Productivity and characteristics of triticale (x*Triticosecale* Wittm.) dihaploid lines

Hristo Stoyanov*, Valentin Baychev, Ivan Belchev
Dobrudzha Agricultural Institute – General Toshevo
*E-mail: *hpstoyanov@abv.bg*

Abstract

Stoyanov, H., Baychev, V. & Belchev, I. (2018). Productivity and characteristics of triticale (x*Triticosecale* Wittm.) dihaploid lines. *Rastenievadni nauki*, *55*(4), 3-13

The biotechnology methods are a major part of the contemporary breeding. The anther culture method plays a particularly important role for the acceleration of the breeding process in cereals and for achieving fast uniformity of the hybrid material. The double haploid (DH) lines of triticale developed by this method are especially important in the breeding of triticale, as well, due to the complex genome of the crop and the respective higher complexity of the breeding process. In order to determine the efficiency of the anther culture method for production of high-yielding and stable lines of triticale, 1448 DH lines were tested and evaluated under field conditions during 2013-2016. Out of this number, 145 lines were investigated in a control nursery, 12 lines – in a preliminary varietal trial, and 8 – in a competitive varietal trial. The grown DH lines possessed high production potential; DH line 157T/9-4 yielding more than 800 kg/da during harvest years 2015 and 2016 and exceeding the mean standard with over 10%. This line was characterized with very high ecological plasticity and stability in comparison to the contemporary Bulgarian triticale cultivars. Lines 161T/4-2 and 158T/1-1 were also with very high production potential, their yield exceeding 700 kg/da. Such data are indicative for the high efficiency of the anther culture method in improving and accelerating the breeding process in winter hexaploid triticale.

Keywords: triticale; DH lines; productivity

The main task of the breeding of cultural plants is to improve their traits (Chahal and Gosal, 2000). In this respect, the yield, which a certain variety, line or hybrid realizes under specific environments, is one of the most important features (Tsenov et al., 2014). High and optimal values of yield are difficult to achieve; such an undertaking is also time-consuming, having in mind the long time necessary for testing of the breeding materials (Stoyanov and Baychev, 2016a). It is necessary to collect a large amount of information that would allow proper and precise evaluation of the productivity potential and stability of a given genotype with regard to its yield (Stoyanov et al., 2017).

Triticale, as a product of wide hybridization, has a number of peculiarities which make the breeding process very difficult (Baychev, 1990). On the one hand, there is the extremely complicated genome of the crop; its expression is significantly influenced by the conditions of the environment (Stoyanov and Baychev, 2016b). On the other hand, there are the peculiarities of the amphidiploid forms related to some deviations from the meiotic process (Baychev, 1996). Such peculiarities increase the time necessary to achieve uniformity of the hybrid materials according to the desired characters. Such difficulties are related to the search for new breeding approaches that would give satisfactory results in a significantly shorter breeding process (Randhawa et al., 2015).

The use of double haploid lines (DH lines) is an efficient biotechnology method for shortening of breeding process (Belchev et al., 2004; Grauda et al., 2010). Its application to cereals, especially to wheat, is well investigated, and numerous protocols and methods have been developed (Bos and Caligari, 2008; Grauda et al., 2010; Hussain et al., 2012; Plamenov et al., 2013; Wedzony et al., 2015).

A comparatively limited number of protocols related to development of DH lines in triticale have been developed during the recent years (Wedzony et al., 2015). This is due to the fact that the methods used in winter wheat are often applied to triticale. Nevertheless, different methods with sufficiently high efficiently are used in practice (Wedzony et al., 2015).

However, different researches point out to a large number of problems related to triticale DH lines: high percent of albino plants, incomplete uniformity of the dihaploid progenies, comparatively lower yields from the DH lines in comparison to the conventional investigations. Thus, for example, Thiemt and Oettler (2008) report DH lines which follow the tendency of lower yields in comparison to the SSD lines from the same crosses. Oleszczuk et al. (2011), on the other hand, point out to the high occurrence of aneuploidy. Such genetic instability of the triticale DH lines is strictly specific. Therefore, in order to achieve higher efficiency, it is necessary to choose properly both the parental forms and the crosses from which the DH lines will be developed.

