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Effects of pollinator line characteristics on quantity and quality of monogerm hybrid seed production in sugar beet (*Beta vulgaris* L.)

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Abstract

A two-year experiment was carried out to study the effects of pollinator line characteristics on the quantity and quality of monogerm hybrid seed production in sugar beet (*Beta vulgaris* L.) and select proper pollinator for five promising sugar beet cytoplasmic male sterile lines (CMSs) during 2012-2013 growing seasons. In this study, four diploid pollinator lines were crossed by five CMSs of sugar beet. It was proved that the concurrence of flowering time between female and male parents and pollen and pollination characters is essential for sugar beet hybrid seed production. Pollinator lines SHR01-P.12 and F-8662 had the largest number of pollens. The duration of pollination for SHR01-P.12 and F-8662 was longer than other pollinator lines. Moreover, the most synchronizationof male and female recipient flowers was related to the pollen donors of SHR01-P.12 and F-8662 by the pollen receptors of 7112×SB36 and SB37×28874. Hybrids derived from crosses of CMS lines with pollinator lines SHR01-P.12 and F-8662 had significantly less empty seed percentage and the highest raw seed yield, saleable seed yield, standard seed percentage, while hybrid seeds derived from crosses of pollinator line S1-88239 by CMS lines had the lowest quantity and quality. Our results showed that unlike CMS lines 7112*436, the CMS lines 7112×SB36 and SB37×28874 419*SB36 and 261*231 produced the highest number of seeds with the highest quality.

Keywords: Paternal line; Pollen supply; Sugar beet; *Beta vulgaris*; Seed quality.

Introduction

Sugar beet (*Beta vulgaris* L.) is a biennial plant which builds a leaf rosette and a beet root in the first year. After a period of cold temperatures (vernalization), bolting starts in the second year and is expected to result in flowering and seed production (Marlander et al., 2011; Milford, 2006). Specific climatic conditions are are necessary for seed production. Favorable conditions are mainly found in Ardabil region in the north-west of Iran, as a focalarea of commercial sugar beet seed production. The tendency to use hybrid seeds (monogerm seeds) has been extensively grown in recent years and related cultivation area has been increased compared to polygerm seeds. Presently, 80% of total cultivated areas in Iran is cultivated by monogram seeds (Farzaneh, 2008).

High seed quality is an important component for realizing yield potential (Christiaan Biemond et al., 2012; Ghassemi-Golezani et al., 2008; Soltani et al., 2002) and the germination performance of seed is one of the critical factors in the production of the sugar beet root crop and a reliable supply of seed which germinates well is essential for successful sugar beet growing (Scott, 1970; Arnold et al., 1984; Sadeghian and Khodaii, 1998). When the monogerm varieties were included in the commercial production process, there was immediate concern because of low germination in many varieties. When precision planted, these varieties germinated poorly, causing irregular stands. Because when monogerm varieties with a 1:1 seed to fruit ratio were planted, poor germination was readily detected (Tekrony and Hardin, 1968). The quality of the seeds is determined to a large extent by the growing conditions during seed production, especially during flowering and maturation phases of the seeds (Kockelmann et al., 2010). Pollen dispersal within seed plots (Stewart and Campbell, 1952), Optimum flowering synchronization and maximum hybridization during flowering (Kockelmann et al., 2010; Kockelmann and Meyer, 2006), always are important in sugar beet seed production and affect the quantity and quality of sugar beet seed.

Studies of pollen dispersal of sugar beet initially were carried out in the middle of the twentieth century in order to maximize seed production (Darmency and Klein, 2009). When conditions are not optimal or pollen tube growth is disturbed, some flowers may remain unpollinated, seed development may be terminated and empty fruits are produced which have to be eliminated during subsequent processing of the seed lot (Scott, 1970; Alcaraz et al., 1998). Pollen is being recognized as an important research tissue in genetics, breeding, physiology and germplasm preservation (Smith and Moser, 1985; Willing et al., 1984). The number of pollen grains per anther is estimated at about 17000 grains (Marlander et al., 2011). This would coincide with 85000 grains per flower and, given 10000 flowers per bush, almost one billion per plant (OECD, 2001). Scott (1970) found favorable atmospheric conditions combined with peak pollen release times an hourly concentration of 50000 grains m^{-3} can account for long distance dispersal. Hecker (1988) had estimated that each plant, presumably field-grown monogerm diploid and tetraploid plants produced about 1.5×10^9 and 1.9×10^9 pollen grains per plant, respectively. When beet pollen stored in cold and dry condition can remain viable for 50 days, but does not survive wetting by dew (Treu and Emberlin, 2000). When conditions are not suitable or pollen tube growth is disturbed, some flowers may remain unpollinated, seed development may be terminated and empty fruits are produced which was being eliminated during processing of the seed lot (Scott, 1970; Alcaraz et al., 1998).

This study was aimed to study the effects of pollinator lines and concentration of pollen within monogerm sugar beet seed crops, on the quantity and quality of monogerm hybrid seed production in sugar beet also selection reasonable pollinator for five promising (advanced) sugar beet seed plants (CMS) lines.

Materials and Methods

The study was carried out in Agricultural Research Station of Ardabil, Iran (longitude of 38^o 15′ N., Latitude of 48^o 17′ E., Altitude of 1314 m a.s.l) during the growing season of 2012 and 2013. Ardabil region with a total precipitation of 250.12 mm and annual mean of minimum and maximum temperatures is 3.5 and 15.5 °C respectively and based on the climatic coefficient of Koppen identified the region as cold and semi-arid (Hemayati, 2009).

