Identification of Resistant Pollinator Lines to Soil-Borne *Rhizoctonia solani* J.G. Kühn, 1858 in Sugar Beet Using SIIG Technique

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Summary

Introducing resistant hybrids as a low-cost and environment-friendly strategy is an effective approach to disease management. The first step in developing resistant hybrids is to identify pollinator lines that are resistant as the paternal parent. The study aimed to diagnose *Rhizoctonia* rot disease resistance in 47 lines derived from five different populations of pollinators. The lines were evaluated for their resistance to *Rhizoctonia* under mini-plot conditions. *Rhizoctonia solani* AG-2-2 isolate was used to artificially infect the roots. The number of plants and roots were counted, and disease and harvest indexes were calculated to assess the resistance rate using the selection index of ideal genotype (SIIG) method. To compare with the SIIG output, biplot and cluster analysis statistical techniques were utilized to validate the results. Population P.107 and P.121 illustrated desirable potential resistance to *Rhizoctonia* across various pollinator populations. Based on SIIG criterion, the pollinator lines No.19 (S1-980022), No.3 (S1-980004), No.1 (S1-980002), No.20 (S1-980025) and No.25 (S1-980032) were identified as the most resistant lines, which was in accordance with the findings from cluster and biplot analysis. In essence, these pollinator lines were introduced as resistant paternal parents to provide resistant hybrids for future breeding programs.

Key words

artificial inoculation, hybrid, paternal parent, resistance, rot

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Introduction

In Iran, sugar production is primarily linked to the cultivation of sugar beet (*Beta vulgaris* L.), which accounts for around 60% of the country's total sugar production. The annual average of harvested area of sugar beet in Iran is about 120,000 ha which produces nearly 800,000 tons of sugar (Ministry of Agriculture-Jahade, 2022). Pathogens can disturb sugar beet cultivation, resulting in a loss of both quantity and quality of the yield. *Rhizoctonia solani* J.G. Kühn, 1858 is a soil-borne plant pathogen that leads to crown and root rot on sugar beet (Bartholomaus et al., 2017). The majority of sugar beet farms in Iran suffer from *R. solani* rot and it is the crucial cultivation challenge that growers face in some regions (Ebrahimi kolaei et al., 2019). The disease symptoms include brown to black lesions that merge and envelop large areas of the root surface. Abrupt drooping of the leaves, complete rotting of the root and damping-off thoroughly could occur under great severity of the disease (Strausbaugh et al., 2011; Harveson et al., 2009). The yield losses vary extremely from farm to farm and sometimes result in significant and considerable crop failure (Strausbaugh, 2016). Agronomic practices such as crop rotation, fungicide utilization and using resistant cultivars are the main disease control strategies (Liu et al., 2019). Development of varieties resistant to diseases is one of the main approaches to disease management due to low cost and being environment-friendly method (Peressotti et al., 2010). Initiation of breeding programs for developing resistant varieties to *R. solani* in sugar beet was begun in the late 1960s (Gaskill, 1968; Hecker and Ruppel, 1977) and two multigerm pollinator lines including FC709-2 and FC727 were identified and introduced as the sources of resistance to *R. solani* (Panella, 1999). Developing resistant cultivars heavily depends on identifying of resistant pollinator lines as paternal parent of sugar beet hybrids (Vahedi et al., 2016). Success in the screening of sugar beet genotypes for resistance to disease needs to evaluate them under infection conditions. Plant disease severity fluctuates annually due to varying crop management practices and climate conditions (Chiu et al., 2022). Hence evaluating lines under field conditions to assign disease ratings is extremely difficult by reason of the non-uniform and unpredictable environments that lead to patchy patterns of the disease (Bhuiyan et al., 2023). Therefore, the utilization of artificial inoculation can supply more uniformity in the evaluation procedure and allow for making accurate decisions on the elimination or selection of lines from the breeding programs (Shekhar and Kumar, 2012). Mahmoudi et al. (2003) report that artificial inoculation under mini-plot conditions can effectively assist in the evaluation of the resistance to *Rhizoctonia* rot in sugar beet genotypes. In mini-plot conditions, impregnated cereal grains (such as barley, corn and sorghum) with fungal inocula are applied to inoculate sugar beet roots, and disease severity is recorded on the basis of a rating scale (Mahmoudi et al., 2003). On the other hand, using powerful techniques is an absolutely essential part for the selection of resistant lines. The Selection Index of Ideal Genotype (SIIG) is a helpful method that considers multiple traits to identify the best lines (Zali et al., 2015). The study aimed to identify resistant pollinator lines to *Rhizoctonia* rot in sugar beet using the SIIG technique under artificially inoculated mini-plot conditions.

