**Bangladesh J. Pl. Breed. Genet., 29(1): 31-38, 2016**

# USE OF MALE STERILITY AND SYNTHESIS OF MAINTAINER LINE FOR HYBRID SEED PRODUCTION IN ONION (*ALLIUM CEPA* L.)

**G. M. Mohsin[1], Farruk Ahmed, M. S. Rahman and M. S. Islam[2]**

Research and Development Wing, Lal Teer Seed Limited, Gazipur, Bangladesh
[1] Department of Agriculture, Noakhali Science and Technology University, Noakhali 3814, Bangladesh
[2] Plant Breeder, BRAC, Bangladesh

## ABSTRACT

A field experiment was carried out and one cytoplasmic genetic male sterile line (Smsms) and two fertile lines were identified as maintainer lines (Nmsms). These two crossed materials namely 004 (Shallot x Red creole) and 008 (Shallot x Red pinoy) produced 100 per cent male sterile progeny in full sib and backcross generations. The Shallot x Taherpuri/Suksagor and Shallot x Hazera-202/Hazera-203 cultivar produced both male fertile and male sterile segregating progenies. It indicated that these materials are probably determined by dominant and recessive independently acting genes which was resulting the genetically impure lines. All other crossed materials produced 100 per cent male fertile progeny upon crossing with shallot. Thus, the materials Red creole and Red pinoy can be used as maintainer line for "Shallot". The performance of 904 F1 and 905 F1 hybrids over check (Taherpuri) and better parent was found to be preferably better using these CMS system.

**Keywords**: *Allium cepa* L., cytotoplasmic male sterility, hybrid

## INTRODUCTION

Onion (*Allium cepa* L.) is an important spice around the world and grown as a winter crop in Bangladesh. Average yield 3.45 t/ha in Bangladesh and 45 t/ha in developed country (FAO 2002). Low yielding variety and Inadequate supply of quality seed is the main constraint to its higher production. Due to lack of high yielding variety, Bangladesh importing onion bulb worth of about BDT 400-500 crores in every year (Prothom Alo, July 11, 2004). It is highly cross pollinated crop and sensitive to day length and temperature (Pike 1986). Early stage for vegetative growth needs cooler weather and formation of bulb is enhanced by high temperature. Hybrid varieties play a vital role in increasing vegetable production due to their high yield potential, early maturing, superior quality, disease and pest resistance attributes (Nagaraju et al. 2017). MS mechanism is one of the tools for hybrid variety development and it is the most significant mechanism for improving the yield and higher returns of onion (Pike, 1986, Kalloo, 1988). First CMS plant was reported within the progenies of an onion cultivar Italian Red (Jones and Emsweller, 1936) and male sterility was under the control of single recessive nuclear restorer locus (Jones and Clarke, 1943). A good male sterile line can

be obtained by repeated backcrossing, where local maintainer is very essential (Chatterjee, 1986). Red color onion bulb is not preferred by the Bangladeshi farmers and consumers. So, this offers a great scope for improving the production of this crop in Bangladesh through CMS breeding. In this consequence however, the present study was conducted for searching the maintainer line from distance heterotic pole for the development of hybrid onion variety which could be performed as better yielder than our local cultivar and to fight against different agro-climatic condition.

**Materials and Methods**

Bulbs of "shallot" (Fig. 2) and its probable maintainer (Nmsms) were collected from the open market of Philippines, Bangladesh and Thailand in November 2006. Shallot bulbs were vernalized at $10^{o}$C for four weeks as this cultivar do not flower or poorly flower under Bangladesh conditions. Total eleven male fertile both local and exotic cultivars viz. Taherpuri, Suksagor, Batanes, Red pinoy, NZ-2, Red creole, Hazera-202, Hazera-203, Nueventa, Light red and Nasik red were grown at the same time. At flowering stage the "Shallot" was crossed with fertile pollen during the winter season of 2007-2008 at Research and Development wing (R&D) of Lal Teer Seed Ltd., Gazipur. In 2009-2010 at flowering stage both Male Sterile (MS) and Male Fertile (MF) plants were identified through the study of flower morphology and pollen viability test. Performances of MS plants from F1 families in respect of days to bolting and days to flowering were recorded for synchronization. In addition few F1 plants of 004 (Shallot x Pinoy) and 008 (Shallot x Red Creole) were back crossed with their recurrent and expected pollen parents. Total six crosses as F1 were evaluated during 2012-2013 in respect of yield performance against check variety (Taherpuri). Total methodologies were cited as below:

**1st year: Bulb to Seed**

- Growing the selected bulbs of expected MS lines and expected B -lines in alternate rows
- Selection of male sterile plants based on pollen sterility using 1% acetocarmin dye.
- Crossing between MS (A-line) and expected B-lines.

