China. Since this system eliminates the requirement of B-line, it is popularly called 'two-parent hybrid' breeding system. In pigeonpea, the TGMS system has already been identified and the prevailing temperatures (Table 4) at lower latitudes such as Patancheru (17° N) can be successfully used to develop high yielding hybrids. The two-parent hybrid breeding system has the following advantages that make the hybrid breeding easy for adoption:

- The male sterile or A-line (TGMS line) can be multiplied on a large scale without its maintainer or B-line and more importantly, there will be no need of cross-pollinating insects for pod setting. Thus, the quality seed can be produced economically and with ease.
- This system also eliminates the need of fertility restoring (R-) line for hybrid seed production. Any line which lacks the TGMS gene will produce fertile hybrids. This will enhance the number of prospective parents entering into hybrid combinations with TGMS lines.
- Since cytoplasm has no role in determining the male sterility, the potential deleterious effects of cytoplasm on the expression of pollen fertility or any other trait of economic importance is totally eliminated.
- In TGMS-based hybrid system, the problem of narrow genetic variability can be overcome with ease, because lines can be easily converted to TGMS and any line can be used as male parent.
- A large number of hybrid combinations can be tested in a short time with ease, thus it enhances the probability of identifying high yielding hybrid combinations.

4. Breeding New TGMS Lines

(i) Facilities required for breeding and characterization of TGMS selections

Pigeonpea has special traits that make it very different from other pulses; these are partial natural out-crossing and perennial growth habit. The natural out-crossing helps in hybrid seed production, while the perennial growth habit of the plants permits their assessment with great accuracy using the same plants across different temperature regimes. The selections when planted in middle of June (high temperature) can easily survive for more than one year and hence remain in the field through the warm and cool seasons and keep flowering continuously. They complete the cycle of male sterility (June to October), followed by male fertility (November to February), and again male sterility (March to May) at ICRISAT condition. These events allow assessment of the plants critically and the observations on thermo-sensitivity of the genotypes can be confirmed on the same genotype within a single year. The partial natural out-crossing that is mediated by a variety of pollinating insects, supports economic hybrid seed production. This trait, however if not handled carefully, can adversely affect the selection programme by pollinating the male sterile buds and it will be impossible to distinguish the pod set caused by male sterility reversion or by cross-pollination. Therefore, it is mandatory to grow all the research materials related to TGMS under mosquito nets; this will keep the pollinating insects away and the selections will have high heritability. In addition to the requirement of insect-proof cages, facilities to confirm and characterize TGMS lines will also be required. This will help in identifying seed production locations of A-line and hybrids. Besides this, trained human resource will always remain an asset for the program.

(ii) Detection of natural mutants from germplasm, breeding and panmictic populations

Nature has been very kind to human beings by providing genetic variability that has directly or indirectly benefited the human race by directly or indirectly providing food for masses. The primary (cultivated), secondary and tertiary (wild relatives) gene pools of germplasm harbour vast genetic variability and it is believed that till date only a fraction of it has been exploited in breeding programmes, and a lot is still unexplored. The exhaustive review of literature on various aspects of male sterility by Kaul (1988) showed that both TGMS as well as PGMS natural mutants have been selected in crops like rice, maize, barley, sorghum, pearl millet etc. To start a programme on hybrid breeding using environment-sensitive male sterility in pigeonpea, a similar approach for identifying targeted mutants can be adopted.

Methodology:

For selecting the chance natural TGMS mutants, the breeders should watch carefully various breeding populations that are sown in the experiment station under different projects for the single plants with a few or no pods. In the absence of self-pollen production, one can observe scars of unfertilized fallen flowers on the racemes of the plant (Fig 1). Such plants should be examined for the presence/absence of pollen grains using 2% aceto-carmine solution. After establishing their male sterility, the plants should be bagged after removing all the open flowers and pods, if any. This is an essential step due to inherent natural out-crossing. Each selfed male sterile plant should be carefully observed for pod set inside the bag for periods extending over a few months. Any plant found setting self-pollinated pods should be marked as 'suspected TGMS' and pursued further for reconfirming its fertility/sterility reaction under diverse temperatures.

(iii) Detection of natural variability for TGMS through multiple plantings

In this approach, the available elite germplasm and advanced breeding lines are evaluated for their response to temperature with respect to pollen fertility/sterility under sowings undertaken under different dates representing diverse temperature regimes.

Methodology:

Since the objective is to find out if any elite line or germplasm has thermo-sensitive gene, select a genetically diverse set of about 200 lines, and plant them in a single un-replicated rows to accommodate about 50 plants each. Repeat this sowing fortnightly, starting June 15 up to September 30 under insect-proof cage. A total of eight plantings will cover a good temperature range from warm to cool at flower initiation. Record observations on pod set in each planting date, compare data, and identify lines showing differential behaviour with respect to pod set. Observe these plants/rows for pod setting in low temperature period. Select the male sterile plants showing pod set late in the season under cool climate for further study.