Materials and Methods

Preparation of Pollinator Lines

Pollinator lines were provided from various genetic sources by the Sugar Beet Seed Institute (SBSI). 48 pollinator lines were gained from five different populations of sugar beet, including 36 full-sib families (self-pollinated, S1 lines) derived from four populations (P.107, P.165, P.121 and P.201), and 12 half-sib families (HSF) derived from P.724 population (table 1). Seeds from each population were planted in the field in mid-August 2018 to develop sugar beet stecklings. To avoid the risk of freezing during winter, stecklinges were covered with wheat straw 100 days after planting. After undergoing vernalization in winter, the roots were sorted based on their size and health. The selected roots were then cut lengthwise into two parts. Two parts of each root were planted side by side, 40 cm apart in March 2019. The planting line spacing was set at 2 meters between roots. In order to acquire S1 lines, the plants were enclosed by fabric cages before they entered the flowering stage. The cages serve the purpose of preventing the spread of pollen, which results in complete isolation of the plants placed under them. A drip system was used to irrigate plants under the cage. Seeds were harvested in end-July 2019 under each cage and considered as S1 pollinator lines. To gain HSF lines, winter-vernalized stecklings of the P. 724 population were sown in four 50 cm spaced rows. The distance between stecklings was 20 cm. Using tarpaulin fabrics around bolted plants allowed them to pollinate together under isolated conditions and harvested seeds of them were regarded as HSF pollinator lines. All agronomic operations to produce HSF lines were the same as S1 lines production.

Mini-Plot Experiment

Mini-plot site is located at the Hamedan Agricultural Research Centre, Hamedan, Iran. Most basic studies about plant diseases especially *Rhizoctonia* root and crown rot are carried out on this site and it plays an important role in selecting resistant lines to diseases. The site includes mini-plots of concrete that are 2 m long, 1 m wide and 2 m deep to prevent the spread of disease among the plots. There is 50 cm distance between the plots. In late April 2020, two rows 2 meters long were used to sow 48 pollinator lines and three check treatments in each mini-plot. Experimental design was based on a randomized complete block with three replications. Therefore, 77 mini-plots were occupied to perform the experiment. The required fertilizer was calculated and applied in accordance with the soil test. Weed control was exclusively used by manual weeding. No herbicides or fungicides were used in the mini-plots. Thinning crowded plants was done after the proper establishment of seedlings as there were about 20 plants in each planting line. During the growth season, the plants were irrigated using a drip system and did not experience any drought stress.

Inoculation of Roots with *Rhizoctonia*

To inoculate sugar beet roots to *Rhizoctonia*, a highly aggressive isolate (AG-2-2) was used as *Rhizoctonia* inocula (Windels and Brantner, 2011). Sterilized corn grains were inoculated with pure culture of *R. solani* AG-2-2 isolate. Impregnated corn grains were directly used to infect roots when plants were 60 days old and roots had grown enough to be infected (Mahmoudi et al., 2003). Plant number (PN) in all rows was counted before inoculating.

Table 1. The treatment number and origin of pollinator lines derived from five various sugar beet populations

Pollinator population with code 107		Pollinator population with code 165		Pollinator population with code 121		Pollinator population with code 201		Pollinator population with code 724	
P. 107		**P. 165**		**P.121**		**P. 201**		**P. 724**	
No	Origin	No	Origin	No	Origin	No	Origin	No	Origin
1	S1-980002	11	S1-980013	19	S1-980022	26	S1-980033	37	HSF-980001
2	S1-980003	12	S1-980014	20	S1-980025	27	S1-980034	38	HSF-980002
3	S1-980004	13	S1-980016	21	S1-980027	28	S1-980039	39	HSF-980003
4	S1-980005	14	S1-980017	22	S1-980028	29	S1-980040	40	HSF-980004
5	S1-980006	15	S1-980018	23	S1-980030	30	S1-980042	41	HSF-980005
6	S1-980007	16	S1-980019	24	S1-980031	31	S1-980044	42	HSF-980006
7	S1-980008	17	S1-980020	25	S1-980032	32	S1-980045	43	HSF-980007
8	S1-980009	18	S1-980021			33	S1-980046	44	HSF-980008
9	S1-980010					34	S1-980047	45	HSF-980009
10	S1-980011					35	S1-980049	46	HSF-980010
						36	S1-980054	47	HSF-980011
								48	HSF-980012

Note: Check treatments: No.49: Check1 (FC-709; resistant), No.50: Check2 (Novodoro, resistant), No.51: Check3 (Mass-191, susceptible)

The *R. solani*-infested corn grains should be placed nearby crown area of roots according to the process explained by Windels et al. (1995). For this purpose, the soil around the crowns was put aside and made a 5 cm depth furrow. Five infected corn grains were placed 1 cm away from the crowns in the furrow and then covered with the soil. To ensure adequate spreading of the inoculum, the plants were irrigated daily for seven days after inoculation to maintain moisture. Thenceforward, plants were adequately irrigated until the onset of the disease symptoms. Disease rating of individual pollinator lines was conducted one month after inoculation by evaluating all roots of the rows using a 1-9 rating scale, as described by Buttner et al. (2004). Therefore, all roots of each line were entirely pulled out of the soil, and the disease score was recorded on the basis of the following scale after washing and counting the roots. The score of 1 indicates that there is no infection in the plant, while a score of 9 indicates that the plant has died due to the infection (Fig. 1).

After scoring, disease index (DI) and harvest index (HI_3) were computed by following formulas (Buttner et al., 2004).

$$DI = \frac{\sum_{i=1}^{9}(S_i \times n_i)}{N} \qquad HI_3 = \frac{n_{1,3}}{N} \times 100$$

where S_i and n_i are disease score and the number of roots with that score (i = 1, …, 9), N represents the total number of roots and $n_{1,3}$ means the number of roots with scores 1, 2 and 3. In Buttner et al. (2004), plants with scores up to 3 were considered resistant.

