Bulgarian Journal of Agricultural Science, 18 (No 4) 2012, 565-570
Agricultural Academy

POSSIBILITY TO SELECT HETEROZYGOUS GENOTYPES BY POLLEN FERTILITY IN SEGREGATION HYBRID AND BACKCROSS PROGENIES CREATED BASED ON NUCLEAR MALE STERILITY

V. NIKOLOVA and V. TODOROVA
Maritsa Vegetable Crops Research Institute, BG - 4003 Plovdiv, Bulgaria

Abstract

NIKOLOVA, V. and V. TODOROVA, 2012. Possibility to select heterozygous genotypes by pollen fertility in segregation hybrid and backcross progenies created based on nuclear male sterility. *Bulg. J. Agric. Sci.,* 18: 565-570

A successful attempt for separation of heterozygous (*Msms*) and dominant homozygous (*MsMs*) fertile genotypes based on pollen fertility in the segregating pepper backcross progenies with gene male sterile (*ms8ms8*) female parent was made. The study was done during 2007 – 2010 at the Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria. The selected *Msms* plants were included in pollination with the new male sterile forms to confirm their heterozygosis, to receive the segregation 1:1 male sterile (*msms*): male fertile (*Msms*) plants and to define fertile analogues. As a criteria in the determination of the fertile analogous plants – the pollinator with pollen fertility below 40% shows correct ratio near to the expected theoretical one 1:1 in maintenance of new male sterile forms. This method is faster and easier choice of fertile analogous and could be used in other plant species for selection of heterozygote genotypes by pollen fertility in segregation hybrid and backcross progenies based on nuclear male sterility.

Key words: Capsicum annuum L., cytology, fertile analogue

Introduction

Recently commercial utilization of pepper nuclear and nuclear-cytoplasmic male sterility for hybrid seed production is popular in all over the world. Over a dozen single *ms* gene mutants have been found or obtained by mutagenesis (X – rays, gamma rays and EMS) (Daskaloff, 1971; Deshpande et al., 1983; Meshram et al., 1992; Pathak et al., 1983; Pockard, 1970; Prakash et al. 1987 and Shifriss, 1973) and by remote hybridization (Rusenova - Kondareva, 1968; Dumas de Vaulx and Pitrat, 1977 and Andrastalvy and Csillery, 1983), as they were widely used.

One of the major aims of research work in the *Capsicum annuum*'s breeding programmes is to produce new nuclear male sterile lines with different fruit shape. Receiving of new sterile lines with suitable characteristics is necessary: to transfer the gene determined sterility into desirable pepper genotypes; to obtain uniform backcross progenies with good agronomic traits by multiple backcrossing and as a next step to find pollinator (fertile analogues), which after pollination of new male sterile forms will manifest segregation of 50% male sterile (*msms)* and 50% heterozygous male fertile (*Msms)* plants. Until now, in the breeding practice the fertile analogues was selected occasionally that require expensive and multiple crosses. One of the problems in the occasionally pollinator (fertile analogues) selection in some cases is significant deviation of the segregation *msms*: *Msms* genotypes

E-mail: vesseto_nik@abv.bg

from the theoretical correct one 1:1. The other one is the loss of segregation in the *msms* x *Msms* progeny, as all plants produced fertile pollen in the anthers, result from the wrong pollinator choice possessing homozygous (*MsMs*) genotype.

The purpose of the study was to make separation of the heterozygous (*Msms*) from dominant homozygous (*MsMs*) fertile genotypes by pollen fertility in the segregating backcross progenies obtained on the nuclear male sterile base.

Materials and Methods

The study was done during 2007 – 2010 at the Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria. New male sterile forms with different fruit shape and good agrobiological quality were received in pepper breeding program, after several backcrossing (BCP_2) of hybrids between nuclear male sterile line No 1647 Zlaten medal ms_8 (Daskalov, 1973) and pepper varieties - Byal Kalinkov, Stryama, Buketen 50 and Gorogled 6.

The type of segregation (fertile: sterile plants, sterile: heterozygous fertile: homozygous fertile plants) was defined in the second progeny of the hybrids and backcrosses (BC_2P_2, BC_3P_2) of sterile line No1647 (ms_8ms_8) and the chosen varieties cultivated in 2007. Pollen fertility and respectively the absent of pollen grains in the anthers were observed in more then 50 plants (from 50 to 90) several times at different dates. For slides preparation were used three to five flowers per plant. Sterility and pollen fertility were evaluated by staining with 4% acetocarmine and glycerol (1:1). The percentage of the colored pollen was determined by counting more then 100 pollen grains from each studied flower. Data were processed by hypothesis testing with χ^2.

