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RESEARCH ARTICLE

Identification of Owen-Type Male Sterility Maintainers Carrying Resistance Against Rhizoctonia Crown and Root Rot (Rcrr) Disease in Sugar Beet Germplasm

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Abstract Propagation and maintaining cytoplasmic male-sterile (CMS) lines are prerequisite of hybrid production programs in sugar beet. The identification of Owen-type (O-type) source materials is important to maintain CMS plants that accelerate crosses between single plants. The objectives of the present study were to assess a base beet germplasm (SB19) to identify O-type plants for use in hybrid production programs and test for resistance against rhizoctonia crown and root rot (Rcrr) disease. A family was developed from each two identified candidate monogerm O-types. A number of 100 plants of each family were crossed with FC708 (male sterile) in insulated cages, and the progenies of hybrids were tested for the frequency of male sterility. Progenies of candidate O-types crossed with FC708 revealed various levels of male sterility. Type I and Type II male sterility had lowest frequency, while completely sterile type was the most frequent. The results demonstrated that 6 plants in each family were fully male sterile and their parallels S_1 were selected as O-type and male sterility maintainer. The mean for male sterility frequency was 10.5%. The mean disease index (DI) in the selected O-types was 2.22 that was lower than DI in SB19 as a resistant check. Mean comparison for Rcrr demonstrated that all O-types and SB19 plants were discriminated form Jolgeh as Rcrr-susceptible check. In conclusion, the identified O-types can be tested for combining ability for the development of Rcrr-resistant sugar beet hybrids with respect to root and sugar yield traits.

Keywords Owen-type · FC708 · Rhizoctonia · Root rot · Sugar beet

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Introduction

Contamination of sugar beet fields with rhizoctonia crown and root rot (Rcrr) disease leads to above 50% yield losses, affects sucrose content in roots and complicates sugar extraction during sugar processing (Büttner et al. 2004; Kiewnick et al. 2001; Strausbaugh et al. 2011). *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris*) that cause Rcrr is a soilborne basidiomycete and a pathogen for a wide range of crops and plant species (Harveson et al. 2009). Geographical distribution of Rcrr disease is extended to most beet-growing areas, i.e., China, Chile, Iran, European countries (Spain, Germany, Belgium, the Netherlands) and North America (Buhre et al. 2009; McGrath et al. 2015). One of the environment-friendly and

effective strategies to combat with Rcrr disease is to develop resistant hybrids. The practical importance of heterosis encourages breeders to consider utilization of hybrid vigor for the improvement of disease resistance and sugar traits in sugar beet (*Beta vulgaris* L.). Commercial sugar beets are three-way hybrids, and access male-sterile plants are prerequisite in hybrid production programs (Biancardi 2005). Male-sterile cytoplasm has no effect on Rcrr resistance, and triploid hybrids should be advantageous in breeding programs (Hecker and Ruppel 1976). Due to bearing of anthers and stigma in the same flower, emasculation is almost tedious and time-consuming and needs technical training in most crop plants (Duvick 1959). Cytoplasmic male sterility (CMS) enables breeders to produce a population with all individuals which are effectively emasculated.

Owen (1942, 1945) described CMS in sugar beet as resulted from the combined action of at least two nuclear restorer-of-fertility (*Rf*) genes, known as X and Z and sterilizing cytoplasm (S). The genes X and Z now are termed as *Rf* genes. In Owen theory, completely male-sterile plants have the genotype [S]xxzz, with the other genotype combinations ([S]XXZZ, [S]XXZz, [S]XXzz, [S]XxZZ, [S]XxZz, [S]Xxzz, [S]xxZZ and [S]xxZz) usually showing a varying degree of pollen fertility (Oldemeyer 1957; Bosemak 2006). Maintenance of CMS lines requires a genotype with recessive alleles for X and Z in nucleolus and fertile conferring alleles (N) in cytoplasm (Moritani et al. 2013). Such maintainer genotypes are rare with the frequency of less than 5% in sugar beet germplasm that constraints production of hybrid varieties in breeding programmes (Bosemak 2006; Moritani et al. 2013; Arakawa et al. 2019). Cross between maintainer and CMS genotypes and analysis of frequency of male sterility in F_1 generation is the only way for the development of CMS lines. This demonstrates development and identification of maintainers are of high priority in breeding hybrid varieties (Hagihara et al. 2005). Owen (1952) developed backcross population of US 35/2 that was segregated for 50% male sterility in sugar beet. In another study, progeny test for the cross between diploid and tetraploid plants was used to identify CMS ratio and assess the frequency of O-types in sugar beet (Koç 2005). Two sugar beet germplasms FC709-2 and FC727 have been released by the USDA in cooperation with the Beet Sugar Development Foundation, Denver, CO, but these were non-O-type (Panella 1999). Recently, DNA markers have been used to test linkage with *Rf* genes and to test whether selection of plants with recessive nuclear alleles (xxzz) is effective for identifying maintainer genotypes in beet germplasms (Moritani et al. 2013; Arakawa et al. 2019).