Five diploid cytoplasmic male sterile (CMS) lines (7112×SB36, SB37×28874, 7112×436, 419×SB36 and 261×231) of sugar beet were crossed with four diploid, pollinator lines (SHR.1-P12, F-8662, FC709-2/24 and S_1-88239) to evaluate effects of pollinator lines on quantity and quality of hybrid seed production. In this experiment, crosses between pollinator and CMS lines were carried under strict isolated situation in the field. In order to perform isolation, canvas tents with a height of 2.5 m were used as a barrier between isolates (Yosefabadi et al., 2008). Each plot included eight rows which two rows were considered for pollinators on either side and 6 internal rows were considered as CMSs.

To measure the percentage of flowering, all flowered plants of both pollen donor and recipient seed plants (the percentage of flowering) were recorded within each replicate (three replicates) in two-day intervals throughout the flowering period (between 20 June and 20 July in 2012 and 2013). The first bloom appearance in plants was considered as flowering stage. During the flowering period, the potential pollen dispersal and the concentration of pollen grains within plots for all pollen donor lines was recorded by microscope sticky slides (to create a sticky surface on the slides, glycerin was used) established in the height of one meter (because most flowering branches are located at 1 meter height) in 2012 and 2013 from 20 June to 20 July. Slides were located on anchors were positioned in each plot. In two-day intervals, the slides were picked up and washed using ethylic alcohol. Then 1 mL of extracted material was placed in a hemocytometer to count pollen number per cm^3 (Waller et al., 1998). When the flowers opened, staining of pollens was done using acid fushin and pollen diameter was measured with an internal microscope (×40).

This experiment was conducted using a three replicated split-plot experiment on the base of randomized complete block design. The main plots were four diploid pollinator lines and five diploid cytoplasmic male sterile were arranged in subplots. But to compare each pollinator line with other pollinator lines and determine pollen concentration a four replicated randomized block design was performed. To determine pollen concentration, eight microscope slides were used for each replication.

Sugar beet seeds were harvested in the second half of August 2012 and 2013. Seed lots from CMS lines were hand-harvested (in all the text, 'seed' term refers to the fruit as the sugar beet dispersal unit (Hermann et al., 2007)). The standard laboratory seed testing methods of the International Seed Testing Association (ISTA, 2005) were conducted to evaluate the germination percentage, empty seed percentage (empty fruits), different seed sizes distribution [i.e. standard seed percentage (with diameters of 3.5-4.5 mm Ø), oversized seeds (with diameters of >4.5 mm Ø), undersized seeds (with diameters of <3.5 mm Ø)] and percentage of monogerm seeds. For this, three samples were taken from each plot. Then from each sample, four samples were taken again. Then, data for hybrids were analyzed by a three replicated RCB design.

All statistical analyses and least significant differences (LSD) were performed by SAS software statistical program (SAS Institute, 2001) and all graphs were drawn by Microsoft Excel (2007). It should be noted that the data were transformed to $\sqrt{x+0.5}$ for the total of pollens caught during flowering, non-bolted plants and empty seed and male sterile plants in pollinator plants as needed. Data of ANOVA analysis presented based on transformed data, while mean comparisons results are based on back-transformed data.

Results

The temperature, relative humidity, precipitation and wind speed during the flowering period (between 20 June and 20 July) in 2012 and 2013 are shown in figure 1. The weather conditions during the flowering period differed in the 2 years. The average temperature during flowering period was 18.3 °C in 2012 and 17.5 °C in 2013. In 2012 the warmest temperatures occurred in 29 June and 10 July (about 20.6 °C). In 2013 the highest temperatures occurred in 1 July and 2 July with the values of 22.6 and 23.3 °C, respectively. Average relative humidity during the flowering period was approximately 76% and 63.7% in 2012 and 2013, respectively. In 2012, the minimum relative humidity occurred in the 25 and 28 June (65%). The relative humidity during the flowering season in 29-30 June and 9-10 July was lower in 2013 and it was approximately 47%. The total amount of precipitation during the flowering period in 2012 was 29.2 mm which happened in 29 June (2.8 mm), 30 June (2.9 mm), 2 July (0.1 mm), 3 July (0.6 mm), 4 July (0.2 mm), 16 July (2 mm), 20 July (7.1 mm) and 21 July (5.2 mm). The total amount of precipitation during the flowering period in 2013 was only 0.7 mm which occurred on 25 June. Average wind speed during the flowering period in 2012 and 2013 was 9.7 and 9.93 m s^{-1}, respectively.

According to the result of this two-year study, there were significant differences among four male pollinator lines with respect to total pollen collected during the flowering period, length of pollination period, non-bolted plants and without-pollen plants with white anthers, while there was no significant difference in respect to pollen diameter among the four pollinator lines. ANOVA results on pollinator lines characteristics are given in Table 1. The average of total pollens caught during flowering stage in the male pollinator lines varied from 167×10^5 (pollen m^{-3}) for S1-88239 to 503×10^5 (pollen m^{-3}) for F-8662 (Table 2). The mean of pollination period for SHR01-P.12, F-8662, FC709-2/24 and S1-88239 were 20, 21.5, 14 and 11.87 days, respectively (Table 2). The mean of pollen diameter ranged from 20.39 µm for SHR.1-P.12 to 22.58 µm for S1-88239 (Table 2). The average percentage of non-bolted plants among four male pollinator lines varied from 0.5 to 21.62 percent and the highest percentage of non-bolted plants was related to FC709-2/24. Male sterile plants were just seen in the pollinator line S1-88239 (16.5%). The total caught pollens during the flowering period and the percentage of male sterile plants were different in the two years. The value for total caught pollens during the flowering period was lower in 2013 than in 2012. Mean values for the male sterile plants in 2012 and 2013 were 5.50 and 6.87 percent, respectively (Table 2).

