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# BIPLOT ANALYSIS OF MORPHOLOGICAL, QUALITY, AND DISEASE RESISTANCE TRAITS IN SUGAR BEET HYBRIDS

## SUMMARY

The breeding of yield, quality, and resistance to disease in sugar beet (*Beta vulgaris* L.), relies on understanding the interrelationships among agronomic traits. In current research, sixteen sugar beet hybrids were evaluated under field conditions in Miandoab, Iran, for morphological, physiological, sugar quality, and rhizomania disease-related traits. Hybrids were assessed for leaf width, leaf length, petiole length, plant height, canopy coverage, plant number, growth score, root uniformity, sugar content, sodium, potassium, α-amino nitrogen, alkalinity coefficient, white sugar yield, and rhizomania infection parameters. The dataset was analyzed using a biplot model, which enabled visualization of hybrid-trait interactions, exploration of trait associations, and evaluation of discriminative potential of each trait. The first two principal components explained 73% of variation. Leaf length, leaf width, plant height, and canopy coverage exhibited the highest discriminative potential, while sugar content, alkalinity coefficient, and white sugar yield were critical for extractable sugar. Positive associations were observed between sugar content, alkalinity coefficient, and white sugar yield, whereas sodium, potassium, and α-amino nitrogen were negatively associated with these key quality traits. Traits associated with vegetative vigor grouped together, highlighting their primary role in biomass production rather than direct sugar accumulation. In contrast, disease-related traits formed a distinct cluster, illustrating the negative effects of rhizomania on root quality and yield. The biplot facilitated the identification of ideal entries: hybrids H1, H2, and H3 performed best across most traits, while in terms of rhizomania tolerance, H16, H10, H11, H13, H14, and H15 exhibited low infection levels. Hybrids H15 and H16 are recommended as superior candidates for ideotype-based breeding programs.

**Keywords:** white sugar yield, rhizomania tolerance, ideotype selection, hybrid-by-trait interaction

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## INTRODUCTION

As one of the twelve major crops, sugar beet (*Beta vulgaris* L.), provides food and energy for people worldwide. It is a high-yielding crop with substantial economic importance, particularly as a source of sugar (Ćirić *et al.*, 2024). The development of sugar beet has been closely tied to advances in genetics, with significant progress achieved through scientific selection during two recent centuries. Nevertheless, conventional agricultural practices have greatly diminished crop diversity, as only about 30 of the 30,000 edible plant species are now major contributors to the global human diet (Ray *et al.,* 2020). Recognizing and utilizing genetic diversity is therefore crucial for crop improvement and sustainable production. Genetic variation is the base of breeding efforts, as the ability to select superior genotypes depends directly on the presence of variability within breeding populations.

Genetically diverse parents produce hybrids with higher heterosis, increasing the probability of obtaining offspring with desirable traits (Labroo *et al.,* 2021). Despite some spontaneous crosses with leaf beet and wild beet (Beta maritima), genetic diversity of sugar beet remains relatively limited compared to many other crops. Nonetheless, research on sugar beet and related species has demonstrated considerable variation in important agronomic traits, such as leaf dimensions (length, width, and thickness), petiole length, stem diameter, overall biomass, root size, and plant height (Michalska-Klimczak *et al.,* 2024; Sabaghnia *et al.,* 2024). These traits are crucial for selection programs aimed at improving root yield, sugar content, and stress tolerance.

Genetic variability in sugar beet directly influences the efficiency of selection for both quantitative and qualitative traits. Root yield, sugar content, sugar extraction efficiency, seed germination potential, and resistance to diseases and mechanical damage are primarily quantitative traits, requiring complex breeding approaches (Zhuzhzhalova *et al.,* 2020; Bojović *et al.,* 2024). Leaf number and leaf thickness are positively correlated with sugar content and gross sugar yield (Leilah and Khan, 2021), whereas traits such as petiole length and root diameter may show an exchange between root weight and sugar content (Fitters *et al.,* 2022). Impurity elements such as α-amino nitrogen, sodium, and potassium strongly influence sugar quality, as they interfere with sucrose crystallization in molasses and reduce extractable sugar yield.

Selection for low concentrations of these elements can significantly improve syrup purity and sugar content (Aksu and Altay, 2020; Lamlom *et al.,* 2025). The presence of substantial genetic variation for morpho-physiological traits, and root characteristics, in sugar beet germplasm highlights the potential for breeding programs to simultaneously improve yield, and sugar quality. By evaluating and utilizing such genetic diversity, breeders can develop new cultivars that show the best performances under environmental circumstances. Peng *et al.,* (2024), assessed 129 sugar beet genotypes and reported high variations for petiole length and petiole width, while principal component analysis extracted five components which were represented as shoot traits, root morphology, leaf anatomical, root and leaf type, and fascicled leaf type features, so they demonstrated considerable morphological and genetic diversity within the evaluated sugar beet genotypes, providing a valuable foundation for breeding and genetic improvement. Rhizomania, caused by Beet necrotic yellow vein virus (BNYVV), is one of the most destructive diseases of sugar beet and can cause severe yield losses and reduced sugar quality in infected fields.