Figure 1. The various disease severities in sugar beet roots and their score based on 1-9 rating scale (Buttner et al., 2004)

Therefore, $n_{1,3}$ reflects this resistance. To evaluate the severity of infection in pollinator lines, it was necessary to include standard checks for resistance and susceptibility in all measurements. Therefore, two resistant checks, namely FC-709 and Novodoro cultivar, and a susceptible check called Mass-191 were considered as control treatments (Panella, 1999; Hamze et al., 2022). Plants with a disease score less than 3 are usually resistant. However, under severe conditions, even resistant cultivars may lose their resistance. As part of our experiment, we assessed the intensity of infection in mini-plot conditions. To do this, we used the disease score of resistant controls. The scores obtained were 3.21 and 3.57, indicating a high level of disease severity. This is a commonly used criterion to evaluate the intensity of infection in such conditions. Therefore, to improve the accuracy of our evaluation, we calibrated the threshold score for resistance based on the special conditions of our experiment. Assuming that scores less than 4 indicate

resistant plants, we used an experiment-specific formula modified from the standard formula, as HI_4 as described below:

$$HI_4 = \frac{n_{1,4}}{N} \times 100$$

$n_{1,4}$ implies the number of roots with a score between 1 and 4. The study separately analyzed and reported both standard HI (HI_3) and experiment-specific HI (HI_4). Diagnosis and identification of the cause of disease were conducted through morphological traits to ensure that *Rhizoctonia* was the cause of the rot. Accordingly, some infected roots were randomly sampled and the cause of the disease was identified under the microscope in the laboratory.

Applying Selection Index of Ideal Genotype (SIIG) to Identify Resistant Lines

SIIG technique was used to select resistant S1 pollinator lines in the study. SIIG method was recently developed by Zali et al. (2015) and defined as:

$$SIIG = \frac{d_i^-}{d_i^+ + d_i^-}$$

where d_i^+ is the Euclidean distance of each line from the ideal line and d_i^- is the Euclidean distance from the non-ideal line obtained by:

$$d_i^+ = \sqrt{\sum_{j=1}^{m}(r_{ij} - r_j^+)^2} \quad i = 1, \dots, n \qquad d_i^- = \sqrt{\sum_{j=1}^{m}(r_{ij} - r_j^-)^2} \quad i = 1, \dots, n$$

r_{ij} represents normalized data of i trait (i = PN, Root Number (RN), DI and HI_4) and j line (j = 1, … , 50), which can be obtained as follows. r_j^+ is the maximum value and r_j^- is the minimum value of each trait among lines.

$$r_{ij} = \frac{x_{ij}}{\sqrt{\sum_{i=1}^{n} x_{ij}^2}} \qquad i = 1, \dots, n; \quad j = 1, \dots, m$$

where, x_{ij} implies the actual value of trait 'i' and line 'j'. Referring to SIIG score, the ideal line is equal to 1. This line has the shortest distance from the positive ideal traits and certainly the longest distance from the negative ideal traits. Finally, the lines with SIIG scores close to 1 were considered as the resistant lines.

Biplot and Cluster Analysis

Biplot and cluster analysis were utilized for validating SIIG results. In this context, resistant lines selected using the SIIG method should be matched with biplot and cluster findings. Therefore, a biplot was used as a simple descriptive graphic tool to display situation of each line in response to DI and HI_4 variables. Other variables such as PN and RN were not considered in the biplot analysis due to the limitation of the method. In addition, cluster analysis was applied for processing data, and pollinator lines were separated into groups on the basis of their similarity in all traits including PN, RN, DI and HI. It means that each group's lines had similar trait values and were close together. The clustering procedure and computing of similarity were grounded on the Euclidean distance. The main outcome of cluster analysis was a dendrogram, which was used to visualize how clusters were developed and illustrated the lines belonging to each group.

Statistical Analysis

Data were analyzed using two-way analysis of variance (ANOVA) and the difference of means among pollinator lines was separated by least significant difference method (LSD) at the significance level of $P \leq 0.05$. In addition, an orthogonal comparison was accomplished to determine the difference among five pollinator populations. SAS version 9.4 was applied to data analysis and Minitab 16 software was used to draw diagrams of biplot and cluster analysis.

Results

Infected Roots Characteristics (PN, RN, DI and HI)

The laboratory evaluation indicated that *Rhizoctonia* was the primary cause of root rot, while other potential causes such as fusarium, pythium and phytophthora were negligible. *Rhizoctonia* was approximately affecting all plants so rot symptoms obviously appeared in the roots, especially in susceptible plants. For the precise rating, plants that had not grown enough and showed no disease symptoms in their roots were not included in the disease rating process. The ANOVA output revealed statistically significant differences between means for all measured traits ($0.01 \leq P$ - value ≤ 0.05 for PN and RN; P - value ≤ 0.01 for DI, HI_3 and HI_4), indicating desirable genetic diversity among treatments in terms of resistance to *Rhizoctonia*. The average PN value for all lines was 16.34, indicating suitable plant establishment. The number of evaluated roots for each line, on average, was 15.72 which was eligible for the assessment. The highest and lowest root numbers were related to line No.19 (S1-980022) and No.41 (HSF-980005) by 21 and 8, respectively (table 2). Because line No.33 (S1-980046) did not have enough root numbers to evaluate (5 roots), it was excluded from treatment.

