

1 **ACCEPTED ARTICLE**

2 **Analysis of the Genotype by Environment Interactions of Sugar Beet**
3 **Genotypes under Rhizomania Contamination**

4
5 Parviz Fasahat^{1*}, Javad Rezaei², Mastane Sharifi³, Heydar Azizi⁴, Keyvan Fotuhi⁴, Parviz
6 Mahdikhani⁴, Adel Pedram⁴, Ali Jalilian⁵, and Babak Babaei¹

7
8 **Running title:** Evaluation of Sugar Beet Genotypes Stability

9 **ABSTRACT**

10 The sugar beet crop has always been attacked by various pests and diseases. Rhizomania
11 viral disease, which has spread in different regions of sugar beet cultivation, has become of
12 prime importance disease of the crop in the last three decades. Resistant cultivar usage is the
13 only reliable way to manage rhizomania disease. In order to identify promising genotypes,
14 eleven sugar beet genotypes in a company with three controls were assessed in a randomized
15 complete block design (RCBD) with four replications in experimental fields with natural
16 infection to rhizomania in six research stations of Karaj, Khoy, Kermanshah, Mashhad,
17 Miandoab, and Shiraz for two cropping seasons (2020 and 2021). Based on the rhizomania
18 score, all genotypes had acceptable resistance to the disease. The additive main effects and
19 multiplicative interaction (AMMI) stability analysis illustrated that the first five principal
20 components were significant and specified 88.8% of the total genotype by environment
21 interaction variance. Gen-7, Gen-10, Gen-11, and Gen-2 were selected as stable genotypes
22 based on the AMMI model. Genotype plus genotype by environment interaction (GGE) biplot
23 results also confirmed the superiority of Gen-10 and Gen-11 regarding sugar yield and
24 stability in disease-infected environments. According to the results of the multi-trait
25 stability index (MTSI), genotypes Gen-4, Gen-1, Gen-2, and Gen-11 were identified as stable
26 genotypes under rhizomania-infected conditions. By applying different stability measurement
27 methods, in addition to identifying the genotype's adaptation to different environments,
28 accurate decisions for future breeding or cultivar registration can be achieved.

¹Sugar Beet Seed Institute (SBSI), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Islamic Republic of Iran.

²Agricultural and Natural Resources Research Center of Khorasan Razavi, Agricultural Research, Education and Extension Organization (AREEO), Islamic Republic of Iran.

³Agricultural and Natural Resources Research Center of Fars, Agricultural Research, Education and Extension Organization (AREEO), Islamic Republic of Iran.

⁴Agricultural and Natural Resources Research Center of West Azerbaijan, Agricultural Research, Education and Extension Organization (AREEO), Islamic Republic of Iran.

⁵Agricultural and Natural Resources Research Center of Kermanshah, Agricultural Research, Education and Extension Organization (AREEO), Islamic Republic of Iran.

*Corresponding author; e-mail: parviz.fasahat@gmail.com

29 **Keywords:** Environment, Genotype Selection, Stability parameters, Sugar beet.

30
31 **INTRODUCTION**

32 Sugar is a global bulk commodity that can be stored without loss and transported easily. In
33 2020-21, global sugar production was about 181 million tons, approximately 26% was
34 obtained from sugar beet (ISO, 2022; Statista, 2022). Global sugar production has risen by
35 nearly 1.5% per year, with vast fluctuations over the years for more than 20 years (Jurgen,
36 2019). The growth in global consumption is principally due to developing countries with an
37 annual consumption of less than 10 kg of sugar per capita. In developed countries, sugar
38 consumption ranges from 25 to 50 kg per person based on eating habits and appetite. In the
39 majority of countries, sugar prices are determined by national import and export regulations
40 and sugar price policies. Therefore, the national profitability of sugar production from sugar
41 beet and its cultivated area varies to a great extent. Sugar beet cultivation is commonly related
42 to agreements between sugar producers and farmers. For sugar beet as an annual crop, there is
43 more flexibility in the cultivated area than sugarcane (Fasahat and Kakueinezhad, 2021;
44 Hoffmann *et al.*, 2021). For decades, the sugar beet crop has been the cornerstone of the
45 activities and income of many farmers and sugar industries around the world. Breeding
46 activities have contributed to maintaining the competitive position of this crop. Continuous
47 increases in yield and improving the crop tolerance to the biotic and abiotic stress are
48 of its development over the years.

49 Rhizomania is one of the main diseases of sugar beet. The disease is caused by the sugar
50 beet necrotic yellow vein virus, which itself is transmitted to sugar beet through the root
51 fungus *Polymyxa betae*, a soil-borne pathogen. The pathogen mainly attacks the roots of the
52 plant, causing the proliferation of lateral roots along the main root (Norouzi *et al.*, 2017).
53 About half of the lands under sugar beet cultivation in Iran are infected with rhizomania, and
54 the severity of infection in the fields is different from each other. The damage caused by
55 rhizomania differs depending on the cultivar and virus strain and can reduce the crop yield by
56 90%. Over the past few decades, plant breeders have worked to improve the productivity and
57 quality of rhizomania-resistant cultivars. By 2008, the genetic progress was such that the vast
58 majority of sugar beet growers in Iran, France, Belgium, and the Netherlands planted
59 rhizomania-resistant cultivars in their fields (Norouzi *et al.*, 2017). Nowadays, most
60 commercial sugar beet cultivars carry resistance genes to rhizomania, including *Rz1* and *Rz2*,
61 as a priority. Other resistance sources, such as *Rz3*, *Rz4*, and *Rz5*, were also identified
62 (Biancardi and Tamada, 2016).