In spite of the above problems, Thiemt and Oettler (2008) expressly underline the fact that the DH lines allow selecting high-yielding elite genotypes. Older investigations on dihaploid triticale lines (Charmet and Branlard, 1985) showed that the yields from them can be both identical to and higher than the yields from the conventional lines. Therefore it is necessary the triticale lines developed in the different breeding programs to be properly evaluated and selected by both level of yield and by yield stability to achieve high efficiency and applicability of the anther culture method.

The aim of this investigation was to characterize dihaploid triticale lines developed at Dobrudzha Agricultural Institute by their yield and stability at different stages of the breeding process and to evaluate the efficiency of the applied method for breeding of triticale.

MATERIALS AND METHODS

In order to realize the above aim, 1448 DH lines were investigated; they were tested and evaluated under field conditions during 2013-2017. The investigation involved the following:

- 1448 DH lines were evaluated in a breeding nursery; they were planted in single rows each 2 m long, with 30 cm interspacing;
- 145 lines were assessed in a control nursery in a whole-surface crop field in experimental plots each of 6.25 m^2, in one replication;
- 12 DH lines were included in a preliminary varietal trial in a whole-surface crop field in experimental plots each of 10 m^2, in one replication;
- 8 DH lines were involved in a competitive varietal trial in experimental plots of 10 m^2, in four replications of a standard block design.

Planting in the experimental plots was done with agricultural machines within the standard dates for sowing of triticale (10th – 15th October) at density 550 seeds per square meter.

The lines in the breeding nursery were evaluated on the basis of morpho-phenotypic expression and uniformity, without using qualitative traits. The selected lines were subsequently evaluated by grain quality. The best lines were transferred to a control nursery. The lines in the control nursery were estimated by their uniformity, and absolute and relative yield according to an applicable standard variety. The best lines were further transferred to a preliminary varietal trial.

In the preliminary varietal trial, the lines were evaluated in a manner identical to that of the control nursery. The best lines were transferred to a competitive varietal trial, while some were again grown at the same breeding stage for additional estimation during the next year. The evaluation in the competitive varietal trial was identical to that of the preliminary varietal trial, this time based on 5 replications. The lines were assessed at this stage for a minimum of two years. The experimental plots were harvested at full maturity, recording each individual yield separately. The data on the absolute yield from each breeding stage were summarized by calculating the means for each line and for each year. The relative yield was calculated according to the applicable standard for each breeding stage by line and by year. Based on the selection carried out at the separated breeding stages and on the end yield, the efficiency of the used breeding method was estimated.

The effect of the environment and the genotype on the yield of the promising lines was determined in a competitive varietal trial by performing two-factor dispersion analysis. An AMMI analysis was carried out according to the index yield, analyzing the specific environment x genotype interaction. The analysis also involved data from the competitive varietal trials for the respective year of contemporary Bulgarian triticale cultivars and the respective standards (AD-7291, Vihren and Rakita), and of the world standards Lasko and Presto. Comparative characterization was done of the cultivars and the investigated lines by their yield and its stability.

The software Microsoft Excel 2003 was used to summarize the data and to perform variation analysis, the AMMI analysis was done with IRRISTAT 4.0.2., and the ANOVA was performed applying IBM SPSS Statistics 19.

RESULTS

The data on the selection carried out and the performance of the DH lines in the individual stages of the breeding process are shown in Table 1. It can be seen that out of the large number of lines tested in the breeding nursery (1448), an average of 10% passed to the next breeding stage (Table 2); this percent varied from 2 to 19 according to the specific year and the phenotypic expressions of the respective investigated lines. The selection was strictly dependent on the purely morphological expression and uniformity of the DH lines, and on the physical properties of the grain: plumpness, presence of germinating seeds. The amount of the grain obtained was also important due to purely empirical considerations.