The new sterile forms from the BC_3P_2 progeny of the crosses between line No1647 and varieties Gorogled 6 (red pepper for grinding), Byal Kalinkov and Viktoria (green pepper) were hand pollinated with the selected on the basis of the pollen fertility intervals, genotypes (fertile analogues) in 2008 - 2009. In botanical maturity the fruits from the successful crosses were collected separately from each plant, their seeds were extracted and preserved for the next vegetation.

The segregation sterile: heterozygous fertile plant was evaluated in their first and second intraline progenies.

Results

The expression of male sterility determined by gene ms_8 in line No1647 Zlaten medal ms_8 was confirmed to be completed and stable in the performed study. No pollen grains were produced and there was complete absence of pollen in the anthers of the male sterile plants in all investigated segregation progenies. Phenotypic expression of the recessive gene was not changed in sterile genotypes of the mother line № 1647 and of hybrid and backcross progenies, as the anthers were without pollen grains, brown and deformed.

In previous cytological investigation (Nikolova et al., 2001; 2002), it was defined incomplete expression of the dominant *Ms* gene in the heterozygous (*Msms*) genotypes of the line №1647 and in the F_1 hybrids with different pepper varieties. Based on this suggestion was created a new hypothesis for separation of the fertile plants to heterozygous (*Msms*) and homozygous (*MsMs*) genotypes by establishing pollen fertility in F_2 hybrids and in second backcross progenies. According to this investigation, the studied plants were classified in three hypothetic variants with following intervals of the stained pollen grains:

- heterozygous *Msms* plants - with 0.1 to 60% stained pollen grains and homozygous *MsMs* plants - with from 60 to 100% pollen fertility;
- heterozygous *Msms* plants - with 0.1 to 70% stained pollen grains and homozygous *MsMs* plants - with from 70 to 100% pollen fertility;
- heterozygous *Msms* plants - with 0.1 to 80% stained pollen grains and homozygous *MsMs* plants - with from 80 to 100% pollen fertility.

The data showed that when the obtained ratio fertile: sterile plants was near to expect one (3:1) in the segregating hybrid and backcross progenies, the suitable interval of the pollen fertility, determined *Msms* genotype, is between 0.1 и 70% (the II variant). The homozygous *MsMs* plants were classified in the interval of the pollen fertility from 70 to 100%. According to these two intervals, it was established the following

segregation ratio – sterile ms_8ms_8: heterozygous fertile *Msms:* homozygous fertile *MsMs* plants:

- in F_2 hybrids (line № 1647 x varieties Byal Kalinkov and Stryama) - 1 : 1.3 : 0.7 and 1 : 1.3 : 0.9, respectively.

- in the second backcross progeny BC_2P_2 (line № 1647 x variety Stryama) и BC_3P_2 (line № 1647 x varieties Buketen 50 and Gorogled 6) - 1 : 1.4 : 0.9 and 1 : 2.7 : 0.8 and 1 : 1.6 : 1, respectively.

Probably the deviation of the ratio *Msms*: *MsMs* in the one hand and sterile: fertile genotypes in the other, in the studied crosses and progenies are because of occasional factors. The established values of the function χ^2 demonstrated, that in the degree of freedom 1 these values were acceptable in the level of the significance 5 % and the differences between practical received and theoretical expected results had occasional character.

If the fertile heterozygous *Msms* and homozygous *MsMs* plants from the same crosses and progenies were classified in pollen fertility intervals between 0.1- 60% and respectively 60 – 100% (I variant), between 0.1-80%, respectively 80 – 100% (III variant) the ratio sterile: heterozygous fertile: homozygous fertile plants could be considerable deviate from the theoretical expected one 1:2:1.

The part of the heterozygous *Msms* fertile genotypes were ignored in the I-st variant or some homozygous *MsMs* plants pass on to the group of heterozygous fertile *Msms* plants (III variant), which can define the above mentioned problem – the wrong choice of the pollinator with *MsMs* genotype, resulting in the loss of segregation in the progeny with sterile mother, as all plants produced fertile pollen in the anthers.