63 Evaluation of the adaptability and stability of cultivar production under different
64 environmental conditions is of particular importance in breeding programs. Due to the
65 different responses of the cultivars to environmental changes, their performance varies from
66 one environment to another. Typically, each genotype has the maximum production potential
67 in a particular environment; however, by assessing the stability and adaptability of the
68 genotypes under various environments, it is possible to identify genotypes with acceptable
69 performance in all environments (Fasahat et al., 2015). Since traditional statistical methods of
70 analysis, such as using combined ANOVA tables, provide only limited information on the
71 interaction of genotypes in the environment, different methods are used for the stability
72 assessment. Using regression-based equations is one of the first methods used (Finlay and
73 Wilkinson, 1963; Eberhart and Russell, 1966). Other statistical multivariate methods such as
74 AMMI and GGE-biplot have also been widely used (Yan, 2001; Fasahat *et al.*, 2015). The
75 AMMI method is a multivariate statistical method that assess the cumulative effects of
76 genotype, environment, and G×E multiplicative effects and interprets G×E interaction (Ebdon
77 and Gauch, 2002). The AMMI method is a combination of ANOVA and principal component
78 analysis (PCA) (Fasahat *et al.*, 2014). The GGE-biplot method graphically illustrates G×E
79 interaction to help breeders simply check the stability of genotypes and combines stability
80 with a genotype's performance in different environments. It also evaluates the relationships
81 among environments to identify target environments in breeding programs (Yan et al., 2001).

82 Since rhizomania is a soil-borne disease and the ineffectiveness of conventional methods
83 (such as chemical and agronomical) in managing soil-borne diseases are reported, genetic
84 resistance has been proven as the most effective way to control the disease. Therefore, it is
85 essential to evaluate the genetic diversity of breeding lines to distinct disease-resistant
86 genotypes. In this study, sugar beet genotypes were assessed in terms of the effects of
87 different environmental conditions on resistance to rhizomania disease and analysis of
88 genotype by environment interaction for the use of resistant genotypes in breeding programs
89 as well as to recommend them for cultivation in contaminated environments in Iran.

90

91 MATERIALS AND METHODS

92 This study was performed under the Breeding Department, Sugar Beet Seed Institute,
93 Karaj, Iran. Eleven sugar beet genotypes accompanied by three controls were sown across six
94 agricultural research stations in two cropping seasons (2020 and 2021). The selected
95 environments (combination of year and location) covered considerably different conditions

96 regarding temperature, rainfall, and soil properties. Geographical characteristics and rainfall
 97 amounts of the experimental sites across the two growing seasons are brought in Table 1.

98 Trials were performed in a randomized complete block design (RCBD) with four
 99 replications in each environment (Table 1). The name and the given code of each genotype
 100 are listed in Table 2. The susceptible cultivar Sharif was sown around the trials in order to
 101 confirm the field infection to rhizomania. After reaching the necessary base temperature for
 102 germination, seeds were sown at 20 cm within rows. The experimental units consisted of
 103 three-row plots, 8 m long and spaced 50 cm apart. Irrigation was performed immediately after
 104 planting and adjusted for subsequent irrigation intervals according to the region's thermal
 105 regime and water evaporation potential. At the 2-leaf stage, thinning was done, and weeds
 106 were controlled manually. The experimental fields were managed according to local
 107 agronomic practices. At harvest, to eliminate marginal effects, the first row, the last row, the
 108 beginning, and the end of each row (one m long) were removed.

109

110 **Table 1.** Geographical characteristics and rainfall of the research stations during 2020-21
 111 seasons.

Locations	Codes	Cropping season	Rainfall (mm)	Altitude (m)	Coordinate		Temperature (C°)			Soil type
					Longitude	Latitude	Min	Max	Ave	
Karaj	KJ20	2020	252.3	1244	50°52 E	35°50 N	10.4	26.5	18.5	Clay-loam
Karaj	KJ21	2021	51.6				12.1	27.9	19.9	
Kermanshah	KH20	2020	319.2	1362	46°48 E	34°15 N	10.8	28.5	19.7	Silty-clay
Kermanshah	KH21	2021	71.3				10.7	28.9	20	
Khoy	KY20	2020	240.2	1147	44°56 E	38°22 N	11.0	24.9	17.9	Silty-loam
Khoy	KY21	2021	154.4				11.7	25.9	18.8	
Mashhad	MD20	2020	214.9	998	60°48 E	35°12 N	12.3	25.7	19.0	Silty-loam
Mashhad	MD21	2021	62.7				13.2	27.4	20.3	
Miandoab	MB20	2020	166.8	1294	46°06 E	36°57 N	9.0	25.3	17.6	Silty-loam
Miandoab	MB21	2021	107.3				10.7	26.3	18.6	
Shiraz	SZ20	2020	207.3	1598	52°42 E	29°46 N	11.1	28.9	20.0	Clay-loam
Shiraz	SZ21	2021	28.2				13.0	30.5	21.8	

112

113

Table 2. List of the studied sugar beet genotypes.

Genotype	Code	Genotype	Code
F-21236	Gen-1	F-21276	Gen-8
F-21237	Gen-2	F-21277	Gen-9
F-21238	Gen-3	F-21278	Gen-10
F-21239	Gen-4	F-21279	Gen-11
F-21242	Gen-5	BTS310	Gen-12
F-21243	Gen-6	Denzel	Gen-13
F-21244	Gen-7	Macumba	Gen-14

114

115 The disease score was given to the roots at harvest in accordance with the Luterbacher et
 116 al. (2005) on the basis of 1-9 scale (score 1 shows plants with healthy roots and 9 as dead
 117 plants) at two agricultural research stations of Shiraz and Mashhad. Although the trial in
 118 Miandoab was also performed under disease-infected conditions, the data on infection

119 severity was not recorded. Harvested roots were weighed, washed, and pulp samples were
 120 taken. Quality analysis was conducted via a Betalyser (Anton Paar, Germany) automatic beet
 121 laboratory system based on standard procedures (ICUMSA, 2009). Quality characteristics
 122 such as sugar content, sodium (Na⁺), potassium (K⁺), and amino-Nitrogen (N) were measured.
 123 Their values were used to estimate sugar yield, white sugar yield, white sugar content,
 124 molasses sugar, and extraction coefficient of sugar on the basis of Equations (1-5) (Cook and
 125 Scott, 1993; Reinfeld et al., 1974).

$$SY = RY \times SC \quad (1)$$

$$WSY = RY \times WSC \quad (2)$$

$$WSC = SC - (MS + 0.6) \quad (3)$$

$$MS = 0.0343(K^+ + Na^+) + 0.094(N) - 0.31 \quad (4)$$

$$ECS = \left(\frac{WSC}{SC} \right) \times 100 \quad (5)$$

126 where SY is sugar yield (t ha⁻¹), RY is root yield (t ha⁻¹), SC is sugar content (%), WSY is
 127 white sugar yield (t ha⁻¹), WSC is white sugar content (%), MS is molasses sugar (%), K⁺ is
 128 potassium (meq 100 g⁻¹), Na⁺ is sodium (meq 100 g⁻¹), amino-Nitrogen is N (meq 100 g⁻¹),
 129 and ECS is extraction coefficient of sugar (%).

