Indian J. Genet., 68(1): 44-46 (2008)

Development of male sterile lines of tomato and assessment of their utility in hybrid development

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(Received: December 2007; Revised: January 2008; Accepted: February 2008)

Abstract

High cost of hybrid tomato seeds is attributed to labour involved for emasculation and pollination processes. Emasculation can be avoided and labour saved if male sterile line is used as the female parent. Efforts were made to incorporate functional male sterility conditioned by *ps2* gene and sporogenic male sterility conditioned by *ms2* and *ms33* genes into locally adapted and horticulturally superior genotypes. The lines *viz. 'ps2* L 3841', `*ps2* NS 101' `*ps2* San Pedro' and `*ps2* UC 82-B' (all functional male sterile); and *'ms2* IPA' and `*ms33* IPA' (both sporogenic male sterile) were stabilized after six cycles of alternate selfing and backcrossing. It is suggested that the utility of functional male sterile lines can be enhanced if it is combined with exserted stigma conditioned by the gene *ex.* The stigma position of '*ms33* IPA' is exserted and is, therefore, accessible for hand-pollination for F_1 hybrid seed production without disturbing the anther cone. It was estimated that while using *'ms33* IPA' as a female parent, 54.4 per cent time was saved over its fertile counterpart for F_1 hybrid seed production. This indicated the potential of exploiting male sterility for heterosis breeding in tomato.

Key words: Tomato, functional male sterility, sporogenic male sterility, heterosis breeding

Introduction

Hybrids are very popular in tomato (*Lycopersicon esculentum* Mill.) especially in the developed world and appear to be the primary reason for high productivity [1]. An important factor limiting more extensive use of hybrid tomatoes is high cost of hybrid seed, which is produced through manual emasculation and pollination. Incorporation of male sterility can avoid emasculation and reduce the labour necessary for F_1 hybrid seed production, and thus reduce cost of F_1 hybrid seed. There are five types of male sterility in tomato but only pollen abortive type (sporogenic) and functional sterility have been exploited for hybrid seed production. Other male sterility mechanisms are associated with defective fruit development in the hybrid progeny [2]. In pollen abortive type, the pollen grains are non-functional whereas in functional type, pollen grains are functional but anther dehiscence prevents self-fertilization. Functional male sterility can be maintained by manual pollination and offers production of homozygous stand of female parent. The pollen abortive type is maintained by backcrossing homozygous male sterile plants (*msms*) with heterozygote male fertile (*Msms*) plants. The progeny segregates into male fertile and male sterile in 1:1 ratio. For commercial exploitation of male sterility, it is important that the sterility genes are transferred into horticulturally desirable and superior combining parents. The project was, therefore, initiated to;

1. Transfer of trait through back cross
2. Evaluation of new stocks
3. Assessment of labour needs for hybrid seed production using new stocks.

Material and methods

The line *'ps2* CA 3631' was crossed with 'NS 101', 'L 3841', 'San Pedro' and 'UC 82-B' to broaden genetic base of functional male sterility. 'IPA', 'NS 10F, 'San Pedro' and 'UC 82-B' are improved lines possessing desirable fruit and plant characteristics, and 'L 3841' is an extra early line capable of setting fruits under sub-optimal temperature conditions [3, 4]. Both pollen abortive and functional male sterile lines were developed and established by alternate selfing of heterozygote fertile to recover homozygote sterile and then backcrossing to the respective recurrent parent. The lines were stabilized after six cycles of selfing and backcrossing. The progenies were advanced in off-season to cut short time requirement.

For evaluation of newly developed male sterile lines for important horticultural traits, nursery was sown on October 26, 2006 and seedlings were transplanted

in the field on December 6, 2006 following randomized block design with three replications. Bed x plant spacing was maintained at 1.35 m x 30 cm. Each genotype in each replication was represented by a single row accommodating ten plants. Observations on five representative fruits in each replication were recorded on polar diameter (cm), equatorial diameter (cm), fruit shape index (polar/equatorial diameter), number of locules, pericarp thickness (mm), total soluble-solids (^{o}Brix, using hand refrectometer) and fruit weight (g). The data were analyzed using the computer software package 'CPCS' developed by Singh and Cheema [5].

