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Application of genotype by yield* trait biplot approach for selection of winter barley varieties

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Abstract

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The aim of the study was to use the genotype by yield trait (GYT) biplot method to select superior winter barley varieties, based on yield-trait combinations under rain-fed conditions. In the experimental field of the Institute of Agriculture – Karnobat, Bulgaia, 17 varieties of winter barley were evaluated in a complete block design with four replications in three growing seasons (2019/2019, 2019/2020, and 2020/2021). Based on the GT biplot and Pearson's correlations, a positive correlation of grain yield with lodging resistance and the number of spikes per 1 m² and a negative correlation of grain yield and plant height and 1000-grain weight were found. The result of polygon view of GYT biplot showed that varieties IZ Bori (G4), Casino (G6), and Zemela (G17) had the high levels of most of the studied yield by trait combinations. The Average Tester Coordination view of the GYT biplot identified as the best-ranked varieties Casino (G6), IZ Bori (G4) followed by Zemela (G17), and Bojin (G3). The results of the present study showed that the GYT biplot approach can help visual identification of the genotypes with the best combination of yield with other traits and could improve the genotype selection, based on multiple traits in the feed barley breeding program.

Keywords: barley; GYT biplot; yield-trait combinations

Introduction

Barley (*Hordeum vulgare* L.), is mainly used for animal feed and malt production and it has been the fourth most produced cereal crop after corn, wheat, and rice in the world. Therefore, it is essential to develop high-yielding varieties with the appropriate grain quality for the particular grain end-use. In Bulgaria, the barley is cultivated exceptionally under rain-fed conditions so the development of varieties with high and stable yield and tolerance to abiotic stresses is one of the main challenges for barley breeding.

Improvement of a single trait, disregarding all others, is relatively simple nevertheless, this is unlikely to result in developing a useful new variety. Simultaneous improvement of all traits of interest is one of the main challenges of plant breeding. This task is particularly difficult, when there are negative correlations between different traits, which may be due to physical linkage between genes in the chromosomes, to pleiotropic effects of genes, or to population genetic structure (Hartl & Clark, 1997).

According to Yan et al. (2019), the economic value of any trait in a genotype depends upon the level of the main target trait i.e., yield. For example, lodging resistance has economic value, only when it is coupled with high grain yield. Similarly, a genotype possessing a high level of quality traits, but with poor grain yield has low economic value due to the fact that this genotype cannot be recommended as a variety.

Therefore, the main purpose of multiple trait selection is to pyramid the desirable traits with higher grain yield in a single genotype. Various breeding approaches allowing the selection of genotypes with optimal combination between grain yield with other desirable agronomic traits, under favorable environmental conditions, as well under different biotic and abiotic stresses have been developed. Methods, such as independent culling (Godshalk et al., 1988), tandem selection (Simmonds & Smartt, 1999), and index selection (Bos & Caligari, 2008), are routinely used in breeding programs for multiple trait selection. Nevertheless, all these approaches are comparatively subjective, because certain weights, or truncation points are given to each trait depending on the breeder and the economic importance of the trait (Yan &Frégeau-Reid, 2018).

Recently, Yan & Fregeau-Reid (2018) proposed the genotype-by-yield trait (GYT) biplot approach for the selection of superior genotypes based on multiple traits. The GYT biplot ranks genotypes, based on the combination of yield with other target traits, such as grain quality, disease resistance, and other agronomical important traits. The several views of GYT biplot enable not only the ranking of genotypes, according to the superiority of yield trait combinations, but also the present the trait profile of each genotype to reveal its weaknesses and strength (Yan et al., 2019).

After the introduction of the GYT biplot method (Yan & Fregeau-Reid 2018), this approach was applied for genotype selection, based on multiple traits in different cereal crops, such as bread wheat (Hamid et al., 2019; Merrick et al., 2020), durum wheat (Kendal, 2019; Mohammadi, 2019; Faheem et al., 2022), and barley (Kendal, 2020; Karahan & Akgun, 2020; Hudzenko et al., 2021; Bakhshi & Shahmoradi, 2022).

The aim of this study was to use the genotype by yield \times trait (GYT) biplot method to select superior winter barley varieties, based on yield-trait combinations under rain-fed conditions.

Materials and Methods

Experimental site location

The study was conducted during the period of three concessive years from 2018/2019 to 2020/2021, in the experimental field of the Institute of Agriculture – Karnobat, Southeastern Bulgaria. The soil of the experimental field was slightly acid (pH is 6.2) Pellic Vertisol.

