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# ACCEPTED ARTICLE

# Analysis of the Genotype by Environment Interactions of Sugar Beet Genotypes under Rhizomania Contamination

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## Running title: Evaluation of Sugar Beet Genotypes Stability

# 9 ABSTRACT

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

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## 31 INTRODUCTION

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

Rhizomania is one of the main diseases of sugar beet. The disease is caused by the sugar 49 50 beet necrotic yellow vein virus, which itself is transmitted to sugar beet through the root fungus *Polymyxa betae*, a soil-borne pathogen. The pathogen mainly attacks the roots of the 51 52 plant, causing the proliferation of lateral roots along the main root (Norouzi *et al.*, 2017). About half of the lands under sugar beet cultivation in Iran are infected with rhizomania, and 53 the severity of infection in the fields is different from each other. The damage caused by 54 rhizomania differs depending on the cultivar and virus strain and can reduce the crop yield by 55 56 90%. Over the past few decades, plant breeders have worked to improve the productivity and quality of rhizomania-resistant cultivars. By 2008, the genetic progress was such that the vast 57 58 majority of sugar beet growers in Iran, France, Belgium, and the Netherlands planted rhizomania-resistant cultivars in their fields (Norouzi et al., 2017). Nowadays, most 59 commercial sugar beet cultivars carry resistance genes to rhizomania, including  $R_{Z_1}$  and  $R_{Z_2}$ . 60 as a priority. Other resistance sources, such as  $R_{z_3}$ ,  $R_{z_4}$ , and  $R_{z_5}$ , were also identified 61 62 (Biancardi and Tamada, 2016).

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

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

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## **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 agricultural research stations in two cropping seasons (2020 and 2021). The selected 94 95 environments (combination of year and location) covered considerably different conditions regarding temperature, rainfall, and soil properties. Geographical characteristics and rainfall
amounts of the experimental sites across the two growing seasons are brought in Table 1.

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

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

Logations	Codes	Cropping	Cropping Rainfall		Coord	inate	Temj	perature	$e(C^{o})$	Soil turno
Locations	Codes	season	(mm)	(m)	Longitude	Latitude	Min	Max	Ave	Son type
Karaj	KJ20	2020	252.3	1244	50052Æ	25050 N	10.4	26.5	18.5	Clay loam
Karaj	KJ21	2021	51.6	1244	30°32 E	33°30 N	12.1	27.9	19.9	Clay-IOall
Kermanshah	KH20	2020	319.2	1260	16019 T	24015 1	10.8	28.5	19.7	Ciltre alare
Kermanshah	KH21	2021	71.3	1302	40 48 E	54 15 N	10.7	28.9	20	Sitty-clay
Khoy	KY20	2020	240.2	1147	11056 TE	200221	11.0	24.9	17.9	Silty loom
Khoy	KY21	2021	154.4	114/	44 JO E	30 22 IN	11.7	25.9	18.8	Siny-Ioan
Mashhad	MD20	2020	214.9	008	60019 TE	25012 N	12.3	25.7	19.0	Silty loom
Mashhad	MD21	2021	62.7	990	00 40 E	55°12 N	13.2	27.4	20.3	Sitty-Ioan
Miandoab	MB20	2020	166.8	1204	16006 T	260571	9.0	25.3	17.6	Cilty loom
Miandoab	MB21	2021	107.3	1294	40 00 E	50'57 IN	10.7	26.3	18.6	Sitty-Ioan
Shiraz	SZ20	2020	207.3	1509	50010 TE	20046 N	11.1	28.9	20.0	Clay loom
Shiraz	SZ21	2021	28.2	1398	32'42 E	29'40 IN	13.0	30.5	21.8	Clay-Ioall

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Table 2. List of the studied sugar beet genotypes.

		0 0	71
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

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The disease score was given to the roots at harvest in accordance with the Luterbacher et al. (2005) on the basis of 1-9 scale (score 1 shows plants with healthy roots and 9 as dead plants) at two agricultural research stations of Shiraz and Mashhad. Although the trial in Miandoab was also performed under disease-infected conditions, the data on infection severity was not recorded. Harvested roots were weighed, washed, and pulp samples were
taken. Quality analysis was conducted via a Betalyser (Anton Paar, Germany) automatic beet
laboratory system based on standard procedures (ICUMSA, 2009). Quality characteristics
such as sugar content, sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), and amino-Nitrogen (N) were measured.
Their values were used to estimate sugar yield, white sugar yield, white sugar content,
molasses sugar, and extraction coefficient of sugar on the basis of Equations (1-5) (Cook and
Scott, 1993; Reinfeld et al., 1974).

$SY = RY \times SC$	(1)
$WSY = RY \times WSC$	(2)
WSC = SC - (MS + 0.6)	(3)
$MS = 0.0343(K^{+} + Na^{+}) + 0.094(N) - 0.31$	(4)
$ECS = (WSC / SC) \times 100$	(5)

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

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## 131 Statistical analysis

Bartlett's test (Bartlett, 1937) was calculated to check the homogeneity of the variances of experimental errors. After confirming the homogeneity of error variance for each trait (RY= 0.7073, SY= 0.6909, SC= 0.0867, WSC= 01768, WSY= 0.4540, Na= 0.6608, K= 06673, N= 0.5138, MS= 0.8691, and ECS= 0.9933), a combined variance analysis was performed. The genotypes were considered as fixed variables, while the environments were treated as random variables.

