

## FREQUENCY OF MALE STERILITY FACTORS IN 'BEHAIRY RED' ONION.

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### ABSTRACT

*Frequency of cytoplasmic and genetic factors controlling male sterility in the Egyptian onion (Allium cepa L.) cv. Behairy Red was studied during 2001-05. In the first season (2001/02), 8 male-sterile plants were found out of 5240 plants examined at El-Gemmiza Research Station.*

*These male sterile plants were left for open pollination. In the following season (2002/03), seeds of open pollinated male sterile plants were sowed in commercial fields, Giza Governorate for bulb production. Bulbs obtained were planted in the 2003/04 season for seed production of these progenies. The progenies of male sterile plants were inspected in April 2004. Out of 102 progenies examined, only 31 were found to be male sterile plants. Inspection of 'Behairy Red' population was repeated at El-Gemmiza Station during the 2002/03 season. Out of 7560 plants inspected, 48 plants were found to be male sterile, and they were left for open pollination. In the following season (2003/04), seeds of open pollinated male sterile plants were sowed in commercial fields, Giza Governorate for bulbs production. Bulbs were planted in the 2004/05 season for seed production of these progenies. The progenies of male sterile plants were inspected in April 2005. Out of 979 progenies examined, only 426 were found to be male sterile plants. Average frequency of male sterile plants was 0.0034, while average frequency of the ms locus was estimated as 0.3695. Therefore, average frequency of the Ms locus was estimated as 0.6305. Average frequency of the sterile S cytoplasm was estimated as 0.0222, while that of the N cytoplasm was 0.9778. The frequency of the N ms ms genotype was estimated as 0.13745.*

Key words: *Onion, Allium cepa L., Cytoplasmic male sterility, Cytoplasmic-genetic male sterility.*

### INTRODUCTION

Male sterility is an important character in onion as it contributes greatly to production of hybrid onion. The frequency of male sterility had been studied in local Egyptian onion cvs., viz., Giza 6 Mohassan, Behairy, Shandaweel1, and Giza 20. (El-Shafie and Ahmed 1976, El-Shafie and El-Kafoury 1977, Ahmed and Hanna 1987, Ahmed and El-Shafie 1988 and Yaso 2002) and was found to range from 0.0017 to 0.009. Similar studies have been also conducted on foreign cvs.e.g., Scott County Globe, Primeur, Wijbo, Rijnsburger, Brigham Yellow Globe, Mountain Danvers, Sapporo-

Ki, B2215C, and Texas Grano1015Y (Peterson and Foskett 1953, Banga and Petiet 1958, Van der Meer and Van Bennekom 1971 and Havey 1993, 1995).

The present investigation was conducted to determine the frequency of male sterility factors in the Egyptian onion cv. Behairy Red as a prerequisite for the detection and establishment of B-Line in this cv..

## MATERIALS AND METHODS

Selection and multiplication of male sterile 'Behairy Red' plants were conducted at El-Gemmiza research Station, Field Crops Research Institute, Agriculture research Center during the 2001/02 and 2002/03 seasons. Consequently surveys of progenies of male sterile plants were conducted in commercial fields, Giza Governorate during 2002/05.

In April 2001, when at full bloom, 5240 plants were examined visually for male sterility. Pollen grain from flower samples of each detected male sterile plant were examined in the laboratory for aceto carmine stainability. Non-stainable pollen grains were considered sterile. Plants, which proved male sterile, were tagged in the field and left for open pollination. The seeds of open-pollinated male sterile plants were individually harvested in June 2001.

Seeds of each male sterile plant were sown in the seed bed in commercial fields, Giza Governorate in September 2002 and seedlings were transplanted in the permanent field in December 2002 for the production of the S<sub>1</sub> bulbs (progeny of the open-pollinated male sterile plants), which were harvested in June 2003. In December 2003 bulbs obtained were planted for seed production. In April 2004 all progenies were visually examined for male sterility. This was confirmed by the laboratory test, i.e., non-stainability with aceto carmine.

Similar measures were taken during the second selection and multiplication season (2002/03) and its subsequent surveys (2004/05).