DI values for resistant checks were 3.21 and 3.57 for FC-709 and Novodoro, respectively and it was gained as 8.11 in susceptible check (table 2). The lowest DI (=2) among pollinator lines was found in line No.3 (S1-980004) and recognized as the most resistant line in the context of DI. No.30 (S1-980042) and No.37 (HSF-980001) lines demonstrated the greatest values of DI with 8.66 and 8.50, respectively, which had a higher DI than susceptible check. About 33% of all pollinator lines (16 lines) showed higher DI than the resistant check (table 2). As mentioned above, concerning the DI values of resistant checks (DI < 4), which indicate a high level of disease intensity, we set the threshold for identifying resistant plants as DI < 4 in our study. Accordingly, 24 pollinator lines (50% of all lines) were found to be resistant based on DI.

Data revealed that line No.3 (S1-980004) had the maximum value of HI_3 by 92.50% among others. Lines No.19 (S1-980022), No.10 (S1-980011), No.1 (S1-980002) and No.17 (S1-980020) were in the next ranks with 90.8%, 90.6%, 88.6% and 88.5% HI_3, respectively (table 2). For resistant checks, HI_3 values were obtained at 62.50% and 41.27% for FC-709 and Novodoro, respectively. Upon comparing the HI_3 gained through resistant checks, it was found that roughly one-third (compared to FC-709) to half (compared to Novodoro) of the pollinator lines exhibited more HI_3 than the checks. The HI_3 of susceptible check showed a rate of 2.63%, while most pollinator lines had higher HI_3 compared to the susceptible check, except for No.30, No.35 and No.37 which had an average of zero (table 2).

Table 2. Plant Number (PN), Root Number (RN), Disease Index (DI) and Harvest Index (HI) of pollinator lines in sugar beet

HI_4*	HI_3*	DI	RN	PN	Origin	No	HI_4*	HI_3*	DI	RN	PN	Origin	No
13.2	10.5	6.45	15.5	16.0	S1-980033	26	91.8	88.6	2.24	20.5	20.5	S1-980002	1
3.60	3.60	7.67	13.0	13.5	S1-980034	27	86.1	65.9	3.22	14.5	15.5	S1-980003	2
5.00	5.00	7.24	12.0	13.0	S1-980039	28	97.5	92.5	2.00	20.0	20.0	S1-980004	3
20.3	14.1	5.74	16.5	17.0	S1-980040	29	89.3	75.0	2.83	17.5	17.5	S1-980005	4
0.00	0.00	8.66	11.0	13.0	S1-980042	30	90.0	65.5	2.74	18.0	19.0	S1-980006	5
15.4	3.10	5.93	16.5	16.5	S1-980044	31	71.9	23.0	4.05	17.5	17.5	S1-980007	6
13.3	8.30	6.21	11.0	11.5	S1-980045	32	67.8	51.6	3.61	18.5	19.0	S1-980008	7
40.0	17.5	5.58	20.0	20.0	S1-980047	34	83.2	71.9	2.67	18.5	19.0	S1-980009	8
8.60	0.00	7.48	12.0	13.0	S1-980049	35	78.9	66.2	3.16	19.5	20.5	S1-980010	9
23.2	13.6	5.27	10.5	12.5	S1-980054	36	94.4	90.6	2.30	15.5	16.6	S1-980011	10
3.80	0.00	8.50	16.5	17.5	HSF-980001	37	53.5	34.5	4.40	17.5	18.5	S1-980013	11
10.9	3.40	6.59	13.0	13.5	HSF-980002	38	68.2	39.1	3.90	17.0	18.0	S1-980014	12
69.1	47.3	3.50	15.5	16.0	HSF-980003	39	83.8	74.2	2.77	15.5	15.5	S1-980016	13
52.9	38.2	4.03	17.0	17.0	HSF-980004	40	44.4	19.0	5.13	15.5	16.0	S1-980017	14
31.7	13.3	5.58	8.00	9.00	HSF-980005	41	91.7	50.8	3.23	16.0	17.0	S1-980018	15
49.2	34.2	4.46	13.5	15.5	HSF-980006	42	39.0	13.2	5.74	15.5	16.0	S1-980019	16
42.1	23.7	5.09	19.0	19.5	HSF-980007	43	97.2	88.5	2.69	17.0	18.0	S1-980020	17
32.1	21.0	5.06	14.0	14.5	HSF-980008	44	90.6	45.1	3.43	15.0	15.5	S1-980021	18
36.1	20.6	4.94	19.0	19.0	HSF-980009	45	95.7	90.8	2.17	21.0	21.5	S1-980022	19
55.0	35.0	4.00	13.5	14.5	HSF-980010	46	97.5	84.7	2.63	19.5	19.5	S1-980025	20
59.1	39.0	4.02	18.5	19.0	HSF-980011	47	84.4	81.3	2.59	15.0	16.0	S1-980027	21
48.4	22.9	4.36	17.5	18.0	HSF-980012	48	80.1	73.9	2.79	13.5	14.0	S1-980028	22
75.0	62.5	3.21	11.0	11.5	Check1 (FC709)	49	86.2	56.2	3.20	14.5	16.0	S1-980030	23
73.0	41.3	3.57	15.0	15.5	Check2 (Novodoro)	50	91.7	70.8	2.80	12.0	12.0	S1-980031	24
5.30	2.60	8.11	13.0	13.5	Check3 (Mass-191)	51	92.5	76.2	2.50	19.5	19.5	S1-980032	25
44.5	44.3	2.24	8.54	7.85	LSD 1%		44.5	44.3	2.24	8.54	7.85	LSD 1%	
33.4	33.2	1.68	6.40	5.89	LSD 5%		33.4	33.2	1.68	6.40	5.89	LSD 5%	

Note: Check treatments: No.49: Check1 (FC-709; resistant), No.50: Check2 (Novodoro, resistant), No.51: Check3 (Mass-191, susceptible).