130

131 **Statistical analysis**

132 Bartlett's test (Bartlett, 1937) was calculated to check the homogeneity of the variances of
 133 experimental errors. After confirming the homogeneity of error variance for each trait (RY=
 134 0.7073, SY= 0.6909, SC= 0.0867, WSC= 0.1768, WSY= 0.4540, Na= 0.6608, K= 0.6673, N=
 135 0.5138, MS= 0.8691, and ECS= 0.9933), a combined variance analysis was performed. The
 136 genotypes were considered as fixed variables, while the environments were treated as random
 137 variables.

138 The weight of sugar beet root and the sugar content are the two main components of yield
 139 formation in sugar beet. A combination of high values obtained from root yield and sugar
 140 content will result in a high sugar yield per hectare. Therefore, owing to the importance of
 141 sugar yield as the main criterion to distinguish sugar beet cultivars, multivariate stability
 142 analysis was conducted graphically on the basis of GGE biplot for this trait using GGE biplot
 143 software (Yan, 1999, 2001) and AMMI analysis by GEA-R (v. 4.0, CIMMYT, Mexico).
 144 Different statistics from the AMMI model, including AMMI based stability parameter
 145 (ASTAB), AMMI stability index (ASI), AMMI stability value (ASV), sum across
 146 environments of absolute value of G×E interaction modeled by AMMI (AV_{AMGE}),
 147 Annicchiarico's D parameter (DA), Zhang's D parameter (Dz), Average of the squared
 148 eigenvector values (EV), stability measure based on fitted AMMI model (FA), modified

149 AMMI stability index (MASI), modified AMMI stability value (MASV), sums of the absolute
150 value of the IPC scores (SIPC), absolute value of the relative contribution of IPCAs to the
151 interaction (ZA) (Sneller *et al.*, 1997; Zhang *et al.*, 1998; Purchase *et al.*, 2000; Raju, 2002;
152 Rao and Prabhakaran, 2005; Zali *et al.*, 2012; Ajay *et al.*, 2018) were calculated to identify
153 stable genotypes. All statistical analysis was performed using R Statistical Software 4.0.3 (R
154 core Team 2020).

155 To estimate the average yield and simultaneous stability of RY, SY, WSY, SC, WSC, K⁺,
156 Na⁺, N, MS, and ECS, the MSTI index was computed based on Equation (6) (Olivoto, 2019)
157 using R Statistical software 4.0.3 (R core Team 2020).

$$MSTI_i = \left[\sum_{j=1}^f ((\gamma_{ij} - \gamma_j)^2) \right]^{0.5} \quad (6)$$

158 Where, $MSTI_i$ is the multi-trait stability index of the genotype i , γ_{ij} is the score of the
159 genotype i in the factor j , and γ_j is the score of the ideal genotype in the factor j .

160

161 RESULTS AND DISCUSSION

162 Combined analysis of variance

163 After confirming the uniformity of error variances in all trials by performing Bartlett's test
164 (Bartlett, 1937), a combined analysis of variance was performed to determine $G \times E$
165 interaction (Table 3). There was a highly significant difference among genotypes for all traits,
166 and the location had a significant effect on most traits such as root yield, sugar content, white
167 sugar content, sugar yield, white sugar yield, and K⁺. The year \times location interaction showed
168 significant differences in all studied traits, except for the sugar content trait. The genotype \times
169 location interaction had significant differences for Na⁺, K⁺, N, and the extraction coefficient
170 of sugar. The genotype \times year \times location ($G \times E$), as a three-way interaction, showed the
171 significance of this effect only for root yield, sugar yield, white sugar yield, and N.

172 To better understand the $G \times E$ interaction, the partitioning of interaction percentage was
173 calculated from the total sum of squares for sugar yield. A remarkable scale of discrepancy
174 was because of location (46.9%), followed by genotype \times location (9.6%), and $G \times E$
175 interaction (7.7%). A large difference between locations results in higher variability in
176 genotype performance. Such location effects are in congruence with the results of Oladosu *et al.*
177 *et al.* (2017) and Khan *et al.* (2021). The genotype effect accounted for 5.4% of the total sum of
178 squares, and the genotype \times year, location \times year, and the year effect contributed 1.3%, 1%,
179 and 0.3% of the variation, respectively. The low contribution of year showed that the
180 evaluated years in this study were similar. In addition, the lower percent of the sum of squares

181 for the location \times year effect than the location effect indicates that there was no variation
 182 across locations over the two years. Significant variations in the response of genotypes to the
 183 impact of environments demonstrate the right choice of experimental sites for $G \times E$
 184 interaction assessment (Hassani et al. 2018).

185
 186 **Table 3.** Results of ANOVA for the studied traits of sugar beet genotypes across 12
 187 environments.

Source of variation	df	Mean of squares				
		Root yield	Sugar yield	Sugar content	White sugar content	White sugar yield
Year	1	15.4	20.3	29.7	2.4	3.2
Location	5	16629.4**	643.6**	335.1*	320.1	688.4**
Year \times Location	5	1219.1**	14.0	56.1**	94.6**	16.4*
Error 1	36	242.4	8.4	3.3	4.8	6.3
Genotype	13	1081.6**	28.5**	9.7**	15.7**	24.9**
Genotype \times Year	13	189.1	6.6	1.4	1.5	5.3
Genotype \times location	65	272.9	10.1	1.5	2.2	7.2
Genotype \times Year \times location	65	245.1**	8.1	1.1	1.4	6.2**
Error 2	468	86.7	3.4	1.0	1.3	2.6

188 ns, *, **: non-significant and significant at five and one percent probability levels, respectively.

