

## **Genotype by yield × trait (GYT) biplot analysis for the identification of the superior winter and facultative barley breeding lines**

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**Abstract.** In the present study, in a panel of promising winter and facultative barley (*Hordeum vulgare* L.) breeding lines, the peculiarities of yield performance and its combination with resistance (tolerance) to the most common under conditions of the Ukrainian Forest-Steppe abiotic and biotic stresses have been determined. In 2016–17–2018–19 the breeding lines were differentiated based on grain yield, thousand kernel weight, frost resistance, leakage of electrolytes, relative drought tolerance, lodging resistance, and resistance to powdery mildew (caused by *Blumeria graminis* (DC.) E. O. Speer, f. sp. *hordei* emend. É. J. Marchal (anamorph *Oidium monilioides* Link)), spot blotch (caused by *Cochliobolus sativus* (anamorph *Bipolaris sorokiniana* [Sacc.] Shoem.)), and leaf rust (caused by *Puccinia hordei* Otth.). GYT (genotype by yield × trait) biplot model was used for comprehensive evaluation of the breeding lines by a combination of yield with a complex of traits. As a result, the winter breeding line ‘Pallidum 5096’ and facultative breeding line ‘Pallidum 5110’ superior to others in terms of yield × traits combinations have been identified. These breeding lines as new varieties ‘MIP Atlas’ and ‘MIP Yanus’ accordingly have been submitted to the Ukrainian Institute for Plant Variety Examination

for further State Qualification Examination. The winter ('Pallidum 5134', 'Pallidum 5097', 'Pallidum 5024', 'Pallidum 5090', and 'Pallidum 5130') and facultative ('Pallidum 5153', 'Pallidum 5102', 'Pallidum 5126', and 'Pallidum 5131') breeding lines can be used as valuable genetic sources in breeding programs in Ukraine and some other Central and Eastern European countries.

**Key words:** *Hordeum vulgare* L., frost resistance, disease resistance, drought tolerance, yield × trait combination.

## INTRODUCTION

Barley (*Hordeum vulgare* L.) is an important food and feed crop. Ukraine is one of the leading producers and exporters of barley grain in the world (FAOstat, 2023). In recent years, significant changes have taken place in Ukraine both in the total barley area and in the ratio of autumn and spring sowings (Demydov et al., 2016a). Until recently, spring barley occupied the main part of the barley area in Ukraine. Currently, the winter barley area has significantly increased, while spring area has decreased. One of the prerequisites for the extension of winter barley sowings is the softening the weather conditions during winter period in the central and northern regions of Ukraine. Along with that, winter barley is characterized with a number of biological and agronomic advantages as compared with other grain crops (Linchevskyi & Legkun, 2020). For example, if winter barley overwintered well enough, it forms as a rule higher yield than spring barley, especially in years with severe early spring drought. Winter barley ripens 10–14 days earlier than winter wheat (*Triticum aestivum* L.) and spring barley, which makes it possible to reduce the pressure on the harvesting machinery during the harvest and to receive early funds for further harvesting in advance. Due to the early harvest, winter barley also is a good forecrop for the following crops. In turn, winter barley can be sown later as compared with winter wheat, which makes it more matches to forecrops harvested late (sunflower (*Heliantus annuus* L.), maize (*Zea mays* L.), soybean (*Glycine max* L)). The prospect of developing facultative barley varieties, especially in circumstances of climatic change have also been highlighted (Muñoz-Amatriaín et al., 2020). The advantage of facultative barley is the possibility of transferring sowing to the early spring period, if the conditions in the autumn sowing dates were extremely unfavorable. Facultative growth habit gives the opportunity to quickly restore the seed production of the variety by spring sowing in the same year, if the autumn crop was winter killed. As a rule, facultative barley varieties restore the spring vegetation a little bit earlier than winter ones and thereby can more effectively use the soil moisture reserves accumulated during the winter.

Barley growth habit (winter, spring and facultative) is largely determined by resistance to low temperatures, vernalization requirement and photoperiod sensitivity (Hill & Li, 2016; Fernández-Calleja et al., 2022). Winter barley varieties can differ among themselves by the level of frost resistance and photoperiod sensitivity, but necessarily require vernalization for earing (Monteagudo et al., 2019; Fernández-Calleja et al., 2021; Ochagavía et al., 2022). Facultative genotypes do not have vernalization requirement and can have different frost resistance (Cockram et al., 2015). However, for successful overwintering, facultative genotypes must be sensitive to short day. In turn,

spring barley genotypes have no frost resistance and vernalization requirement, but may differ in response to photoperiod.

In spite of the growth habit, one of the main characteristics of a commercial barley variety is grain yield. Yield is a complex trait, the manifestation level of which is determined by the genetic potential of the variety and its realization in the interaction with a number of environmental and anthropogenic factors (Bailey-Serres et al., 2019; Panfilova et al., 2019; Heil et al., 2021). Thus, a modern barley variety should combine a high yield potential with resistance (tolerance) to the most common stresses under certain environmental conditions (Panfilova et al., 2020; Sadok et al., 2022). As a result of a long-term research, carried out on different genetic material, the main unfavorable factors which the most affect winter barley yield formation under conditions of Ukrainian Forest Steppe have been determined (Gudzenko & Vasylykivskyi, 2016; Demydov et al., 2017). Accordingly, it has been established that under these environmental conditions the critical adaptive traits of winter and/or facultative barley are: maximum possible level of winter hardiness and frost resistance as its main part, drought tolerance, lodging resistance and resistance to a complex of pathogens (powdery mildew (caused by *Blumeria graminis* (DC.) E. O. Speer, f. sp. *hordei* emend. É. J. Marchal (anamorph *Oidium monilioides* Link)), spot blotch (caused by *Cochliobolus sativus* (anamorph *Bipolaris sorokiniana* [Sacc.] Shoem.)) and leaf rust (caused by *Puccinia hordei* Otth.).

Based on the above, the purpose of this study was to determine the peculiarities of yield manifestation and its combination with resistance (tolerance) to a complex of abiotic and biotic stresses in a panel of promising winter and facultative barley breeding lines under conditions of the Ukrainian Forest-Steppe and to identify the superior ones as candidates for new varieties.

## MATERIALS AND METHODS

The study was carried out in 2016-17–2018-19 at the V.M. Remeslo Myronivka Institute of Wheat of National Academy of Agrarian Sciences of Ukraine (MIW). The MIW is located in the Central part of the Ukrainian Forest-Steppe (latitude 49°64', longitude 31°08', altitude 153 m). Soil is deep, little humus, slightly leached chernozem (Berezhniak & Pasichnyk, 2015). Humus content is 3.8%, alkaline hydrolysed nitrogen (N) – 59.0 mg kg<sup>-1</sup>, phosphorus (P<sub>2</sub>O<sub>5</sub>) – 220.1 mg kg<sup>-1</sup>, potassium (K<sub>2</sub>O) – 96.0 mg kg<sup>-1</sup>, pH (KCL) = 5.8.

