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Biplot Analysis of the Morphological Variability in a Number of *Secale cereale* L. Genotypes

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Abstract

The morphological variation of 64 *Secale cereale* L. accessions was evaluated based on several traits using a lattice design with two replications over two years (2016 and 2017). The first two principal components of the entry \times tester interaction biplot accounted for 76% (46% and 30% for PC1 and PC2, respectively) of the variability across both years. Six vertex genotypes for the first year and eight vertex genotypes for the second year were identified. Important traits, such as grain yield and seed number per spike, were favorable in the sector of genotype 23, and this vertex was the same in both years. For cultivar release, genotype 23, followed by 21, 29, and 32, can be considered after being tested under multi-environmental trials for adaptability. Evaluation of grain yield indicated that genotypes 20, 21, 23, 28, 29, and 32 were the best genotypes across the two years. According to the discriminative potential of traits, seed width, awn length, spike weight, and thousand-seed weight were more discriminative. The ideal entry biplot indicated that the same genotypes identified for obtaining high grain yield had the ability to evaluate traits in *S. cereale*, and they can be assumed as ideotypes. Given the importance of seed number per spike, seed weight per spike, and seed width on grain yield in the current study, these traits should be considered in *S. cereale*. The identified variations demonstrated a high degree of diversity within *S. cereale*, for genetic improvement programs.

Keywords: Genotype by Trait Interaction, Genetic Variation, Graphic Analysis.

Introduction

Secale cereale L. is a vital cereal crop primarily grown in Europe, valued for baking and livestock feed due to its significant winter hardiness. In the past decade, it has also gained importance for biogas production. Currently, *S. cereale* is cultivated on approximately 4.4 million hectares, with global production around 13 million tons (FAOSTAT, 2022). The crop's origin is believed to be southwestern Asia, with two primary centers of origin: one extending from northwest Iran towards the Black Sea, and the other from eastern Iran towards Afghanistan. Despite its relatively recent history in cultivation, with the earliest known cultivation occurring about 4,000 years ago in regions of Iran and Turkey (Schreiber et al., 2021), *S. cereale* has become widespread. Most of *S. cereale* production worldwide is used for bread, but some is utilized for animal feed or industrial purposes like alcohol production. Although the use of *S. cereale* for bread-making has declined, other market segments, particularly those involving feed, ethanol processing, and biogas production, have expanded (Nayebi-Aghbolag et al., 2022).

For *S. cereale* breeding, an awareness of genetic diversity is valuable for enhancing the selection of genetically controlled traits in breeding lines. Phenotypic evaluations of accessions have revealed significant diversity in important traits like bread-making ability

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(Mir-Jalili et al., 2023; Sabaghnia et al., 2023). *S. cereale* exhibits wide genetic variation due to significant geographical changes across its various growing regions and its centers of origin. Extensive collections exist in numerous countries, encompassing international cultivars and landraces from different areas, with more than 22,200 accessions documented across 94 international gene banks (Schlegel, 2013). Propagation of *S. cereale* genotypes is typically conducted on experimental plots, allowing for the evaluation of around 200 genotypes annually. These evaluations focus on performance characteristics such as grain yield and quality traits. However, the viability of *S. cereale* seeds can decline during storage, primarily due to natural aging processes, leading to substantial genetic changes and decreased heterozygosity in different populations.

In poor soils, *S. cereale* stands out as the most economical crop due to its low production costs, winter hardiness, nitrogen efficiency, and yield stability. Recent breeding objectives for *S. cereale* focus on achieving optimal plant height, yield, thousand grain weight, and resistance to biotic and abiotic stresses (Hackauf et al., 2022). These goals have fluctuated yearly due to various stresses, and despite these challenges, breeding efforts have mitigated negative trends, with yield potential on poor soils being influenced by outbreeding. Effective evaluation and utilization of *S. cereale* accessions necessitate detailed knowledge of germplasms, like their phenomic profiles and categorization (Eshghi-Malayeri et al., 2015; Arabsalehi et al., 2016). Vendelbo et al. (2020) evaluated total 376 inbred lines with SNP markers and found 4,419 polymorphic SNPs, confirming the genetic separation of parental populations and validating this germplasm's suitability for hybrid breeding.