**2nd year: Seed to bulb**

- Growing the bulbs of the cross between A-line and expected B-line
- Selection of bulbs based on desirable characters like color
- Selection of bulbs including pungency and storability

**3rd year: Bulb to Seed**

- Planting of selected bulbs based on storage quality and pungency
- Identifying MS plants which will be repeatedly backcrossed with B-line (maintainer line)
- Crossing between A-line with expected C-line (Restorer line).

**4rth year: Seed to bulb**

- Growing and selecting bulbs based on desirable characters.
- Storing the selected bulbs in ambient conditions.

**5th year: Bulb to seed**

- The progeny from A*C will show male sterility or fertility with desirable heterosis

## RESULTS AND DISCUSSION

The plants of all the crossed families flowered. The percentage of male sterile (MS) plants (Fig. 3) in the families resulting from the crosses between shallot and male fertile (MF) plants varied from 0 to 100 (Table 1.). Two exotic cultivars (Red Pinoy and Red creole) were identified as maintainer lines as these cultivars were found to posses' recessive chromosomal gene (msms) responsible for male sterility. These results revealed that the pollen parents which possessed msms genes could be used as a maintainer line of "Shallot". The exotic cultivars viz. Batanes, NZ-2, Nueventa, Light red and Nasik red were found to posses the dominant gene (MsMs) responsible for restoration of male fertility in the sterile plants. On the other hand exotic cultivar Hazera-202 and 203 as well as our local cultivar Taherpuri and Suksagor were found both male fertile and male sterile segregating progenies in a certain extent which indicated that these materials were probably determined by dominant and recessive independently acting genes resulting the genetically impurity or the effect of temperature. The materials Hazera-202 and Hazera-203 produced 50 percent male fertile and 50 percent male sterile progenies upon crossing with shallot plants. Furthermore, our local cultivar Taherpuri exhibited 90 percent male fertile and 10 percent male sterile along with Suksagor which was in 75:25 fertility sterility ratios indicating the presence of Msms genes in their cytoplasm.

The performance of 904 F1 and 905 F1 hybrids (Table 2 and Fig. 4) over check and better parent was found to be preferably better using CMS system. The local pollen parent has the potentiality to maintain the sterility of shallot. The flowering period of shallot and local lines are naturally well synchronized. Almost similar findings were also reported by Mohsin (1996) and Hossain (1995) who used the "Ogura radish" which is male sterile lines.
For the production of hybrid, A, B and C lines will be required (Fig.1 & 5). Line A is with shallot (Smsms), Line B is the Red Creole and Red Pinoy (Nmsms) and line C is the (Taherpuri).

### Genetic constituents of CMS and maintainer lines

CMS line or A- line: S msms (S is sterile)
Maintainer or B-line: N msms (N is fertile)
Genetic constituents of A and B line is identical except the fertility which is govern by a single gene presence in B line.
By the way of illustration A-line is genetically male sterile with recessive gene consequently B-line is genetically male fertile with recessive gene

### How to develop a maintainer line, if not available in the nature

- **1st :** Smsms * NMsMs…….>SMsms (all male fertile)
- **2nd:** NMsMs * SMsms……..> NMsMs, NMsms (all male fertile)
- **3rd:** First Progeny test:

3.1: Smsms * NMsMs…….>SMsms (all male fertile)
3.2: Smsms * NMsms…….>SMsms,Smsms (1/2 fertile + ½ sterile)
3.3: NMsms selfed……>NMsMs, NMsms, Nmsms (all male fertile)