Average temperatures and sums of precipitation during the experimental period are shown in Table 1. The sum of precipitation for the vegetation period, compared to the longterm average precipitation for the location was lower in the first two growing seasons (with 56.8 mm in the first year and with 120.5 mm in the second year) and higher in the third growing season (with 197.4 mm). The average temperatures of the three growing seasons were higher than the long-term average monthly temperatures.

Experimental Materials

Seventeen six-rowed varieties of winter barley were used in this study. Varieties Bojin, IZ Bori, Izgrev, Vaslets, Zemela are developed at the Institute of Agriculture – Karnobat, Bulgaria. Varieties Casino ("KWS"), Giga ("KWS"), and Paso ("Limagrain") were offered to Bulgarian producers in recent years. The rest 9 varieties originate from different European countries: Attiki and Banteng – France; Colonia, Monika, and Dea – Germany; Noveta and Videt – Netherland; Hampus – Sweden; Brucker Vielzeilige – Austria.

Experimental Design

The experiments were organized in a Complete Block Design with 4 replications on plots of 10 m² with sowing rate 450 seeds/m². During the growing seasons, standard plant protection practices were used.

Months	2018	/2019	2019/2020		2020/2021		LT	
	T, °C	P, mm	T, ℃	P, mm	T, °C	P, mm	T, ℃	P, mm
Х	14.0	15.4	14.9	19.3	15.8	70.7	12.5	44.3
XI	7.4	68.3	12.4	53.8	7.4	25.5	7.1	53.7
XII	2.4	27.3	4.7	9.6	6.6	94.5	2.6	51.2
Ι	2.5	38.9	2.5	13.1	3.7	142.8	0.6	36.5
II	4.3	15.6	5.7	33.0	4.9	22.1	2.2	35.8
III	8.6	8.9	8.4	29.6	4.9	47.4	5.3	34.1
IV	10.3	52.9	10.4	19.5	9.5	86.0	10.5	45.3
V	17.1	44.9	16.3	54.3	16.6	15.6	15.6	58.5
VI	22.6	95.6	20.7	71.9	19.3	117.4	19.6	65.2
T, °C	9.9		10.7		9.9		8.4	
P, mm		367.8		304.1		622.0		424.6

Table 1. Average air temperature, monthly sums of precipitation and long-term average data in Karnobat, Southeastern Bulgaria during (2018/2019–2020/2021)

T - average air temperature; P - sum of precipitation; LT - long-term average air temperature, or sum of precipitation (1931-2022)

Data Collection

Days to heading (DH), the number of spikes per 1 m^2 , plant height, cm (PH), lodging score (L, scale 9 – 1, where 9 = no lodging and 1 = 100% lodging) and grain yield, t/ha (Y) were determined on plot bases.

1000-grains weight, g (TGW) and protein content, % (P, Kjeldahl method) were analyzed. Total carbohydrate content (TC) of grain was estimated as follow: TC = Moister + Ash + Fat + Fiber + Protein - 100 (Sakhawat et al., 2014).

Statistical analysis

Data were graphically analyzed by the genotype × trait (GT) biplot method according to Yan & Thinker (2005), and the genotype × yield × trait (GYT) biplot according to Yan & Frégeau-Reid (2018). A superiority index (SI) combining all yield-trait interactions was calculated based on the standard-ized GYT data (Yan & Frégeau-Reid, 2018).

The GYT table was standardized so that the mean for each trait or yield-trait combination was 0 and the variance a unit. The GT and GYT biplots were carried out in RStudio, R version 4.2.1 by using "metan" R package (Olivoto & Lúcio, 2020).

Results and Discussion

Genotype by trait (GT) biplot

The means of studied trait across three growing seasons of 17 barley varieties are presented in Table 2. Comparison of average grain yield of varieties for three years showed that genotypes IZ Bori (G4), Casino (G6), Zemela (G17), and Bojin (G3) had the highest grain yield 4.43, 4.41, 4.35 and 4.33 t/ha, respectively. Higher protein content was found in Monika (G12) – 12.97%, Hampus (G10) – 12.74%, and Izgrev (G11) – 12.65%. The highest 1000-grain weight was found in varieties B. Vielzeilige (G5) – 43.66 g, Attiki (G1) – 42.52 g, and Gigga (G9) – 41.13 g.