Targońska et al. (2016) examined 367 *S. cereale* accessions, representing wide geographical diversity and capturing over 93% of SSR alleles, demonstrating that the SSR markers effectively detected genetic diversity patterns. Multivariate methods are valuable for evaluating genetic resources when multiple genotypes are assessed for several morphological traits. The effectiveness of multivariate tools in characterizing and classifying morphologic diversity in genotypes has been demonstrated in many plants (Eshghi-Malayeri et al., 2015; Arabsalehi et al., 2016). This information can identify groups of genotypes with desirable traits for purposes such as crossbreeding or understanding aspects of crop evolution. However, few studies have explored the morphological variation in international *S. cereale* germplasm. The goal of the current study was to characterize the structure of diversity for ten traits of sixty-four *S. cereale* accessions from IPK's GenBank, collected from different geographical areas and categorize similar genotypes.

Materials and Methods

The 64 *S. cereale* accessions were cultivated over two years (2016 and 2017) in Maragheh. The soil was clay loam with a pH of 7.3, and plots were formed according to an 8×8 lattice scheme with two replicates, each with six rows, 2 m in length, 0.25 m row spacing, and 0.05 m within-row spacing. The whole plot size was 3.0 m², and the four central rows were harvested as 1.5 m². Sowing was performed in October, and NPK (nitrogen, phosphorus, and potassium) fertilizers were broadcast at a rate of 120:60:30 kg ha⁻¹. Irrigation was conducted at 60% of field capacity based on local practices. Ten traits of *S. cereale* were assessed, including awn length (AL), seed width (WS), seed length (LS), seed number per spike (SNS), first internode weight (FIW), spike weight (SW), weight of seeds per spike (WSS), and straw weight per spike (SWS). These traits were recorded from ten randomly selected plant samples from each plot. Grain yield (GY) was recorded from the harvested area of each plot at physiological ripening and adjusted to 12% seed humidity, expressed as kilograms per hectare. Thousand-seed weight (TSW) was recorded from three randomly chosen samples of harvested seeds.

The data was evaluated for normality using the Shapiro-Wilk test with Minitab (Minitab Ins. USA) and then analyzed through an entry by tester biplot model via the GGEBiplot application which shows graphic grasp from interaction structure of measured traits (testers) across genotypes (entries) as:

$$\frac{Y_{ij} - \bar{Y}_j}{SD_j} = \sum_{n=1}^2 \Phi_n \Psi_{in} \Omega_{jn} + R_{ij}$$

Y_{ij} is the mean of entry (genotype) i for tester (trait) j , \bar{Y}_j is the mean of genotypes for tester j , SD_j is the standard deviation of tester j for genotypes, Φ_n is the eigenvalue for PC n , Ψ_{in} and Ω_{jn} are values for entry i and trait j on PC n , R_{ij} is the error term of the fitted equation related to entry i for tester j . To obtain symmetrical scales of testers and entries, the eigenvalue is corrected through vectors' absorption, so a normal presentation of testers and entries ($\Psi_{in}^* = \sqrt{\Phi_n \Psi_{in}}$ and $\Omega_{jn}^* = \sqrt{\Phi_n \Omega_{jn}}$) is happened. The graphs of entry by tester interaction biplot method are generated by these symmetric scales, and each genotype (entry) or trait (tester) represented by a unique symbol. Thus, a graphic interpretation of the relations among genotypes and traits as well as their interaction can be provided.

Research Findings

The first and second components derived from principal component analysis, accounted for 76% of the variability across both years, whereas the first PC contributed 46%, and the second PC contributed 30% to the fitted variance. This fitted variability of the entry by tester interaction indicated the role of non-crossover and crossover types of interactions (Yan & Frégeau-Reid, 2018), meaning that ranks of *S. cereale* genotypes across traits change, which is in accordance with the report of Sabaghnia et al. (2024) in safflower, emphasizing the problem of getting an indirect reaction to selecting process across genotypes without reading the effect of the entry by tester interaction. Given the role of the entry by tester interaction in our *S. cereale* genotypes, this research benefits biplot representation facility, as a suitable procedure, because it provides the effective exploration of genotypes and traits. Fig. 1A indicates visual behavior of *S. cereale* genotypes in the first year and shows which genotypes were better than others in target traits. Six traits, consist on awn length (AL), length of seed (LS), first internode weight (FIW), spike weight (SW), straw weight of spike (SWS), and thousand-seed weight (TSW), were grouped in the section of genotype 23 while seed numbers per spike (SNS), grain yield (GY) weight of seeds per spike (WSS), and width of seed (WS), were located in a distinct section, with the genotype 19 as the winning genotype.