The objects of the study were 34 winter and facultative barley breeding lines developed at the MIW (Table 1). The winter barley variety ‘Zherar’ was used as a standard. The field trial was laid out with randomized complete blocks in four replications. The size of the individual plot was 10 m<sup>2</sup>.

Assessment of resistance to diseases and lodging was performed under natural field conditions. The breeding lines were scored on a scale of 1–9 points. A score of 9 points corresponds to the maximum resistance and score of 1 point to the minimum resistance. The evaluations were carried out in accordance with generally accepted methodical recommendations (Babaian et al., 1988; Volkodav, 2003).

Frost resistance of the breeding lines was evaluated after artificial freezing (Kyrychenko et al., 2008; Demydov et al., 2016b). Seeds of the breeding lines were sown in the sowing wooden boxes, which in advance had been filled with soil mixture. Sowing was carried out on 1<sup>st</sup> October. Then, sowing boxes were placed on the open area for

passing all phases of hardening under natural conditions. Artificial freezing was carried out in the last decade of January in the modernized KNT-1 camera (constructed in the former German Democratic Republic as a part of Phytotron complex (the specific manufacturer is unknown) at -14 °C. Living and frost killed plants were counted after two weeks growing.

**Table 1.** Winter and facultative barley breeding lines involved in the study

Code	Variety, breeding line	Growth habit	Pedigree
G1	'Zherar' – St	winter	'Manas'/'M-93'
G2	'Pallidum 5154'	winter	'M-93'/'Kromoz'
G3	'Pallidum 4031'	winter	i. s. 'M-87'
G4	'Pallidum 5024'	winter	'Borysfen'/'Seim'
G5	'Pallidum 5041'	winter	'Borysfen'/'Apollon'
G6	'Pallidum 5155'	facultative	'HVW 1072'/'Mirena'/'Kovchek'
G7	'Pallidum 5156'	winter	'HVS 56863'/'M-92'/'Borysfen'
G8	'Pallidum 5082'	winter	'M-87' /3/ 'Erfa'/'Radikal'/'Kromoz'
G9	'Pallidum 5159'	winter	'M-93' /3/ 'Leut 19/77/796'/'K 27129'/'Opera'
G10	'Pallidum 4897'	facultative	'Seim'/'M-87'
G11	'Pallidum 5090'	winter	'Strimki'/'Borysfen'
G12	'Pallidum 5096'	winter	'Strimki'/'Borysfen'
G13	'Pallidum 5097'	winter	'Strimki'/'Borysfen'
G14	'Pallidum 5102'	facultative	'Kovchek'/'Narcis'/'P-77' /3/ 'IBON-CN-CW'
G15	'Pallidum 5104'	winter	'Odeski 165'/'Manas'/'Borysfen'
G16	'Pallidum 5106'	winter	'Osнова' /3/ '2211/76'/'Bemir 2'/'Bemir 2'
G17	'Pallidum 5110'	facultative	'Kovchek'/'Seim'/'Bugar'
G18	'Nutans 5113'	winter	'Fighter' /3/ '2211/76'/'Bemir 2'/'Bemir 2'
G19	'Pallidum 5118'	winter	'P-77'/'P-107'/'Vezha' /3/ 'M-93'/'Kromoz'
G20	'Pallidum 5121'	facultative	'Kovchek'/'M-93'/'Manas'
G21	'Pallidum 5124'	facultative	'Kovchek' /4/ 'Manas' /3/ 'HVW 1089'/'Erfa'/'R-735'
G22	'Pallidum 5125'	facultative	'Kovchek' /4/ 'Manas' /3/ 'HVW 1089'/'Erfa'/'R-735'
G23	'Pallidum 5126'	facultative	'Kovchek'/'Pallidum 4718'
G24	'Pallidum 5127'	facultative	'Kovchek'/'Pallidum 4718'
G25	'Pallidum 5128'	winter	'Zherar'/'Borysfen'
G26	'Pallidum 5129'	winter	'Cinderella'/'Seim'
G27	'Pallidum 5130'	winter	'Cinderella'/'Seim'
G28	'Pallidum 5131'	facultative	'Cinderella'/'Seim'
G29	'Pallidum 5134'	winter	'Zherar'/'Paladin Myronivskiy'
G30	'Pallidum 5141'	winter	'Zherar'/'Paladin Myronivskiy'
G31	'Pallidum 5142'	winter	'Atlant Myronivskiy'/'Seim'
G32	'Pallidum 5143'	winter	'Atlant Myronivskiy'/'Seim'
G33	'Pallidum 5149'	winter	'Meteor'/'Zherar'
G34	'Pallidum 5151'	winter	'Meteor'/'Zherar'
G35	'Pallidum 5153'	facultative	'Meteor'/'Zherar'

Note. Code – the genotype code.

To determine the tolerance to dehydration (drought and heat stresses), the resistance of cell membranes was evaluated by the leakage of electrolytes (Dorofeev et al., 1974). At the beginning of earing, 15–20 flag leaves of barley plants had been cut in field condition, placed in plastic bags and transferred to the laboratory. The leaves had been

dried to 50% moisture loss, then crushed and infused in test tubes filled with distilled water. The electrical conductivity of the solution was determined using a rheochord bridge. To differentiate of the breeding lines the following scale was used: low resistance (leakage of electrolytes is more than 81%), average resistance (leakage of electrolytes is 61–80%), and high resistance (leakage of electrolytes is less than 60%).

Relative drought tolerance was determined using the method of seed germination in a sucrose solution with osmotic pressure of 1,215.9 kPa (Kozhushko, 1988). The seeds of breeding lines were grown in the Petri dishes in three replications (100 seeds in the dish). The dishes were placed in the thermostat at +22 °C. The seeds ability to germinate was determined on the seventh day. As a control there was used germination of seeds in distilled water. The percentage of germinated seeds in the osmotic solution was compared to the control. For differentiation of the breeding lines according to relative drought tolerance, the following scale was used: high tolerance (more than 81% of seeds germinated), above average tolerance (61–80% of seeds germinated), average tolerance (41–60% of seeds germinated), low tolerance (21–40% of seeds germinated), and very low tolerance (less than 20% of seeds germinated).