**Schematic diagram of Hybrid seed production using CMS system**

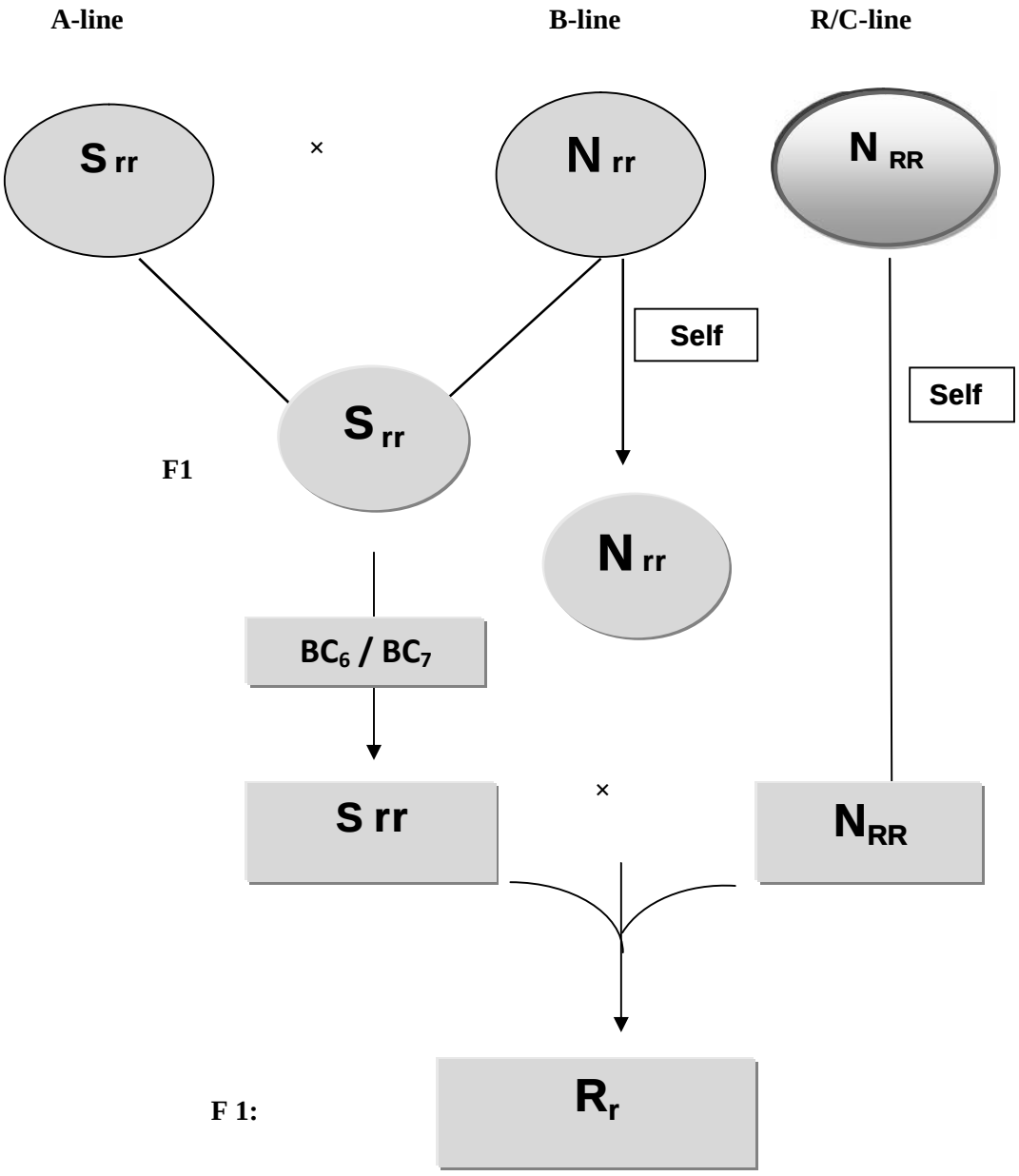


**Fig 1. Schematic diagram of hybrid variety of onion development using CMS mechanism.**

- **4rth :** Second progeny test:

4.1: Smsms * NMsMs……>SMsms (all male fertile)
4.2: Smsms * NMsms……..>SMsms, Smsms (1/2 fertile + ½ sterile)
4.3: Smsms * Nmsms……> Smsms (all male sterile)

So, Nmsms is identified as maintainer which is able to maintain the CMS mechanism. CMS line and new B line (Nmsms) to be planted in isolation for repeated backcrossing to transfer all characters of B line except fertility.