The other four vertex genotypes (12, 13, 22 and 44) did not perform exceptionally for any of the measured traits of *S. cereale* in the first year (Fig. 1A).

In the second year, eight vertex genotypes (12, 13, 19, 20, 22, 23, 38 and 44) were identified. Similar to the first year, genotype 23 was the best for SNS, GY, WSS, and SW; genotype 12 was the best for AL and WS; and genotype 19 was the best for LS, FIW, SWS, and TSW (Fig. 1B). Additionally, the remaining empty sections for five vertex genotypes (13, 20, 22, 38 and 44) were not excellent for any of the characters in *S. cereale* in the second year (Fig. 1B), so they were not good in the measured traits and maybe good in the unmeasured traits. It seems that grain yield performance in *S. cereale* is more related to the weight of seeds per spike and width of seed following to seed numbers per spike, instead of the other traits like hundred seed weight. Similarly, Nayeby-Aghbolag et al. (2022) found the positive relation of the weight of seeds per spike and width of seed following to seed numbers per spike on grain yield of *S. cereale*. However, the entry by tester biplot method prepared clear insights into the response of genotypes, revealing their potential across measured traits and facilitated the detection of the best genotypes. Thus, for commercial cultivar release, regarding the results of both years, genotype 23, followed by other genotypes in this section, such as 21, 29, and 32, can be considered after their testing under multi-environmental trials to ensure their adaptability and yield stability.

The distinguishing ability of a genotype for the measured traits and the potential to demonstrate typical properties based on traits for *S. cereale* genotypes can be identified by an assumed ideal genotype position (Fig. 2A). The best genotypes are those closest to this ideal position, while genotypes on the opposite side are the least desirable. In the first year, based on Fig. 2A, genotypes 21 and 29, followed by 1, 23, 28, and 32, were ideal, as they were the closest entries to the ideal position, while genotypes 5, 13, and 44 were on the other side and far from this position, making them the most undesirable genotypes regarding distinguishing ability and typical potential in the first year. According to Fig. 2B, in the second year, genotypes 21, 23, and 29, followed by 12, 24, 28, and 32, were ideal, given their proximity to the ideal position, while genotypes 5, 13, and 44 had the greatest distance from this position, making them the least desirable in terms of distinguishing and typical properties in the second year. Results over two years indicated that 21, 23, 28, 29, and 32 were the best candidates for trait evaluation in *S. cereale* and can be assumed as ideotypes. Additionally, these genotypes exhibited high values for most traits, although trait associations were not always significant. These issues are critical in *S. cereale* breeding projects, where grain yield and other components are essential. For genetic improvement efforts, strategies that incorporate multivariate tools, particularly with graphical presentations, are highly valuable for ideotype determination in *S. cereale*. Similarly, Nayeby-Aghbolag et al. (2022) utilized the ideal genotype concept in their study of genetic diversity in 22 *S. cereale* genotypes, identifying and introducing two top-performing genotypes (G9 and G22) as commercial varieties based on agronomic and morphological traits. They also noted that the entry-by-tester biplot method greatly enhances data analysis efficiency.