GYT biplot model was used for comprehensive evaluation of the breeding lines by a complex of traits (Yan & Fregeau-Reid, 2018). At the first stage of the GYT biplot analysis, the experimental genotype by trait data (it is given in the Table 2) is modified by the yield. If a larger numerical value of the characteristic is desired, it multiplies ( $\times$ ) by the yield, but it should be used division ( $/$ ) operator if a smaller numerical value of the trait is better (in our case it is the leakage of electrolytes). The division ( $/$ ) operator means the values of the trait were reversed before being multiplied by the yield. From the original genotype by trait data a GYT table is derived, in which each column is a yield  $\times$  trait combination (it is not shown in the paper). Based on obtained intermediate GYT data (numerical value of the yield and traits multiplication or division), the standardized (index) data are obtained (it is given in the Table 3). This can be done by subtracting the mean and dividing the centered value by the standard deviation within the yield  $\times$  trait combination. The general (average) GYT index, which characterizes the comprehensive assessment of each genotype, is defined as the arithmetic average of all index indicators. The graphical GYT biplot analysis is performed based on the standardized index data. The basic principles of construction and interpretation of the GYT biplot are similar to the GGE biplot, except that the term ‘environment’ is replaced by the combination of yield  $\times$  trait. GGE biplot combines analysis of variance for additive parameters and singular value decomposition for multiplicative parameters, the principal components (Yan et al., 2007; Gauch et al., 2008). These results are graphically displayed as a scatter plot which was first described as biplot by (Gabriel, 1971). It approximates and graphically shows a two-way table by both its row and column factors such that relationships among the row factors, relationships among column factors, and the interactions between the row and column factors can be visualized simultaneously. Mathematically, a GGE biplot is regarded as a graphical display of matrix multiplication (Yan & Tinker, 2006).

The graphical analysis was performed with non-commercial software GEA-R, version 4.1 ((Pacheco et al., 2015) CIMMYT, Mexico). The principal and peculiarities of the experimental data analysis with free software based on R-programing is described by (Frutos et al., 2014).

The differences among the values of the experimental variants given in the Table 2 were calculated using ANOVA and were considered significant at *LSD* 0.05. The computer program Statistica 12 (TIBCO, USA) was used for statistical analysis.

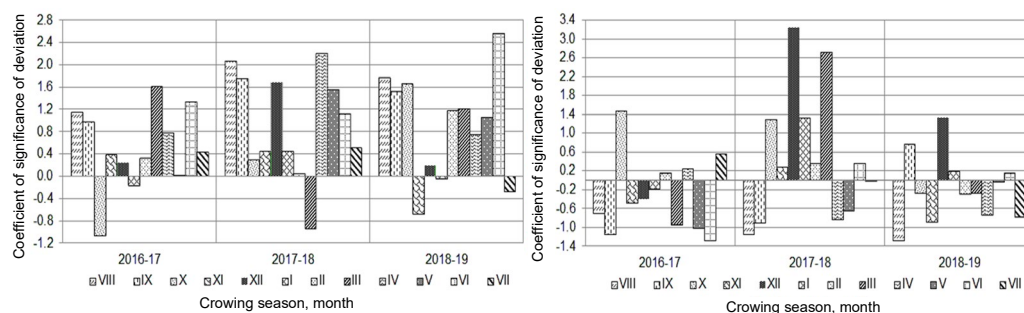
**Table 2.** Characteristics of winter and facultative barley breeding lines by a complex of traits, 2016-17–2018-19

Code	Yld	TKW	FR	LE	RDT	LD	PM	SB	PH
G1	3.84	40.87	68.00	75.57	16.00	8.00	6.33	6.33	6.67
G2	4.26*	41.97*	67.00	81.20	45.00*	8.00	6.33	7.00*	6.67
G3	4.17*	40.33*	61.00*	64.78*	35.60*	8.00	7.00*	6.33	6.67
G4	4.59*	39.93*	77.00*	68.10*	60.90*	8.00	7.00*	7.33*	7.00
G5	4.05	40.50	73.00	81.51*	50.00*	8.00	7.00*	7.33*	6.00*
G6	3.94	40.57	64.00	72.94	29.50*	8.00	7.00*	8.00*	6.00*
G7	4.06	41.03	49.00*	73.79	44.20*	8.00	7.00*	6.33	6.00*
G8	4.13	37.80*	39.00*	61.72*	38.50*	8.00	7.00*	6.33	7.00
G9	4.19*	40.20*	50.00*	54.56*	19.60	8.00	7.00*	6.33	7.00
G10	4.30*	39.67*	63.00	79.21	16.40	8.00	7.00*	8.00*	7.00
G11	4.24*	43.30*	73.00	53.44*	42.60*	9.00*	7.00*	7.00*	7.67*
G12	4.93*	43.93*	68.00	42.66*	60.10*	9.00*	7.00*	7.00*	7.67*
G13	4.44*	42.47*	65.00	57.09*	22.60*	9.00*	7.33*	7.00*	7.67*
G14	4.41*	42.23*	33.00*	48.37*	49.90*	8.00	7.00*	7.00*	7.00
G15	4.08	40.37*	59.00*	65.09*	32.10*	8.00	7.00*	6.33	7.00
G16	3.93	39.57*	41.00*	59.83*	26.90*	8.00	7.33*	5.67*	7.00
G17	4.83*	42.40*	70.00	47.15*	60.20*	8.00	7.00*	7.33*	7.33*
G18	3.88	51.00*	47.00*	73.70	25.60*	8.00	7.00*	6.33	6.00*
G19	3.76	40.27*	61.00*	85.98*	27.30*	8.00	6.33	7.33*	6.00*
G20	4.16*	39.50*	60.00*	77.17	73.10*	8.00	7.00*	5.67*	7.67*
G21	4.05	43.57*	20.00*	64.72*	42.60*	8.00	6.33	7.00*	7.00
G22	4.28*	39.43*	25.00*	72.58	50.90*	8.00	7.00*	5.33*	7.33*
G23	4.70*	41.07	49.00*	57.38*	56.30*	8.00	7.00*	5.33*	7.00
G24	4.32*	40.20*	55.00*	66.91*	42.60*	8.00	7.00*	5.33*	7.00
G25	3.93	40.67	62.00*	61.43*	51.30*	8.00	7.00*	5.33*	7.00
G26	3.90	40.27*	77.00*	55.99*	52.00*	8.00	7.00*	6.33*	6.33
G27	4.23*	38.43*	64.00	51.22*	49.20*	9.00*	7.00*	7.00*	7.00
G28	4.39*	44.93*	71.00	49.70*	15.90	9.00*	7.00*	7.00*	6.33
G29	4.59*	38.03*	68.00	50.21*	50.30*	8.00	7.00*	7.00*	6.33
G30	4.15*	38.40*	62.00*	49.53*	60.40*	8.00	7.00*	5.67*	6.00*
G31	4.18*	39.47*	60.00*	53.64*	47.40*	8.00	7.00*	5.67*	6.33
G32	4.00	38.80*	53.00*	64.35*	25.60*	8.00	7.00*	5.67*	6.00*
G33	4.20*	39.00*	49.00*	63.61*	15.60	8.00	7.00*	6.33*	6.33
G34	4.08	42.53*	59.00*	57.52*	28.60*	8.00	7.00*	7.33*	6.33
G35	4.72*	42.07*	63.00	56.12*	53.60*	9.00*	6.67	7.00*	7.00
$\sigma$	0.29	2.44	13.68	11.24	15.53	0.38	0.24	0.76	0.54
<i>LSD</i> <sub>0.05</sub>	0.30	0.40	5.24	5.82	6.36	0.28	0.35	0.42	0.34