**Table 1. Flowering behavior and sterility status of different crosses in onion**

| Acc. no. | Description | Bulbs | 1st bolting (DAS) | 1st dof (DAS) | Fertility (%) | Sterility (%) |
|---|---|---|---|---|---|---|
| 001 | Shallot X Batanes | 35 | 66 | 110 | 100 | 0 |
| 002 | Shallot X Taherpuri | 25 | 59 | 100 | 90 | 10 |
| 003 | Shallot X Suksagor | 50 | 58 | 99 | 75 | 25 |
| 004 | Shallot X Red pinoy | 5 | 97 | 136 | 0 | 100 |
| 005 | Shallot X NZ-2 | 9 | 57 | 99 | 100 | 0 |
| 006 | Shallot X Hazera 202 | 7 | 57 | 100 | 50 | 50 |
| 007 | Shallot X Hazera 203 | 8 | 59 | 101 | 50 | 50 |
| 008 | Shallot X Red creole | 5 | 68 | 104 | 0 | 100 |
| 009 | Shallot X Nueventa | 8 | 75 | 117 | 100 | 0 |
| 010 | Shallot X Light red | 10 | 63 | 103 | 100 | 0 |
| 011 | Shallot X Nasik red | 14 | 64 | 104 | 100 | 0 |

**Characteristics of A, B and C/R lines**

- Components of three lines for hybrid development:

1. A-line (Male sterile line or CMS line)
2. B-line (Male fertile line)
3. C/R-line (Male fertile)

Genetic makeup of components lines:
A-line: This CMS is genetically pure and male sterile. It possesses nuclear recessive gene and sterile cytoplasm (Smsms)
B-line: Identified B-line is fertile and possesses nuclear recessive gene with fertile cytoplasm (Nmsms)
Before backcross A-line and B-line is morphologically dissimilar

**Development of A-line**

- Backcross breeding method is applied for development of A-line using identified B-line and CMS line.
- By the way B-line should be selfed for its maintaining
- After 5-6 backcross CMS line will be converted as expected A-line. 1st yr: 50%, 2nd yr: 75%, 3rd yr: 87.5%,4th yr: 93.75, 5th yr: 96.875, 6th yr: 98.4375, 7th yr: 99.21875
- A and B line is isogenic. Difference is only male fertility and sterility.

**Fig 2. Sprouted bulb of shallot Onion**

**Fig 3. Male sterile flowers (left) and male fertile (right) flowers in umbel of onion**

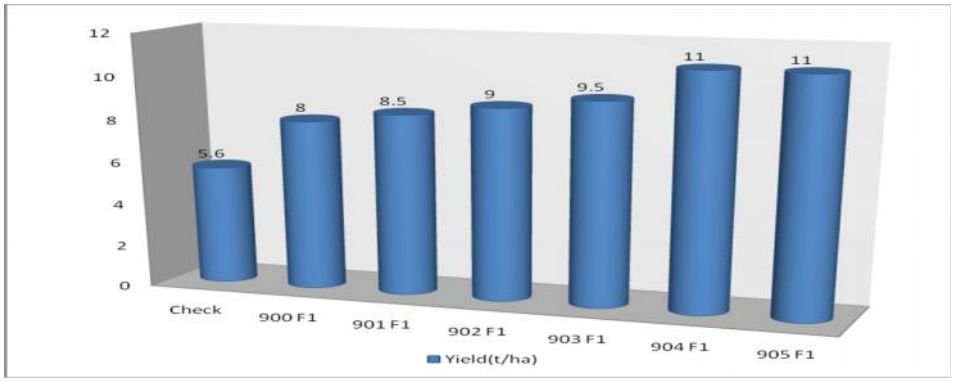


**Fig 4. Comparative yield (t/ha) of different CMS hybrids with check var. of onion.**

**Clearance of A-line**

**Field:**

1. Through selfing no seed,
2. Petal has no color,
3. Long stigma compare to anther
4. Anther shriveled as thread , Pale and no pollen