The responses of *S. cereale* genotypes in terms of grain yield as a final target trait were evaluated by analyzing their yield response (GY), where a horizontal axis was defined as the line of GY with an arrow indicating the axis direction. Genotypes 23, 29, and 32, followed by 20, 21, and 28, were the most desirable genotypes regarding yield performance, while genotype 19 was the least desirable in GY during the first year (Fig. 3A). The distance from the horizontal axis indicates the standard deviation: low values are preferable for selection, so the identified top genotypes can be recommended with greater confidence due to their low variability. These traits can therefore be included in selection indices of breeding programs, resulting in notable genetic gains. In the second year (Fig. 3B), the same genotypes from the first year (20, 21, 23, 28, 29, and 32) remained the most favorable in terms of grain yield. Their low distance from the horizontal axis made them desirable selections due to their low variation. Genotypes 13 and 19 had yields even lower than average (relative to the vertical axis) and greater distances from the horizontal axis, indicating high variability and making them the least desirable genotypes across both years. Consequently, genotypes 20 (Kustro from Germany), 21 (Chrysanth from Austria), 23 (unnamed from Slovakia), 28 (M 8a from Poland), 29 (DSV from Poland), and 32 (Fin K/63 from Finland) can be recommended for cultivar release after verification in multi-environment trials to confirm their stability and adaptability. Notably, traits such as seed numbers per spike, thousand-seed weight, and plant density per area are critical for improving grain yield in cereals. Therefore, developing breeding procedures aimed at yield increase should focus on these traits (Sirat, 2023). The high yield component values of these genotypes and the process of identifying desirable genotypes observed in this study through the entry-by-tester biplot method align with findings in other crops, such as wheat (Sabaghnia & Janmohammadi, 2014), and safflower (Ebrahimi et al., 2023).

The discriminative potential of a trait is the magnitude of standard deviation, so the higher discriminative potential is assumed an assumptive position as ideal location of traits (Fig. 4A), so the best traits are close to this position; but traits of the other side are the worst. Thus, it can be proposed that width of seed (WS) following to awn length (AL), spike weight (SW), and first internode weight (FIW), thousand-seed weight (TSW) are more discriminative traits in the first year. However, the discriminative potential of the other remained traits of *S. cereale* were higher than average of the discriminative potential, so they can discriminate the differences among *S. cereale* genotypes (Fig. 3A). Similarly, Yari et al. (2018) examined 18 *S. cereale* genotypes with the entry by tester biplot and found that grain yield following to number of spikes were the best trait for discriminative potential as well as typical ability. Also, the typical potential a trait is measured by the its angle with the axis of the average trait, and the small angle demonstrate the more potential of the trait, so they had a small angle with this axis and have more typical potential. In contrast some traits like seed numbers per spike (SNS) had large angles with the axis of the average trait and indicated relatively lower typical potential in the first year (Fig. 3A). In the second year (Fig. 3B), width of seed (WS) and awn length (AL), following to spike weight (SW) and thousand-seed weight (TSW) are more discriminative traits and this potential of the other remained traits were higher than average, so they can discriminate the differences among *S. cereale* genotypes. Also, these traits had a small angle with horizontal axis, so they had more typical potential while similar to results of the first year, seed numbers per spike (SNS) indicated large angles with this axis and indicated relatively lower typical potential (Fig. 3B).

Various studies have explored the variability of crop landraces and genotypes using morphological and molecular markers (Sabaghnia et al., 2024), but such comprehensive investigations, particularly focused on morphological traits, have been limited for *S. cereale* germplasm. Most research has highlighted significant variation in measured traits among accessions from different geographical regions, and our findings corroborate this, showing substantial variation in *S. cereale* plant materials across various traits.

In our study, ten traits were used to assess variability in *S. cereale* landraces, revealing notable differences in grain yield performance, yield components and agronomic traits which suggests that the *S. cereale* germplasm possesses significant potential for enhancing yield, making it valuable for breeding programs focused on yield improvement. According to Kottmann (2015), reductions in the number of spikes and seed number per spike decreases grain yield, underscore the strong impact of these yield components on *S. cereale* yield. Slafer et al. (2014) note that thousand-seed weight primarily serves as a fine regulator of seed yield in cereals, with a large seed number being crucial for high yield. Our study found high variability in yield components, providing a promising basis for breeders. The greater variability in these traits offers significant opportunities for developing high-yielding *S. cereale* cultivars through selective breeding in segregating generations.

The identified *S. cereale* genotypes, distinguished by their potential yield components and notable genetic divergence, present significant opportunities for breeding through crossing and selecting. Thus, there is substantial potential to exploit genetic variation. It is important to recognize, however, that traits of each group might be influenced by the presence of accessions within each section (Sabaghnia et al., 2023). Therefore, while selecting candidate accessions for hybridization, it is advantageous to consider both the inter-group distance and the amount of divergence. The behavior of the first and the second principal components has been proved in multi-environmental trials while it may be change in other two-way datasets, thus Yan & Frégeau-Reid (2018), for overcoming this problem, another model, which is used in this investigation, was suggested, in which the first principal component is substituted by predication of the linear regression model of the tester-centered data on the genotype effects so that the original scores are the genotype effects and make the model more explicable. For understanding of variation and improve classification, molecular markers should be employed to provide deeper insights into the genetic differences among accessions and to design effective conservation strategies.