Note. Code – the genotype code according to the Table 1; Yld – yield, t ha<sup>-1</sup>; TKW – thousand kernel weight, g; FR – frost resistance, %; LE – leakage of electrolytes, %; RDT – relative drought tolerance, %; LD – lodging resistance, score; PM – resistance to powdery mildew, score; SB – resistance to spot blotch, score; PH – resistance to leaf rust, score;  $\sigma$  – standard deviation; \* – the difference is significant as compared to the standard ‘Zherar’ at the level *LSD* 0.05.

## RESULTS AND DISCUSSION

The weather conditions of the pre-sowing period and during the growing season of winter (facultative) barley differed every year, as well as varied relative to the long-term data. The coefficient of significance of deviations of the actual meteorological data from long-term average for monthly air temperature pointed on a general trend of its increase in most months (Fig. 1, a). For the monthly precipitation, on the contrary, there was observed significant unevenness during the growing season (Fig. 1, b).



**Figure 1.** The coefficient of significance of deviation of actual weather data from long-term average during growing season of winter barley, 2016-17–2018-19: a – monthly air temperature; b – monthly precipitation.

If a very briefly to characterize, 2016-17 was very harsh and dry, 2017-18 was rather wet, and 2018-19 was relatively average between the two ones. As a result, such the weather conditions led to a different combination of abiotic and biotic stresses in different years, as follows: drought in 2016-17, small score of lodging resistance and high level of spreading of pathogens in 2017-18, above average spreading of pathogens in 2016-17. Actually, all mentioned above made it possible to comprehensive evaluate and differentiate the breeding lines by yield and a complex of other adaptive traits.

In the Table 2 there are given characteristics (on average in 2016-17–2018-19) of winter and facultative barley breeding lines by yield, thousand kernel weight, frost resistance, leakage of electrolytes, relative drought tolerance, lodging resistance score, and score of resistance to three the most common diseases (powdery mildew, spot blotch, and leaf rust).

As mentioned, yield (Yld) is the main trait that determines the suitability of a variety for use in agricultural production. Therefore, a significant number of scientific works are devoted to the study of winter barley yield performance under different growing conditions (Zhong et al., 2019; Zavalypich et al., 2021; Czembor et al., 2022). In our study, on average in 2016/17–2018/19 only the breeding line ‘Pallidum 5118’ (G19) had yield unreliable lower ( $3.76 \text{ t ha}^{-1}$ ) than the standard ‘Zherar’ ( $3.84 \text{ t ha}^{-1}$ ). The other studied breeding lines were superior to standard or were statistically at the same level.

The breeding lines ‘Pallidum 5096’ (G12), ‘Pallidum 5110’ (G17), ‘Pallidum 5153’ (G35), ‘Pallidum 5126’ (G23), ‘Pallidum 5024’ (G4), and ‘Pallidum 5134’ (G29) had the highest average yield ( $4.59\text{--}4.93 \text{ t ha}^{-1}$ ).

Thousand kernel weight (TKW) is one of the key structural components of barley yield (Bekele et al., 2020; Zhou et al., 2020). At the same time, it can be used as a measure for elucidation of tolerance to different stresses, especially to high temperatures and deficit of soil moisture during kernel formation and filling (Djuric et al., 2018). The two-rowed breeding line ‘Nutans 5113’ (G18) had the maximal level of TKW in three years (51.00 g).

There were also 12 six-rowed breeding lines (‘Pallidum 5131’ (G28), ‘Pallidum 5096’ (G12), ‘Pallidum 5124’ (G21), ‘Pallidum 5090’ (G11), ‘Pallidum 5151’ (G34), ‘Pallidum 5097’ (G13), ‘Pallidum 5110’ (G17), ‘Pallidum 5102’ (G14), ‘Pallidum 5153’ (G35), ‘Pallidum 5154’ (G2), ‘Pallidum 5126’ (G23), and ‘Pallidum 5156’ (G7)) with TKW (41.03–44.93 g) being higher than the standard (40.87 g) and other ones. The first of mentioned above breeding lines prevailed over the standard significantly (*LSD* 0.05).

Frost resistance (FR) is the ability of a plant to withstand against negative temperatures during the wintering. It is one of the main components of winter hardiness, which determines the suitability of winter crops for cultivation (Stockinger, 2021; Guerra et al., 2022). It is important to note that the maximum FR of plants is formed after sufficient hardening (a period of specific light-temperature combination), during which the upregulation or downregulation of certain genes take place (Ahres et al., 2020; Kovács et al., 2020). It is also known that during the transition from the vegetative to the generative development, FR significantly decreases (Pociecha et al., 2020; Wójcik-Jagła et al., 2021). In this study, the percentage of the survived plants after hardening under natural conditions and following artificial freezing at -14 °C varied from 20.00% in the breeding line ‘Pallidum 5124’ (G21) to 77.00% in the breeding lines ‘Pallidum 5024’ (G4) and ‘Pallidum 5129’ (G26). The latter two breeding lines were reliable more frost resistant than the standard ‘Zherar’ (68.00%). The breeding lines ‘Pallidum 5090’ (G11), ‘Pallidum 5041’ (G5), ‘Pallidum 5131’ (G28), ‘Pallidum 5110’ (G17), ‘Pallidum 5096’ (G12), and ‘Pallidum 5134’ (G29) had FR statistically unreliable higher than the standard or at the same level. It should be highlighted that the breeding lines ‘Pallidum 5124’ (G21), ‘Pallidum 5131’ (G28), and ‘Pallidum 5110’ (G17) are facultative. Thus, there were identified facultative breeding lines with FR higher or at least at the same level as winter breeding lines. Similar results were obtained by other researchers (Rizza et al., 2016). Therefore, despite the well-known relationship between the vernalization and FR of winter barley (and other winter cereals), for facultative barley, vernalization is not necessary for the formation of a high (maximum possible as for barley) level of tolerance to low temperatures.