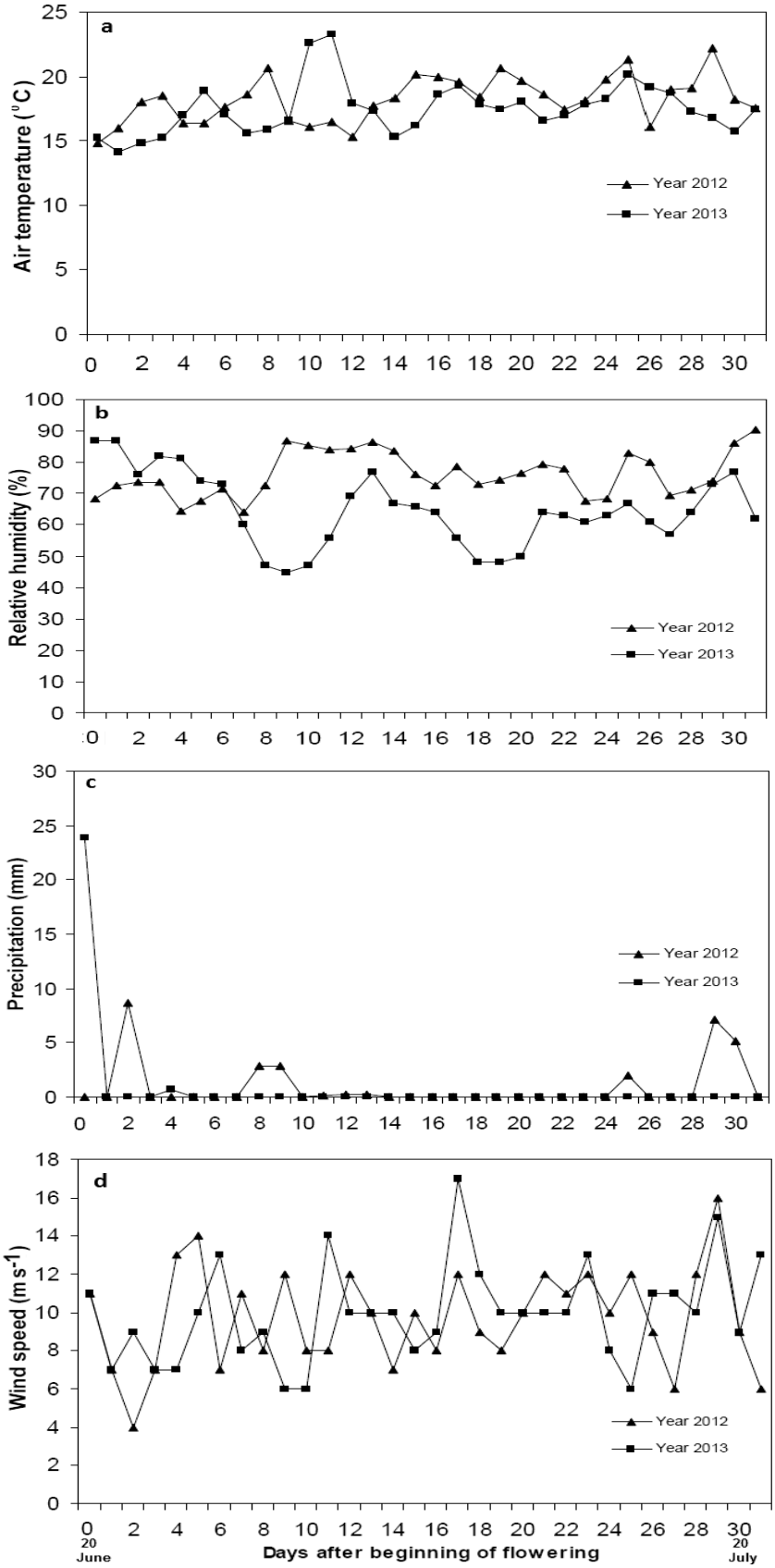

Figure 1. Relative humidity (a), air temperature (b), precipitation (c) and wind speed (d) during the flowering period (20 June to 20 July) over two growing seasons (2012 and 2013).

Table 1. Results of the analyses of variance (means of squares) for traits related to pollinator lines for the two years of experimentation.

Source of Variation	df	Total of pollen collected during flowering	Length of pollination period	Pollen diameter	Non-bolted plants	Male sterile plants in pollinator lines
Year (Y)	1	2103254.35*	0.058^{ns}	0.0098^{ns}	0.332^{ns}	0.398*
Replication (R)	6	1737036.70	0.602	7.6187	0.117	0.064
Male pollinator lines (F)	3	17105712.11**	3.200**	7.5708^{ns}	25.09**	22.66**
F ×Y	3	119241.70^{ns}	0.326^{ns}	0.6382^{ns}	0.153^{ns}	0.398*
Error (E)	18	430874.32	0.147	5.0747	0.259	0.060
CV(%)		12.09	9.44	10.37	25.03	16.32

**, * and ns show means differences are significant at 1 and 5% level of probability and are non-significant, respectively.

Table 2. The mean values of traits related to pollinator lines for the two years of experimentation.

Male pollinator lines	Total pollen collected during flowering (grain m^{-3})	Length of pollination period (day)	Pollen diameter (µm)	Non-bolted plants (%)	Male sterile plants in pollinator lines (%)
SHR01-P.12	39453125	20	20.39	0.75	0.0
F-8662	50390625	21.5	22.28	0.5	0.0
FC709-2/24	20234375	14	21.59	21.62	0.0
S1-88239	16796875	10.87	22.58	1.87	16.5
LSD%5	6680000	2.99	2.37	2.86	2.01
Year					
2012	34179688	16.00	21.70	5.50	3.25
2013	29257813	17.18	21.73	6.87	5.0
LSD%5	4720000	2.12	1.67	2.02	1.42

Pattern of pollen release during the flowering period for pollinator lines differed between years. In 2012, the first pollen release for F8662, SHR.1-P.12, FC709-2/24 and S1-88239 was approximately took place in June 21-22, June 23-24, June 25-26 and June 29-30, respectively (Figure 2). Related dates in 2013 were at June 21-22, June 23-24, June 25-26 and July 7-8, respectively. The maximum (peak value) of pollen releases for SHR.1-P.12 and FC709-2/24 approximately occurred in July 5-6, while it was as July 7-8 and July 11-12 for F8662 and S1-88239, respectively. In 2013, the maximum of pollen release for SHR.1-P.12, F8662 and FC709-2/24 occurred in July 6-7, but it happened in July 11-12 for S1-88239. Therefore, peak of pollen release for S1-88239 took place several days later than other lines. (Figure 2).