It should be emphasized that the use of quantitative parameters is inappropriate (Genchev et al., 1975) since each line was sown in a single row, without replications. At this stage, the lines were characterized with good phenotypic uniformity by height, spike morphology, earliness and phenotypic

Table 1. Number of DH lines in different breeding stages by years

Breeding stage	Number of DH lines	2013	2014	2015	2016	2017
Breeding nursery (BN)	Studied	320	358	343	131	296
	Harvested	104	57	62	7	40
	Tranferred to CN	60	51	30	2	29
Control nursery (CN)	Studied	20	60	53	30	2
	Harvested	4	10	16	11	2
	Grown again in CN	0	2	0	0	0
	Transferred to PVT	2	4	2	0	1
	Transferred to CVT	0	3	0	4	0
Preliminary variety trial (PVT)	Studied	4	2	4	5	3
	Harvested	4	2	4	3	3
	Grown again in PVT	0	0	1	3	2
	Transferred to CVT	0	0	1	0	0
Competative variety trial (CVT)	Studied	0	0	3	4	7
	Harvested	0	0	3	4	7
	Grown again in CVT	0	0	3	3	5

Table 2. Scheme of the selection process

Grown again in the same breeding stage
Transferred to the next breeding stage
Transferred to the following the next breeding stage
Rejected

expression of disease resistance. Additionally, only those lines were transferred to the control nursery (CN), which possessed plump grain. This is an indication that the average 10% of selected DH lines have very high complex scores. Therefore, the subsequent selection in the later breeding stages should be comparatively easier.

At the next stage of the breeding process, the control nursery (CN), the mean number of lines transferred to the upper level varied from 4 to 50% out of all planted lines. The main criterion here was also phenotypic uniformity, although strictly controlled by phenophases. Especially important was the uniform occurrence of heading and maturation of the investigated lines. Speaking from a morphological point of view, the DH lines should be uniform both by height and by phenotypic expression of the spikes. It should be pointed out that all lines with biological contamination were discarded.

In 2013, two lines were left in CN for yet another growth season. This was due to their contradictory results obtained with regard to the phenotypic expression of the lines and their phenological characteristics. In 2016, all lines with high scores were transferred directly to a competitive varietal trial (CVT). This was due to their exceptional properties making their evaluation in a preliminary varietal trial (PVT) practically unnecessary. In 2017, the high percent of lines transferred to the next breeding stage was due to the fact that out of the two lines grown, only one passed to PVT. On the other hand, the number of DH lines in the CN during that period was low because the greater part of the lines in the breeding nursery failed as a result from lodging in 2016.

The yields in CN registered over years (Table 3) demonstrated a high variety of phenotypic yield expression. This was due both to the investigated genotypes and to their expression over the periods of investigation. The data from harvest year 2013 showed that the DH lines realized rather low yields, only two out of the four tested lines exceeding the mean standard. Lines 148т/44-1 and 151т/31-1 passed to PVT although their exceeding of the standard cultivar Vihren was not significant. In 2014, the lines exceeding the standard Vihren with 50-80% passed to PVT. Out of the harvested 10 lines, 3 exceeded the standard even with more than 80%, the exceeding of line 161т/4-2 amounting to 109%. In spite of the extremely unfavorable meteorological conditions for growing of triticale in 2014, all 10 harvested DH lines (out of 60) had exceptional values of yield. Worth mentioning is line 156т/7-1, which realized a very high yield but was nevertheless discarded. This was a result from the high level of lodging registered at harvesting. In 2015, out of the 16 harvested lines (from a total of 53 tested lines), only two were transferred to PVT for testing, the rest were discarded. Although a large number of lines gave yields exceeding the standard Vihren, the exceptionally favorable year for development of triticale required exceptionally strict selection. Therefore, only the lines with yields at the highest level of significance of the differences according to the standard were transferred to the next breeding stage. Year 2016 was also unfavorable for growing of triticale. This was the reason why only a small part of the grown lines managed to realize the properties necessary to pass to the next breeding stage. Out of the harvested 11 lines, only 4 realized yields exceeding the standard Kolorit. Since Kolorit significantly exceeds Vihren with regard to the productivity potential, the 4 selected lines were transferred directly to CVT. Concerning the two lines tested during 2017, only the better one (177т/57-1), with a relative yield of 157%, was transferred to PVT.