* HI_3 reflexes to standard formula of harvest index ($n_{1,3}$ / N), with N representing total number of roots and $n_{1,3}$ meaning number of roots with score 1,2 and 3. HI_4 reflexes to experiment-specific formula of harvest index ($n_{1,4}$ / N), which n1,4 meaning number of roots with score from 1 to 4.

In agreement with HI_3, two pollinator lines comprised No.3 (S1-980004) and No.20 (S1-980025) and acquired the greatest value among other lines (table 2). The lines list that achieved a higher rate in reference to HI_4 was relatively similar to the case of HI_3; thereby No.17 (S1-980020), No.19 (S1-980022) and No.10 (S1-980011) lines were arranged in the high grade of HI_4. The three pollinator lines with the lowest HI_4 values were No.30, No.37, and No.28, all of which had lower HI_4 values than the susceptible check. The HI_4 value for susceptible check was 5.27%. As observed, when considering the harvest index criterion by 4 score, lines with high HI were the same for both standard and experiment-specific conditions, with only the value of HI differing. Therefore, we relied mainly on HI_4 to avoid losing some lines that were highly resistant but might appear susceptible under HI_3 setting due to higher disease severity in the experiment.

Cluster Analysis

47 pollinator lines along with 3 checks were grouped respecting 95% similarity in PN, RN, DI and HI_4 traits. Consequently, all lines were placed in three clusters. The first cluster was formed by 23 pollinator lines and also two resistant checks that were considered as resistant cluster. The most studied lines were classified in this group so that the cluster comprised half of the lines (Fig. 2). Lines in the first group provided less DI (with an average of 2.95) and also greater HI_4 (averaged value of 85.10%) than the two others. About one-fourth of lines, 13 lines, were categorized in the second cluster and most of them were commonly referred to the P.724 population. The average value of DI and HI_4 for the second cluster was 4F.74 and 47.17%, respectively, with higher DI and lower HI_4 compared to the mean of groups. Finally, the remaining lines consisted of 11 pollinator lines and the susceptible check created the third cluster and defined it as susceptible cluster. All pollinator lines of the P.201 population, except No.34 line (S1-980047), were placed in the third group, thus it can be concluded that P.201 population had no potential for resistance to *Rhizoctonia* rot disease by use of cluster analysis technique. The mean for DI in third cluster (6.99) was less and for HI_4 (10.22%) was more comparable to two other clusters.

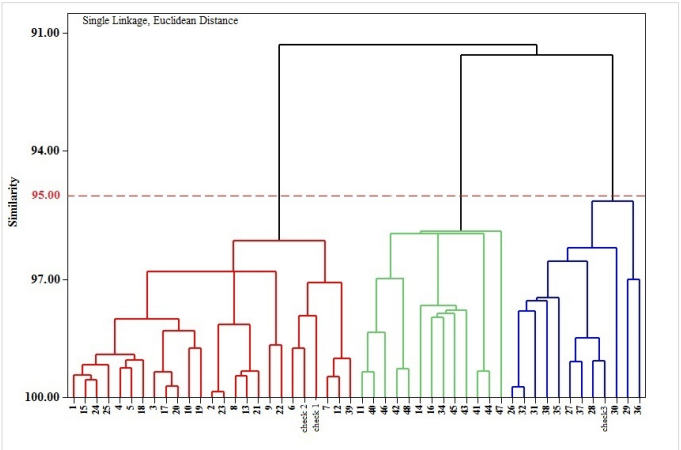

Figure 2. The various disease severities in sugar beet roots and their score based on 1-9 rating scale (Buttner et al., 2004)

Note: Each selected cluster is shown in a different color: red (Cluster 1), green (Cluster 2) and blue (Cluster 3)

Biplot Analysis

Fig. 3 presents the position of each line in reaction DI and HI_4 variables via biplot analysis results. As experiment conditions, DI = 4 and HI_4 = 50% were considered as criteria for classifying the biplot into four distinct zones (Fig. 3). Lines with more DI and HI_4 values than 4 and 50%, respectively, were located in zone A, which was restricted to five pollinator lines: No.11 (S1-980013), No.40 (HSF-980004), No.46 (HSF-980010), No.47 (HSF-980011) and No.6 (S1-980007). In zone B, with established lines with DI > 4 and HI_4 < 50%, there were 20 pollinator lines and susceptible check. This zone was defined as susceptible to disease. No lines were found in zone C, which is characterized by DI < 4 and HI_4 < 50%. It implied that there was an opposite relationship between DI and HI_4. In other words, when a line had a low DI, potentially it would be high HI_4 and vice versa. Biplot analysis also demonstrated that the most studied lines (22 pollinators) as well as two resistant checks were allocated to zone D as described by DI < 4 and HI_4 > 50%. This area could be considered a resistant zone to *Rhizoctonia* disease. Ten pollinator lines, namely No.3 (S1-980004), No.19 (S1-980022), No.10 (S1-980011), No.1 (S1-980002), No.20 (S1-980025), No.17 (S1-980020), No.25 (S1-980032), No.24 (S1-980031), No.4 (S1-980005) and No.5 (S1-980006), were identified as resistant lines based on their minimum DI and maximum HI_4 values. These lines were marked by a red circle in the biplot diagram, which was generated as a result of biplot analysis. It is necessary to remark that the ten above-mentioned lines were categorized in the first group as resistant cluster. This denoted that the results of cluster analysis were closely matched with biplot observations.