An attempt for selection of pollinators (fertile analogous) with pollen fertility not upper of 40% was made on the basis of the created hypothesis, in 2008 in the second BC_3P_2 plant populations (with morphological uniformity and good agro-biological quality) of the crosses between line № 1647 (ms_8ms_8) and varieties Gorogled 6 - red pepper for grinding, Victoria and Byal Kalinkov - green pepper (Figures 1, 2, 3, 4). After intraline pollinations (ms_8ms x *Msms*), the seeds were extracted from the obtained fruits and progenies were cultivated in the next 2009 year.

In 2009 the segregation was studied cytologically in the first intraline progeny of 96 plants - breeding numbers P5/08 and P6/08 (red pepper for grinding) and respectively 38 from No 115/08, 141/08, 142/08 and 143/08 (green pepper) to ensure the created hypothesis and the expected segregation (1:1 – Msms: ms_8ms_8 plants) in maintenance of the new male sterile lines.

In the lines No P5/08 and P6/08, 53 plants formed fertile pollen, as 43 – were without pollen grains in the anthers (segregation in ratio1.2:1). The ratio fertile: sterile green pepper plants were 1.1:1 according to the same scheme as that one used in red pepper breeding.

In order to be confirmed the possibility for selection of fertile analogous, using as a criteria low level of the pollen fertility and to check again the expected ratio (1:1) in maintenance of the new male sterile lines, in 2010 year 142 red pepper plants (No P4/09, P8/09, P10/09, P11/09, P12/09, P13/09 и P14/09) were investigated (Table 1). The cultivated plants were progeny from the second intraline pollination (ms_8ms x *Msms*) in the lines No P5/08 and P6/08. The obtained data showed 1:1.1 and 1:1.3 *Msms*: ms_8ms_8 ratio, respectively which again is close to the expected one 1: 1.

Table 1
Segregation fertile: sterile plants after second intraline pollination (ms_8ms x *Msms*) in lines No P5/08 and P6/08

Progenies	Studied plants			Fertile: Sterile plants ratio
	total number	fertile number	sterile number	
P4/09 – of line P5/08	19	10	9	1.1 : 1
P8/09	18	9	9	1 : 1
P10/09	10	4	6	1 : 1.5
P11/09	29	17	12	1.4 : 1
P12/09	22	7	15	1 : 2.1
Total	**98**	**47**	**51**	**1 : 1.1**
P13/09 – of line P6/08	20	11	9	1.2 : 1
P14/09	24	8	16	1 : 2
Total	**44**	**19**	**25**	**1 : 1.3**