Drought is the most damaging abiotic stress, which prevents the realization of the barley productivity potential on a global scale (Wójcik-Jagła et al., 2018; Pham et al., 2019; Cai et al., 2020). The aggravation of this problem in recent years is quite obvious. Drought tolerance is mainly provided by the synthesis of proteins to detoxify free radicals and to support molecules and membranes in cells (Wendelboe-Nelson & Morris, 2012; Al Abdallat et al., 2014). Another negative abiotic stress is high air temperatures (heat stress), especially, if they are supplemented by a soil moisture deficit (Zandalinas, 2018). The resistance of cell membranes under the action of mentioned stressful abiotic factors (drought and heat) can be estimated by electrical conductivity (leakage of electrolytes (LE)). In the present study we found a high level of stability of membranes (LE was 42.66–59.83%) in 16 breeding lines, as follows: ‘Pallidum 5096’ (G12), ‘Pallidum 5110’ (G17), ‘Pallidum 5102’ (G14), ‘Pallidum 5141’ (G30), ‘Pallidum 5131’



(G28), ‘Pallidum 5134’ (G29), ‘Pallidum 5130’ (G27), ‘Pallidum 5090’ (G11), ‘Pallidum 5142’ (G31), ‘Pallidum 5159’ (G9), ‘Pallidum 5129’ (G26), ‘Pallidum 5153’ (G35), ‘Pallidum 5097’ (G13), ‘Pallidum 5126’ (G23), ‘Pallidum 5151’ (G34), and ‘Pallidum 5106’ (G16). The other 15 breeding lines, as well as the standard ‘Zherar’ (G1) had average resistance (LE was 61.43–79.21%). Among them, 13 breeding lines had lower (better) LE than the standard (75.57%). There were only three breeding lines which had low resistance (LE was 81.20–85.98%).

The other important aspect of drought tolerance is the ability to germinate with soil moisture deficit. Therefore, considerable attention is paid to evaluation of drought resistance in juvenile period (Thabet et al., 2018; Moursi et al., 2020). We found that among breeding lines the percentage of seeds germination in the osmotic solution (relative drought tolerance (RDT)) varied from 15.60% to 73.10%. Thus, there were no breeding lines with high RDT (more than 81.00% of seeds germinated). The breeding line ‘Pallidum 5121’ (G20) had higher than average RDT (73.10% of seeds germinated). There were 19 breeding lines with average RDT (42.60–60.90% of seeds germinated). Among them, the breeding lines ‘Pallidum 5024’ (G4), ‘Pallidum 5141’ (G30), ‘Pallidum 5110’ (G17), and ‘Pallidum 5096’ (G12) should be highlighted as better (60.10–60.90% of seeds germinated) than others. The standard ‘Zherar’ (G1) and other 15 breeding lines had low ability to germinate in the osmotic solution (15.60–38.50% of seeds germinated).

Lodging resistance (LD), even under circumstances of climate change, remains a rather critical characteristic of barley in many areas of its cultivation (Dockter & Hansson, 2015; Yu et al., 2021; Zhang et al., 2022). In our case, differentiation among breeding lines by LD was insignificant. However, there were 6 breeding lines (‘Pallidum 5096’ (G12), ‘Pallidum 5131’ (G28), ‘Pallidum 5130’ (G27), ‘Pallidum 5090’ (G11), ‘Pallidum 5153’ (G35), and ‘Pallidum 5097’ (G13)) that had not lodging at all each year.

The resistance of a winter barley variety to pathogens is also one of its most important properties from both an economic and ecological point of view (Çelik Oğuz & Karakaya, 2021). Powdery mildew (PM) caused by fungus *Blumeria graminis* (DC.) E.O. Speer, f. sp. *hordei* emend. É. J. Marchal (anamorph *Oidium monilioides* Link) is one of the most widespread worldwide foliar disease of barley which can leads to yield loses up to 30–40% (Dreiseitl, 2020; Czembor & Czembor, 2021). The highest level of resistance score to powdery mildew (7.33 points) was found in the breeding lines ‘Pallidum 5106’ (G16) and ‘Pallidum 5097’ (G13). Most of breeding lines were characterized by resistance score of 7.00 points. A very damaging disease of barley is spot blotch (SB) which caused by necrotrophic fungus *Cochliobolus sativus* (anamorph *Bipolaris sorokiniana* [Sacc.] Shoem.) (Clare et al., 2020) We found a significant range of variation among breeding lines in resistance score to spot blotch (from 5.33 to 8.00 points). The breeding lines ‘Pallidum 5155’ (G6) and ‘Pallidum 4897’ (G10) had a very high level of resistance to SB (8.00 points). The breeding lines ‘Pallidum 5024’ (G4), ‘Pallidum 5110’ (G17), ‘Pallidum 5041’ (G5), ‘Pallidum 5151’ (G34), and ‘Pallidum 5118’ (G19) with resistance score of 7.33 points should be also highlighted. Another quite hazardous pathogen in many barley growing area of the world, which under favorable conditions, may cause losses of up to 60% is biotrophic fungus *Puccinia hordei* Otth., which is the causal agent of barley leaf rust (PH) (Sandhu et al., 2016; Fazlikhani et al, 2019). The breeding lines ‘Pallidum 5097’ (G13), ‘Pallidum 5096’ (G12), ‘Pallidum 5090’ (G11), and ‘Pallidum 5121’ (G20) had maximal level of score

of resistance (7.67 points) to PH in the experiment. Slightly inferior to the mentioned, but as better compared with others were the breeding lines 'Pallidum 5110' (G17) and 'Pallidum 5125' (G22) (7.33 points).

Thus, winter and facultative breeding lines were differentiated based on manifestation level of individual traits. However, the ones with single resistance to one or another stress factor can only be used as genetic sources in the breeding process for creation a new initial material. For commercial use, the variety must have an optimal combination of resistance (tolerance) to a number of the most common stress factors with the highest possible yield (Dawson et al., 2015; Laidig et al., 2021; Panfilova et al., 2021). For this purpose, it is important to use effective statistical tools to differentiate genotypes based on a set of traits. GYT (genotype by yield  $\times$  trait) biplot model is one of such statistical and graphical approaches, which makes it possible to evaluate genotypes and select the best ones based on a combination of yield with other important characteristics (Yan & Fregeau-Reid, 2018). Recently, there were publications reported about successful use of the GYT biplot (Karahana & Akgün, 2020; Gubatov et al., 2021; Hudzenko et al., 2022). The authors of the GYT biplot method characterized it as graphical, objective, effective, and straightforward (Yan & Fregeau-Reid, 2018). This makes it possible to rank genotypes based on their levels in combining yield with other important traits and at the same time described their trait profiles.