The objectives of the present study were to identify male sterility maintainer genotypes (O-type) in a sugar beet germplasm and assess resistance to Rcrr disease in candidate O-types. The identified O-types could be involved in hybrid production programs in sugar beet.

Materials and Methods

Plant Materials

SB19 which was a diploid multigerm germplasm with resistance against Rcrr was used as a source of O-type. The scheme associated with the identification of O-type genotype in the base population and crosses between CMS and O-type candidates is presented in Fig. 1. Two monogerm genotypes were selected from the SB19 germplasm. To confirm whether the selected monogerms were O-type, single plants of each were test crossed with FC708 as an international CMS line (Hecker and Ruppel 1981). The experiment was conducted at the Hamedan Agricultural and Natural Resources Research and Education Center, AREEO, Hamedan, Iran. Seeds of two test cross families and FC708 line were sown in the field for steckling development. Each experimental plot was consisted of three 40 m rows with 50 cm row spacing. During winter, developed seedlings were vernalized in the field.

Development of S_1 and F_1 Families

After winter vernalization, 100 single plants of each test cross families and FC708 were sown with 40 cm between plant spacing under cage (Fig. 1). Bolted plants were examined for monogermity. To produce self-pollinated (S_1) and hybrid (F_1) plants, each of the monogerm maintainer candidates and FC708 single plants were covered with fabric cages prior to flowering (Table 1). In each cage, maintainer candidates were checked for fertility. Male fertility was characterized via two ways: first by assessment for the presence or absence of pollen, its color (white to yellow), fullness and dehiscence, and second, the estimation of pollen viability through a staining procedure that discriminated viable pollen grain from non-viable (Alexander 1969). Three buds per plant were sampled just before anthesis. Two stamens were selected from each bud and squashed together into a drop of Alexander's stain. When pollen grains were present, a minimum of 300 grains per sample were scored for viability (Touzet et al. 2004).

Male fertility was classified into four distinct types: fully fertile, semi-fertile (Type I), semi-fertile (Type II) and completely sterile as described by Arakawa et al. (2019). Each plant was controlled for male sterility under insulated cages throughout flowering season. Male fertility indexing was evaluated as described above, and plants were scored more than four times on different days as follows: a score