There was a substantial range in the timing and pattern of flowering among five CMS lines (seed parent) and four pollen donor lines (Figure 3). Pattern of flowering, for pollinator lines and CMS lines during the flowering period, was comparable between years. There was not any exact coincidence in flowering time between parents (Figure 3). But, anthesis concurrency between some pollinator lines with a number of CMS lines was significant. The most pronounced flowering synchrony was seen between the

pollen donor of F-8662 and pollen receptors of 7112×SB36 and, SB37×28874. Beginning of flowering time in SHR.1-P.12 occurred several days earlier than another pollen donor and CMS lines. Flowering time for FC709-2/24 and CMS lines 7112×SB36 and SB37×28874 was synchronized well, but reducing of maximum final percentage of bolted plants (21.62%) and the loss of some plants (14.5%) in FC709-2/24 (as a consequence of infection by downy mildew (*Peronospora farinosa* f. sp. *Betae*)) led to less synchrony in flowering time of FC709-2/24 and its related receptor in the late flowering stage. Due to late flowering in the S_1-88239 and early flowering in CMS lines, the pollinator of S_1-88239 had the the least concurrency in flowering time with CMS lines than the other male parents (Figure 3).

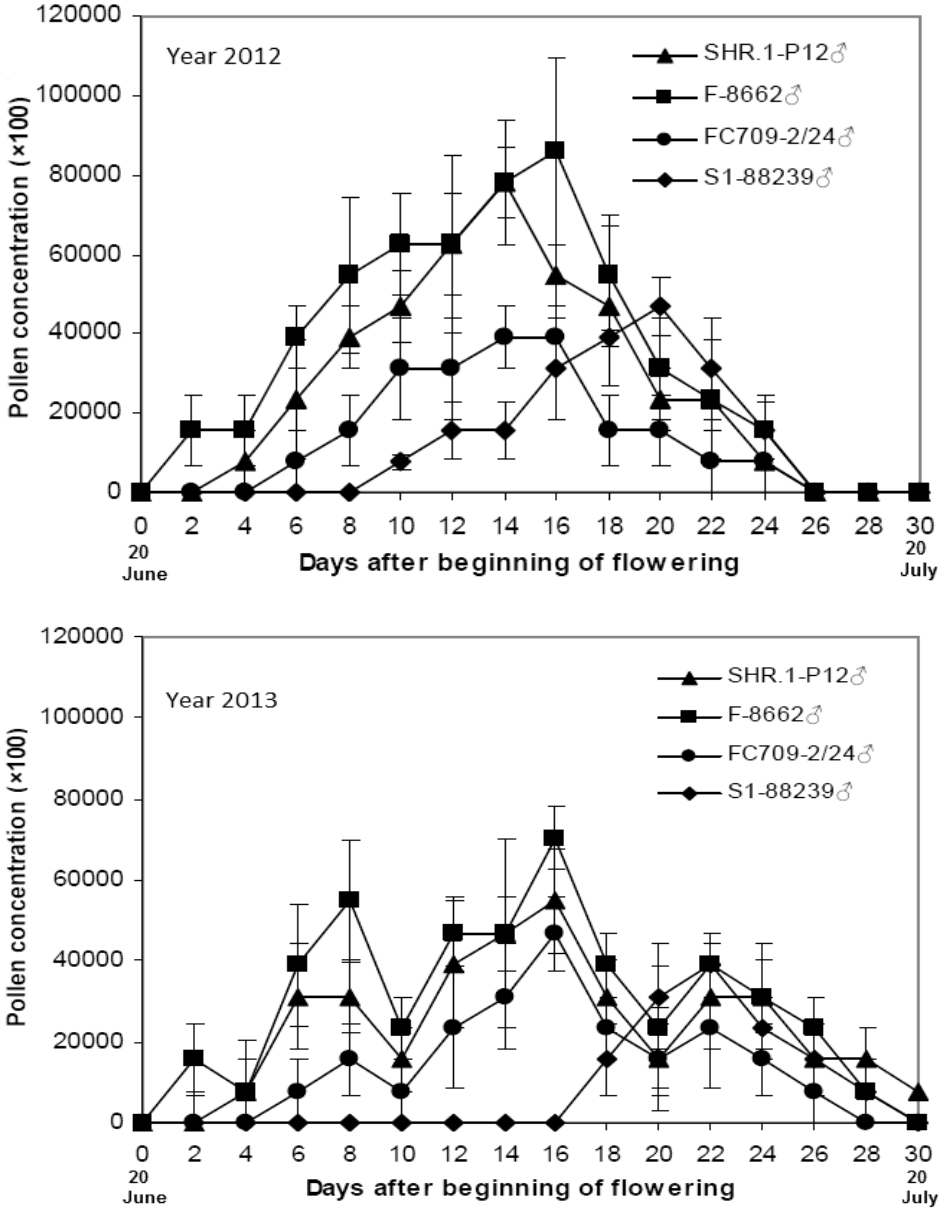

Figure 2. Averaged pollen concentration (pollens m^{-3} suspension) and pattern of pollen release during the flowering period between 20 June and 20 July for different pollinator lines (F8662, SHR01-P.12, SB19-S1-24 and S1-88239) over two growing seasons (2012 and 2013).