For assessing suitability of newly developed ms line *"ms33* I PA' for hybrid seed production, five trained workers were engaged to perform emasculation and pollination work. Emasculation and pollination was required in male fertile line *'Ms33-* IPA' (MF) whereas, only pollination was required in male sterile line '*ms33* IPA' (MS). Time required to emasculate and pollinate 50 flower buds by each of the five workers was recorded. The process was repeated for ten days and the time saved in MS line over the MF line was worked out.

Results and discussion

The male sterile lines, both functional and pollen abortive types, developed by alternate selfing and backcrossing with their respective recurrent parents have been listed in Table 1. The lines developed include '*ps2* NS 101', `*ps2* L 3841', '*ps2* San Pedro' and '*ps2* UC 82-B' (all functional sterile); and `*ms33* IPA' and '*ms2* IPA' (both pollen abortive types). The functional MS lines developed have reached stability and do not set fruit without manual pollination. This indicated that the sterility mechanism is strong that is important for producing genetically pure hybrid seed. This is contrary to the earlier report of Bullard [6] who reported up to 50 per cent selfing in John Bear sterile mutant under field conditions of Indiana, the USA. However, Atanassova [2] suggested that strict control of the per cent of self-fertilization and elimination of plants showing more than five percent selfing was necessary throughout the breeding process to develop lines inherited with strong functional male sterility mechanism.

The stigma in all the lines remains hidden in the anther cone and is not accessible for pollination without disturbing the anther cone. This amounts to emasculation and defeats the very purpose of use of male sterility in heterosis breeding. The initial studies indicated that undesirable selfing and the necessity of emasculation were recognized as the main two disadvantages that limited the use of *ps2* sterile seed parents in tomato hybrid seed production [7, 8]. However, Atanassova [2] subsequently reported that disadvantages in using *ps-2* male sterility in hybrid tomato seed production were exaggerated. He opined that expression of gene *ps-2* varies with the genotype and it is, therefore, possible to select genotypes with very low levels of selfing. Regarding their evaluation for important horticultural traits; '*ps2* NS 101' has round fruit shape, '*ps2* San Pedro' and '*ps2* L 3841' have slightly flattened fruit shape, and '*ps2* UC 82-B' has oval fruit shape (Table 1). The '*ps2* San Pedro' has fruit weight of more than 100 g whereas '*ps2* NS 101', '*ps2* L 3841' and '*ps2* UC 82-B' have fruit weight of around 60 g. All the four MS lines have pericarp thickness of 6.00 mm or more. Least number of locules per fruit were recorded in '*ps2* NS 101'. This was followed by '*ps2* San Pedro' (3.83) and '*ps2* L 3841'. Since the lines developed have desirable horticultural traits, their utility in heterosis breeding can be enhanced if functional male sterility is combined with exserted stigma conditioned by the recessive gene *ex*. This not only makes the stigma accessible for pollination but also reduces chances of selfing, if any.

The important horticultural traits of the two newly developed pollen sterile lines are given in Table 1. The two pollen sterile lines developed *viz*. '*ms2* IPA' and '*ms33* IPA' have recovered the genotype of the recurrent