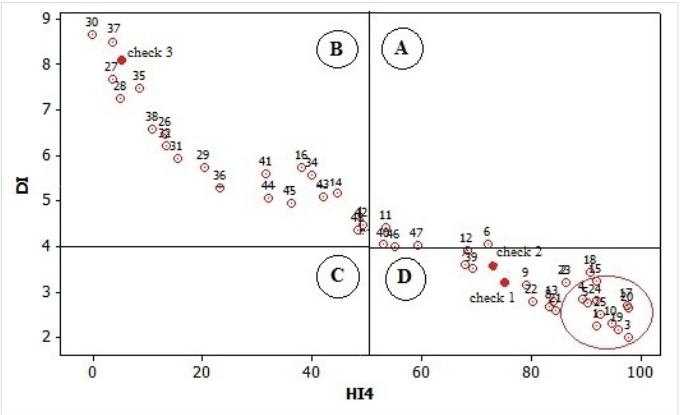

Figure 3. Classifying of studied lines depending upon DI and HI_4 variables using biplot analysis

Note: DI = 4 and HI_4 = 50% were regarded as criteria for separating and classifying of lines into four zones included A (DI > 4 and HI > 50%); B (DI > 4 and HI < 5 0%); C (DI < 4 and HI < 50%) and D (DI < 4 and HI > 50%). Red circle represents selected resistant lines using biplot analysis

SIIG Method

The SIIG technique results were detailed in table 3. The data revealed that the No.19 (S1-980022), No.3 (S1-980004) and No.1 (S1-980002) lines held the minimal deviation from the positive ideal factor with d^+ values of 0.006, 0.016 and 0.017 respectively. On the other hand, these lines had the maximal deviation from the negative ideal factor with d^- values of 0.324, 0.323 and 0.313 respectively.

Table 3. Distance of pollinator lines from ideal (d^+) and non-ideal line (d^-) and the ranking of lines referring to Selection Index of Ideal Genotype (SIIG) scores

SIIG	d^-	d^+	No	Origin	rank	SIIG	d^-	d^+	No	Origin	rank
0.629	0.232	0.137	49	Check1(FC709)	26	0.981	0.324	0.006	19	S1-980022	1
0.623	0.208	0.126	40	HSF-980004	27	0.954	0.323	0.016	3	S1-980004	2
0.623	0.208	0.126	11	S1-980013	28	0.948	0.313	0.017	1	S1-980002	3
0.599	0.201	0.135	48	HSF-980012	29	0.915	0.309	0.029	20	S1-980025	4
0.575	0.194	0.143	46	HSF-980010	30	0.915	0.304	0.028	25	S1-980032	5
0.557	0.193	0.153	43	HSF-980007	31	0.869	0.290	0.044	5	S1-980006	6
0.541	0.180	0.153	42	HSF-980006	32	0.855	0.283	0.048	8	S1-980009	7
0.537	0.187	0.162	45	HSF-980009	33	0.854	0.297	0.051	17	S1-980020	8
0.535	0.190	0.165	34	S1-980047	34	0.837	0.283	0.055	4	S1-980005	9
0.508	0.168	0.163	14	S1-980017	35	0.833	0.276	0.055	9	S1-980010	10
0.449	0.149	0.183	16	S1-980019	36	0.817	0.294	0.066	10	S1-980011	11
0.436	0.146	0.189	44	HSF-980008	37	0.796	0.275	0.070	15	S1-980018	12
0.403	0.141	0.209	29	S1-980040	38	0.776	0.271	0.078	21	S1-980027	13
0.374	0.132	0.221	31	S1-980044	39	0.770	0.267	0.080	13	S1-980016	14
0.347	0.119	0.224	36	S1-980054	40	0.754	0.264	0.086	18	S1-980021	15
0.327	0.115	0.236	26	S1-980033	41	0.753	0.261	0.086	23	S1-980030	16
0.326	0.115	0.237	41	HSF-980005	42	0.746	0.260	0.089	2	S1-980003	17
0.269	0.105	0.286	37	HSF-980001	43	0.740	0.245	0.086	7	S1-980008	18
0.259	0.088	0.252	38	HSF-980002	44	0.714	0.236	0.095	6	S1-980007	19
0.252	0.086	0.253	32	S1-980045	45	0.714	0.255	0.102	22	S1-980028	20
0.192	0.066	0.276	28	S1-980039	46	0.706	0.233	0.097	12	S1-980014	21
0.190	0.066	0.282	27	S1-980034	47	0.697	0.268	0.117	24	S1-980031	22
0.187	0.063	0.275	35	S1-980049	48	0.696	0.234	0.102	50	Check2 (Novodoro)	23
0.177	0.062	0.288	51	Check3 (Mass-191)	49	0.696	0.233	0.102	39	HSF-980003	24
0.122	0.043	0.312	30	S1-980042	50	0.679	0.227	0.107	47	HSF-980011	25

Note: Check treatments: No.49: Check1 (FC-709; resistant), No.50: Check2 (Novodoro, resistant), No.51: Check3 (Mass-191, susceptible).