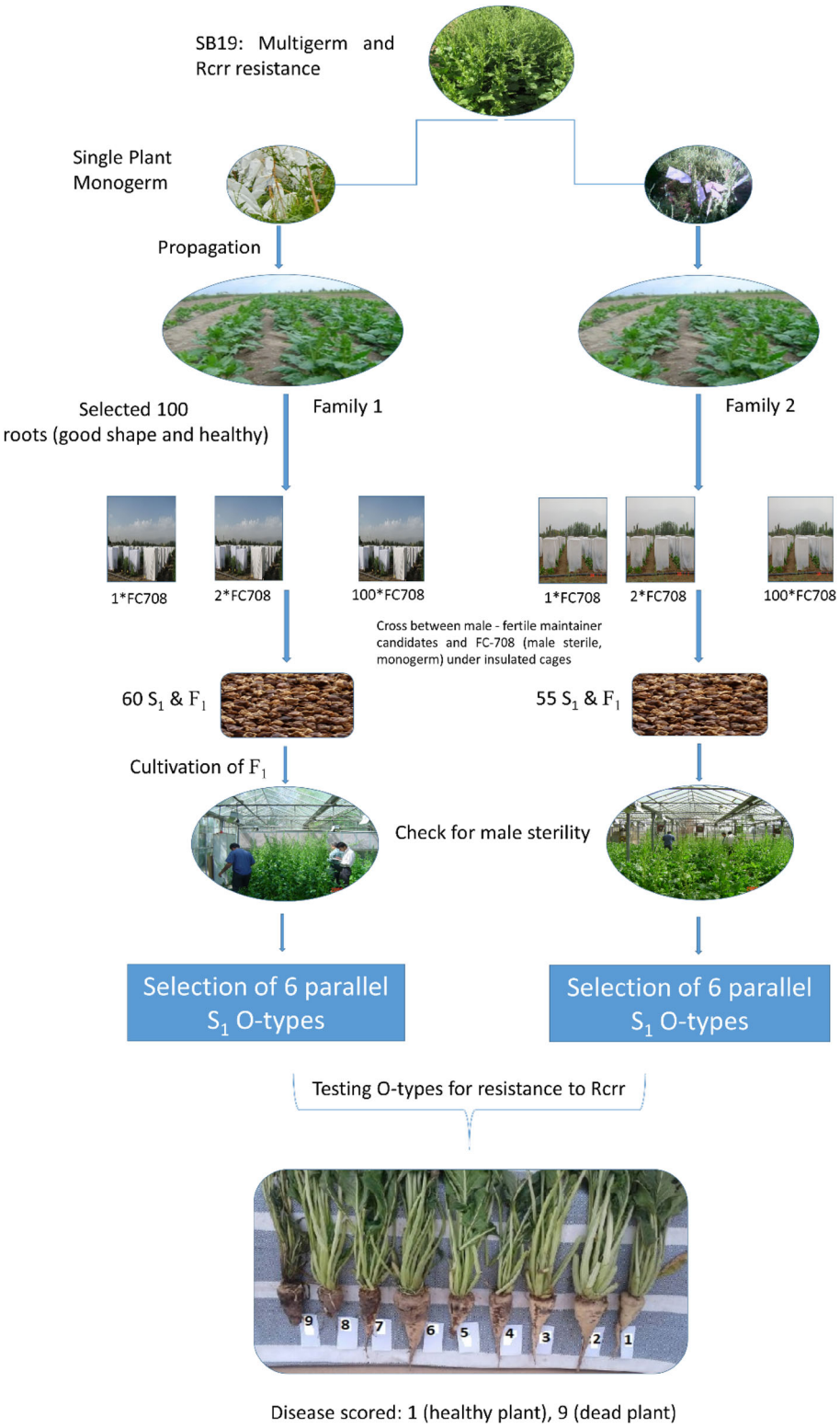

Fig. 1 Scheme for the identification of Owen type (O-type) in the base population (SB19) and cross between sugar beet genotypes under insulated cages. *Rcrr* rhizoctonia crown root rot

of 3 for fully fertile, 2 for Type I semi-fertile, 1 for Type II semi-fertile and 0 for completely sterile, respectively. The average of the scores was the plant's fertility index (Arakawa et al. 2019).

Test for Male Sterility in Hybrids (F_1)

To test whether the selected candidate genotypes were indeed effective for identifying O-types, 115 hybrids (F_1)

with relatively high number of seeds were sown in the field. A number of 100 single plants from each F_1 were produced and the seedlings were winter-vernalized. In the May, bolted plants were examined for monogermity and male sterility. F_1 plants with 100% male sterility were identified, and their S_1 counterparts were selected as O-type.

Infestation of O-types with *R. solani*

Rhizoctonia inocula were prepared from a highly aggressive isolate of *R. solani* (R-9; AG-2-2). The isolate was provided by the SBSI (www.sbsi.ir), Iran. Colonized inoculum was developed on corn grains. Inoculum of the fungus was propagated on corn grains for 3 weeks in 25 °C. Seven weeks after sowing the seeds of the maintainers (S_1), plants were inoculated through adding 6 infested corn grains to the soil surrounding crown area following the instructions described by Windels et al. (1995). In the first week after inoculation, plants were watered every day. Afterward, plants were watered every 7 days until the appearance of the Rcrr disease symptoms. After 6 weeks, rating for Rcrr symptoms was performed on the roots of each individual plant. For Rcrr phenotyping, the commercial hybrids Jolgeh and SB19 were used as susceptible and resistant checks, respectively (Hassani 2018). Rating was performed following a nine-class disease scale (IfZ) defined by Büttner et al. (2004). Accordingly, roots were lifted, gently washed and scored on a scale of 1–9, with 1 for no rot (healthy) and 9 for dead plant. Disease index (DI) for each S_1 was calculated using the below equation (Büttner et al. 2004):

$$\mathrm{DI} = \frac{\sum (\text{Scale} \times \text{number of root})}{\text{Total number of roots}}.$$

Results

Segregation for Male Sterility of F_1 Hybrids

The results of evaluation for male sterility demonstrated that of the 115 F_1 plants, 12 were identified as O-type in the two families tested (Tables 1, 2). All plants in SBH-1-007, SBH-1-032, SBH-1-042, SBH-1-053, SBH-1-070 and SBH-1-076 hybrids in family 1 and SBH-2-002, SBH-2-008, SBH-2-010, SBH-2-012, SBH-2-014 and SBH-2-015 in family 2 were completely pollen sterile (Table 2). For progenies of the remainder hybrids, above 82% male sterility was identified in the two families. The frequency of male sterility maintainers in two families were 10 and 11%. The proportion of male-sterile type white was higher than yellow and semi-sterile types.