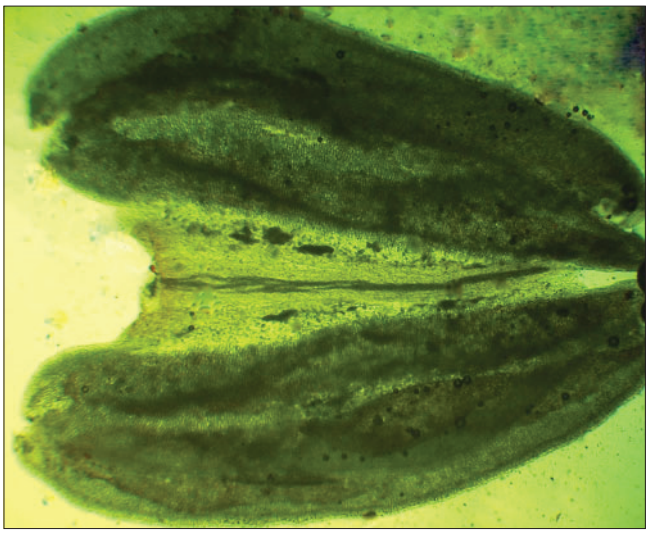

Fig. 1. Anther without pollen grains of sterile plant

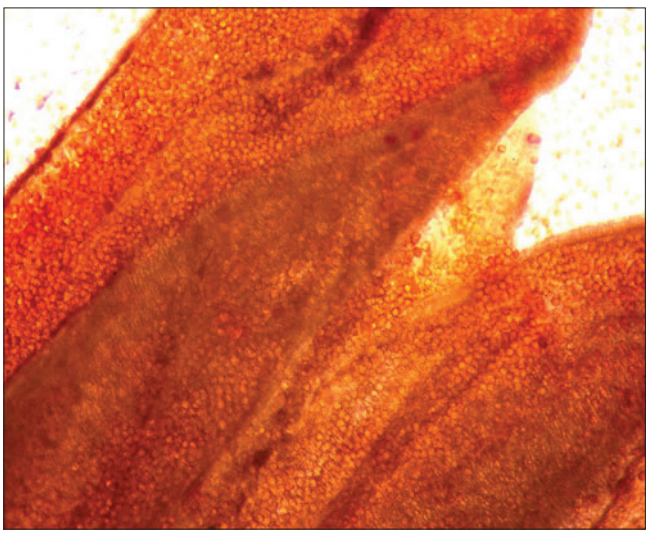

Fig. 2. Anther tissue with fertile pollen of F_2 hybrid plant

Discussion

In the pepper breeding programs based on nuclear male sterility the major problems in creating new lines with suitable agronomic characteristics are to produce new forms with good biological quality and stability of the sterility expression: to incorporate the gene marker for early and easy selection of the sterile genotypes: to choice correct pollinators - fertile analogous (*Msms)*.

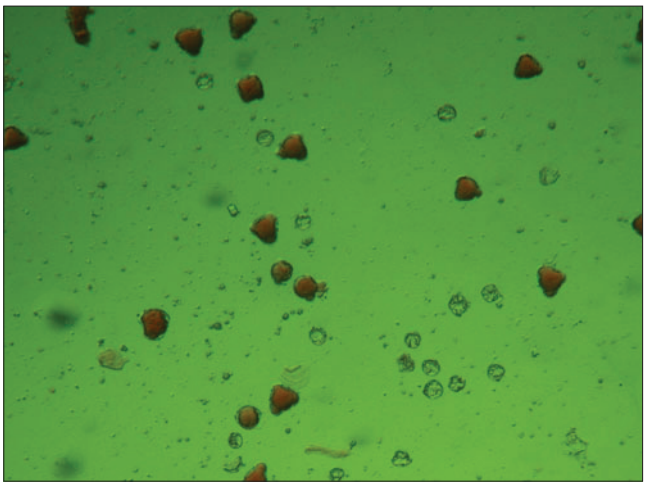

Fig. 3. Pollen of heterozygous F2 plant

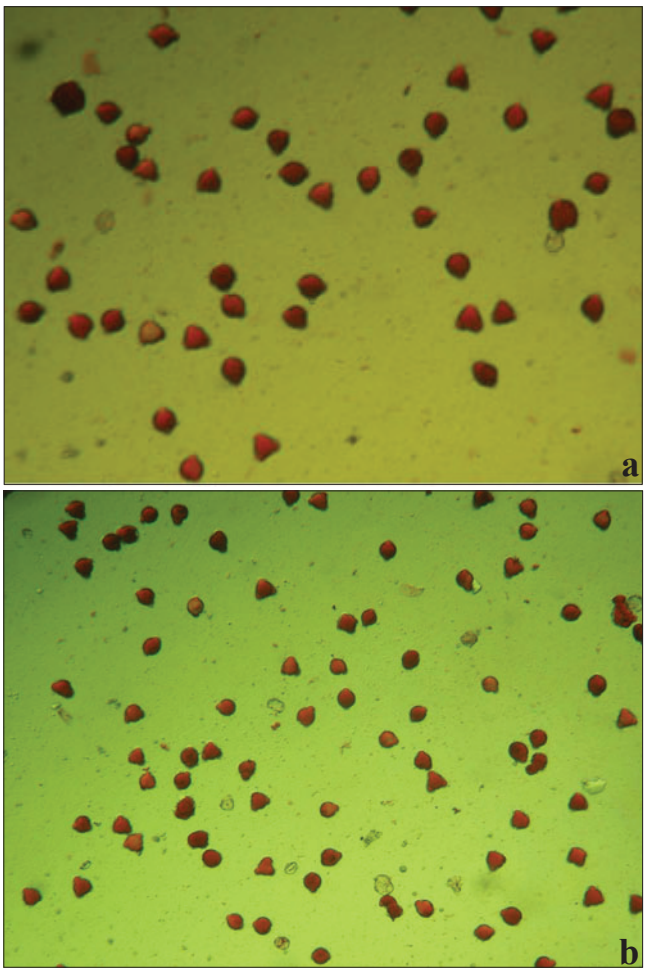

Fig. 4. a, b. High pollen ferility in homozygous F2 plants

Numerous lines with different nuclear *ms* genes, presenting stable expression are known. There is some information about experiments to incorporate the seedling marker linked with *ms* gene for early sterile form selection (Meshram and Narkhende, 1982; Moor, 1986; Patel et al., 2001 and Pathak et al., 1983).