Sabaghnia *et al.*, (2024), in evaluation of twenty sugar beet genotypes, found high variation for root yield, and high correlation of white sugar with sugar percent and coefficient of extraction, and identified G11 as the best genotypes for these characteristics, while they suggested that enhancing growth uniformity, stem diameter, tuber size, and plant number at harvest are important for achieving higher sugar beet yield. Recognizing the essential role of genetic diversity, this research aimed to evaluate the genetic responses of sugar beet hybrids in order to clarify the relationships between key agronomic traits and sugar quality characteristics. Understanding these relationships will provide a foundation for effective selection strategies to improve yield, and sugar content, in sugar beet. This study provides a comprehensive integration of morphological, quality, and disease traits using biplot analysis, offering improved insight into ideotype-based selection in sugar beet compared to previous single-trait evaluations. The aim of this study was to evaluate sugar beet hybrids for morphological, quality, and rhizomania-resistance traits and to identify superior genotypes using biplot analysis.

**Table 1.** Pedigree or name of the 16 sugar beet hybrids.

| Code | Pedigree/Name | Code | Pedigree/Name |
|---|---|---|---|
| H1 | SC§ × SB33 - HSF – 1 | H9 | SC × SB33 - HSF – 9 |
| H2 | SC × SB33 - HSF – 2 | H10 | SC × SB33 - HSF – 10 |
| H3 | SC× SB33 - HSF – 3 | H11 | Pars |
| H4 | SC × SB33 - HSF – 4 | H12 | Sharif |
| H5 | SC × SB33 - HSF – 5 | H13 | 7R26 |
| H6 | SC × SB33 - HSF – 6 | H14 | SBSI 031 |
| H7 | SC × SB33 - HSF – 7 | H15 | Bumerang |
| H8 | SC × SB33 - HSF – 8 | H16 | Novodoro |

**§SC, Single cross 7112 × SB36*



## MATERIAL AND METHODS

Current research was conducted at the Miandoab (36°58′N; 46°90′E), Iran on 16 monogerm hybrids (Table 1). The average yearly rainfall was 289.8 mm, the average yearly temperature was 9.0 °C. It is characterized as a Xeric moisture property, with clay-loam to silty-loam soil properties. Autumn plowing was followed by comprehensive spring land preparation, comprising disk plowing and harrowing, to ensure optimal soil conditions for planting. Based on soil test recommendations, nitrogen and phosphate was applied from $(NH_4)_3PO_4$ as 200 kg

$ha^{-1}$ at planting and kg $ha^{-1}$ urea at a later stage. Also, 150 kg $ha^{-1}$ $K_2SO_4$ was applied at sowing stage for getting sufficient levels. Planting was carried out using a randomized complete block design (RCBD) with six replications, and standard agronomic practices, including thinning, weeding, irrigation, and pest and disease control, were performed throughout the growing season. Six replications were used to increase experimental precision and reduce the impact of environmental variability. Ten hybrids (H1 to H10), were new rhizomania resistant genotypes, two hybrids (H11 and H14), were moderate-resistant (MR) hybrids to rhizomania, three hybrids (H13, H15, and H16), were completely resistant hybrids, and hybrid H12 was susceptible hybrid. The hybrids were planted in a field naturally infested with rhizomania, with confirmed occurrence of beet necrotic yellow vein virus, to evaluate disease response under realistic conditions.

Morphologic traits included leaf width (LW), plant number (PN), petiole length (PL), leaf length (LL), growth score (GS), plant height (PH), root uniformity (RU), and canopy coverage (CC), were recorded as. For assessing rhizomania disease, infected plants (IP), infection incidence (II), and infection severity (IS), were measured according to Büttner *et al.,* (2004). Roots were harvested in early November, washed, and weighed. Brie (pulp) samples were collected, immediately frozen, and transported to the laboratory. Sugar quality traits were measured as sugar content via polarimetry, Na and K via flame photometry, and α-amino nitrogen via the blue number procedure with copper reagent. Sugar quality traits were sugar content (SC, g $kg^{-1}$), sodium (Na, mmol $kg^{-1}$), potassium (K, mmol $kg^{-1}$), α-amino nitrogen (N, mmol $kg^{-1}$). Alkalinity coefficient (AC, mmol $kg^{-1}$ beet), which measures the alkalinity level in the beet roots, is computed via AC=(Na+K) + (α-N/1000). Finally, white sugar yield (WSY, t $ha^{-1}$), which indicates the amount of white sugar extracted per hectare is computed as WSY=(WSC×RY)/100.