(iv) Detection of male sterile segregants from diverse hybrid populations

In active variety development programmes, breeders make efforts to generate new variability to select superior recombinants. To achieve this, crosses are made between diverse genotypes including some wild relatives of the species. In the past, both variety x variety crosses (e g. maize) and variety x wild relative crosses (e.g., pigeonpea) have yielded environment-sensitive male sterile genotypes. Breeders can carefully examine the F_1 and other segregating populations and look for any partial or complete male sterile genotypes. These can be further examined to determine their reaction to environmental factors. The breeding populations should be observed keenly to look for any total male sterile or partial fertile plants. Such plants may be selfed and handled as mentioned earlier.

(v) Breeding new TGMS lines

In order to commence a sustainable TGMS based hybrid breeding programme, it is important to diversify the genetic base of the parents. In this system, the breeder can use a number of male lines without worrying about the presence of fertility restoring gene. Breeding of female parents need attention because the recessive TGMS alleles need to be transferred and sufficient care should be taken that these alleles are not lost during breeding.

Methodology:

A scheme for developing new breeding population using TGMS line as one of the parents is outlined in Table 3. This is based on the observations and experience at Patancheru (17 ^{0}N), where the following three temperature regimes are available.

Temperature regime	Month	Weather class	Mean temperature (°C)	Expression ofTGMS lines
I	June to September	warm	> 25	Sterile
II	October to January	cool	< 24	Fertile
III	February to May	warm	> 25	Sterile

The basic scheme is based on classical pedigree method of breeding. The important feature of this methodology is that the plants carrying recessive thermo-sensitive alleles are selected. Since these alleles are expressed only in the main hot rainy season, the segregating populations should be grown in this very season only. The seed of the selections will be ready for harvest in the following winter season. Once a set of breeding lines are selected, these should be further screened for key agronomical traits and combining ability. The crosses on the male sterile plants can be made in the main season and their evaluation is undertaken in the next main (warm) season. Theoretically, all the progenies should produce pods in the warm season due the presence of dominant alleles. In this crop heterosis can be measured easily for further selection and promotion of hybrids.

4. Studying Critical Fertility and Sterility Points of TGMS lines

In a hybrid breeding programme based on TGMS system, it is very important that critical temperature points that determine the conversion of male sterility to fertility and the vice versa are well defined. To have accurate information on these two parameters, research should be conducted under controlled environment facilities. The sex transition time should be studied carefully and when over 90% of the pollen grains stain dark, it should be considered as 'critical fertility point' (CFP). To identify 'critical sterility point' (CSP), it is good to observe the decline in pollen fertility when temperature starts rising in the month of February; when the male sterility reaches almost zero, the temperature should be noted and identified as CSP. It is also important to study the significance of mean temperature, the maximum temperature, or both in deciding the CFP and CSP in pigeonpea. Information on these aspects will also help in selecting the seed production sites for both A-line and hybrids.

5. Studying Inheritance of TGMS Gene

The unconfirmed results from ICRISAT have shown that the gene conferring temperature-sensitivity in pigeonpea is recessive in nature. Thermo-sensitivity in some pigeonpea lines (IPA 209 and IPA 06-1) has also been observed to be a recessive trait (IIPR Annual Report, 2008-09; Choudhary et al., 2014). However, full studies are needed to understand the nature of inheritance; and it can be built in the on-going breeding activities. Crosses between TGMS and fertile lines can be made in the warm season. During the second year, F_1 population should be grown in the warm season to (i) study dominance relationship, (ii) make backcrosses, and (iii) advance the generation from F_1 to F_2. In the warm season of third year, F_1, F_2 and BC_1F_1 populations should be grown under insect-proof net to study the inheritance. Counts should be made for fertile and sterile plants. Keen observations should be made on the conversion of male sterile plants to fertile ones in the following cool season.

6. Use of TGMS Lines in Hybrid Technology

(i) Site selection for seed production A-line

The seed system involving TGMS pigeonpea genotypes would require two distinct sites, with strict temperature regimes and least fluctuations each with characteristically different temperature regime. This would allow complete expression of the gene(s) responsible for this unique behavior of the genotypes. For multiplication of female parent, the maximum safe mean temperature during crop growth, particularly reproductive phase, should not exceed 20°C (Fig 2). This temperature bar will maintain pollen fertility status of the plants and allow production of fertile flowers and normal pod set.