The correlation coefficients among these traits are shown in Table 3. The GT biplot (Figure 1) is a graphical display of GT data included in Table 2 and Table 3. The GT biplot visually demonstrates the associations among traits and the trait profiles of the genotypes. According to Yan & Frégeau-Reid (2018) in such a biplot, the cosine of the angle between the vectors of two traits approximately corresponds to the Pearson correlation between them. An angle smaller than 90° indicates a positive correlation, an angle greater than 90° indicates a negative correlation, and an angle of 90° indicates zero correlation. The angle between a genotype and a trait indicates the relative level of the genotype for this trait. Hence, an acute angle indicates that the genotype is above-average for the trait, an obtuse angle indicates that the genotype is below-average for the trait, and a right angle indicates that the genotype is average for the trait. The vector length of a trait indicates how well the trait is represented in the biplot. A relatively short vector indicates that the variation of the trait across genotypes is either small, or not well presented in the biplot, which is due to its weak, or lack of correlation with

Table 2. The means of studied trait of 17 winter barley varieties (2018/2019 – 2020/2021)

Nº	Variety	Y	DH	SN	PH	L	Р	TC	TGW
G1	Attiki	2.54	196.00	448.00	84.00	6.33	12.13	70.32	42.52
G2	Banteng	3.10	196.33	520.00	86.67	7.67	12.46	68.96	38.35
G3	Bojin	4.33	195.33	617.33	61.67	8.33	11.97	68.72	34.89
G4	IZ Bori	4.43	195.67	630.67	69.00	8.33	12.43	69.31	35.38
G5	B. Vielzeilige	3.39	196.67	429.33	93.33	6.67	12.04	69.44	43.66
G6	Casino	4.41	195.00	640.00	65.00	8.67	12.28	69.30	40.06
G7	Colonia	3.00	195.00	498.67	72.67	7.67	11.48	70.05	37.96
G8	Dea	3.57	197.00	654.67	76.67	8.67	12.38	68.56	36.26
G9	Gigga	3.59	197.67	442.67	65.67	8.33	12.11	68.32	41.13
G10	Hampus	3.52	197.00	504.00	73.33	8.67	12.74	68.56	40.29
G11	Izgrev	3.69	195.00	480.00	73.33	8.67	12.65	68.22	38.20
G12	Monika	3.96	198.67	493.33	81.67	8.00	12.97	67.50	40.73
G13	Noverta	3.73	199.00	565.33	78.33	8.67	12.39	69.12	39.25
G14	Paso	3.80	195.67	618.67	62.67	8.33	11.94	69.61	38.59
G15	Vaslets	3.68	194.33	601.33	66.67	8.67	12.30	68.10	39.40
G16	Videt	2.90	197.67	373.33	85.00	6.67	12.55	68.34	39.78
G17	Zemela	4.35	196.00	644.00	70.33	8.67	11.97	68.93	35.25

Y - grain yield, DH - days to heading, $SN - number of spikes per 1 m^2$, PH - plant height, L - lodging score, P - protein content, TC - total carbohydrate content, TGW - 1000-grain weight



Fig. 1. Genotype by trait (GT) biplot based on the original genotype by trait data

other traits. The vector length of a genotype shows whether it has clear strengths and/or weaknesses in its trait profile.

Accordingly, grain yield (Y) was positively correlated with lodging resistance (L) and the number of spikes per 1 m^2 (SN) and negatively correlated with plant height (PH) and 1000-grain weight (TGW). Protein content had a negative association with total carbohydrate content, but it was not strongly associated with other traits. 1000-grain weight showed a negative correlation with grain yield, the number of spikes per 1 m^2 , and lodging resistance, which indicated that a high 1000-grain weight was poorly combined with other breeding objectives in the studied set of varieties.

The GT biplot had a relatively high goodness of fit (73.86%) and the found associations between traits were generally in agreement with estimated Pearson's correlation coefficients (Table 3).

The display of genotypes on the GT biplot shows the trait profiles of the varieties. Variety Monika (G12) had high

protein content and low content of total carbohydrates and variety Colonia (G7) was characterized by high total carbohydrates and low protein content. Variety Attiki (G1) had a high 1000-grain grain weight and high content of total carbohydrates. Also, Attiki (G1) had a trait profile similar to variety B. Vielzeilige (G5) while Paso (G14) had a trait profile rather opposite to that of Videt (G16).