189 **Continued Table 3**

Source of variation	df	Mean of squares				
		Na ⁺	K ⁺	alpha-amino nitrogen	Molasses sugar	Extraction coefficient of sugar
Year	1	62.9	159.8	0.2	49.1	929.5
Location	5	93.6	209.8*	32.6	55.3	1407.1
Year \times Location	5	21.9**	29.8*	29.3**	11.0**	508.2**
Error 1	36	1.3	1.0	0.8	0.4	20.3
Genotype	13	12.1**	5.5**	1.1**	1.4**	67.3**
Genotype \times Year	13	0.4	0.3	0.09	0.1	4.6
Genotype \times location	65	0.8**	0.5**	0.3*	0.1	9.3*
Genotype \times Year \times location	65	0.4	0.3	0.2**	0.1	5.8
Error 2	468	0.3	0.3	0.1	0.1	5.1

190 ns, *, **: non-significant and significant at five and one percent probability levels, respectively.

191

192 **The genotype response to rhizomania disease**

193 Table 4 shows the results of the genotype response to rhizomania disease in accord with
 194 the Luterbacher et al. (2005) method. Genotypes evaluation for rhizomania infection in
 195 Mashhad in 2020 showed that all genotypes had a complete resistance with healthy roots and
 196 no hairy root or colour variation. Therefore, all genotypes carry the resistance genes related to
 197 the disease. However, in Mashhad in 2021, only genotypes Gen-4, BTS310 Macumba as
 198 controls had a perfect resistance, and other genotypes accompanied by control Denzel
 199 illustrated a semi-resistant response. This is perhaps because of the environmental situations
 200 and the new pathotypes of the disease development, which resulted in the lack of perfect
 201 genotype resistance (Norouzi et al., 2017). According to the results of genotypes response to
 202 rhizomania infection in Shiraz, the genotypes were grouped in semi-resistant to semi-

203 susceptible with no perfect resistance to the disease during both years of the study. This
 204 indicates that the intensity of genotype infection to the disease in Shiraz was higher than that
 205 of Mashhad.

206
 207 **Table 4.** Resistance score given to sugar beet genotypes against rhizomania in Agricultural
 208 Research Stations of Mashhad and Shiraz.

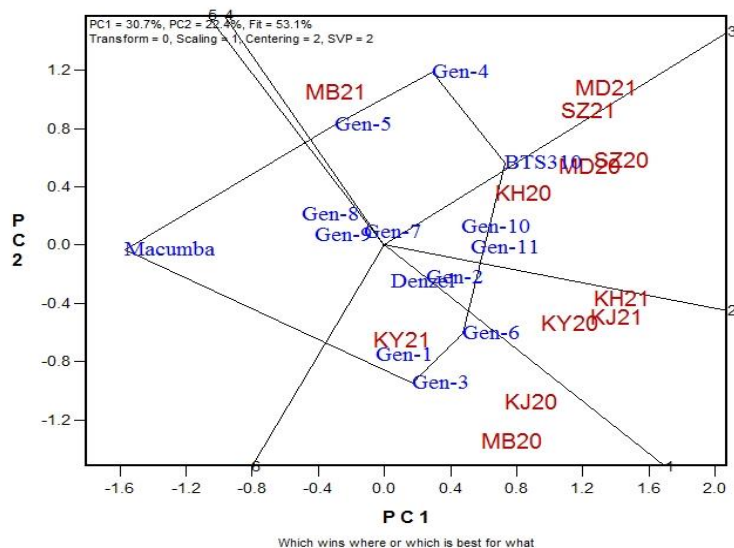
Genotype	Mashhad		Shiraz		Genotype	Mashhad		Shiraz	
	2020	2021	2020	2021		2020	2021	2020	2021
Gen-1	1	2	2	3	Gen-8	1	2	3	3
Gen-2	1	2	2	3	Gen-9	1	2	2	3
Gen-3	1	2	2	3	Gen-10	1	2	2	3
Gen-4	1	1	2	3	Gen-11	1	2	3	3
Gen-5	1	2	2	3	BTS310	1	1	2	3
Gen-6	1	2	2	3	Denzel	1	2	2	3
Gen-7	1	2	2	3	Macumba	1	1	2	4

209
 210 Genotype \times environment interaction causes significant differences in genotype behavior in
 211 different environments, which reduces the relationship between phenotypic and genotypic
 212 values. This interaction effect can be ignored if it does not cause a change in the genotype
 213 ranking, but if it is large enough to cause a change in the rank of genotypes under different
 214 environments, it should be evaluated. Since the conventional statistical methods, like
 215 combined analysis of variance, only provides information about the existence or lack of $G \times E$
 216 interaction, plant breeders are using different stability methods such as GGE-biplot and
 217 AMMI stability analysis (Fasahat *et al.*, 2014; Fasahat *et al.*, 2015).

218
 219 **GGE-biplot analysis**

220 The sum of the first and second principal components in the GGE biplot was 64.3%, which
 221 indicates that these two components explain a large variation in sugar yield variance. Figure 1
 222 shows the polygon biplot (Yan, 1999) to identify mega-environments as well as top genotypes
 223 in different environments. In this biplot, a polygon identifies the top genotypes in each
 224 environment. The environmental indicators are positioned into four sections, with different
 225 genotypes in each section. Based on the 14 genotypes and 12 environments examined here,
 226 the GGE-biplot was divided into six clockwise fan-shaped sections. Genotypes Gen-3, Gen-6,
 227 Gen-4, BTS310, and Macumba were placed at the polygon sides. In KJ20, MB20, and KY21,
 228 Gen-3 was the best genotype, followed by Gen-6 and Gen-1 as the most suitable cultivar in
 229 these environments. Genotype Gen-2 in KJ21, KY20, and KH21, Gen-10 and Gen-11 in
 230 KH20, MD20, and SZ20, Gen-4 and Gen- in SZ21, MD21, and MB21 were identified as the

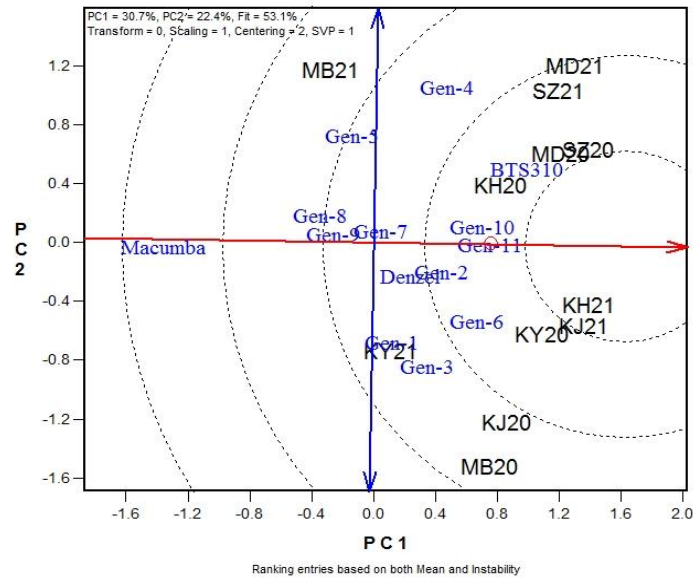
231 best genotypes. Surprisingly, the control Macumba showed no superiority or equality over
 232 other genotypes in any of the studied areas, and was considered a poor cultivar.