To explore the interactions between sugar beet hybrids (entries) and measured traits (testers), the dataset was analyzed using an entry-by-tester biplot model, enabling visualization of interaction patterns and easier interpretation of the complex relationships among hybrids and traits. The GT biplot was constructed using principal component analysis of the standardized genotype × trait matrix. Let Yij represent the value of trait j for genotype i. The PCA decomposes the matrix into principal components:

$$Y_{ij} = \mu + \lambda_1 \gamma_{i1} \delta_{j1} + \lambda_2 \gamma_{i2} \delta_{j2} + \varepsilon_{i1}$$

where μ is the grand mean, λk is the eigenvalue of PC k, γ and δ are the genotype and trait scores for PC k, and εij is the residual. Prior to analysis, data were standardized (mean = 0, standard deviation = 1) to ensure comparability among traits with different measurement scales. The entry-by-trait biplot was generated using singular value decomposition (SVD) of the standardized data matrix. This approach was used to assess genotype–trait interactions, examine relationships among traits, and evaluate the discriminating ability of traits across hybrids.

## RESULTS AND DISCUSSION

### Variance and PCA explanation

The first two components explained 73% of the total variation (Figure 1). The first component accounted for 42%, and the second for 31%. The observed variance captures both additive and crossover interaction effects, suggesting that the ranking of sugar beet genotypes is trait-dependent. Comparable patterns have been documented in previous studies by Hassani *et al.*, (2023) in sugar beet and by Sabaghnia *et al.*, (2016) in spinach. This emphasizes the risk of relying on indirect selection without considering interaction effects. In the current study, hybrid-by-trait interactions were captured effectively by the biplot, as also noted by Mohebodini *et al.*, (2024). The biplot provided a clear visual representation of hybrid performance (Figure 1).

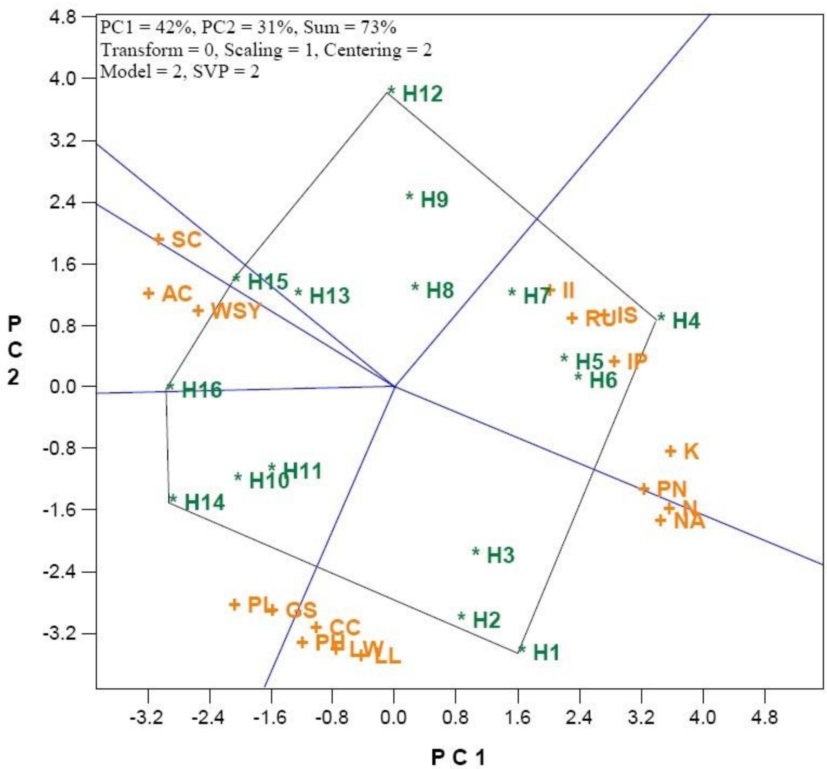


**Figure 1.** Which sugar beet hybrid wins which across which trait (s)

**Traits are leaf width (LW), plant number (PN), petiole length (PL), leaf length (LL), growth score (GS), plant height (PH), root uniformity (RU), canopy coverage (CC), infected plants (IP), infection incidence (II), infection severity (IS), sugar content (SC), sodium (Na), potassium (K), α-amino nitrogen (N), alkalinity coefficient (AC), and white sugar yield (WSY).*

### Hybrid × trait relationships

Some traits; leaf length (LL), canopy coverage (CC), plant height (PH), leaf width (LW), sodium (Na), and α-amino nitrogen (N), clustered around hybrid H1.