In April 2003, when at full bloom, 7560 plants were examined visually for male sterility. Flower samples of each detected male sterile plant were examined in the laboratory for aceto carmine-non stainability. Plants, which proved male sterile, were tagged in the field and left for open pollination. The seeds of open-pollinated male sterile plants were individually harvested in June 2003.

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the  $S_1$  bulbs (progeny of the open pollinated male sterile plants), which were harvested in June 2004. In December 2004 bulbs obtained were planted for seed production. In April 2005 all progenies were visually examined for male sterility. This was confirmed by the laboratory test, i.e., non-stainability with aceto carmine.

The number of male sterile plants in the natural populations and in the progeny of detected male sterile plants was recorded and the frequency of each in the population examined was calculated. The frequency of each of the possible male sterile and male fertile genotypes, viz, N Ms Ms, N Ms ms, N ms ms, S Ms Ms, S Ms ms, and S ms ms, was calculated using the formula:  $a = (S \text{ frequency}) \times (q^2)$  or the frequency of S cytoplasm =  $a/q^2$  which was used by R.DE. Pienaar (1958) as cited after Van Der Meer and Van Bennekom (1971), EL-Shafie and Ahmed (1976), Ahmed and EL-Shafie (1988) and Yaso (2002); where :

a = The frequency of male-sterile plants in the natural population, and

q = The frequency of male-sterile plants in the progeny of the male sterile plant which were left for open pollination.

The frequency of the ms locus was q and the frequency of plants with S cytoplasm was  $a/q^2$ . The frequency of the Ms locus was 1- frequency of ms. Frequency of the N cytoplasm was calculated from the formula:  $N=1-(a)/q^2$  or  $(1- S) (p+q=1)$  after Falconer (1981). Therefore, frequencies of the 6 possible fertility and sterility genotypes were calculated.

## RESULTS AND DISCUSSION

### Frequencies of male sterile plants

Data presented in Table (1) showed that the natural occurrence of male sterile plants in the cv. Behairy Red was 0.0015 and 0.0053 in the first and second seasons, respectively with an average off 0.0034. Similar results were obtained by other investigators as the frequency of male sterile plant was estimated as 0.57% to 1.32% in cv. Giza 6 Mohassan (Ahmed 1963), 0.0023 in the same cultivar (El-Shafie and Ahmed 1976), 0.0016 in cv. Behairy (El-Shafie and El-Kafory 1977), 0.0013 in cv. Shandaweel 1 (Ahmed and Hanna 1987), 0.0017 in cv. Shandaweel 1 (Ahmed and El-Shafie 1988), 0.0958 in cv. Giza 20 (Yaso 2002), 0.002 to 0.005 in Leading Dutch variety Rijnsburger (Meer and Van Bennekom 1971). Meanwhile, Peterson and Foskett (1953) found that the percentage of male sterile plants varied from 0.84% to 0.96 % in cv. Scott Count Globe in two seasons.

**Table 1. Frequency of male-sterile plants naturally occurring in populations of cv. Behairy Red during 2001/02 and 2002/03 seasons.**

Season	No. of Examined plants	Male Sterile plants	
		No.	Frequency
2001-02	5240	8	0.0015
2002-03	7560	40	0.0053
Total	12800	48	0.0034