According to Patel et al. (2001), the recessive gene marker or pleotropic acting gene from female parent can be also used for identification of male sterile plants. However, there are not announcements about any criteria including cytological one for the fertile analogous easier and faster choice. Kumar et al. (2003) studying the genetics of fertile restores and maintainers in cross between stable nuclear - cytoplasmic sterile line "CCA - 4261" and 48 hot and 5 sweet pepper lines (all *Capsicum annum* L.) described lines identified as restores, as 70 % of them were with pollen fertility between 70 and 90%; line defined as maintainers with stained pollen grains from 34.6 to 36.2% and lines segregating for both the traits – with pollen fertility from 33.1 to 82.1%.

On the basis of the suggestion about incomplete expression of the dominant *Ms* gene in the heterozygous (*Msms*) genotypes of the line №1647 (Daskalov, 1973) and in the F_1 plants from the crosses of line №1647ms_8 with different pepper varieties (Nikolova et al., 2001; 2002), it was created a new hypothesis for faster and easier choice of fertile analogous. The attempt to separate the fertile plants from F_2 hybrids and from second progenies of the backcrosses to heterozygous (*Msms*) and homozygous (*MsMs*) genotypes by established pollen fertility intervals gave us possibility for more correct selection of *Msms* forms.

The pollen fertility is a plant characteristic influenced by environmental and technological changes and it was necessary the obtained results to be repeated in the two following years (2009-2010). The find segregations in maintenance of the new male sterile forms, crossed with chosen new heterozygous male fertile plants (according to created hypothesis with low level of pollen fertility) were near to expected one (1:1) in 2009-2010 and that was a proof about the successful selection of fertile analogous, using as a criteria - pollen fertility not up to 40%.

The described method manifested on the one hand higher reality of the heterozygosis of the selected pollinators in comparison to usually using practice - by occasional choice - and on the other – acceleration of the breeding process, avoidance of the numerous crosses and following control of the segregations, witch require extensive plant cultivation.

This method could be used in other plant species for selection of heterozygote genotypes by pollen fertility in segregation hybrid and backcross progenies based on nuclear male sterility.

The results of performed investigations and analysis showed, that when the segregation ratio fertile: sterile genotypes in F_2 hybrids and in second progenies of backcrosses deviated significant from the expected one (3: 1), the selection of heterozygous *Msms* plants was less possible and not very definite on the basis of pollen fertility.

Csillery (1989) and Shifriss and Pilowsky (1993) succeeded in developing a digenic system *ms1 ms1 ms2 ms2* x *Ms1ms1 Ms2ms2* which, due to complementary gene action, yielded 3 male-sterile and 1 fertile progenies. According to Moor (1986), desirable ratio is 2:1 fertile: male sterile individuals in populations for the best results in hybrid seed production.

Conclusions

A successful attempt to separate the heterozygous (*Msms*) from the homozygous (*MsMs*) genotypes in F_2 hybrids and second progenies of the backcrosses (obtained on the base of nuclear male sterility) by establishing pollen fertility was carried out. The suitable tolerance of pollen fertility, which determined *Msms* plants, was defined to be between interval of 0.1 and 70%.

As a criterion in determination of the fertile analogous plants – pollen fertility below 40% shows correct ratio 1:1 in maintenance of new male sterile forms.

When the ratio sterile ms_8ms_8 : fertile plants in segregating hybrid and backcross progenies deviated from the expected (3: 1) it was less possible and not very definite to select the heterozygous *Msms* genotypes on the basis of pollen fertility.

This method could be used in other plant species for selection of heterozygote genotypes by pollen fertility in segregation hybrid and backcross progenies based on nuclear male sterility.