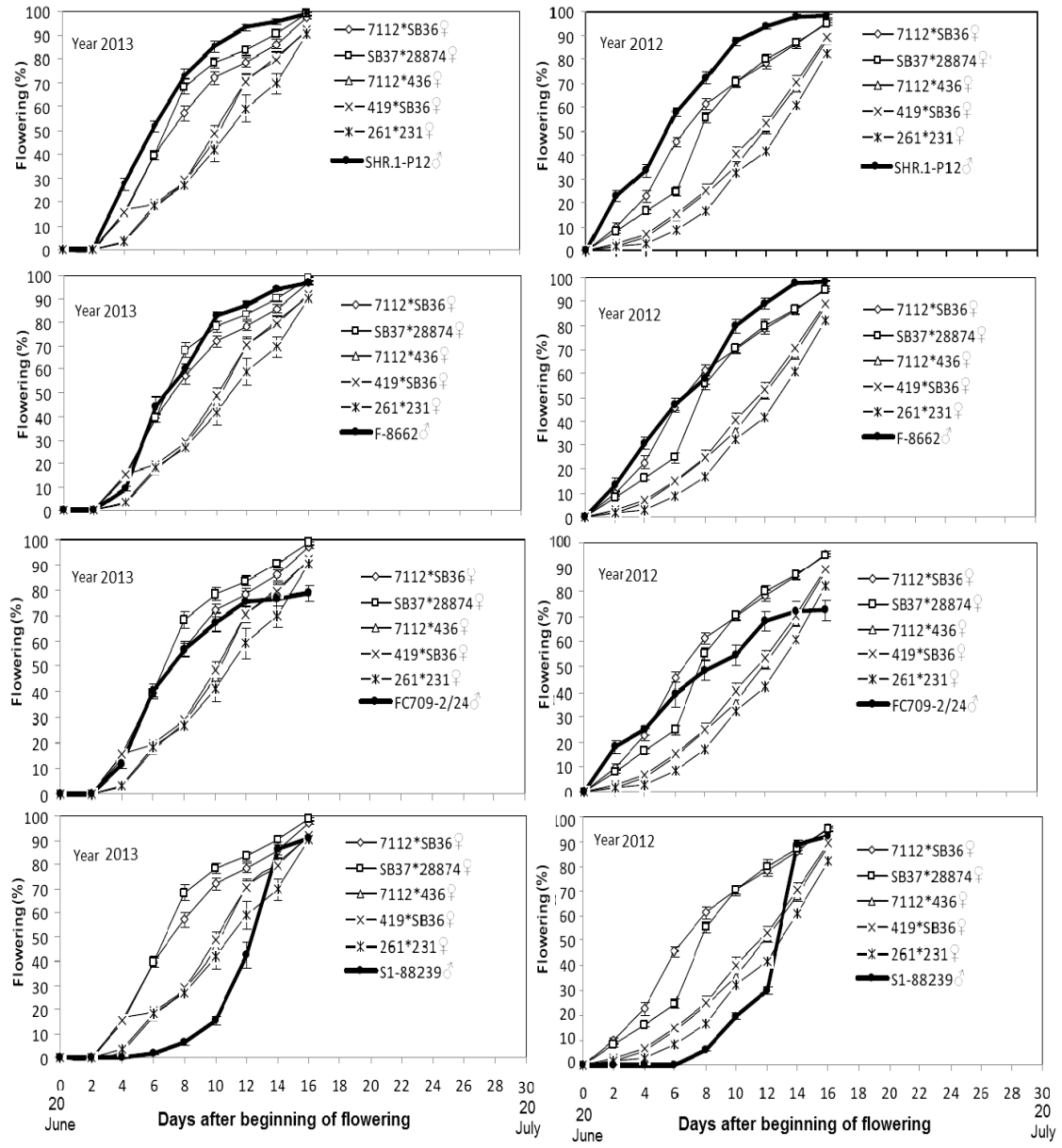

Figure 3. Flowering percentage for pollinator lines (SHR.1-P12 (A), F-8662 (B), FC709-2/24 (C) and S_1-88239 (D) with five CMS lines (seed parent) during flowering period over two growing seasons (2012 and 2013).

The results of combined analyses of variance indicated that male pollinator lines and CMS lines affected the raw seed yield, standard seed percentage, seed germination percentage and empty produced seeds (hybrid seeds) significantly. Year × pollinator line and year × CMS line interactions for raw seed yield and percentage of standard seed were significant. Also, CMS × pollinator line interaction was significant ($P<0.05$) for germination percentage of produced seeds (Table 3).

Table 3. Results of the analyses of variance (means of squares) for effects of pollinator lines and CMS lines on qualitative and quality traits of seed.

Source of Variation	df	Raw seed yield	Saleable seed	Germinated seed	Empty seed	seed sizes distribution		
						Ø <3.5 mm	Ø 3.5-4.5 mm	Ø >4.5 mm
Year (Y)	1	940578.13**	5301025.44**	593.07**	898.76**	142.12**	4542.51**	2.18^{ns}
Error	4	471145.09	168894.42	26.85	55.67	76.14	13.07	2.92
Pollinator lines(F)	3	914048.41**	749846.80**	97.53**	77.11**	220.90**	457.07**	7.20^{ns}
Y×F	3	335476.27**	40753.00^{ns}	0.76^{ns}	2.51^{ns}	3.17^{ns}	121.85**	19.93**
Error	12	16617.03	55504.62^{ns}	43.34	2.73	25.75	19.88	15.54
Cytoplasmic male sterile lines (CMS)	4	4168191.51**	990098.41**	127.70**	37.59**	620.39**	272.61**	214.89**
CMS×F	12	60454.12^{ns}	31133.257^{ns}	18.62*	1.82^{ns}	24.29^{ns}	12.03^{ns}	6.12^{ns}
CMS×Y	4	236239.28**	17461.59^{ns}	14.02^{ns}	3.26^{ns}	44.77*	67.14**	3.29^{ns}
CMS×F×Y	12	25189.72^{ns}	21465.45^{ns}	5^{ns}	3.42^{ns}	24.25^{ns}	13.2^{ns}	4.32^{ns}
Error	64	53736.05	46878.76	9.23	1.89	15.89	12	4.52
CV(%)		10.73	23.4	3.38	15.54	11.83	7.52	23.39