In the Table 3 there are given standardized GYT (genotype by yield  $\times$  trait) data and general (average) GYT superiority index for studied winter and facultative barley breeding lines. It is clearly visible strengths and weaknesses of the breeding lines in terms of combination of yield and individual traits. The mean performance for all set of the studied genotypes is always 0.00. The genotype with positive numerical value for particular yield  $\times$  trait combination is better than average in the trial. If the genotype has minus mark with particular index value it is inferior to the average in the trial. It is also easy to compare the genotype with each other and with the standard 'Zherar' (G1). For more comprehensive differentiation of the breeding lines and better perception of the results, based on the data from the Table 3 the graphical GYT biplot analysis was performed. The first and the second principal components of the GYT biplot (labeled on the Figs 2–5 as AXIS1 and AXIS2, accordingly) explained 73.03% of the yield  $\times$  trait interaction.

Fig. 2 shows discriminating power and representativeness of yield  $\times$  trait combinations. It can be seen that almost all combinations of yield and other traits had very high discriminating power, as evidenced by the length of their vectors. At the same time, the Yld\_RDT and Yld\_FR combinations were the least representative because they were the most distant from average yield  $\times$  trait axis (also called the average tester axis (ATA)). The latter is shown as a thick line crossing the origin of the GYT biplot from left to right in the horizontal plane. The average value for all yield  $\times$  trait combinations is indicated on ATA by an arrow in a circle.

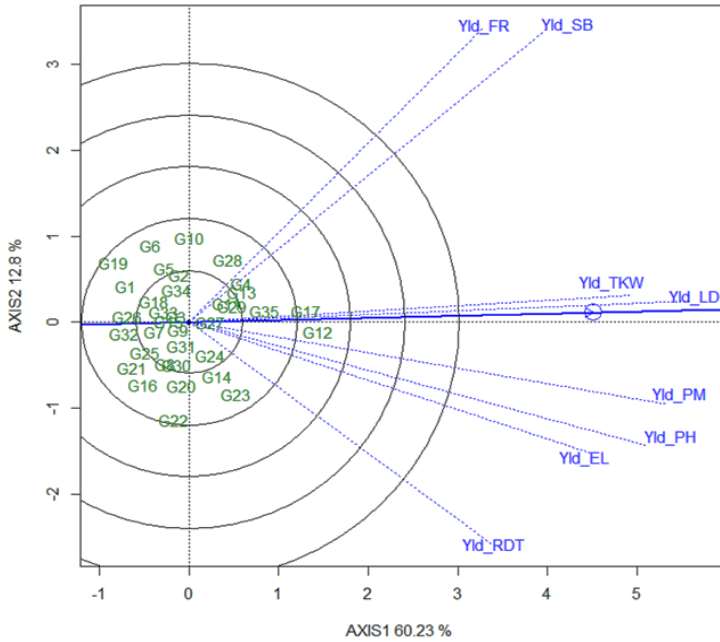
It should be noted that the vectors of the Yld\_RDT and Yld\_FR combinations were the most distant from each other. Thus, the breeding lines differed most among themselves in these combinations. The Yld\_TKW and Yld\_LD optimally combined both high discriminating power and representativeness. In addition, these combination were very close to each other because they had the smallest angle between the vectors. The vectors of the Yld\_FR and Yld\_SB were also close to each other. At the same time, they were significantly distant from other yield  $\times$  trait combinations. The Yld\_EL, Yld\_PH,

and Yld\_PM formed another cluster. The Yld\_RDT placed separate from others yield × trait combinations. The obtained peculiarities indicate the differences among studied breeding lines according to certain yield × trait combinations.

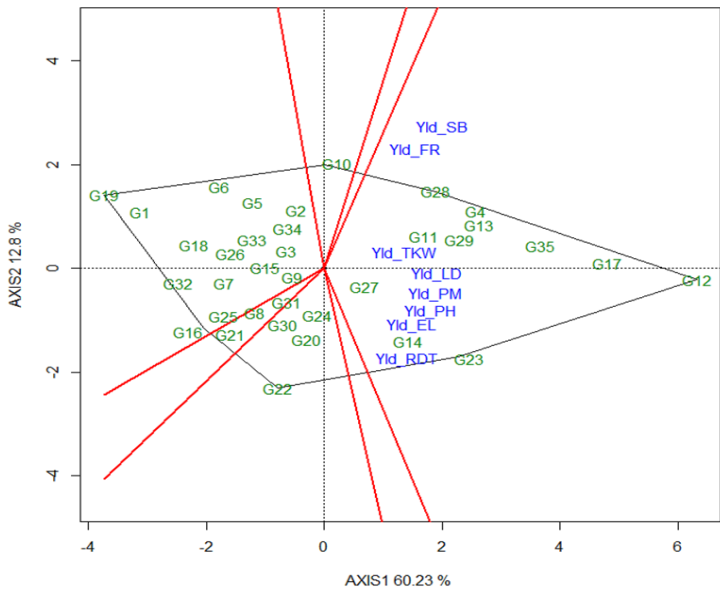
**Table 3.** Standardized genotype by yield × trait data and superiority index for winter and facultative barley breeding lines, 2016-17–2018-19