(ii) Site selection for hybrid seed production

The hybrid seed production involving temperature-sensitive pigeonpea male-sterile lines should generally be done in rainy season when the mean temperatures are well over 25°C (Fig 2) and to avoid low temperatures, the high altitude locations should not be selected. Rouging of the female parent would be essential to eliminate any fertile plant arising due to short spells of temperature alterations. Yuan (1987) proposed its use in rice hybrid breeding program. Since it eliminates the requirement of maintainer 'B' line, this hybrid system, is popularly called as 'two-parent hybrid' breeding. At present the two parent hybrids are being used in China commercially, and more than 15 hybrids are in cultivation with an estimated area of over 1,300,000 hectares with yields as high as 8-9 t/ha (Yin,1999).

(iii) Effect of temperature fluctuations on seed production

For the seed production of A-line, quality control is essential to produce true hybrids. 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 back to male-sterility and get pollinated by neighboring 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.

7. New Research Opportunities

Thermo-sensitivity in pigeonpea is a new and recent finding and very little is known about it. This however, offers unique opportunity for research in the fields of genetics, genomics, breeding and physiology. A number of postgraduate students can do research to unlock the secrets of thermo-sensitivity in pigeonpea. The future research in 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

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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/ QTLs responsible for controlling this trait (thermo-sensitivity) that will facilitate quick transfer of these genes into heterotic hybrid parents.

Table1.Field observations recorded in three months on male-sterility and fertility in four temperature- sensitive selections during 2009*.

Selection	September		November		February	
	Sterile plants	Fertile plants	Sterile plants	Fertile plants	Sterile plants	Fertile plants
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

*Source: Saxena, 2014

Table 2.Segregation for male-sterility and fertility as af fected by date of planting at ICRISAT, Patancheru during 2008*.

Date	June 15 sowing		September 30 sowing	
	%Sterile plants	%Fertile plants	%Sterile plants	%Fertile plants
August 28-29	92.1	7.9	-	-
November 25-30	2.0	98.0	3.9	96.1
March 21-24	92.9	7.1	92.3	7.7

*Source: Saxena, 2014

Table 3. A procedure for breeding new thermo-sensitive A-lines of pigeonpea at lower latitudes.

Year	Generation	Season	Activity
1	-	Rainy (warm)	Plant parents, select male sterile plants, make crosses with elite male line
2	F_1	Rainy (warm)	Grow F_1, examine sterility of each plant, harvest F_2 seed
3	F_2	Rainy (warm) Winter (cool)	Grow 2000 plants, examine each plant for sterility, reject the fertile plants, number the sterile plants. Carry them to winter season. Observe each plant for fertility and pod set. Reject poor pod setting plants. Harvest about 200 single converted plants for evaluation in F_3 progeny rows.
4	F_3	Rainy (warm) Winter (cool)	Grow F_3 rows, evaluate them for male sterility. Reject off-type progenies. Take them to the cool season. Select progenies on the basis of conversion to male fertility. Record data on the rate of conversion. Bulk or single plant harvest after rouging.
5	F_4	Rainy (warm) Winter (cool)	Grow F_4 rows, evaluate them for male sterility. Reject off-type progenies. Take them to the cool season. Select progenies on the basis of conversion to male fertility. Record data on the rate of conversion. Bulk or single plant harvest after rouging.
6	F_5	Rainy (warm) Winter (cool)	Grow F_5 rows, evaluate them for male sterility. Reject off-type progenies. Take them to the cool season. Select progenies on the basis of conversion to male fertility. Record data on the rate of conversion. Bulk harvest and record yield.
7	F_6 (New A- lines-ready; test them for combining ability before using in hybrid program	Rainy (warm) Winter (cool)	Grow F_6 rows, evaluate them for male sterility and other traits. Reject inferior progenies. Take them to the cool season. Select progenies on the basis of conversion to male fertility. Record data on the rate of conversion and yield. Bulk harvest for use in hybrid breeding program.

Table 4. Mean temperatures and photo-periods during critical standard weeks recorded at ICRISAT, Patancheru (17°N).

Standard week	Period	Daylength (h)	Average air temperature (°C)		
			2007-08	2008-09	2009-10
34.0	20-26 Aug	12.6	26.0	26.0	25.8
35.0	27 Aug-02 Sep	12.5	26.0	26.7	25.2
Mean		12.6	26.0	26.4	25.5
47.0	19-25 Nov	11.2	19.1	23.9	22.9
48.0	26 Nov-02 Dec	11.2	20.3	22.3	20.4
Mean		11.2	19.7	23.1	21.7
12.0	19-25 Mar	12.1	25.8	27.0	29.0
13.0	26 Mar-01 Apr	12.2	26.5	28.3	30.3
Mean		12.2	26.2	27.7	29.7

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