Petiole length (PL) and growth score (GS) were associated with hybrid H14. Hybrid H4 performed best for plant number (PN), potassium (K), infected plants (IP), infection incidence (II), infection severity (IS), and root uniformity (RU). Alkalinity coefficient (AC) and white sugar yield (WSY) were highest in hybrid H16. Hybrid H15 was top for sugar content (SC), while H12 did not outperform others in any measured trait. These results suggest that white sugar yield is more closely linked to sugar content and alkalinity coefficient than to other traits. Rašovský *et al., (*2022) and Nowicki *et al.*, (2025) also highlighted the importance of these traits. In contrast, Sayad *et al.*, (2022) found that leaf dimensions and root characteristics influence sugar yield indirectly through yield components and sugar accumulation. The fitted biplot clearly revealed hybrid responses across multiple traits. Also, the biplot analysis aided in identifying superior hybrids. While H15 and H16 emerged as promising candidates, their adaptability should be confirmed through subsequent yield stability trials.

**Trait associations**

To visualize trait relationships, vectors were projected from the origin, showing connections when the model explained sufficient variation. Associations among traits were inferred from the cosine of vector angles: vectors at 0° indicated a strong positive relationship, vectors at 90° indicated no relationship, and vectors at 180° indicated a strong negative relationship. Vector length reflected trait importance, with longer vectors contributing more to the model (Figure 2). Vectors closer to the origin or shorter in length indicate traits with limited influence on hybrid differentiation, whereas long vectors and angles close to 0° or 180° provide practical guidance for selecting hybrids with complementary trait profiles. Strong positive associations were observed among sugar content (SC), alkalinity coefficient (AC), and white sugar yield (WSY); among root uniformity (RU), infected plants (IP), infection incidence (II), and infection severity (IS); among sodium (Na), plant number (PN), potassium (K), and α-amino nitrogen (N); and among leaf width (LW), petiole length (PL), leaf length (LL), growth score (GS), plant height (PH), and canopy coverage (CC).

Negative associations appeared between SC, AC, WSY and Na, PN, K, N, as well as between RU, IP, II, IS and LW, PL, LL, GS, PH, CC. The negative impact of Na, K, and α-amino nitrogen on extractable sugar can be attributed to their interference with sucrose crystallization in molasses, which reduces sugar purity and yield. Weak or near-zero correlations were indicated by near-perpendicular vectors, for example between SC, AC, WSY and LW, PL, LL, GS, PH, CC, and between Na, PN, K, N and LW, PL, LL, GS, PH, CC. The biplot provided a clear visualization of positive, negative, and weak associations among the traits under study (Figure 2). Similar positive associations were reported by Hassani *et al.*, (2023), who found that white sugar yield (WSY) is primarily influenced by sugar content (SC) and technological parameters when impurity levels are within acceptable limits. The positive correlation between alkalinity coefficient (AC) and WSY supports the view that balanced ionic composition enhances extractable sucrose, while excessive alkalinity can reduce sugar purity (El-Mageed *et al.*, 2022).