Code	Yld_TKW	Yld_FR	Yld_LE	Yld_RDT	Yld_LD	Yld_PM	Yld_SB	Yld_PH	GYT index
G1	-1.03	0.23	-1.21	-1.56	-1.14	-2.13	-0.86	-0.83	-1.06
G2	0.35	0.61	-1.46	0.26	-0.15	-1.00	0.53	-0.06	-0.11
G3	-0.32	0.13	-0.22	-0.35	-0.36	-0.05	-0.33	-0.22	-0.21
G4	0.63	1.68	-0.23	1.49	0.63	1.20	1.50	0.97	0.99
G5	-0.58	1.41	-1.56	0.41	-0.64	-0.41	0.50	-1.19	-0.33
G6	-0.85	0.09	-0.97	-0.80	-0.91	-0.74	0.96	-1.37	-0.57
G7	-0.42	-0.75	-0.97	0.09	-0.62	-0.38	-0.51	-1.17	-0.59
G8	-1.08	-1.34	-0.01	-0.20	-0.46	-0.17	-0.40	0.08	-0.45
G9	-0.30	-0.58	0.59	-1.27	-0.31	0.01	-0.30	0.20	-0.25
G10	-0.17	0.39	-1.29	-1.44	-0.05	0.34	1.69	0.41	-0.01
G11	0.65	0.99	0.72	0.10	1.06	0.16	0.49	1.07	0.66
G12	2.72	1.40	2.30	1.72	2.90	2.22	1.71	2.53	2.19
G13	0.96	0.66	0.59	-1.02	1.59	1.39	0.85	1.49	0.82
G14	0.81	-1.58	1.28	0.66	0.21	0.66	0.79	0.62	0.43
G15	-0.54	-0.09	-0.30	-0.59	-0.57	-0.32	-0.48	-0.01	-0.36
G16	-1.12	-1.34	-0.02	-0.94	-0.93	-0.21	-1.38	-0.30	-0.78
G17	1.98	1.44	1.79	1.65	1.20	1.92	1.94	1.87	1.73
G18	1.55	-1.01	-1.05	-1.03	-1.05	-0.92	-0.80	-1.47	-0.72
G19	-1.37	-0.27	-1.97	-0.99	-1.33	-2.35	-0.04	-1.67	-1.25
G20	-0.56	0.05	-1.19	1.83	-0.38	-0.08	-1.05	0.90	-0.06
G21	0.20	-2.60	-0.30	-0.01	-0.64	-1.57	0.16	-0.07	-0.61
G22	-0.28	-2.19	-0.77	0.63	-0.10	0.28	-1.24	0.76	-0.37
G23	1.24	-0.25	0.77	1.28	0.89	1.53	-0.67	1.18	0.75
G24	0.02	-0.14	-0.29	0.15	-0.01	0.40	-1.18	0.45	-0.07
G25	-0.85	-0.04	-0.13	0.40	-0.93	-0.77	-1.71	-0.30	-0.54
G26	-1.02	0.85	0.24	0.42	-1.00	-0.86	-0.76	-1.08	-0.40
G27	-0.67	0.38	0.88	0.49	1.03	0.13	0.48	0.27	0.38
G28	1.51	1.03	1.15	-1.45	1.46	0.61	0.76	-0.22	0.61
G29	0.08	1.03	1.29	0.81	0.63	1.20	1.11	0.13	0.79
G30	-0.87	0.17	0.94	1.09	-0.41	-0.11	-1.06	-1.02	-0.16
G31	-0.52	0.07	0.65	0.35	-0.34	-0.02	-1.02	-0.59	-0.18
G32	-1.14	-0.54	-0.30	-0.99	-0.76	-0.56	-1.28	-1.27	-0.85
G33	-0.60	-0.64	-0.11	-1.51	-0.29	0.04	-0.28	-0.55	-0.49
G34	0.02	-0.09	0.27	-0.79	-0.57	-0.32	0.55	-0.76	-0.21
G35	1.59	0.80	0.90	1.12	2.34	0.92	1.34	1.22	1.28
Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$\sigma$	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-

Note. Code – the genotype code according to the Table 1; Yld\_TKW – is for yield × thousand kernel weight; Yld\_FR is for yield × frost resistance; Yld\_LE is for yield / leakage of electrolytes; Yld\_RDT is for yield × relative drought tolerance; Yld\_LD is for yield × resistance to lodging; Yld\_PM is for yield × resistance to powdery mildew; Yld\_SB is for yield × resistance to spot blotch; Yld\_PH is for yield × resistance to leaf rust; GYT\_index is for superiority index;  $\sigma$  – standard deviation.



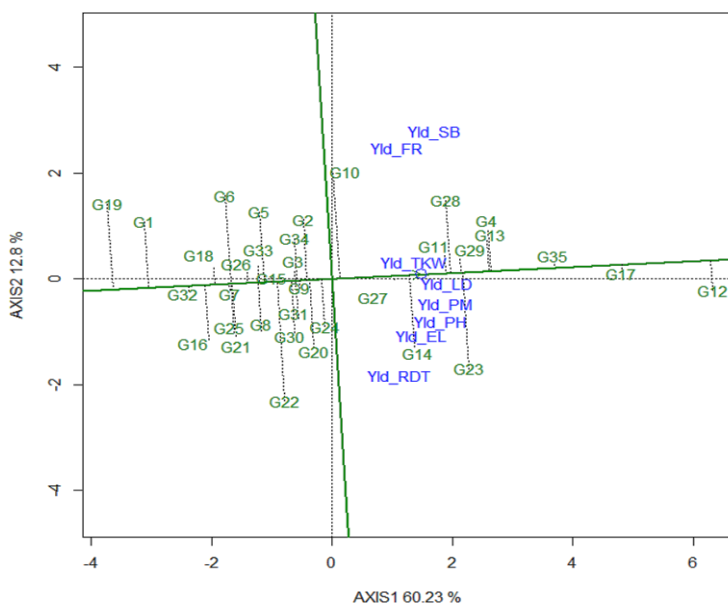
**Figure 2.** GYT biplot discriminating power and representativeness of yield × trait combinations in winter and facultative barley breeding lines, 2016-17–2018-19: AXIS1 – is for the first principal component; AXIS2 – is for the second principal component; the genotype code according to the Table 1; the yield × trait code according to the Table 3.



**Figure 3.** GYT biplot ‘which-won-where’ view for winter and facultative barley breeding lines, 2016-17–2018-19: AXIS1 – is for the first principal component; AXIS2 – is for the second principal component; the genotype code according to the Table 1; the yield × trait code according to the Table 3.

The ‘which-won-where’ view shows that, despite the above-mentioned differences, all yield  $\times$  trait combinations fell into one sector and formed one mega-combination (Fig. 3). This also indicates that there is one genotype dominated the others in this mega-combination. In this case, the undisputed winner was the breeding line ‘Pallidum 5096’ (G12). A group of breeding lines also located in this sector (‘Pallidum 5110’ (G17), ‘Pallidum 5153’ (G35), ‘Pallidum 5134’ (G29), ‘Pallidum 5097’ (G13), ‘Pallidum 5024’ (G4), ‘Pallidum 5090’ (G11), ‘Pallidum 5130’ (G27), ‘Pallidum 5131’ (G28), ‘Pallidum 5102’ (G14), and ‘Pallidum 5126’ (G23)) were significantly superior to others.