References

Andrastalvy, A. and G. Csillery, 1983. Cytoplasmic systems of interspecific hybrids in Capsicum, reconsidered. *Eucarpia Vth Meeting on Genetics and Breeding of Capsicum and Eggplant*, Plovdiv, Bulgaria, pp. 18-20.

Csillery, G., 1989. More Efficient Pepper Hybrid Seed Production by Double Male Sterile Mother Line. *Eucapia VIIth Meeting on Genetics and Breeding of Capsicum and Eggplant*, Kragujevac, Yugoslavia, pp. 129-133.

Daskaloff, S., 1971. Two new male sterile pepper (*Capsicum annuum* L.) mutants. *C R Acad Sci. Agric. Bulg.,* **4**: 291-294.

Daskalov, S., 1973. Three new male sterile mutants in pepper (*Capsicum annuum* L.). C *R Acad Sci. Agric. Bulg.,* **6**: 39-41.

Deshpande, A. A., C. S. Pathak and D. P. Singh, 1983. Tipes of male sterility in chilli pepper (*Capsicum annuum* L.). *Capsicum and Eggplant Newsletter*, **2**: 104-105.

Dumas de Vaulx and M. Pitrat, 1977. Interspecific crosses between *Capsicum annuum* and *Capsicum baccatum. Eucapia IIIth Meeting on Genetics and Breeding of Capsicum and Eggplant* (Avignon-Montfavet, France), pp. 75-80.

Kumar, S., S. K. Rai, Major Singh and G. Kallo, 2003. Genetics of fertility restoration and identification of restorers and maintainers in pepper (*Capsicum annuum* L.). *Capsicum and Eggplant Newsletter*, **22**: 79-82.

Nikolova, V., V. Todorova, S. Daskalov, J. Todorov and V. Stoeva, 2001. Pollen fertility of pepper cultivars and their hybrids on male sterility basis. *Cappsicum and Eggplant Newsletter*, **20**: 50-52.

Nikolova, V., V. Todorova, , S. Daskalov and Y. Todorov, 2002. Study of pollen fertility in some hybrids of pepper (*C. annum* L.) on the basis of nuclear male sterility. *Proceedings of First symposium of horticulture,* Skopie, Macedonia, pp. 324 – 327.

Meshram, L. D. and M. N. Narkhede, 1982. Natural male sterile mutant in hot chili (*Capsicum annuum* L.). *Euphytica,* **31:** 1003-1005.

Meshram, L. D., R. V. Choudhari, B. K. Kudade and M. W. Marawar, 1992. Functinal male sterility in hot chili (*Capsicum annuum* L.). *Eucapia VIIIth Meeting on Genetics and Breeding of Capsicum and Eggplant,* Roma, Italy, pp. 61-65.

Moor, A., 1986. Hybrid seed production by male sterile female lines in Hungry, *VIth Meeting on genetics and breeding on Capsicum and Eggplant, EUCARPIA*, Zaragosa, Spain, pp. 51-54.

Pathak, C. S., D. P. Singh and A. A. Deshpande, 1983. Male and female sterility in chili pepper (*Capsicum annuum* L.). *Capsicum and Eggplant Newsletter*, **2**: 102-103.

Patel, J. A., M. J. Patel, A. S.Bhanvadia, R. R. Acharia and M. K. Bhalala, 2001. Extent of natural cross pollination with cms lines in chilli pepper (*Capsicum annuum* L.). *Capsicum and Eggplant Newsletter*, **20:** 35-37.

Pockard, E., 1970. Obtaining three new male sterile mutants of pepper (*Capsicum annuum* L.) through application of mutagenesis on monoploid material. *Eucarpia,* pp. 93-95.

Prakash, N. S., N. Lakshimi and I. Harini, 1987. Male sterility and manipulation of yield in *Capsicum*. *Capsicum Newsletter,* **6**: 35.

Rusenova - Kondareva, I., 1968. Results of Interspecifc Hybridization in Genus *Capsicum. Bulgarian Academy of Science Press,* Sofia (Bg).

Shifriss, C., 1973. Additional spontaneous male sterile mutant in *C. annuum* L. *Euphytica,* **22:** 527-529.

Shifriss, C. and M. Pilowsky, 1993.Digenic nature of the male sterility in pepper (*Capsicum annuum* L.). *Euphytica,* **67:** 111-112.

Received August, 2, 2011; accepted for printing February, 2, 2012.