**, * and ns show means differences are significant at 1 and 5% levels and non-significant, respectively.

In both years (2012 and 2013), pollen donors SHR.1-P.12 and F-8662 performed better than the other lines regarding to raw seed yield and standard seed percentage. Although, in 2013 the difference between the pollinator lines with respect to these characteristics was not significant (Table 4). Mean comparison of the CMS lines revealed that raw seed yield in 2012 varied from 2600.96 (kg h^{-1}) for 7112×SB36 to 1815.70 (kg h^{-1}) for 261×231, while in 2013 varied from 2651.66 (kg h^{-1}) for 7112×SB36 to 1429.55 (kg h^{-1}) for 261×231. In both years, unlike the other three maternal parents, 7112×SB36 and SB37×28874 exhibited the maximum raw seed yield (Table 5). Mean comparison of CMS lines indicated that average standard seed percent in 2012 was higher than in 2013. In both years, the highest standard seed was obtained from the maternal parents 7112*SB36 and SB37×28874 (Table 5).

Table 4. Intraction of pollinator lines and year on the raw seed yield (kg h^{-1}), seed size with diameters of > 4.5 mm (%) and seed size with diameter of 3.5-4.5 mm (%).

Year	Pollinator Lines	Raw seed yield (kg h^{-1})	seed size with diameters of > 4.5 mm (%)	seed size with diameters of 3.5-4.5 mm (%)
2012	SHR01-P.12	2391.17^{a}	9.07^{b}	53.59^{a}
	F-8662	2540.74^{a}	11.08^{a}	54.35^{a}
	FC709-2/24	2147.86^{b}	8.26^{b}	50.22^{b}
	S1-88239	1914.67^{c}	8.50^{b}	50.59^{c}
2013	SHR01-P.12	2172.78^{a}	8.65^{a}	43.88^{a}
	F-8662	2091.78^{a}	8.57^{a}	45.36^{a}
	FC709-2/24	2058.46^{a}	9.42^{a}	37.55^{a}
	S1-88239	1963.15^{a}	9.17^{a}	32.72^{a}

Table 5. Intraction of CMS lines and year on the raw seed yield (kg h^{-1}), seed size with diameter of < 3.5 mm (%) and seed size with diameter of 3.5- 4.5 mm (%).

Year	Cytoplasmic male sterile lines (CMS)	Raw seed yield (kg h^{-1})	Seed size with diameter of 3.5-4.5 mm (%)	seed size with diameter of < 3.5 mm (%)
2012	7112×SB36	2600.96^{a}	54.80^{a}	28.88^{c}
	SB37×28874	2547.89^{a}	53.55^{a}	28.40^{c}
	7112×436	2077.81^{b}	52.94^{a}	29.82^{c}
	419×SB36	2200.67^{b}	50.15^{a}	35.94^{b}
	261×231	1815.70^{c}	49.47^{a}	39.89^{a}
2013	7112×SB36	2651.66^{a}	46.11^{a}	29.65^{c}
	SB37×28874	2525.26^{a}	43.67^{a}	29.07^{c}
	7112×436	1921.58^{b}	36.00^{b}	36.71^{b}
	419×SB36	1829.65^{b}	38.45^{b}	36.29^{b}
	261×231	1429.55^{c}	35.17^{b}	42.08^{a}

Mean comparison of the 20 hybrids derived from crosses of 5 CMS lines by 4 pollinator lines indicated that there were significant differences among hybrids with respect to raw seed yield, saleable seed yield, standard seed percentage and the empty seed percentage, as the hybrids derived from CMS lines in crossing with pollinator lines SHR01-P.12 and F-8662, had the highest raw seed yield, saleable seed yield and

standard seed percentage (Table 6). The average saleable seed yield of hybrids provided by pollinator lines SHR01-P.12 and F-8662, were 1049.7 and 1068.54 (kg h^{-1}), respectively, while related values for the percentage of germination for the same pollinator lines were as 90.6 and 91.54 percent, respectively. Results revealed that saleable seed yield and germination percentage in those hybrids produced by pollinator line S1-88239, were as 760.54 (kg h^{-1}) and 87.18 (%), respectively. Thus, hybrid seeds derived from pollinator lines SHR01-P.12 and F-8662 in crossing with CMS lines had the highest quantity and the best quality. Mean comparison of CMS lines indicated that CMS lines 7112*SB36 and 28874*SB37, unlike the other three CMS lines, had the most saleable seed yield and germination percentage. There were significant differences among both pollinator lines and CMS lines with respect to empty seed percentage (Table 3). For pollinator lines, the empty seed percentage varied from 7.1 to 10.6 percentage. The empty seed percentage was higher for those hybrid seeds produced by pollinator line S1-88239 in crossing with CMS lines, while lowest value belonged to pollinator line F-8662 (Table 6). Comparison of CMS lines indicated that the empty seed percentage varied from 7.44% for 7112*SB36 to 10.54% for 261*231. Thus, unlike the other three CMS lines, 7112*SB36 exhibited the minimum empty seed percentage (Table 6). Figure 4 shows the interaction of CMS lines by pollinator lines with respect to germination percentage. Also, the difference between CMS lines in crossing with pollinator lines SHR.1-P.12, F-8662 and FC709-2/24 was significant (Figure 4). The highest percentage of germination was related to the condition that maternal parents 7112*SB36 and SB37×28874 crossed by the pollinator lines SHR.1-P.12 and F-8662 or parents 7112*SB36 and 261×231 crossed by the pollinator line FC709-2/24.