233
 234 **Figure 1.** Polygon of GGE biplot method for identification of best genotypes in each
 235 environment.

236
 237 In Figure 2, genotypes were ranked based on the average sugar yield and yield stability in
 238 12 environments. The line that crosses through the biplot's origin and the desired point
 239 (which represents the average of PC1 and PC2 of environmental scores) is called the average
 240 environment coordinate (AEC) (Yan and Kang, 2003). Genotypes that are closer to the center
 241 of the circle on this line have higher yields. The line perpendicular to this line and crosses
 242 through the center of the biplot (line with double arrow) is the criterion for measuring the
 243 stability of genotypes. Genotypes that are far from this line are less stable. Based on the GGE
 244 biplot model, genotypes with more adaptability should be close to the optimal point on the
 245 AEC line and have the least distance from this line. As can be deduced from Figure 2, Gen-11
 246 and Gen-8 had the highest and lowest sugar yield, respectively compared with other
 247 genotypes. Among studied environments, KH20 showed higher stability, followed by MD20.
 248 Such $G \times E$ interaction effects are in congruence with the results of Khan et al. (2021), who
 249 evaluated the stability of Bambara groundnut genotypes in four environments in Malaysia.

250



251 **Figure 2.** Genotype ranking based on average sugar yield and stability.

252
253
254 **AMMI stability**

255 The sugar yield data of genotypes were subjected to AMMI analysis. Results showed that
 256 the $G \times E$ interaction for sugar yield was significant ($P < 0.01$) and explained 25.7% of the
 257 variance (Table 5). In a study conducted on the grain yield of finger millet using the AMMI
 258 method, the $G \times E$ interaction contributed to 37.8% of the variance (Anuradha et al., 2022). In
 259 addition, the analysis unfolded that $G \times E$ interaction was significantly specified by the first
 260 five principal components (PCs). Among them, the first PC contributed to 33.5% of the total
 261 $G \times E$ interaction, while the second to fifth PCs explained 20.1%, 14.3%, 13.2%, and 7.7%,
 262 respectively. In a study on $G \times E$ assessment for grain quality in rice using the AMMI model,
 263 the first principal component significantly contributed 67% toward the total of $G \times E$
 264 interaction (Fasahat et al., 2014).

265
266
267
268
269
270
271
272
273
274
275
276
277
278

279 **Table 5.** Analysis of variance based on AMMI model for sugar yield of sugar beet genotypes.

Source of variation	df	Sum of squares	Mean of squares	Relative variance (%)	Cumulative variance (%)
Environment	11	3308.67	300.78**	-	-
Error 1	36	302.57	8.4	-	-
Genotype	13	371.69	28.59**	-	-
G × E interaction	143	1279.32	8.94**	-	-
PC1	23	428.98	18.65**	33.5	33.5
PC2	21	256.96	12.24**	20.1	53.6
PC3	19	182.71	9.62**	14.3	67.9
PC4	17	168.64	9.92**	13.2	81.1
PC5	15	97.99	6.53*	7.7	88.8
Noise	48	143.71	2.99 ^{ns}		
Error 2	504	1908.07	3.79 ^{ns}		
CV (%)	11.9				

*,**and ns: significant at 5 and 1% probability levels and non-significant, respectively.

280

281

282

283

284

285

286

287

288

289

290

291

292

293

294

295

296

297

298

299

300

301

302

303

304

305

306

307

308

In Table 6, the average sugar yield and various AMMI stability parameters for fourteen sugar beet genotypes in twelve environments are shown. Genotypes Gen-2 and Gen-11 had the highest, and Gen-9 and Gen-8 had the lowest sugar yield with an average sugar yield of 15.4 t ha⁻¹. Based on ASTAB, ASI, ASV, FA, ZA, and AVAMGE stability indices, genotypes Gen-7 and Denzel were the most stable genotypes with the lowest value for these indices. Stability indices of DA, DZ, EV, MASI, MASV, and SIPC showed the same results and identified Gen-10 and Gen-8 as the most stable genotypes. However, Gen-2, Gen-3, Gen-9, and Macumba, with the highest values for these statistics, were the most unstable genotypes. The results are in congruence with those achieved by Yadav et al. (2022) and Anuradha et al. (2022), who reported the importance of the first two principal components in the prediction of the accurate model in AMMI decomposition. Meanwhile, Anuradha et al. (2022) found a strong correlation among the AMMI-based indices. Considering the results of the present study, except Gen-8, the selected genotypes, according to AMMI-based indices, had sugar yield values around the average.

309 **Table 6.** Average sugar yield, and different AMMI stability parameters for 14 sugar beet
 310 genotypes in 12 environments.