Resistance to Rcrr in O-types

The means for Rcrr disease index tested in the check varieties and O-types demonstrated that Jolgeh with the score of 7.95 was sensitive against Rcrr and SB19 with 2.88 was resistant (Table 3). The range for disease index in O-types varied between 1.66 and 2.73 demonstrating high resistance against Rcrr was obtained in male-sterile maintainers. Of 34 individual plants tested in the O-type O-2-010, 21 showed rating 1 for disease index demonstrating resistance against Rcrr. No plant with score above 4 was identified in the O-type O-2-010.

Discussion

Identification of CMS maintainer lines is of high importance in hybrid production systems in open pollinated plants. Assess variation in plasma genes almost involves backcrossing homozygous lines to male-sterile plants suspected of having various plasma genes and analyzes progenies (Oldemeyer 1957). In the present study, a Rcrr-resistant sugar beet germplasm was used as a source for production of O-type lines. The frequency of maintainers is low in sugar beet germplasm demonstrating the need to use appropriate source germplasm to identify and develop O-type lines (Bosemak 2006). SB19 as a multigerm open pollinated germplasm was used to identify monogerm maintainers. The expected frequency of O-type monogerms was relatively low in the SB19 germplasm. In a study, 600 plants from 6 families were crossed with CMS testers and six fertile plants yielding 100% male-sterile offspring were identified as O-type (Erdal 2001). Presently, the results of our study revealed the development of two families with O-type frequency of 10.5% which was in agreement with the frequencies identified in other studies (Koç 2005;

Table 1 Selected monogerm families, sample size and the frequency of the identified maintainers (O-type) in sugar beet

Family	Sample size	Caged plant	Maintainer	Maintainer plant (%)
FAM1	100	60	6	10
FAM2	100	55	6	11
Total	200	115	12	Mean = 10.5

Table 2 Genotypic variations in male sterility in the F_1 progenies and the status of S_1 plants

Family code (FAM)	Hybrid (F_1) code	Male sterility in F_1 (%)				Parallel S_1	Maintainer/non-maintainer
		MS	SMS Type I	SMS Type II	*F*		
FAM1[a]	SBH-1-004	86.7	7.5	5.8	–	S_1-1-004	Retest S_1
	SBH-1-005	93.3	–	6.7	–	S_1-1-005	Retest S_1
	SBH-1-007	100	–	–	–	S_1-1-007	Maintainer
	SBH-1-017	82.53	2.5	14.17	0.8	S_1-1-017	Rejected
	SBH-1-031	95.83	–	4.17	–	S_1-1-031	Retest S_1
	SBH-1-032	100	–	–	–	S_1-1-032	Maintainer
	SBH-1-040	99.2	0.8	–	–	S_1-1-040	Retest S_1
	SBH-1-042	100	–	–	–	S_1-1-042	Maintainer
	SBH-1-053	100	–	–	–	S_1-1-053	Maintainer
	SBH-1-070	100	–	–	–	S_1-1-070	Maintainer
	SBH-1-074	94	–	1	5	S_1-1-074	Rejected
	SBH-1-076	100	–	–	–	S_1-1-076	Maintainer
	SBH-1-100	97.53	0.8	1.67	–	S_1-1-100	Retest S_1
FAM2[a]	SBH-2-001	97.52	–	0.8	1.67	S_1-2-001	Rejected
	SBH-2-002	100	–	–	–	S_1-2-002	Maintainer
	SBH-2-003	96.66	1.67	1.67	–	S_1-2-003	Retest S_1
	SBH-2-008	100	–	–	–	S_1-2-008	Maintainer
	SBH-2-010	100	–	–	–	S_1-2-010	Maintainer
	SBH-2-012	100	–	–	–	S_1-2-012	Maintainer
	SBH-2-014	100	–	–	–	S_1-2-014	Maintainer
	SBH-2-015	100	–	–	–	S_1-2-015	Maintainer
	SBH-2-017	92.7	2	2.3	3	S_1-2-017	Rejected