From that followed that these pollinator lines provided the greatest value of SIIG index (0.981, 0.954 and 0.948, respectively) and were determined as the most resistant pollinator lines in comparison with others. No.20, No.25, No.5, No.8, No.17 and No.4 were in the next ranks of resistance.

As observed, the mentioned lines were placed in the resistant cluster according to cluster analysis and also established into resistant zone (zone D) as a result of biplot analysis. Accordingly, it can be concluded that the findings of biplot and cluster methods confirm the results of SIIG. Novodoro and FC-706 as resistant checks were in the 23th and 26th rank by SIIG = 0.696 and 0.629, respectively, which means 22 pollinator lines had the more SIIG rate than Novodoro resistant check.

On the other hand, 23 pollinator lines recorded a lower SIIG value than FC-706 check (table 3). Across 47 pollinator lines, only No.30 (S1-980042) line (SIIG = 0.122) demonstrated lower SIIG compared to the susceptible check (SIIG = 0.177) and was named as the most susceptible line. It is necessary to point out that the top ten lines as ranked by SIIG score, except for No.17 which ranked 8th, were from P.107 and P.121 populations. This indicates that these populations have the potential to be used as new sources for the preparation of resistant cultivars to *Rhizoctonia* rot.

Orthogonal Comparison among Populations

Comparison among various populations displayed that the highest PN and RN were gained by P.107 and the lowest by the P.201 population (table 4). The populations of P.107 and P.121 illustrated the least value of DI and the highest rates of HI (both of HI_3 and HI_4). Moreover, there was no significant difference between them in terms of all characteristics that confirmed their high resistance level to *Rhizoctonia* compared to other populations. It was observed that the P.201 population was unable to resist *Rhizoctonia* rot disease, as evidenced by the greatest DI score of 6.62 and the lowest values of HI_3 = 7.57 and HI_4 = 14.26. The graph in Fig. 4 shows the position of all the studied lines, which were colored according to five different genetic sources, with respect to DI, HI_4, and SIIG rates. The filled green and red circles that represent P.107 and P.121 populations had high values of HI_4 and SIIG, and a low DI score, indicating that they are more resistant to *Rhizoctonia* disease.

Discussion

Various diseases are major yield-reducing factors in sugar beet. *Rhizoctonia* rot is one of the main and recurrent diseases in sugar beet fields, which is mostly reported to cause yield loss from 2% to 60% based on field conditions (Neher and Gallian, 2011; Strausbaugh et al., 2011; Buhre et al., 2009). Cultural tools, chemical management and genetic resistance are the main strategies used to reduce the disease (Haque and Parvin, 2021). Host resistance is a useful approach of controlling *R. solani* (McGrath et al., 2015). Producing resistant hybrids in sugar beet requires identifying resistant pollinator lines, which is one of the most crucial initial steps (Basati et al., 2013). Determining resistance in sugar beet lines typically involves screening lines following artificial inoculation on roots in a controlled environment (McGrath et al., 2015; Nagendran et al., 2009). Testing resistance in sugar beet lines through artificial infection and monitoring their response in mini-plot environment has been found to be an effective

Table 4. Comparison of various pollinator populations relating to all characteristics by orthogonal comparisons

Pollinator population	Number of lines	PN	RN	DI	HI_3	HI_4
P.107	10	18.50^{a}	18.00^{a}	2.88^{d}	69.09^{a}	85.09^{ab}
P.165	8	16.81^{ab}	16.12^{ab}	3.91^{c}	45.56^{b}	70.92^{b}
P.121	7	16.93^{ab}	16.43^{ab}	2.67^{d}	76.28^{a}	89.72^{a}
P.201	10	14.60^{b}	13.80^{b}	6.62^{a}	7.57^{d}	14.26^{d}
P.724	12	16.04^{ab}	15.42^{ab}	5.01^{b}	24.95^{c}	40.86^{c}

Note: PN: plant number; RN: root number; DI: disease index; HI_3: standard harvest index; HI_4: experiment-specific harvest index. Values in the same column followed by the same letter are not significant according to LSD test at $P \leq 0.05$ level.