Genotype	Mean sugar yield (t ha ⁻¹)	ASTAB	ASI	ASV	AVAMGE	DA	DZ	EV	FA	MASI	MASV	SIPC	ZA
Gen-1	15.8	1.95	0.43	2.12	11.21	4.35	0.46	0.04	18.92	0.43	2.22	2.28	0.19
Gen-2	16.0	3.09	0.08	0.42	13.95	4.59	0.67	0.09	21.04	0.26	1.93	2.22	0.13
Gen-3	15.8	4.81	0.63	3.15	19.51	6.71	0.73	0.11	44.97	0.65	3.68	3.30	0.27
Gen-4	15.9	3.04	0.49	2.45	15.02	5.28	0.59	0.07	27.92	0.50	2.77	3.36	0.26
Gen-5	15.2	1.98	0.31	1.56	10.23	4.01	0.50	0.05	16.05	0.32	2.03	2.74	0.19
Gen-6	15.5	1.81	0.25	1.22	10.24	3.69	0.50	0.05	13.60	0.25	1.83	2.13	0.13
Gen-7	15.2	0.94	0.03	0.14	7.14	2.51	0.38	0.03	6.30	0.14	1.18	1.57	0.09
Gen-8	14.6	1.42	0.04	0.21	8.23	2.70	0.53	0.06	7.29	0.11	1.23	1.77	0.08
Gen-9	14.7	2.47	0.02	0.08	12.00	4.08	0.61	0.07	16.65	0.22	1.78	2.13	0.11
Gen-10	15.8	4.29	0.36	1.80	15.86	5.65	0.78	0.12	31.92	0.40	3.18	4.22	0.27
Gen-11	16.0	2.77	0.16	0.78	11.79	3.90	0.73	0.11	15.21	0.20	1.84	2.75	0.14
BTS310	16.6	1.71	0.33	1.64	11.65	3.86	0.45	0.04	14.87	0.34	2.10	2.35	0.18
Denzel	15.1	1.28	0.20	1.02	8.09	3.14	0.41	0.03	9.86	0.22	1.67	1.85	0.12
Macumba	13.5	5.02	0.43	2.16	16.48	6.27	0.81	0.13	39.29	0.48	3.56	4.36	0.30
LSD (0.05)	1.2												

ASTAB: AMMI based stability parameter, ASI: AMMI stability index, ASV: AMMI stability value, AV_{AMGE}: sum across environments of absolute value of G × E interaction modeled by AMMI, DA: Annicchiarico's D parameter, Dz: Zhang's D parameter, EV: Average of the squared eigenvector values, FA: stability measure based on fitted AMMI model, MASI: modified AMMI stability index, MASV: modified AMMI stability value, SIPC: sums of the absolute value of the IPC scores, ZA: absolute value of the relative contribution of IPCAs to the interaction

311

312 **MTSI and genotype selection**

313 In Table 7, the results of factor analysis on the basis of principal component analysis are
 314 presented. The first factor, with eigenvalues of 4.75 and an explanation of 43.1% of total
 315 variance, had high and positive factor coefficients for root yield, sugar yield, Na⁺, K⁺, alpha-
 316 amino nitrogen, and molasses sugar. The second factor explained 27.1 of the total variance
 317 and had an eigenvalue of 2.98. This factor had high and negative coefficients for root yield,
 318 sugar yield, white sugar yield, and alpha-amino nitrogen. The third factor contributed to
 319 18.2% of data discrepancy, and an eigenvalue of 2, which showed a high and negative factor
 320 coefficient for half of the traits consisting of sugar yield, sugar content, white sugar yield,
 321 Na⁺, and molasses sugar.

322

323

324

325

326

327

328

329

330

331

332

333

334

335 **Table 7.** Eigenvalues, relative, and cumulative variance as well as factor coefficients after
 336 varimax rotation in factor analysis based on principal component analysis.

Traits	Factors		
	First	Second	Third
Root yield	0.41	-0.91	0.08
Sugar yield	0.08	-0.99	-0.02
Sugar content	-0.91	0.22	-0.25
White sugar content	-0.98	0.14	-0.07
White sugar yield	-0.19	-0.97	0.03
Na ⁺	0.72	0.01	-0.51
K ⁺	0.17	0.15	0.01
alpha-amino nitrogen	0.18	-0.05	0.95
Molasse sugar	0.85	0.11	-0.43
Extraction coefficient of sugar	-0.96	0.0	0.23
Eigenvalue	4.75	2.98	2
Relative Variance (%)	43.1	27.1	18.2
Cumulative variance (%)	43.1	70.2	88.4

337

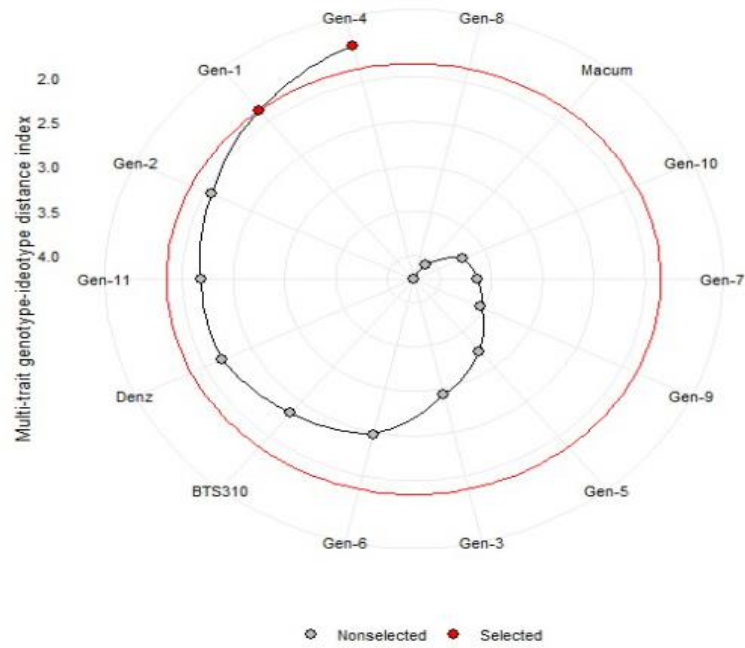
338 The factor scores of the aforesaid factors were used to calculate the MTSI stability index of
 339 the genotypes. In Figure 3, genotypes ranking based on the MTSI stability index is shown in
 340 which Gen-4 and Gen-1 were selected as ideal genotypes using a selection pressure of 20%.
 341 Based on the highest to the lowest value of the MTSI index, genotypes are placed in the
 342 outermost circuit to the center of the Figure, respectively. Macumba had the lowest stability
 343 index score showing poor stability and mean sugar yield in different environmental
 344 conditions. Genotype selection by MTSI is important according to the value of traits in
 345 genotypes, i.e., traits that have a good appearance (Olivoto et al., 2019). The overall results of
 346 the stability analysis of pearl millet genotypes from the previous study (Yadav et al., 2022)
 347 are concordant with the results of this study.