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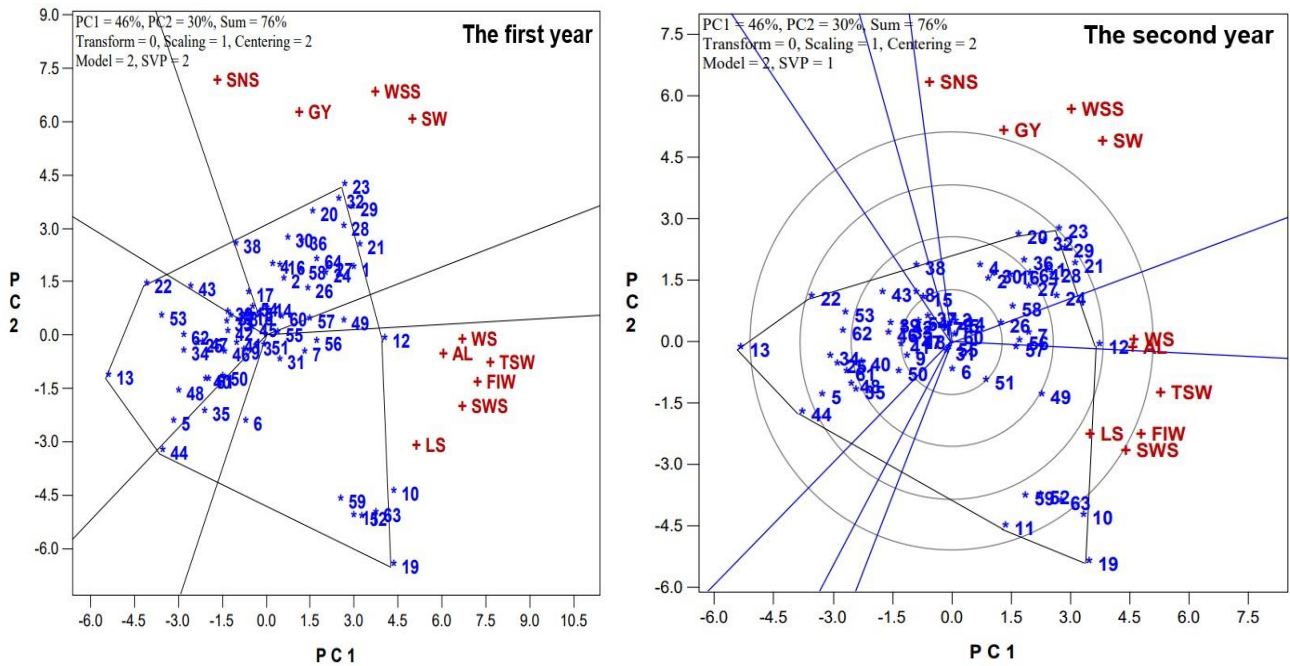


Fig 1: Polygon-view of entry (genotype) by tester (trait) biplot showing the "which-won-where" structure of 10 traits and 64 *Secale cereale* L. genotypes: A in left side for the first year and B in right side for the second year. Traits are: awn length (AL), width of seed (WS), length of seed (LS), seed numbers per spike (SNS), first internode weight (FIW), spike weight (SW), weight of seeds per spike (WSS), straw weight of spike (SWS), grain yield (GY) and thousand-seed weight (TSW).