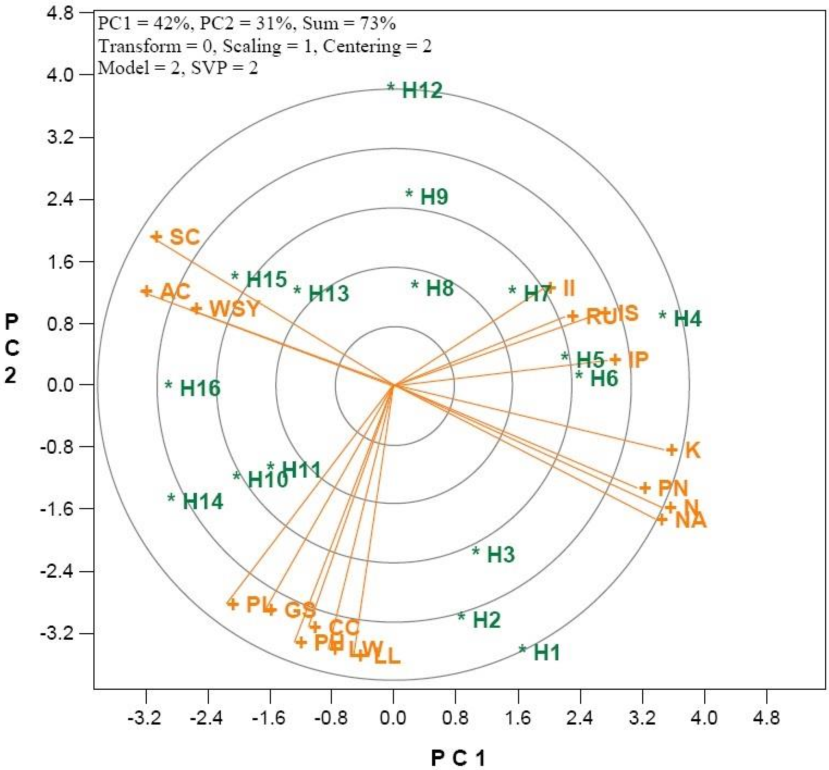


**Figure 2.** Ranking sugar beet hybrids based on traits

**Traits are leaf width (LW), plant number (PN), petiole length (PL), leaf length (LL), growth score (GS), plant height (PH), root uniformity (RU), canopy coverage (CC), infected plants (IP), infection incidence (II), infection severity (IS), sugar content (SC), sodium (Na), potassium (K), α-amino nitrogen (N), alkalinity coefficient (AC), and white sugar yield (WSY).*

The relationship between root uniformity (RU) and disease traits reflects the negative impact of rhizomania on root quality and sugar accumulation, as also noted by Fasahat *et al.*, (2024), who linked infection severity to yield loss, canopy reduction, and lower sugar content. The associations among sodium (Na), plant number (PN), potassium (K), and α-amino nitrogen (N) align with Hamze *et al.*, (2025), who observed that these compounds are chemically linked and can reduce extraction efficiency and WSY. Their position opposite SC, AC, and WSY vectors confirms their antagonistic effect on sugar production. Vegetative vigor traits, in contrast, mainly affect biomass rather than extractable sugar (El-Mageed *et al.*, 2022). Most biplot associations are consistent with the correlation matrix. However, exact one-to-one agreement should not be expected. Although, the biplot reflects multivariate interactions among all traits at once, Pearson correlation coefficients are limited to describing linear relationships between individual trait pairs. Some discrepancies are expected because the biplot accounted for 73% of the total variation, leaving a portion unexplained.

**Ideal hybrid**

The discriminating ability of a hybrid and its capacity to express desired traits can be evaluated by defining an assumed ideal entry (Figure 3). The ideal entry represents a practical breeding target, guiding selection of hybrids that combine favorable traits rather than serving solely as a statistical construct. Hybrids positioned close to this ideal were considered superior, while those farther away were less desirable. In this study, hybrids H1, H2, and H3 were closest to the ideal and can be regarded as ideotypes, showing high values for most measured traits. In contrast, hybrids H12 and H9 were farthest from the ideal and performed poorly in both discrimination and trait expression (Figure 3). Some challenges arise because trait associations were not always significant and, in some cases, were negative. This limitation is relevant for breeding programs, where yield and quality traits are primary targets but often show weak or antagonistic correlations. The ideal entry defined by the biplot goes beyond a statistical concept, providing a practical tool for identifying superior genotypes, emphasizing important traits, efficiently allocating breeding resources, and supporting ideotype-based selection strategies (Peixoto *et al.*, 2022). Graphical multivariate procedures are therefore essential for identifying ideotypes that combine desirable trait profiles.