### Frequency of nuclear factors ms and Ms

Data obtained on the frequency of male sterile and male fertile plants in the progenies of the detected male sterile plants of cv. Behairy Red, left for open pollination, and evaluated in 2003/04 and 2004/05 seasons are presented in Table (2). The frequency of ms (q) was 0.3039 and 0.4351 in the first and second seasons, respectively with an average of 0.3695. The frequency of Ms (p) was 0.6961 and 0.5649 in the first and second seasons, respectively, with an average of 0.6305. Similar results were obtained by Ahmed and El-Shafie (1988) who found that ms and Ms frequency was 0.2117 and 0.7833, respectively, in cv. Shandaweel 1, Havey (1995) reported that the frequency of ms allele was 0.35, 0.35 and 0.41 in cvs B2215C, Mountain Danvers and Sapporo-Ki, respectively. Meanwhile; El-Shafie and Ahmed (1976 ) found a low frequency of 0.0013 for ms and high frequency of 0.987 for Ms in cv. Giza 6 Mohassan. Yaso (2002) found the average frequencies for ms and Ms were 0.031 and 0.69, respectively in cv. Giza 20. Meanwhile, El-Shafie and El-Kafory (1977) found a high frequency of 0.95 for ms and a low frequency of 0.27 for Ms in cv. Behairy. Havey (1995) reported that the frequency of ms was 0.59 in cv. Brigham Yellow Globe, but he detected a low frequency in cv. Texas Grano 1015Y.

**Table 2. Frequency of male-sterile and male-fertile plants in the progenies of the detected male-sterile 'Behairy Red' plants, left for open pollination in 2001/02 and 2002/03 and evaluated in 2003/04 and 2004/05 seasons.**

Seasons	Sterile plants		Fertile plants	
	No.	Frequency of ms (q)	No.	Frequency of Ms (p)
2003/04	31	0.3039	71	0.6961
2004/05	426	0.4351	553	0.5649
Average		0.3695		0.6305

### Frequency of S and N cytoplasm

Data presented in Table (3) showed that the frequency of S cytoplasm was 0.0165 and 0.0279 in the two seasons, respectively with an average of 0.0222. These results are in agreement with those obtained by El-Shafie and El-Kafory (1977) who found that the frequency of S cytoplasm was 0.0003 in cv. Beahiry and Ahmed and El-Shafie (1988) who indicated a low frequency (0.003) of S-cytoplasm in cv. Shandaweel 1. Also, van der Meer and van Bennkom (1971) obtained a frequency of less than 0.01 of S cytoplasm in the leading Dutch variety Rijnsburger. Meanwhile, Havey (1993) reported that the frequency of S cytoplasm was 0.2, 0.02, 0.40 in the cvs. Brigham Yellow Globe, Mountain Danvers, and Sapporo-Ki, respectively. However, El-Shafie and Ahmed (1976) found a high frequency of 1.000 for S cytoplasm in cv. Giza 6 Mohassan. Yaso (2002) found a high frequency of 0.985 for S cytoplasm in cv. Giza 20. Also, Sato (1998) found 20 plants with S Cytoplasm and zero with N cytoplasm in Hayashi strains of onion.

Table 3. Calculations of the frequency of 'Behairy Red' plants with S and N-cytoplasm in 2001/02 and 2002/03 seasons .

Season		Frequency of S cytoplasm <sup>a</sup>	Frequency of N cytoplasm <sup>b</sup>
Selection	Evaluation		
2001/02	2003/04	0.0165	0.9835
2002/03	2004/05	0.0279	0.9721
Total		0.0444	1.9556
Average		0.0222	0.9778

<sup>a</sup> : Frequency of S cytoplasm = Frequency of male sterile plant (a) / (frequency of ms<sup>2</sup>) = a/q2

<sup>b</sup> : Frequency of N cytoplasm = 1- Frequency of S cytoplasm.

The frequency of of N cytoplasm was 0.9835 and 0.9721 in both seasons, respectively with an average of 0.9778. Similar results were obtained for the frequency of N cytoplasm by El-Shafie and El-Kafory (1977) who reported a frequency of 0.997 in cv. Behairy, Ahmed and El-Shafie (1988) indicated a frequency of 0.9665 in cv. Shandaweel 1 and Gokce and Havey (2002) reported frequency of 0.77, 0.76, 0.76, respectively, in cvs. Brigham Yellow Globe, Mountain Danvers, Sapporo-Ki. On the contrary, El-Shafie and Ahmed (1976) found that the frequency of N Cytoplasm was Zero in cv. Giza 6 Mohassan, and Yasso (2002) found that the average frequency of N cytoplasm was 0.015 in cv. Giza 20.