The raw seed yield, saleable seed yield, standard seed percentage, empty seed percentage and undersized seed percentage of hybrid seeds differed between years (2012 and 2013) (Table 6). Mean comparison for the hybrid seeds indicated that average raw seed yield, saleable seed yield and standard seed percent was more in 2012 than 2013, While, undersized seed percentage and empty seed percentage in the 2013 was more than in 2012.

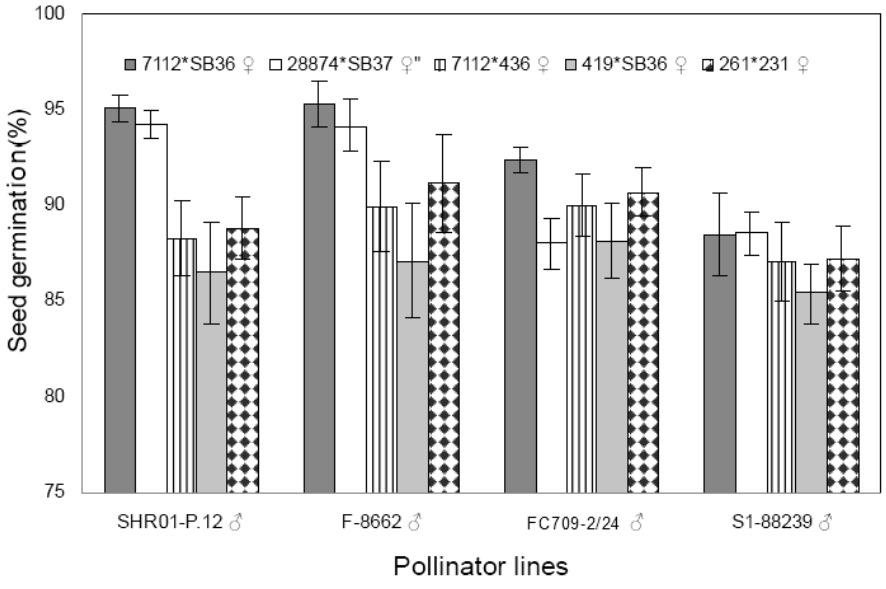

Figure 4. Interaction effect of pollinator lines and CMS lines on percentage of germination.

Table 6. The mean values of raw seed yield (kg h^{-1}), saleable seed yield (kg h^{-1}), empty seed (%), Oversized seed (%), standard seed (%) and undersized seed (%) for 20 hybrids derived from crosses of 5 CMS lines with 4 pollinator lines.

♂	♀	Raw seed yield (kg h^{-1})	Saleable seed yield (kg h^{-1})	Empty seed (%)	Oversized seed (%)	Standard seed (%)	Undersize seed (%)
SHR01-P.12	7112*SB36	2798.0	1325.1	6.17	10.26	53.38	28.89
	SB37*28874	2739.3	1314.2	6.50	12.04	51.66	28.32
	7112*436	2113.5	966.1	8.92	10.31	47.94	29.83
	419*SB36	2091.7	835. 6	7.86	7.37	45.60	37.45
	261*231	1667.3	807.4	10.23	4.35	45.11	37.79
F-8662	7112*SB36	2697.6	1346.8	6.13	11.31	55.10	24.85
	SB37*28874	2706.6	1167.3	6.07	15.20	54.00	23.27
	7112*436	2317.1	1060.6	8.09	9.25	46.64	32.42
	419*SB36	2049.4	966.8	7.55	8.26	47.54	33.82
	261*231	1810.7	801.2	7.71	5.12	45.99	38.59
FC709-2/24	7112*SB36	2620.6	1149.0	7.92	9.96	47.27	31.27
	SB37*28874	2500.0	877. 4	9.04	11.16	44.43	31.98
	7112*436	1842.5	758.9	10.03	10.17	44.39	33.25
	419*SB36	1970.8	743.0	10.11	8.87	42.39	36.24
	261*231	1582.0	553.8	11.51	4.07	40.94	41.60
S1-88239	7112*SB36	2389.1	971.6	9.53	10.29	46.08	32.05
	SB37*28874	2200.5	836.0	9.61	11.01	44.36	31.39
	7112*436	1725.7	696.2	10.49	9.77	38.91	37.58
	419*SB36	1948.7	761.1	10.69	9.17	41.68	36.94
	261*231	1430.6	537.8	12.72	3.96	37.24	45.97
LSD%5		251.6**	252.56**	1.63**	2.88**	4.18**	4.80**
Year							
2012		2248.61	1133.97	6.10	9.23	52.19	32.59
2013		2071.54	713.61	11.58	8.96	39.88	34.76
LSD%5		79.56**	79.86**	3.75**	0.91^{ns}	1.32**	1.52**

Discussion

In this study, we opted diploid pollinator lines, because the tetraploid plants produce less pollen than diploids and the pollen of tetraploid genotypes is less competitive than that of diplpid genotyps. This is because the pollen grains of tetraploid genotypes are larger and the pollen is hardly released from the anthers (Hecker, 1988, Scott and longden, 1970) and it may be easier to produce high quality triploid seed (Scott, 1968).