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

- AMMI stability index (MASI), modified AMMI stability value (MASV), sums of the absolute 149 value of the IPC scores (SIPC), absolute value of the relative contribution of IPCAs to the 150 interaction (ZA) (Sneller et al., 1997; Zhang et al., 1998; Purchase et al., 2000; Raju, 2002; 151 Rao and Prabhakaran, 2005; Zali et al., 2012; Ajay et al., 2018) were calculated to identify 152 stable genotypes. All statistical analysis was performed using R Statistical Software 4.0.3 (R 153
- core Team 2020). To estimate the average yield and simultaneous stability of RY, SY, WSY, SC, WSC, K<sup>+</sup>, 155 Na<sup>+</sup>, N, MS, and ECS, the MSTI index was computed based on Equation (6) (Olivoto, 2019) 156
- 157 using R Statistical software 4.0.3 (R core Team 2020).

$$MSTI_{i} = \left[\sum_{j=1}^{f} \left( (\gamma_{ij} - \gamma_{j})^{2} \right)^{1/2} \right]^{1/2}$$
(6)

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

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#### **RESULTS AND DISCUSSION** 161

#### **Combined analysis of variance** 162

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

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

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

interaction assessment (Hassani et al. 2018). 184

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

		Mean of squares							
Source of variation	df	Root vield	Sugar	Sugar content	White sugar	White sugar			
		Root yield	yield	Sugar content	content	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×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×Year	13	189.1	6.6	1.4	1.5	5.3			
Genotype×location	65	272.9	10.1	1.5	2.2	7.2			
Genotype×Year×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			

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ns, \*, \*\*: non-significant and significant at five and one percent probability levels, respectively. 189 **Continued Table 3** 

		Mean of squares						
Source of variation	df	$Na^+$	<b>K</b> +	alpha-amino	Molasses sugar	Extraction coefficient of		
		INA	K	nitrogen	Wolasses sugar	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×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×Year	13	0.4	0.3	0.09	0.1	4.6		
Genotype×location	65	0.8**	0.5**	0.3*	0.1	9.3*		
Genotype×Year×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.

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#### 192 The genotype response to rhizomania disease

Table 4 shows the results of the genotype response to rhizomania disease in accord with 193 194 the Luterbacher et al. (2005) method. Genotypes evaluation for rhizomania infection in Mashhad in 2020 showed that all genotypes had a complete resistance with healthy roots and 195 196 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 illustrated a semi-resistant response. This is perhaps because of the environmental situations 199 200 and the new pathotypes of the disease development, which resulted in the lack of perfect genotype resistance (Norouzi et al., 2017). According to the results of genotypes' response to 201 rhizomania infection in Shiraz, the genotypes were grouped in semi-resistant to semi-202

- susceptible with no perfect resistance to the disease during both years of the study. This
  indicates that the intensity of genotype infection to the disease in Shiraz was higher than that
  of Mashhad.
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Table 4. Resistance score given to sugar beet genotypes against rhizomania in Agricultural
 Research Stations of Mashhad and Shiraz.

Constant	Mashhad		Sh	iraz	Constant	Mashhad		Shiraz	
Genotype	2020	2021	2021 2020 2021 Genotype		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

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

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# 219 GGE-biplot analysis

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

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



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Figure 1. Polygon of GGE biplot method for identification of best genotypes in each
environment.

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





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

# 254 AMMI stability

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

<b>I uble et</b> i maije	10 01 14	Hanee Subea	on i minin mou	er for bagar freia or i	sugui ecci geneijpesi
Source of	đf	Sum of	Mean of	Relative variance	Cumulative variance
variation	ui	squares	squares	(%)	(%)
Environment	11	3308.67	300.78**	-	-
Error 1	36	302.57	8.4	-	-
Genotype	13	371.69	28.59**	-	-
$G \times E$ 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 <sup>ns</sup>		
Error 2	504	1908.07	3.79 <sup>ns</sup>		
CV (%)	11.9				

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

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

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<sup>-1</sup>. 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. 

genotype		Tonnent	5.										
Ganatuna	Mean sugar	ASTAB	ASI	ASV	AVAMGE	DA	DZ	EV	FA	MASI	MASV	SIPC	ZA
Genotype	yield (t ha <sup>-1</sup> )												
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												

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

ASTAB: AMMI based stability parameter, ASI: AMMI stability index, ASV: AMMI stability value,  $AV_{AMGE}$ : sum across environments of absolute value of  $G \times E$  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

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# 312 MTSI and genotype selection

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

Tarita		Factors	
Traits	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 <sup>+</sup>	0.72	0.01	-0.51
K <sup>+</sup>	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

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.

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

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

## 358 CONCLUSIONS

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

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481	
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