### Frequency of the 6 possible fertile and sterile onion genotypes

Data obtained on the frequency of the 6 possible fertile and sterile onion genotypes are presented in Table (4). The average frequencies of the genotypes N Ms Ms, N Ms ms, N ms ms (B-Line), S Ms Ms, S Ms ms, and S ms ms (A-Line) were 0.3933, 0.4470, 0.1374, 0.0085, 0.0104, 0.0034, respectively.

**Table 4. Calculated frequency of the six possible fertile and sterile 'Behairy Red' genotypes evaluated in 2003/04 and 2004/05 seasons.**

Genotypes	Frequency	Calculated frequency		Average
		2003/04	2004/05	
N Ms Ms	$N p^2$	0.4765	0.3101	0.3933
N Ms ms	$N 2 p q$	0.4161	0.4778	0.4470
N ms ms	$N q^2$	0.0908	0.1841	0.1374
S Ms Ms	$S p^2$	0.0081	0.0089	0.0085
S Ms ms	$S 2 p q$	0.0070	0.0137	0.0104
S ms ms	$S q^2$	0.0015	0.0053	0.0034
<b>Total</b>		<b>1.00000</b>	<b>1.00000</b>	<b>1.00000</b>

These results indicated that for every 100 tests crosses, 14 plants may be detected as B-Line. This situation in cv. Behairy Red is favorable for establishing a hybrid program. Similar results were obtained by Ahmed and El-Shafie (1988) who indicated that the frequency of the B-Line was 0.0433, while the frequency of A-line (S ms ms) was 0.0015 in cv. Shandaweel 1. Yasso (2002) found a low average frequency of 0.0015 for N ms ms genotype (B-Line) and a high average frequency of 0.0958 for S ms ms genotype (A-Line) in cv. Giza 20. Additionally, Havey (1995) reported that the frequencies of maintainer lines were 0.219, 0.097, 0.098, 0.101 in cvs. Brigham Yellow Globe, B2215 C, Mountain Danvers, and Sapporo-Ki, respectively. Also, Cho *et al* (2005) found that the frequencies of maintainer lines were 0.24 and 0.10 in cvs. Manchuhwang and Sapporo-Ki, respectively, while the frequency of the male sterile line was 0.05 and 0.16 in both cultivars, respectively. However El-Shafie and El-Kafory (1977) obtained a high frequency of 0.531 for N ms ms genotype (B-Line) and a low frequency of 0.0016 for S ms ms genotype (A-line) in cv. Behairy. Also, Meer and Van Bennekom (1971) reported that in Leading Dutch variety Rijnsburger and Noordhollandse nearly all plants (95% or more) were of the B-line (N msms).

## REFERENCES

- Ahmed, F.A. (1963). Flower biology and male-sterility in Egyptian onion , *Allium cepa*, L. M.C. Thesis, Fac. Agric., Cairo Univ.
- Ahmed, F.A. and A. B. Hanna (1987). A report on male sterility in the Egyptian onion "Shandaweel 1". Zagazig J. Agric. Res. 14 (1). 167-175.
- Ahmed, F. A. and M. W. El-Shafie (1988). Frequency of male sterility factors in the Egyptian onion variety Shandaweel 1. Zagazig J. Agric. Res. 15 (1). 848-857
- El-Shafie, M.W. and A. A. Ahmed (1976) Frequencies of factors controlling male sterility in the Egyptian onion (*Allium cepa* L.) and their implications for hybrid onion breeding . Egypt. J. Agron. 1 (?) : 283-290.
- El-Shafie, M.W. and A .K. El-Kafory (1977). Male sterility in the Egyptian onion ( *Allium cepa* L. cultivar Behairy ).The Libyan J. Agric. 6 (1): 267-271.
- Banga, O. and J. Petiet (1958). Breeding male sterile lines of Dutch onion varieties as preliminary to the breeding of hybrid varieties. Euphytica 7: 21-30.
- Cho, K.S., S.Y. Hong, Y.S. Kwon, J.G. Woo, J.Y. Moon, S.Y. Ryn, and H. G. Park (2005). Selection of maintainer line in open-pollinated onion (*Allium cepa* L. cv. Manchuhwang) using SCAR marker linked to cytoplasmic male sterile factor . Korean J. Breed. 37 (3) .133-137.
- Falconer, D.S. (1981). Introduction to Quantitative Genetics. (2<sup>nd</sup> ed.). Longman, N.Y. 340 p.
- Gokce, A. F. and M.J. Havey (2002). Linkage equilibrium among tightly linked RFLPs and the *Ms* locus in open - pollinated onion populations J. Amer. Soc. Hort. Sci . 127 (6) : 944-946
- Havey, M.J. (1995) .Identification of cytoplasm using the polymerase chain reaction to aid in the extraction of maintainer lines from open-pollinated populations of onion. Theor. Appl. Genet. 90: 263-268.
- Sato, Y. (1998). PCR amplification of CMS-specific mitochondrial nucleotide sequences to identify cytoplasmic genotypes of onion ( *Allium cepa* L.) . Theor. Appl. Genet. 96: 367-370.
- Van der Meer, Q.P. and J.L van Bennekom (1971). Frequencies of genetical factors determining male sterility in onion ( *Allium cepa* L.) and their significance for the breeding of hybrids. Euphytica 20: 51-56 .
- Yaso, A.A.I. (2002). Studies on the improvement of onion traits. Ph.D. Thesis, Fac. Agric., Alex. Univ.