348

349

350

351



352 **Figure 3.** Genotype ranking and selected genotypes based on multi-trait stability index.
 353 Based on this index, genotypes with lower values of this index are less distant from the ideal
 354 genotype and for the ones with higher MTSI value, more distant from the ideal genotype can
 355 be observed.
 356
 357

358 CONCLUSIONS

359 One of the major accomplishments of plant breeding in sugar beet is the development of
 360 cultivars resistant to rhizomania. Since the 1970s, this disease has spread rapidly throughout
 361 the sugar beet growing areas, and sugar beet breeding companies contributed to the
 362 management of it. Resistance genes pyramiding through the identification of resistance
 363 sources and adding them in breeding programs is a promising way to cope with the disease
 364 evolution. In this study, genetic diversity was found among genotypes regarding sugar yield
 365 under infected environments. The given rhizomania scores indicated a high number of
 366 genotypes with resistance response compared with susceptible ones. Evaluation of genotypes
 367 for yield stability under rhizomania infection using different statistics resulted in the
 368 identification of different stable genotypes from which genotypes Gen-10, Gen-11, Gen-4,
 369 and Gen-2 were common.

370 371 Acknowledgements

372 The authors acknowledge the field and laboratory support (No. 0-02-02-010-990273) from
 373 the Sugar Beet Seed Institute (SBSI), Agricultural Research, Education and Extension
 374 Organization (AREEO), Iran.

375 376 REFERENCE

- 377 1. Ajay, B., Aravind, J. and Abdul Fiyaz, R. 2018. Ammistability: additive main effects
378 and multiplicative interaction model stability parameters. R Package Version 11.
- 379 2. Anuradha, N., Patro, T. S. S. K., Singamsetti, A., Sandhya Rani, Y., Triveni, U.,
380 Nirmala Kumari, A., Govanakoppa, N., Lakshmi Pathy T. and Tonapi, V.A. 2022.
381 Comparative study of AMMI-and BLUP-based simultaneous selection for grain yield
382 and stability of finger millet [*Eleusine coracana* (L.) Gaertn.] genotypes. *Front. Plant*
383 *Sci.*, **12**: 786839.
- 384 3. Bartlett, M. S. 1937. Properties of sufficiency and statistical tests. Proceedings of the
385 Royal Society of London. *Series A-Mathematical and Physical Sciences*, **160(901)**:
386 268-282.
- 387 4. Biancardi, E. and Tamada, T. 2016. Rhizomania. Switzerland: Springer International
388 Publishing.
- 389 5. Cook, D. and Scott, R. 1993. The sugar beet crop: science into practice. New York,
390 USA: Champan and Hall Press.
- 391 6. Eberhart, S. T. and Russell, W. A. 1966. Stability parameters for comparing varieties
392 1. *Crop Sci.*, **6(1)**: 36-40.
- 393 7. Ebdon, J. S. and Gauch, H. G. 2002. Additive main effect and multiplicative
394 interaction analysis of national turfgrass performance trials. *Crop Sci.*, **42(2)**: 497-506.
- 395 8. Fasahat, P. and Kakueinezhad, M. 2021. Evaluation of sugar market in terms of price
396 fluctuations and its global consequences. *Journal of Sugar Beet*, **37(1)**: 131-138.
- 397 9. Fasahat, P., Rajabi, A., Mahmoudi, S.B., Noghabi, M.A. and Rad, J.M. 2015. An
398 overview on the use of stability parameters in plant breeding. *Biometrics &*
399 *Biostatistics International Journal*, **2(5)**: 00043.
- 400 10. Fasahat, P., Muhammad, K., Abdullah, A., Rahman, B. M. A., Siing, N. M., Gauch, J.
401 H. G. and Ratnam, W. 2014. Genotype \times environment assessment for grain quality
402 traits in rice. *Communications in Biometry and Crop Science*, **9(2)**: 71-82.
- 403 11. Finlay, K. W. and Wilkinson, G. N. 1963. The analysis of adaptation in a plant-
404 breeding programme. *Aust. J. Agric. Res.*, **14(6)**: 742-754.
- 405 12. Hassani, M., Heidari, B., Dadkhodaie, A. and Stevanato, P. 2018. Genotype by
406 environment interaction components underlying variations in root, sugar and white
407 sugar yield in sugar beet (*Beta vulgaris* L.). *Euphytica*. **214**: 79.
- 408 13. Hoffmann, C. M., Koch, H. J. and Marlander, B. 2021. Sugar beet. In: *Crop*
409 *physiology case histories for major crops*. 634-672, Academic Press.

- 410 14. ICUMSA. 2009. International commission for uniform methods of sugar analysis.
411 Methods Book. Bartens, Berlin.
- 412 15. ISO. 2022. The Sugar Market, <https://www.isosugar.org/sugarsector/sugar> (Accessed
413 January 28, 2023)
- 414 16. Jurgen, B. 2019. Sugar Economy Europe Zuckerwirtschaft Europa Economie Sucrière
415 Europe 2019. 65. Auflage, revidierte Ausgabe. Berlin: Bartens, A.
- 416 17. Khan, M. M. H., Rafii, M. Y., Ramlee, S. I., Jusoh, M. and Al Mamun, M. 2021.
417 AMMI and GGE biplot analysis for yield performance and stability assessment of
418 selected Bambara groundnut (*Vigna subterranea* L. Verdc.) genotypes under the
419 multi-environmental trials (METs). *Sci. Rep.*, **11(1)**: 1-17.
- 420 18. Luterbacher, M., Asher, M., Beyer, W., Mandolino, G., Scholten, O., Frese, L. and
421 Slyvchenko, O. 2005. Sources of resistance to diseases of sugar beet in related Beta
422 germplasm: II. Soil-borne diseases. *Euphytica*, **141(1)**: 49-63.
- 423 19. Norouzi, P., Stevanato, P., Mahmoudi, S. B., Fasahat, P. and Biancardi, E. 2017.
424 Molecular progress in sugar beet breeding for resistance to biotic stresses in sub-arid
425 conditions-current status and perspectives. *J. Crop Sci. Biotech.*, **20(2)**: 99-105.
- 426 20. Oladosu, Y., Rafii, M. Y., Abdullah, N., Magaji, U., Miah, G., Hussin, G. and Ramli,
427 A. 2017. Genotype \times Environment interaction and stability analyses of yield and yield
428 components of established and mutant rice genotypes tested in multiple locations in
429 Malaysia. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, **67(7)**:
430 590-606.
- 431 21. Olivoto, T., Lúcio, A.D., da Silva, J.A., Sari, B.G. and Diel, M.I. 2019. Mean
432 performance and stability in multi-environment trials II: Selection based on multiple
433 traits. *Agron. J.*, **111(6)**: 2961-2969.
- 434 22. Purchase, J. L. 1997. Parametric analysis to describe $G \times E$ interaction and yield
435 stability in winter wheat. Ph.D. thesis. Department of Agronomy, Faculty of
436 Agriculture, University of the Orange Free State, Bloemfontein, South Africa.
- 437 23. Purchase, J. L., Hatting, H. and van Deventer, C. S. 2000. Genotype \times environment
438 interaction of winter wheat (*Triticum aestivum* L.) in South Africa: II. Stability
439 analysis of yield performance. *S. Afr. J. Plant Soil*, **17**: 101-107.
- 440 24. Raju, B. 2002. A study on AMMI model and its biplots. *Indian Society of Agricultural*
441 *Statistics (India)* **55**: 297-322.
- 442 25. Rao, A. and Prabhakaran, V. 2005. Use of AMMI in Simultaneous Selection of
443 Genotypes for Yield and Stability. *J. Indian Soc. Agric. Stat.*, **59**: 76-82.