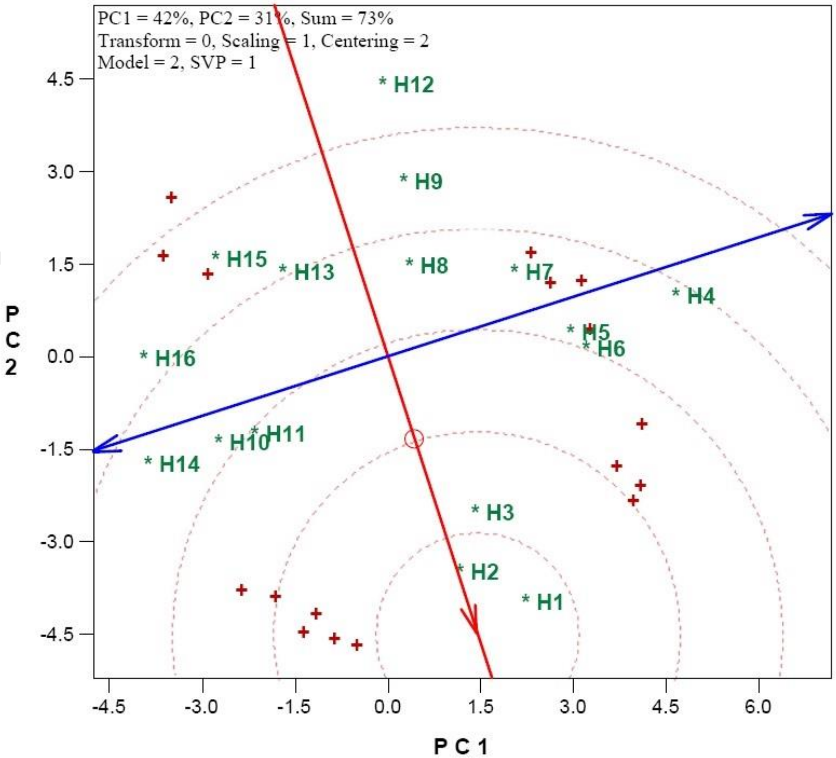


**Figure 3.** Ranking sugar beet hybrids according to discriminative and representativeness potentials

### Discriminative ability of traits

Discriminative potential represents the capacity of a trait to differentiate among hybrids and is directly related to the magnitude of its standard deviation. Traits positioned near the ideal trait (Figure 4) showed the highest discriminative potential, while those on the opposite side contributed less to hybrid differentiation. In this study, leaf length (LL) and leaf width (LW), followed by plant height (PH) and canopy coverage (CC), exhibited the greatest discriminative ability. Although, these vegetative traits effectively differentiate hybrids, they primarily contribute to biomass and canopy development rather than directly to sugar accumulation, explaining their indirect effect on WSY. In contrast, sugar content (SC), alkalinity coefficient (AC), and white sugar yield (WSY) were the least discriminative (Figure 4). Most other traits, except root uniformity (RU) and infection incidence (II), had discriminative potentials above the overall mean, indicating their usefulness for evaluating sugar beet hybrids.

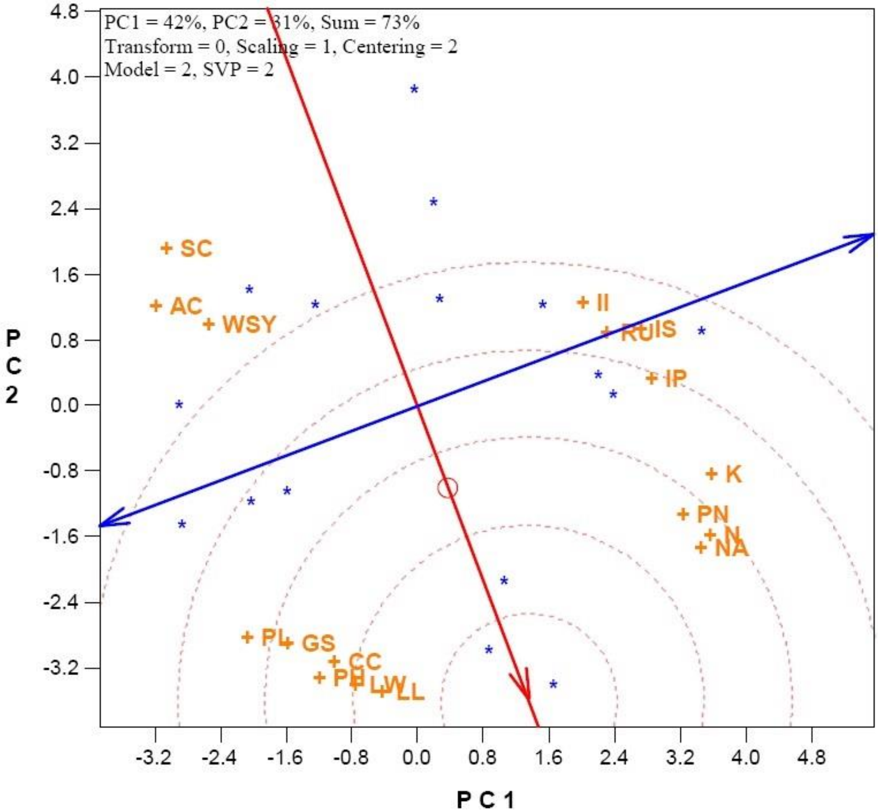


**Figure 4.** Ranking sugar beet traits based on discriminative and representativeness potentials

**Traits are leaf width (LW), plant number (PN), petiole length (PL), leaf length (LL), growth score (GS), plant height (PH), root uniformity (RU), canopy coverage (CC), infected plants (IP), infection incidence (II), infection severity (IS), sugar content (SC), sodium (Na), potassium (K), α-amino nitrogen (N), alkalinity coefficient (AC), and white sugar yield (WSY).*

Ramazi *et al*., (2024) reported varying discriminative potentials among sugar beet traits, emphasizing the importance of selecting highly discriminative traits for hybrid evaluation. Similarly, Xinwang *et al.*, (2025) found that leaf length and leaf width had high discriminative potential in certain genotypes. Multivariate tools are valuable for genotype evaluation and selection, particularly when trait associations are complex or non-linear. Identification of traits with high discriminative potential enables breeders to more efficiently target superior genotypes, thereby improving the effectiveness of breeding programs. Although, sugar content, alkalinity coefficient, and WSY show lower discriminative potential, they remain primary target traits for breeding due to their direct impact on extractable sugar yield.