## تكرار عوامل العقم الذكري في صنف البصل بحيرى أحمر

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أجريت هذه الدراسة للتعرف على مدى تواجد عوامل العقم الذكري الوراثي السيتوبلازمي في صنف البصل بحيرى أحمر. استمرت الدراسة من عام ٢٠٠١ إلى ٢٠٠٥. حيث فحص ٥٢٤٠ نبات في موسم ٢٠٠٢/٢٠٠١ في محطة بحوث الجميزة حيث -بكثر الصنف بحيرى أحمر- وذلك لمعرفة عدد النباتات العقيمة الذكر الموجودة طبيعياً ، وقد تأكد من تلك العينة وجود ٨ نباتات عقيمة تركت للتلقيح المفتوح ، ثم زرعت البذور المتحصل عليها في حقل إكثار في محافظة الجيزة في موسم ٢٠٠٢/٢٠٠٣ ، بهدف إنتاج أبصال من نسل هذه النباتات . زرعت الأبصال المتحصل عليها - بدورها - في موسم ٢٠٠٣/٢٠٠٤ لإنتاج بذور منها. وفي أبريل ٢٠٠٤ فحصت أزهار هذه النباتات حيث تبين وجود ٣١ نبات عقيم و ٧١ نبات خصب في هذا النسل .

وعندما تكرر البحث عن العقم الذكري في عشيرة الصنف بحيرى أحمر في موسم ٢٠٠٢/٢٠٠٣ ، حيث فحص ٧٥٦٠ نبات ، تبين أن ٤٨ نباتاً منها كانت عقيمة الذكر طبيعياً ، وهي التي تركت للتلقيح الطبيعي في الحقل . زرعت البذور المتحصل عليها من هذه النباتات في حقل إكثار بمحافظة الجيزة في موسم ٢٠٠٣/٢٠٠٤ لإنتاج الأبصال ، وهي التي زرعت - بدورها - موسم ٢٠٠٤/٢٠٠٥ لإنتاج بذور من نسل النباتات العقيمة ، حيث تبين وجود ٤٢٦ نبات عقيم و ٥٥٣ نبات خصب . وبحساب تكرار عوامل العقم الذكري ، وجد أن متوسط تكرار النباتات العقيمة كان ٠,٠٠٣٤ ، بينما كان متوسط تكرار العامل  $ms$  ٠,٣٦٩٥ ، ومتوسط تكرار العامل  $MS$  ٠,٦٣٠٥ ، ومتوسط تكرار العامل  $S$  ٠,٠٢٢٢ ، ومتوسط تكرار العامل  $N$  ٠,٩٧٧٨ ، ومتوسط تكرار التركيب  $(B-Line) N ms ms$  ٠,١٣٧٤ .