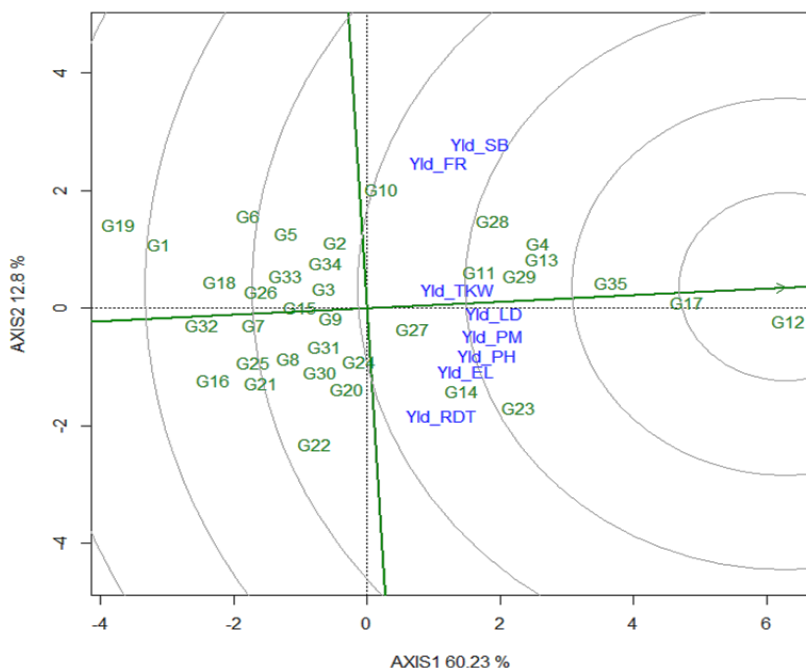
Fig. 4 is the average tester coordination view of the GYT biplot. The breeding lines are ranked based on their overall superiority and their strengths and weaknesses in descending order from right to left. Accordingly, the maximum values of the studied yield  $\times$  trait combinations were in the breeding line ‘Pallidum 5096’ (G12), and the lowest values were in the breeding line ‘Pallidum 5118’ (G19). In addition, it is clearly visible that only the breeding line ‘Pallidum 5118’ (G19) was inferior to the standard ‘Zherar’ (G1). The other breeding lines prevailed over the standard. In the vertical plane, the origin of the biplot is crossed by a thick line which represents the average value of all yield  $\times$  trait combinations for all studied breeding lines (average in the experiment). The breeding lines located on the left of it were inferior to the average in the experiment. The distance of the breeding line from ATA, which is shown by dashed lines, indicates that it differed significantly in terms certain yield  $\times$  trait combinations from the general trend in the experiment. That is, in some yield  $\times$  trait combinations the breeding line was much better and in others worse than expected.



**Figure 4.** GYT biplot of the average tester coordination view for winter and facultative barley breeding lines, 2016-17–2018-19: AXIS1 – is for the first principal component; AXIS2 – is for the second principal component; the genotype code according to the Table 1; the yield  $\times$  trait code according to the Table 3.

The breeding lines ‘Pallidum 5110’ (G17), ‘Pallidum 5153’ (G35), ‘Pallidum 5096’ (G12), ‘Pallidum 5134’ (G29), and ‘Pallidum 5130’ (G27) located close to ATA. The breeding lines ‘Pallidum 4031’ (G3), ‘Pallidum 5159’ (G9), ‘Pallidum 5104’ (G15), ‘Pallidum 5149’ (G33), ‘Pallidum 5129’ (G26), ‘Pallidum 5156’ (G7), ‘Nutans 5113’ (G18), and ‘Pallidum 5149’ (G32) also located close to ATA, but they were inferior to the average in the experiment. The breeding lines ‘Pallidum 5125’ (G22), ‘Pallidum 4897’ (G10), and ‘Pallidum 5155’ (G6) were the most distant from ATA.

Accordingly to the GYT biplot model the ‘ideal genotype’ for studied panel of breeding lines must be located in the center of the centric circles which is pointed on the ATA with an arrow (Fig. 5). Thus, the breeding line ‘Pallidum 5096’ (G12) was as close as possible to the ‘ideal genotype’. That is, it had the most optimal combination of yield and a complex of other traits. The breeding line ‘Pallidum 5110’ (G17) was quite close to the ‘Pallidum 5096’ (G12). The breeding line ‘Pallidum 5153’ (G35) placed in the next circle. The breeding lines ‘Pallidum 5134’ (G29), ‘Pallidum 5097’ (G13), ‘Pallidum 5024’ (G4), ‘Pallidum 5090’ (G11), and ‘Pallidum 5130’ (G27), located in the next two circles, were inferior to the three mentioned above, but superior to other ones. Three breeding lines (‘Pallidum 5102’ (G14), ‘Pallidum 5126’ (G23), and ‘Pallidum 5131’ (G28)) were also in third and fourth circles, but more distant from the ATA in different directions. The other breeding lines were poorer as compared with mentioned ones. Some of them can be only used as a source of individual traits for involvement in crossing when creating new initial material.



**Figure 5.** GYT biplot ranking of winter and facultative barley breeding lines relative to the ‘ideal genotype’, 2016-17–2018-19: AXIS1 – is for the first principal component; AXIS2 – is for the second principal component; the genotype code according to the Table 1; the yield × trait code according to the Table 3.

## CONCLUSIONS

As a result of our study, in a panel of promising winter and facultative barley breeding lines, the peculiarities of yield performance and its combination with resistance (tolerance) to the most common under conditions of the Ukrainian Forest-Steppe abiotic and biotic stressful factors have been determined. The winter breeding line ‘Pallidum 5096’ (G12) and facultative breeding line ‘Pallidum 5110’ (G17), which the most optimally combined yield with the complex of adaptive traits (thousand kernel weight, frost resistance, cell membranes resistance, relative drought tolerance, lodging resistance, and resistance to powdery mildew, spot blotch, and leaf rust) have been identified. These breeding lines as new varieties ‘MIP Atlas’ and ‘MIP Yanus’ accordingly have been submitted to the Ukrainian Institute for Plant Variety Examination for further investigation under conditions of different agro-climatic zones of Ukraine with the aim to determine their suitability for agricultural production and State Registration. The rest of the breeding lines have practical interest only in breeding process as parental components in crossings when creating new initial material. In particular, the winter breeding lines ‘Pallidum 5134’ (G29), ‘Pallidum 5097’ (G13), ‘Pallidum 5024’ (G4), ‘Pallidum 5090’ (G11), and ‘Pallidum 5130’ (G27), as well as facultative breeding lines ‘Pallidum 5153’ (G35), ‘Pallidum 5102’ (G14), ‘Pallidum 5126’ (G23), and ‘Pallidum 5131’ (G28) can be used as genetic sources of combination of yield with several other traits. Some of the other breeding lines could be used as genetic sources of individual traits. It should be mentioned, that highlighted breeding lines are of practical interest not only under condition of Ukrainian, but also can be used in breeding programs of some other countries, especially Central and Eastern European ones.

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