Table 1. Fruit characteristics of tomato male sterile lines

S.No.	Genotype	Type of sterility	Polar diameter (P)	Equatorial diameter (E)	Number of locules	Pericarp thickness (mm)	TSS (%)	Fruit weight (g)	Fruit shape index (P/E)
1.	*ms33* IPA	Pollen abortive	5.13	4.56	2.33	6.33	4.60	59.00	1.12
2.	*ms2* IPA	Pollen abortive	5.09	4.43	2.00	6.33	4.75	62.33	1.15
3.	*ps2* L 3841	Functional	4.26	4.73	4.66	6.00	4.16	61.33	0.89
4.	*ps2* NS 101	Functional	4.40	4.36	3.00	6.00	4.60	59.33	1.00
5.	*ps2* San Pedro	Functional	5.53	6.23	3.83	6.66	4.63	112.66	0.88
6.	*ps2* UC 82-B	Functional	5.15	4.70	2.10	6.30	4.80	67.00	1.10
C.D. at P=0.05			0.34	0.29	2.82	0.82	0.25	7.19	0.09

parent and are stable. The lines represent 50 per cent male fertile and 50 per cent male sterile plants. The sterile plants to be used as a female parent can be identified only after flower initiation. They have similar fruit characteristics. Since the two ms genes *viz.*, *ms2* and *ms33* have been transferred into the same genetic background (IPA), their phenotypic similarity is an indication of recovered recurrent parent genotype. Both the lines possess desirable fruit characters. The fruits are oval with thick pericarp and lesser number of locules. All these characters contribute towards firmness of fruits. Further the lines possess TSS content of more than 4.5 per cent. TSS is one of the important biochemical constituents of processing tomatoes. However, '*ms2* IPA' has stigma hidden inside the anther cone whereas, the stigma position of '*ms33* IPA' is exserted and stable. This would facilitate out-crossing and hence F_1 hybrid seed production. Considering the stigma position of '*ms33* IPA', its suitability for hybrids development was assessed.

This study indicated that the time required for emasculation and pollination of flower buds of male fertile and male sterile lines varies with the individual performing the task (Table 2). On an average, 30.7 and 41.7 minutes were required to emasculate and pollinate 50 flower buds of male fertile line. To pollinate the same number of flower buds on male sterile line '*ms33* IPA', 32.8 minutes were required thus saving 54.4 per cent time required for hybrid seed production if male sterile line is used as a female parent. Earlier report indicated that cost price of hybrid seed was reduced by 20 per cent when *ms10*[35] was used as a female parent [9]. This clearly indicated the potential of exploiting male sterility for hybrid seed production in tomato. Georgiev [9] further reported that the exserted stigma position is stable in small-fruited types and unstable in large-fruited ones Atanassova [10] concluded that the manifestation of exerted stigma was not conditioned solely by the *ex* gene but also by genotype of the plant.

Table 2. Time (minutes) required for crossing 50 flower buds on male fertile *'Ms33* IPA' (MF) and male sterile *'m.s33* 1PA' (MS) plants in tomato

Worker	Activity				
	Emasculation	Pollination on MF	Emasculation + pollination on MF	Pollination on MS	Time saved in MS over MF,%
1	22.0	44.0	66.0	37.7	42.9
2	26.1	34.9	60.5	26.3	56.5
3	38.7	45.1	83.8	33.3	60.3
4	37.3	43.8	75.5	34.2	54.7
5	32.8	41.1	73.9	32.7	55.8
Mean	30.7	41.7	71.9	32.8	54.4
CD at P=0.05	1.52	1.60	1.95	1.10	-

It is important to mention that some time was spent for identification of male sterile plants from the mixed progeny of *'ms33* IPA'. The time spent for this purpose was not considered while working out labour cost saved in *'ms33* IPA' over male fertile '*Ms33*-IPA'. The problem of identification of ms plants from the mixed population can be overcome by the use of linked genetic markers expressing at the seedling stage. This was demonstrated effectively by linkage of male sterile gene *ms10*[35] with the marker gene *'aa'* responsible for absence of anthocyanin pigment in seedlings. Introduction of seedling markers into the female parent would also help in eliminating non-hybrid (selfed) seedlings from the F_1 hybrids prior to transplanting in the field. This will ensure 100% genetic purity of the hybrids in the field.

Refereces

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