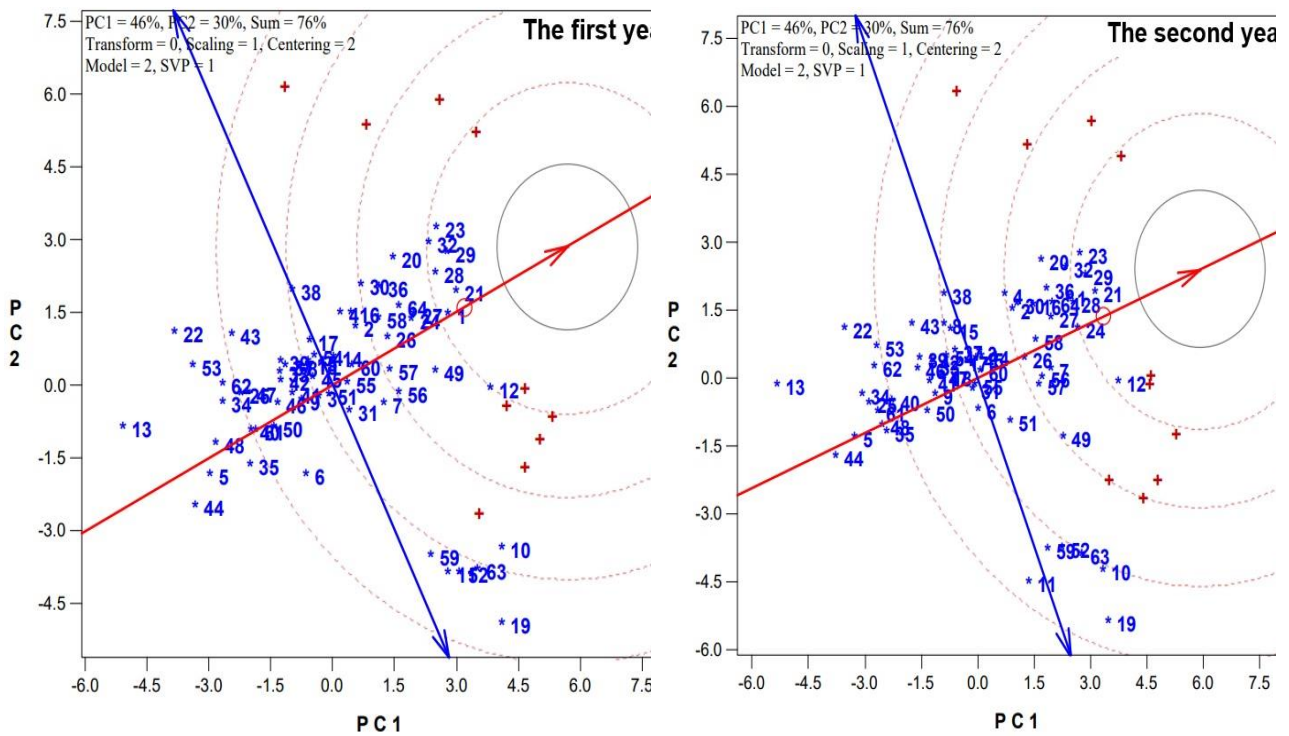


Fig 2: Ideal genotype-view of entry (genotype) by tester (trait) biplot showing the best genotypes of 64 *Secale cereale* L. genotypes: A in left side) for the first year and B in right side) for the second year.