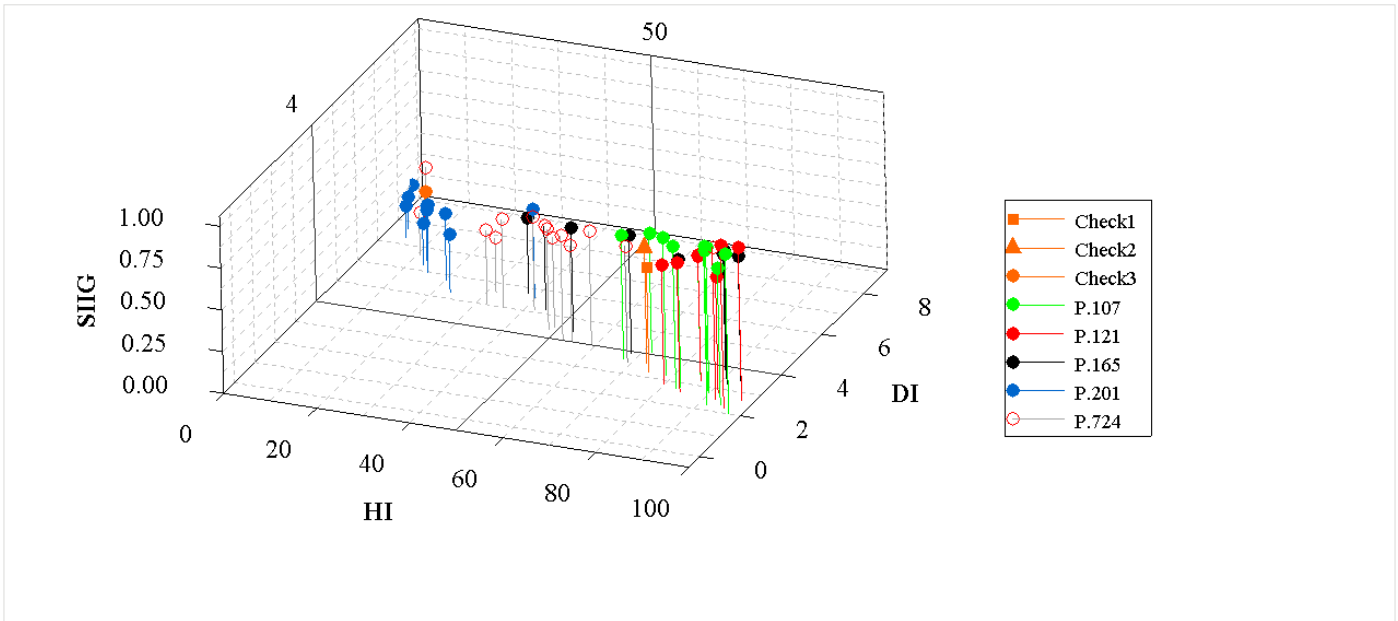

Figure 4. Distribution of pollinator lines derived from five various populations along with checks by 3D scatter plot according to SIIG, DI and HI values

Note: Check1: FC-709 (resistant); Check2: Novodoro (resistant); Check3: Mass-191 (susceptible)

approach. (Mahmoudi et al., 2003). Ebrahimi Kolaei et al. (2019 b) released the first Iranian resistant cultivar to *Rhizoctonia* by using this approach. They identified SB19 as a resistant pollinator line to *Rhizoctonia* under mini-plot conditions and introduced it as the paternal parent to provide a resistant cultivar. Finally, the Ekbatan cultivar was released in 2015. On the other hand, it is essential to apply powerful tools to make precise decisions on the selection of the resistant lines. SIIG is a very useful technique that simultaneously considers different variables to select the best lines (Zali et al., 2015). Zali et al. (2019) used the SIIG method to identify drought-tolerant genotypes of canola and suggested that it was an effective tool for recognizing the best lines based on the simultaneous selection of several variables. Given this, we used five various populations of sugar beet as new genetic sources for identifying resistant pollinator lines with the help of the SIIG approach. In general, 47 lines were derived from these populations and evaluated for resistance to *Rhizoctonia*. The results of the study displayed that there was proper diversity among lines in response to *Rhizoctonia*. The results of SIIG were mostly in agreement with the results of biplot and cluster analysis. Comparing SIIG with biplot and cluster methods they provided evidence that selecting resistant lines via SIIG was reliable. In order to identify resistant pollinator lines, the top five lines in the SIIG system that coincided with findings of the biplot and cluster methods were selected as the resistant pollinator lines. Thereby No.19 (S1-980022), No.3 (S1-980004), No.1 (S1-980002), No.20 (S1-980025) and No.25 (S1-980032) were identified as the most resistant pollinator lines through SIIG criterion. These lines were introduced as paternal parents for producing new hybrids in future breeding programs. The selected lines illustrated appropriate responses for resistance against *Rhizoctonia* inoculation so that they revealed low DI and high HI_4, PN and RN compared to other lines. Since the PN was counted before inoculation, it can be inferred that high PN indicates proper vigor and germination of the lines. On the other hand, RN, which is considered at harvest time, indicates the reliability of results in terms of sample size. Therefore identification of pollinator lines based on lower DI and greater HI_4, PN and RN, with the help of SIIG, denotes their potential to provide resistant hybrids to *Rhizoctonia* in supplementary breeding programs.

Conclusion

Among other lines, pollinator lines S1-980022, S1-980004, S1-980002, S1-980025, and S1-980032 were identified as the most resistant. Two populations, P.107 and P.121, were potentially confirmed to have resistance to *Rhizoctonia* rot. They can be used as new genetic resistance sources for future research.

CRediT Authorship Contribution Statement

Hamed Mnsouri: Supervised the work, analyzed the data and drafted the manuscript. **Hamze Hamze:** Performed some of the experiments and contributed to the editing of the manuscript. **Mahdi Hassani:** Conceptualization, performed some of the experiments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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