The results of the study revealed that the effect of both pollinator lines and CMS lines on the raw seed yield, saleable seed yield, standard seed percentage, germination seed percentage and empty seed percentage of produced seeds (hybrid seeds) was significant and there were significant differences among 20 hybrids derived from crosses of 5 CMS lines by 4 pollinator lines with respect to raw seed yield, saleable seed yield, standard seed percentage and empty seed percentage. Those hybrids derived from CMS lines in crossing with pollinator lines SHR01-P.12 and F-8662 had the lowest empty seed percentage and the highest raw seed yield, saleable seed yield and standard seed percentage, while hybrid seeds derived from pollinator line S1-88239 in crossing with CMS lines had the lowest quantity and quality (Table 6). Our results showed that the CMS lines 7112×SB36 and SB37×28874, unlike CMS lines 7112*436, 419*SB36 and 261*231, produced the seeds with the highest quantity and the best quality. It seems that in the case of sugar beet seed production, seed yield and quality not only depends on the genotype, but also depends on the condition of flowering synchronization with male parent and pollen dispersal within seed plants. So that the pollinator lines SHR01-P.12 and F-8662, had the highest pollen and also the mean of pollination period (compared with other pollinator lines) (Table 2). Moreever, the higher coincidence of male and female recipient flowers observed between the pollen donors of SHR01-P.12 and F-8662 with the pollen receptors of 7112×SB36 and SB37×28874 (Figure 3). Most likely, the high quality of the hybrid seeds derived from crosses of pollinator lines SHR01-P.12 and F-8662 with CMS lines (7112×SB36 and SB37×28874) is for the reasons mentioned above. The poor quality and quantity of harvested seeds in crossing with the pollinator line S1-88239 may be related to pollen and pollination characters and the coincidence in time of flowering between pollinator S1-88239 and seed parent lines. So that unlike other pollinator lines, the pollinator of S1-88239 had minimum released pollens, length of pollination period and maximum of white anthers. Also the pollinator of S1-88239 had the least synchronization at the time of flowering which resulted from its later flowering compared with CMS lines (Table 2 and Figure 3). Also, there was an optimal coincidence in flowering. Between pollinator line FC709-2/24 and 7112×SB36 and SB37×28874. Butlack of bolting and losses by downy mildew infection in FC709-2/24 (36%) led to poor concurrency in the later stages of flowering. Hybrid seed production requires close concurrency in flowering time between female and male parents or the exact synchronization between the receptive pistil on the female and pollen shed by male parents. There are many reports on the beneficial effects of matching of flowering between male sterile and pollinator plants for hybrid seed production (Kockelmann et al., 2010; Bannert and Stamp, 2007; Langhof et al., 2008). Flowering time in the pollinator of S1-88239 generally occurred later than female parents. Since in hybrid seed production, parental lines could be very different in their flowering behavior or since there is no synchronization between flowering time of female and male parents, the topping technique (cutting of primary shoots) ensures that as many flowers will pollinat as possible and any cross-pollination from the outside of

the crop is minimized (Kockelmann et al., 2010; Kockelmann and Meyer, 2006). In addition, the timing and intensity of topping are also key factors for an improved synchronization of flowering (Kockelmann et al., 2010). In sugar beet seed production, the empty seed rate could reach more than 20% (Kockelmann et al., 2010). One of the reasons could be deficient fertilization due to little dispersion of pollen in sugar beet seed plots and low pollen tube lengthening capacity, which is related to climatic factors. When weather conditions are not optimal or pollen tube growth is disturbed, seed development may be terminated and producing of empty fruits increases (Alcaraz et al., 1998). Therefore, all measures in seed production are directed to ensure a healthy and uniform development of male sterile seed plants and male pollinator plants and an optimal matching of flowering between male sterile and pollinator plants. This ensures a high level of fecundation, seed formation in seed plants, high seed yields and minimizes cross-pollination with other varieties or other *Beta* species and support optimal development of the seeds along with uniform maturation (Kockelmann et al., 2010).

Our results showed that year × pollinator lines and year × CMS lines interactions for raw seed yield and percentage of standard seed were significant. Under field conditions, the seed produced by plants in a given sugar beet variety may vary considerably. Relatively little is known about the causes of variations, but changes in specific environmental factors from year to year seem to be involved (Snyder and Hogaboam, 1963). Temperature and relative humidity appear to be responsible for a part of the variance.

Raw seed yield, saleable seed yield and percentage of standard seed in 2012 were significantly greater than 2013, but percentage of empty seeds in 2013 was more than 2012. The most suitable flowering conditions is at an average temperature of 15-20 °C with a maximum at 35 °C (Wood et al., 1982) and an average relative humidity of 75% (Scott, 1970). As Figure 1, in 2012, climatic limitations during the flowering period are not a concern for sugar beet seed production in this area. But, in 2013 conditions were not favorable for suitable flowering and pollination, because the relative humidity during the following season in June 29, 30 and July 9, 10, (which was within the peak of anthesis ranges), was less (approximately 46%). Also, average air temperature was higher. Therefore, in 2013 the climatic conditions in this area in the peak of anthesis was accompanied by much lower relative humidity and higher temperatures, which reduced the pollen concentration and it changed the patterns of pollen releasing during the flowering period which led to reduced seed yield and increased percentage of empty seeds. In this regard, it was reported that air humidity should not be too low during the flowering period (Marlander et al., 2011) and maximum pollens are released under the relative humidity of 60-70% (Wood et al., 1982) When relative humidity decreases to 42% (despite apparently ideal weather for further release), little pollen produces in the day (Scott, 1970). According to the German experts, the pollen ability of survival is limited to maximum 24 hours. This depends on the environmental conditions, especially moisture state (OECD, 2001). Therefore, air temperature and relative humidity are two important factors for sugar beet seed production so that affect sugar beet seed yield at all growth stages, particularly from pollination to seed ripening. In general, in addition to favorable climatic conditions needed during blooming and seed ripening, the optimal coincidence of flowering time between pollen parents and seed parents and pollen dispersal within seed plants always are important for sugar beet seed production because affect the quantity and quality of sugar beet seed.

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