- 444 26. Reinfeld, E., Emmerich, A., Baumgarten, G., Winner, C. and Beiss, U. 1974. Zur
445 Voraussage des Melassez zuckersaus Ruben analysen Zucker. London, UK.: Chapman
446 & Hall, World Crop Series.
- 447 27. Sneller, C., Kilgore-Norquest, L. and Dombek, D. 1997. Repeatability of yield
448 stability statistics in soybean. *Crop Sci.*, **37**: 383-390.
- 449 28. Statista. 2022. [https://www.statista.com/statistics/249679/total-production-of-sugar-](https://www.statista.com/statistics/249679/total-production-of-sugar-worldwide/)
450 [worldwide/](https://www.statista.com/statistics/249679/total-production-of-sugar-worldwide/) (Accessed on January 28, 2023)
- 451 29. Yadav, C.B., Gangashetty, P.I., Beckmann, M., Mur, L.A. and Yadav, R.S. 2022.
452 Genotype-by-Environment Interaction Analysis of Metabolites in Pearl Millet
453 Genotypes with High Concentrations of Slowly Digestible and Resistant Starch in
454 Their Grains. *Cells*, **11(19)**: 3109.
- 455 30. Yan, W. 1999. A study on the methodology of cultivar evaluation based on yield trial
456 data with special reference to winter wheat in Ontario. Ph.D. thesis, University of
457 Guelph, Canada.
- 458 31. Yan, W. 2001. GGEbiplot- a windows application for graphical analysis of
459 multienvironment trial data and other types of two-way data. *Agron J.*, **93**: 1111.
- 460 32. Yan, W. and Kang, M. S. 2003. GGE biplot analysis: A graphical tool for breeders,
461 geneticists, and agronomists. CRC Press, Boca Raton, FL.
- 462 33. Zali, H., Farshadfar, E., Sabaghpour, S. H. and Karimizadeh, R. 2012. Evaluation of
463 genotype× environment interaction in chickpea using measures of stability from
464 AMMI model. *Ann. Biol. Res.*, **3**: 3126-3136.
- 465 34. Zhang, Z., Cheng, L. and Zhonghuai, X. 1998. Analysis of variety stability based on
466 AMMI model. *Zuo wu xue bao*, **24**: 304-309.

467
468
469
470
471
472
473
474
475
476
477

478 بررسی اثرات متقابل ژنوتیپ در محیط ژنوتیپ های چغندر قند تحت آلودگی به ریزومانیا

479
480 پ. فصاحت*، ج. رضایی، م. شریفی، ح. عزیزی، ک. فتوحی، پ. مهدیخانی، ع. پدرام، ع. جلیلیان، و ب. بابایی

481 چکیده

482 گیاه چغندر قند همواره مورد حمله آفات و بیماری های مختلف بوده است. بیماری ویروسی ریزومانیا که در مناطق
483 مختلف کشت چغندر قند گسترش یافته است، در سه دهه اخیر به مهمترین بیماری این محصول تبدیل شده است. استفاده
484 از رقم مقاوم تنها راه قابل اعتماد برای مقابله با بیماری ریزومانیا است. به منظور شناسایی ژنوتیپ های امیدوارکننده،
485 11 ژنوتیپ چغندر قند به همراه سه شاهد در قالب طرح بلوک های کامل تصادفی با چهار تکرار در مزارع آزمایشی با
486 آلودگی طبیعی به ریزومانیا در شش ایستگاه تحقیقاتی کرج، خوی، کرمانشاه، مشهد، میاندوآب و شیراز برای دو سال
487 (1399 و 1400) مورد ارزیابی قرار گرفتند. بر اساس امتیازدهی ریشه ها نسبت به آلودگی به بیماری ریزومانیا،
488 ژنوتیپ ها مقاومت قابل قبولی را به بیماری نشان دادند. تجزیه و تحلیل پایداری AMMI نشان داد که پنج مؤلفه اول
489 معنی دار بوده و 88/8 درصد از اثرات متقابل را بیان می کنند. ژنوتیپ های Gen-7، Gen-10، Gen-11 و Gen-2
490 به عنوان ژنوتیپ های پایدار بر اساس مدل AMMI انتخاب شدند. نتیجه حاصل از روش گرافیکی GGE-biplot نیز
491 برتری Gen-10 و Gen-11 را از نظر عملکرد شکر و پایداری در محیط های آلوده به بیماری تایید کرد. نتایج
492 به دست آمده از شاخص MTSI ژنوتیپ های Gen-4، Gen-1، Gen-2 و Gen-11 را به عنوان ژنوتیپ های پایدار
493 تحت شرایط آلوده به ریزومانیا شناسایی کرد. با بکارگیری روش های مختلف اندازه گیری پایداری، علاوه بر شناسایی
494 سازگاری ژنوتیپ ها با محیط های مختلف، می توان تصمیم دقیقی برای بهنژادی یا ثبت رقم در آینده گرفت.
495