Genotype-by-yield trait (GYT) biplot

The GYT data was generated by the combination of each trait and grain yield (Table 2), as developed by Yan & Frégeau-Reid (2018). In GYT biplot analysis, the breeder has the responsibility to identify if a trait has a positive, or negative influence over variety performance, and to decide if grain yield should be multiplied, or divided by a specific trait. In this way, for a particular trait, a positive selection in the case of multiplying and a negative selection in the case of dividing were applied. Therefore, the selection results in GYT biplot analysis depend strongly on the traits that are included. According to Yan & Frégeau-Reid (2018), it is advisable to include GYT biplot analysis only those traits that are essential for the success of a variety. All included in our study varieties were six-rowed and in Bulgaria six-rowed barley is used mainly for feed. Grain yield is undoubled the most important trait for feed barley. Another important trait is lodging resistance, because lodging still is an actual problem, especially for six-rowed barley in agro-ecological conditions in Southeastern Bulgaria. From grain quality traits of particular interest are protein and carbohydrate content, and 1000-grain weight, when barley grain is used for feed. Therefore, the GYT table was obtained as follows: for the number of spikes per 1 m², plant height, lodging score, protein content, total carbohydrate content, and 1000-grain weight the values for each trait was multiplied (*) with the yield. While for traits of days to heading and plant height, in which the high values are undesirable, the value for each trait was divided (/) by the yield. The GYT biplot graphically displays the GYT data (Table 4), and the different views of the

Table 3. Pearson correlations between traits of 17 six-rowed varieties of winter barley

Trait	DH	SN	PH	L	Р	TC	TGW
Y	-0.140	0.722**	-0.626**	0.710**	0.082	-0.280	-0.533*
DH		-0.393	0.451	-0.166	0.432	-0.297	0.336
SN			-0.625**	0.706**	-0.166	0.068	-0.693**
PH				-0.694**	0.284	0.063	0.521*
L					0.159	-0.399	-0.544*
Р						-0.683**	0.155
TC							0.077

*, **: significant at the 0.01 and 0.05 level, respectively; Y – grain yield, DH – days to heading, SN – number of spikes per 1 m², PH – plant height, L – lodging score, P – protein content, TC – total carbohydrate content, TGW – 1000-grain weight

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No	Variety	Y/DH	Y*SN	Y/PH	Y*L	Y*P	Y*TC	Y*TGW	SI
G1	Attiki	-1.97	-1.50	-1.70	-2.11	-1.93	-1.93	-1.80	-1.85
G2	Banteng	-0.97	-0.67	-1.24	-0.90	-0.99	-0.99	-1.21	-0.99
G3	Bojin	1.25	1.17	1.63	0.95	1.23	1.23	0.53	1.14
G4	IZ Bori	1.39	1.39	1.14	1.08	1.49	1.49	0.84	1.26
G5	B. Vielzeilige	-0.48	-0.95	-1.19	-1.07	-0.41	-0.41	0.38	-0.59
G6	Casino	1.40	1.44	1.44	1.33	1.45	1.45	1.92	1.49
G7	Colonia	-1.11	-0.87	-0.78	-1.01	-1.08	-1.08	-1.48	-1.06
G8	Dea	-0.17	0.59	-0.34	0.20	-0.18	-0.18	-0.65	-0.10
G9	Gigga	-0.16	-0.71	0.34	0.01	-0.15	-0.15	0.35	-0.07
G10	Hampus	-0.26	-0.39	-0.22	0.13	-0.27	-0.27	0.02	-0.18
G11	Izgrev	0.11	-0.40	-0.03	0.36	0.01	0.01	-0.02	0.01
G12	Monika	0.45	-0.08	-0.18	0.30	0.42	0.42	1.08	0.34
G13	Noverta	0.04	0.19	-0.25	0.41	0.17	0.17	0.27	0.14
G14	Paso	0.28	0.62	0.84	0.28	0.36	0.36	0.30	0.43
G15	Vaslets	0.13	0.37	0.38	0.36	-0.01	-0.01	0.21	0.20
G16	Videt	-1.38	-1.60	-1.38	-1.58	-1.41	-1.41	-1.40	-1.45
G17	Zemela	1.25	1.40	0.94	1.25	1.30	1.30	0.66	1.16
	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	St.deviation	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 4. Standardized genotype by yield*trait (GYT) data and superiority index (SI) for the genotypes

Y - grain yield, DH - days to heading, $SN - number of spikes per 1 m^2$, PH - plant height, L - lodging score, P - protein content, TC - total carbohydrate content, TGW - 1000-grain weight

GYT biplot (Figures 2, 3 and 4) allow the data to be investigated from different angles. The GYT biplot represented 97.03% of total variation.