**Lab:**

1. Sterile pollen is taking no dye, become wrinkle shaped at the time of 1% acetocarmin dye test.

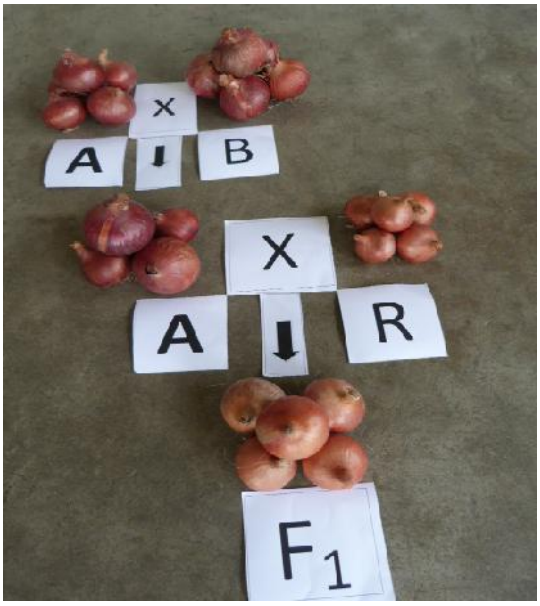


**Fig 5. Hybrid variety of onion (*Allium cepa* L.) development using CMS mechanism.**

**Table 2. Mean performance and percent heterosis over check variety (CV) and better parent (BP) for yield in onion (*Allium cepa* L.)**

| Hybrid | Parentage (A X C) | 10 bulbs wt.(g) | | | % of Heterosis | |
|---|---|---|---|---|---|---|
| | | Mean (F1) | Check (Taherpuri) | BP | Check | Over BP |
| 900 F1 | Red Pinoy X Suksagor | 570 | 223 | 800 | 156 | -29 |
| 901 F1 | Red Creole X Suksagor | 550 | 223 | 800 | 147 | -31 |
| 902 F1 | Red Pinoy X Taherpuri-S | 440 | 223 | 350 | 97 | 26 |
| 903 F1 | Red Pinoy X Taherpuri-M | 410 | 223 | 350 | 84 | 17 |
| 904 F1 | Red Creole-M X Taherpuri-M | 625 | 223 | 350 | 180 | 79 |
| 905 F1 | Red Creole-S X Taherpuri-S | 640 | 223 | 350 | 187 | 83 |

Careful attention using the knowledge of genetics it could be identifies the sterility maintainer gene in our local cultivar which is more adaptable to our agro edaphic factor. The

shallot and local lines are of different genetic back ground, thus they differ for many traits. The characteristics of the “local lines” are to be transferred in the “shallot” by backcrossing. For this, another three to four backcrosses to “local lines” will be required. Certain precautions such as frequent insect pollination should be allowed and rouging of male fertile plants from CMS line should be done timely to avoid unexpected out crossing. So, considering the genetics of the male sterility mechanism, further studies should be taken towards synthesis of maintainer line from local cultivar as well as hybrid variety development for better stability along with better yield and to combat climatic fluctuation in Bangladesh.

**REFERENCES**


Chatterjee, S. S. 1986. Cole Crops in T.K. Bose and M.G. Som (Eds.), Vegetable Crops in India. Naya Prokash, Calcutta6. PP. 236-237.

FAO (Food and Agricultural Organization). 2002. FAO Production Year Book, Rome **54**: 152-154.

Hossain, M. S. 1995. Utilization of self incompatibility and male sterility in hybrid seed radish production. Ph.D thesis. Dept. Genet. Pl. Breed., IPSA, Gazipur, Bangladesh.

Jones, H. A and Clark, A. E. 1943. Inheritance of male sterility in the onion and the production of hybrid seed. Proc. Amer. Soc. Hort. Sci. 43. PP. 189-194

Jones, H. A. and Emsweller, S. L. 1936. A male sterile onion. *Proc. Am. Soc. Hort. Sci.* 63: 443.

Kallo. 1988. Vegetable Breeding. Vol. 1, CRC Press Inc, Boca Raton, Florida. PP. 106-111

Mohsin, G. M. 1996. Performance of backcross populations in hybrid seed production of radish (*Raphanus sativus* L.). MS thesis. Dept. Genet. Pl. Breed., IPSA, Gazipur, Bangladesh.

Nagaraju, M. M.,Thomson, T., Rao, G. K. and Siva, M. 2017. Role of Male Sterility in Vegetable Hybrid Seed Production. *Int. J. Curr. Microbiol. App. Sci. 6*(7): 134-141

Pike, L. M. 1986. Onion Breeding. In: M.J. Basset (Ed). Breeding vegetables crops published by AVI publishing company, Inc., Westport, Connecticut. PP. 357-394.

Prothom Alo, the daily news, July 11, 2004, Dhaka, Bangladesh.