**WSY performance and stability**

The performance of sugar beet for white sugar yield (WSY), the primary target trait, is shown in Figure 5. In this biplot, the horizontal axis represents WSY, with the arrow indicating its direction. Hybrids H16, H15, and H14 had the highest WSY, followed by H13, H10, and H12, while H4 and H1 were the least favorable. The vertical distance of each hybrid from the horizontal axis represents its variability, with smaller distances indicating greater stability and consistency. Hybrid H15, followed by H13, showed the lowest variability and were therefore the most stable entries. In contrast, hybrid H12 had above-average WSY but a large distance from the axis, indicating high variability and making it less desirable for selection. The findings highlight the effectiveness of biplot analysis in evaluating both performance and stability concurrently, thereby aiding breeders in identifying superior and consistently reliable genotypes.

**Rhizomania response**

The reaction of sugar beet hybrids to rhizomania disease, measured by infected plants (IP), is shown in Figure 6. In this biplot, the horizontal axis represents IP, with the arrow indicating its direction. Hybrids H4, H5, and H6 had the highest number of infected plants and were the most susceptible, while H10, H11, H13, H14, H15, and H16 showed lower infection and were more resistant. The vertical distance of each hybrid from the horizontal axis represents variability. Hybrid H16 was the most stable in disease tolerance, followed by H10, H11, H13, H14, and H15, which showed moderate stability. In contrast, H1, H2, and H12, despite below-average IP, exhibited greater distances from the axis, indicating high variability and reduced suitability. These results demonstrate the effectiveness of the biplot in simultaneously evaluating performance and stability, facilitating the identification of superior and reliable hybrids for breeding programs.

**Ideotype selection**

Leaf length (LL), leaf width (LW), plant height (PH), and canopy coverage (CC) showed the highest discriminative potential, reflecting vegetative vigor and contributing to biomass accumulation, light interception, and overall plant growth, which indirectly affect sugar yield (Fasahat *et al*., 2022). In contrast, traits directly related to product quality, such as sugar content (SC), alkalinity coefficient (AC), and white sugar yield (WSY), had lower discriminative potential, highlighting the complex physiological and environmental factors that regulate sugar accumulation. Positive correlations among SC, AC, and WSY indicate that these traits collectively determine extractable sugar, while impurities such as Na, K, and α-amino nitrogen negatively affect sugar yield and content, confirming their antagonistic role in sugar processing efficiency. Vegetative traits (LL, LW, PH, CC) clustered together, suggesting that morphological vigor enhances growth but does not directly improve sugar extraction (Shah *et al*., 2023).