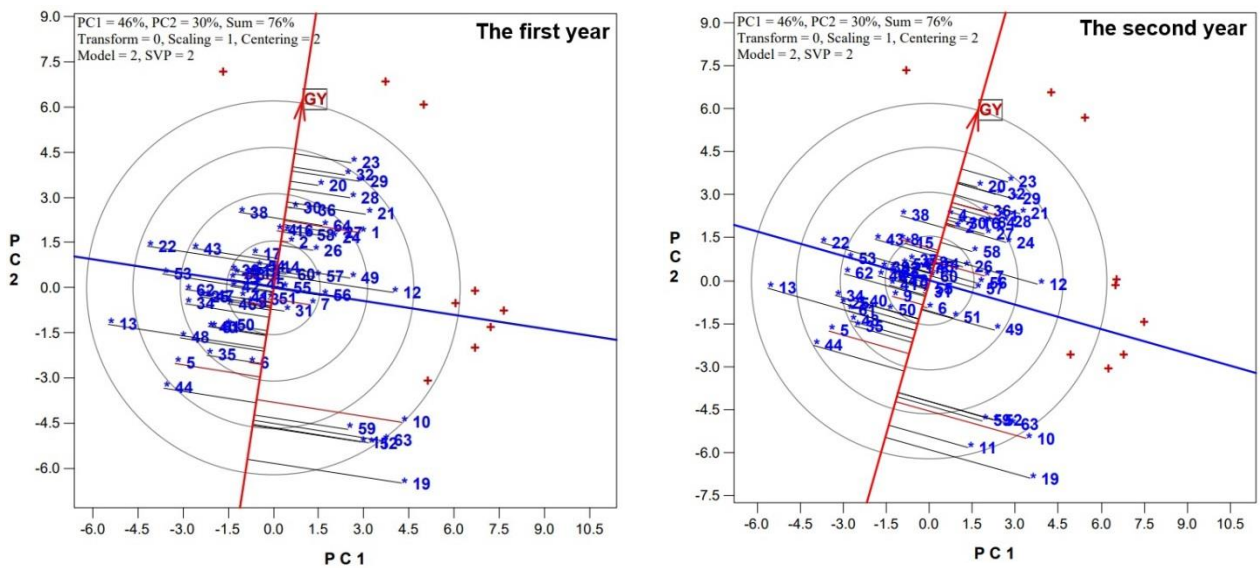


Fig 3: The entry (genotype) by tester (trait) biplot showing the ranking of 64 *Secale cereale* L. genotypes based on grain yield (GY): A in left side) for the first year and B in right side) for the second year.

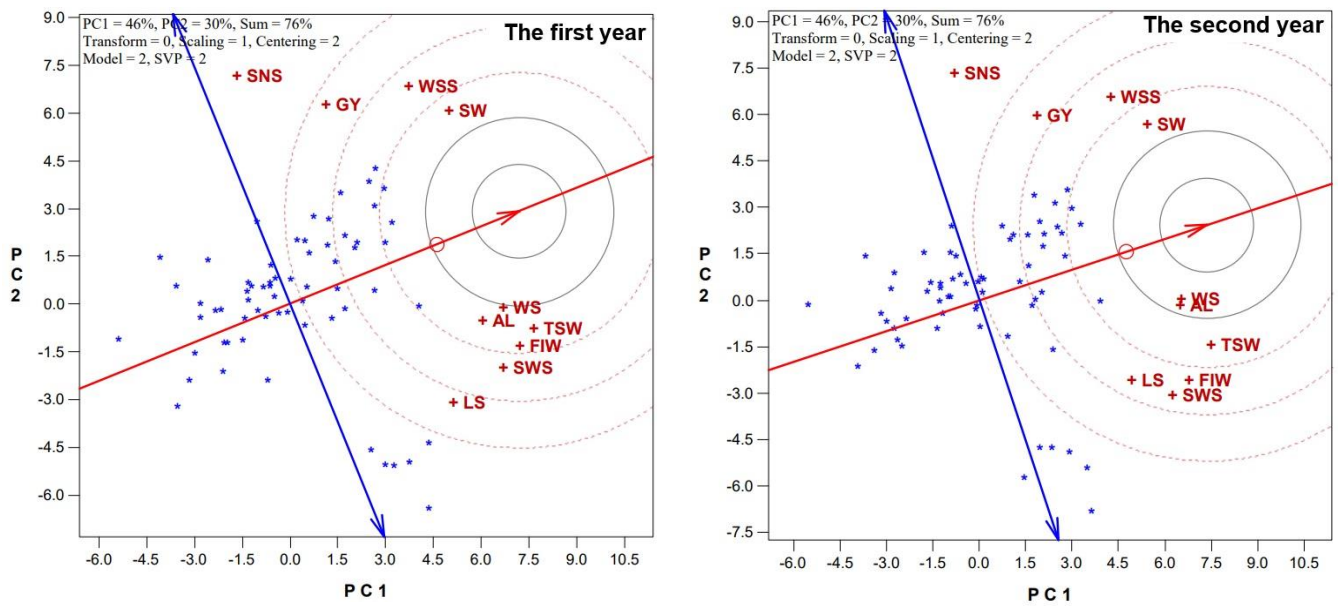


Fig 4: Ideal trait-view of entry (genotype) by tester (trait) biplot showing the best destinating traits of 64 *Secale cereale* L. genotypes: A in left side) for the first year and B in right side) for the second year. Traits are: awn length (AL), width of seed (WS), length of seed (LS), seed numbers per spike (SNS), first internode weight (FIW), spike weight (SW), weight of seeds per spike (WSS), straw weight of spike (SWS), grain yield (GY) and thousand-seed weight (TSW).



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