Associations among yield-trait combinations

Due to the fact that all yield-trait combinations in the GYT biplot included grain yield as a component, most of them were positively associated, as exhibited by the acute angles in the biplot (Figure 2). Nevertheless, some of the strong associations between traits observed in the GT biplot (Figure 1), such as the negative correlation between 1000-grain weight and the number of spikes per 1 m², plant height, and lodging score can still be seen in the GYT biplot, as shown by the magnitudes of angles among between Y*TGW and Y*SN, Y*L and Y/PH (Figure 2).

Trait profiles of the genotypes

Figure 3 is the polygon view, or "which-won-where" of yield-traits combinations and genotypes. This view of GYT biplot is used to highlight genotypes with the best performance in combining yield and other traits. Hence, variety Bojin (G3), had the largest value for Y/PH meaning that this variety has the best combination of high grain yield and short stem. Varieties IZ Bori (G4), Casino (G6), and Zemela (G17) had high levels of most of the studied yield by trait combinations.



Fig. 2. The Tester Vector view of the genotype by yield*trait (GYT) biplot

Superiority rank of the genotypes based on their yieldtrait combinations

The Average Tester Coordination view of the genotype by yield*trait biplot (Figure 4), ranks the genotypes based on their overall superiority or usefulness. The average tes-



Fig. 3. The which-won-where view of the genotype by yield*trait (GYT) biplot

ter axis (ATA), separates genotypes of better than average (placed on its right), from those poorer than average (placed on the left side) (Yan & Frégeau-Reid, 2018). The bestranked varieties based on the yield-trait combinations were Casino (G6), IZ Bori (G4), followed by Zemela (G17), and Bojin (G3). While varieties Attiki (G1), Videt (G16), Colonia (G7), and Banteng (G2) placed on the far-left side of the biplot were ranked as the worst.

The strengths and weaknesses of each variety can be evaluated also by examining the data in Table 4 and the su-



Fig. 4. The Average Tester Coordination view of the genotype by yield*trait (GYT) biplot

periority index (SI) for ranking the varieties as it integrates all yield-trait combinations. However, according to Yan & Frégeau-Reid (2018), the GYT biplot is much more informative than the GYT table.

The genotypes placed close to ATA tend to have balanced trait profiles, whereas those placed away from the ATA in either direction tend to have obvious strengths and/ or weaknesses (Yan & Frégeau-Reid, 2018). Thus, IZ Bori (G4), Paso (G14), Noverta (G13), and Veslets (G15) were balanced for the studied traits. While Monika (G12) had high TGW, but a relatively height stem and low spike number per 1 m². Moreover, varieties placed above the ATA (IZ Bori, Zemela, Bojin, Paso, and Vaslets) have relatively high spike number per 1m², short plant stem, and good lodging resistance, and the opposite is true for varieties placed below the ATA. Varieties positioned below the ATA (Casino, Monika, and Noverta) were characterized with a better combination of grain yield and studied grain quality traits as 1000-grains weight, g (TGW), protein content (P), and total carbohydrate content (TC).

The results, obtained in the present study demonstrate the utility of the GT biplot method for revealing associations between traits and trait profiles of genotypes. However, it was difficult to select genotypes with the best combination of desirable traits based on the GT biplot alone. The genotype by yield*trait (GYT) biplot approach proved to be more useful for this purpose. This method identified the strengths and weaknesses of each variety. Superiority index (SI) also allowed accurate assessment and ranking of genotypes according their performance base on multiple traits.

GYT biplot analysis prioritize the yield relative to other breeding targets. Indeed, other traits as grain quality traits, or improved tolerance to abiotic and biotic stresses are valuable to producers, only when they are combined with sufficiently good yield levels. Thus, magnitudes of yield-trait combinations are more significant than levels of individual traits in the selection of superior varieties, but this is not always true for the selection of parents for hybridization.

The GYT biplot approach allows the selection of high-yielding barley genotypes with good agronomic characteristics and it may be used to assist the development of new barley varieties with high economic value for the rainfed conditions of Bulgaria.

Conclusions

GT and GYT biplot captured 76.61 and 99.08% of the total variation, respectively. The associations between traits found by GT biplot analysis were generally in agreement with estimated Pearson's correlation coefficients and

revealed a positive correlation of grain yield with lodging resistance and the number of spikes per 1 m² and a negative correlation of grain yield and plant height and 1000-grain weight. The best-ranked varieties based on the yield-trait combinations were Casino, IZ Bori, Zemela, and Bojin. The results of the present study showed that the GYT biplot approach can help visual identification of the best genotypes in combining yield with other traits and could improve the genotype selection, based on multiple traits in the feed barley breeding program.

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