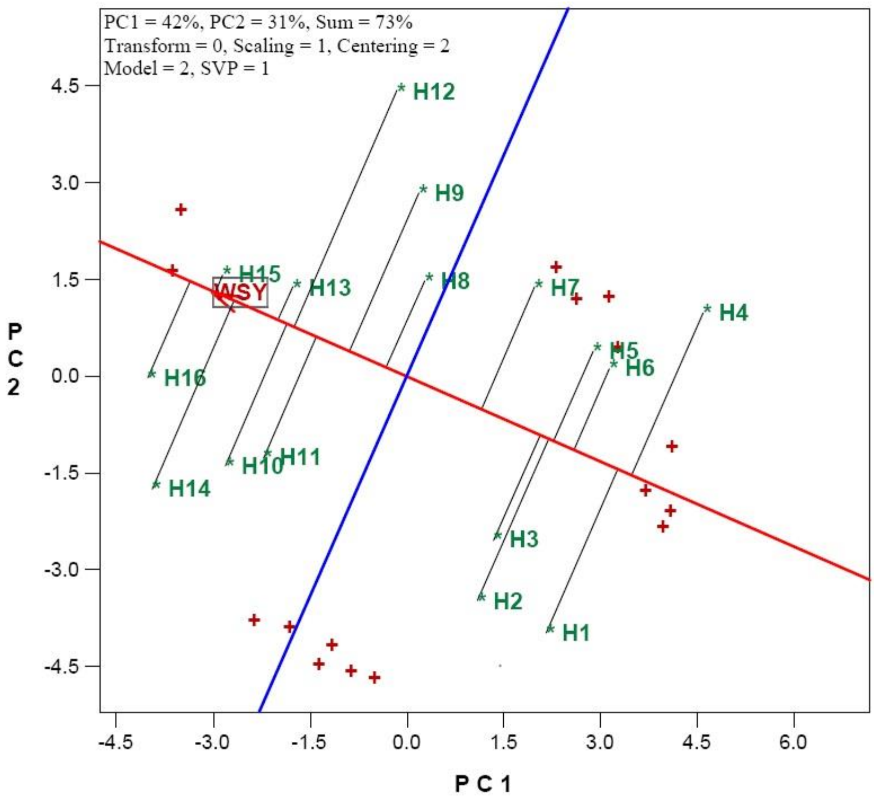


**Figure 5.** Testing the performance of/at white sugar yield (WSY)

Disease-related traits (RU, IP, II, IS) grouped separately, emphasizing the impact of rhizomania on root quality and sugar accumulation. The findings emphasize that evaluating both agronomic performance and disease resistance is crucial for identifying hybrids that achieve high yield potential while maintaining tolerance to rhizomania (Sayad *et al*., 2022). The ideal entry concept provided a practical framework for selecting superior hybrids. Hybrids H1, H2, and H3 emerged as ideotypes for overall trait performance, while H12 and H9 were least favorable. For WSY, H16, H15, and H14 had the highest performance, with H15 showing both high yield and stability. Biplot analysis also effectively assessed rhizomania tolerance, identifying H16, H10, H11, H13, H14, and H15 as low-infection and stable, while H4, H5, and H6 were highly susceptible. The entry-by-tester biplot is a powerful tool to visualize complex trait interactions, identify high-performing genotypes, and prioritize traits for selection (Sabaghnia *et al*., 2015).

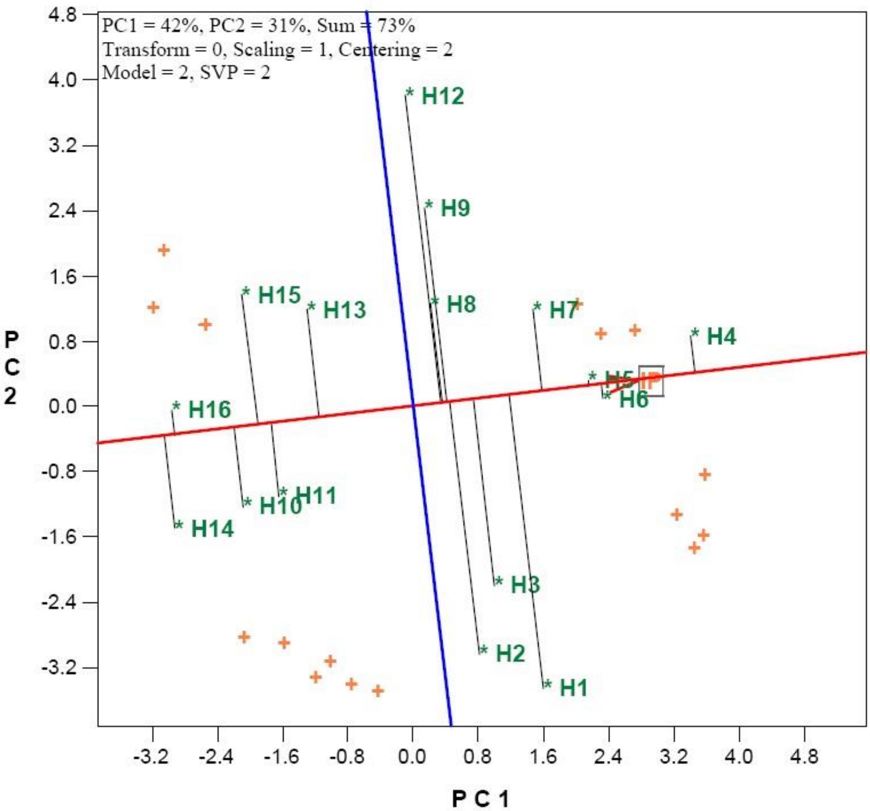


**Figure 6.** Testing the performance of/at infected plants (IP) across rhizomania disease.

Observed relationships between vegetative vigor, sugar accumulation, and disease susceptibility underscore the value of ideotype-based breeding, which simultaneously considers multiple target traits. Hybrids with high WSY and small vertical distances are both high-performing and stable, making them ideal candidates for breeding programs. Similarly, hybrids with low IP and minimal vertical variation demonstrate both resistance and stability, informing selection priorities. Vegetative traits primarily contribute to biomass accumulation and canopy development, whereas quality traits (SC, AC, WSY) directly determine extractable sugar yield. Disease traits negatively affect root quality and productivity. These results provide a practical framework for selecting superior hybrids combining yield, quality, and disease resistance in sugar beet breeding program.

## CONCLUSIONS

The evaluation of sugar beet hybrids revealed genetic variability and complex trait interactions affecting yield, sugar quality, and rhizomania tolerance. Entry-by-tester biplot effectively identified superior hybrids, with H15 and H16 demonstrating high white sugar yield, stable performance, and low disease susceptibility. Although, vegetative traits; including leaf length, leaf width, plant height, and canopy coverage, demonstrated high discriminative potential, sugar content, alkalinity coefficient, and WSY were the main factors influencing extractable sugar. However, the study was conducted in a single environment; therefore, multi-location and multi-year trials are recommended to confirm the stability of the identified hybrids.

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