[a]47 and 46 F_1 plants with 100% male fertility were discarded in FAM1 and FAM2, respectively. SBH-1 and SBH-2 codes stand for sugar beet hybrids of families 1 and 2, respectively. *MS* male sterile, *SMS I* semi-male sterile (Type I), *SMS II* semi-male sterile (Type II), *F* fertile

Moritani et al. 2013; Arakawa et al. 2019). Several fully male-sterile hybrids were observed in the progenies of the cross between FC708 and candidate O-types demonstrating the reliability of these O-types selected from the SB19 germplasm. The number of progenies needed to identify at least two O-types accounts a binomial dispersion (Koç 2005). The sample size for progeny tests used in our study was quit large (100 progenies) demonstrating the reliability of the O-type ratio identified in both families. Plant with 100% male sterility is expected to have S cytoplasm provided their nuclear genes are homozygous recessive (Sxxzz). The semi-sterile plants (Type I) identified in the present study might have $S_{X\text{-}zz}$ or S_{xxZ} genotypes (Koç 2005; Moritani et al. 2013). The results demonstrated that several progenies were identified as semi-sterile (Type II). In Type II male-sterile plants, both nuclear alleles are dominant (XxZz). The proportion of various male sterility types differed demonstrating sugar beet restore fertility (*Rf*) genes has a series of multiple alleles with variable abilities to restore fertility and are reflective of the complexity of *Rf* evolution (Arakawa et al. 2019).

Rcrr is problematic in sugar beet-growing regions worldwide, and germplasm enhancement efforts over the past four decades have resulted in USDA–ARS germplasm releases with improved resistance (Panella 1999; Vagher et al. 2014). One of the most effective strategies to reduce adverse effects of Rcrr as an aggressive pathogen in sugar beet fields is to subject to consistent germplasm screening, to identify resistance sources and transfer of resistance genes through hybridization programs to elite germplasms. Breeding for resistance involved the selection of individual plants and the evaluation of their progenies under relatively severe *Rhizoctonia* conditions (Gaskill 1968). In the present study, FC708 was used as a male sterility conferring line in progeny test crosses. FC708 has been resulted from two cycles of mass selection for Rcrr in the segregating generations and shows high resistance against Rcrr (Hecker and Ruppel 1981). The results showed that hybrids with completely sterile progenies belonged to O-types with lower DI for Rcrr. Such O-types were more resistant against Rcrr compared with the SB19 as a check demonstrating most of the CMS lines harbored resistance genes for Rcrr. Identification of O-types with lower DI for Rcrr

Table 3 Score for disease index (DI) in the selected S_1 maintainers (O-type) of sugar beet

Maintainer	Number of plant	DI[a]									DI
		1	2	3	4	5	6	7	8	9	
O-1-007	10	–	3	7	–	–	–	–	–	–	2.7
O-1-032	24	9	2	7	5	–	–	1	–	–	2.54
O-1-042	20	8	6	3	1	2	–	–	–	–	2.15
O-1-053	25	11	7	1	5	–	1	–	–	–	2.16
O-1-070	30	12	10	5	3	–	–	–	–	–	1.97
O-1-076	29	15	3	3	1	–	3	2	–	1	2.62
O-2-002	3	2	–	1	–	–	–	–	–	–	1.66
O-2-008	26	5	4	10	7	–	–	–	–	–	2.73
O-2-010	34	21	5	8	–	–	–	–	–	–	1.62
O-2-012	23	10	1	6	2	1	–	–	–	–	2.23
O-2-014	13	6	1	4	1	–	–	–	–	–	2
O-2-015	21	8	4	3	5	–	–	–	–	–	2.25
											Mean DI = 2.22
Susceptible check (Jolgeh)											7.95
Resistant check (SB19)											2.88
											LSD (5%) for DI means = 1.72

[a]1 denotes healthy and 9 dead plants, *LSD* least significant differences, O-1 and O-2 codes stand for maintainer in families 1 and 2, respectively

demonstrated their merits for further use in breeding for Rcrr resistance in sugar beet hybrids.

Author Contributions MH conducted the research and experimental works and wrote the initial draft of the manuscript; BH completed the initial draft of the manuscript, supervised and edited the final draft of the manuscript; SBM, DFT and PS edited the final draft of the manuscript

Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

Human and Animal Rights The research does not include human participants or animals.

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