The selection carried out in the preliminary varietal trial differed from the previous breeding stage by its relatively lower intensity. This was so because the lines with poor productivity and properties were discarded in the previous breeding stages. Therefore, at this higher breeding level, the lines were evaluated by a large number of qualitative traits as well, yield being of high importance. Only one line - 159т/109-1 passed to a higher breeding stage during the entire investigated period. The rest of the lines which reached this breeding stage were either discarded or left for additional investigations during the next growth season. Out of the total of 12 lines tested within the three years of PVT, 9 were discarded and 2 repeated their tests in the same breeding stage. This was an indication that the testing of the promising lines on a larger area, taking into account the overall complex evaluation and the yield according to a standard cultivar, did not give unambiguous results during the individual growth seasons.

The tested lines did not demonstrate the necessary properties to pass to the next level. This is very important from a breeding point of view, because

Table 3. Absolute and relative yield of DH lines in control nursery

Harvest year	DH line	Yield, kg/da	Relative yield, %
2013	148т/44-1	1012.70	101.75
	151т/31-1	1015.87	102.07
	153т/8-1	917.46	92.19
	154т/7-2	803.17	80.70
2014	156т/7-1	447.62	129.95
	157т/9-4	665.08	193.09
	158т/1-1	601.59	174.65
	159т/21-3	542.86	157.60
	159т/22-2	420.63	122.12
	159т/71-2	561.90	163.13
	159т/74-3	404.76	117.51
	159т/78-2	563.49	182.05
	159т/109-1	568.25	183.59
	161т/4-2	634.92	205.13
2015	159т/22-2	744.44	150.80
	159т/74-3	730.16	147.91
	166т/35-2	539.68	107.94
	166т/44-1	674.60	134.92
	166т/45-2	611.11	122.22
	168т/5-3	693.65	138.73
	168т/8-2	574.60	114.92
	168т/16-2	703.17	140.63
	168т/57-2	741.27	148.25
	168т/69-1	649.21	129.84
	168т/77-1	665.08	133.02
	169т/1-1	603.17	120.63
	169т/2-2	642.86	128.57
	169т/3-3	663.49	132.70
	169т/8-1	598.41	119.68
	170т/6-2	641.27	131.17
2016	172т/19-1	619.05	111.43
	172т/48-3	606.35	109.14
	173т/49-2	357.14	64.29
	173т/50-1	325.40	58.57
	173т/52-2	582.54	104.86
	174т/11-2	412.70	74.29
	177т/1-2	349.21	62.86
	177т/7-2	428.57	77.14
	177т/12-2	587.30	105.71
	177т/23-1	400.00	72.00
	177т/29-2	438.10	78.86
2017	173т/81-1	606.35	126.91
	177т/57-1	726.98	152.16

the results have to be duly verified and not taken as absolutely valid. The DH lines, although possessing comparatively high theoretical uniformity and stability (Belchev et al., 2004), showed in practice contradictory results during the separate periods of growing. This requires conducting further investigations until registering a certain tendency. Thus, the tested DH lines will be able to unfold their properties under contrasting periods and would allow the evaluation of their efficiency and suitability as potential candidate-varieties.

The results on the yield from the DH lines in the preliminary varietal trial were also extremely variable with regard to the investigated genotypes and the harvest years (Table 4). The conditions of 2013 were highly favorable for growing of triticale. Therefore a great part of the lines realized high absolute yields above 900 kg/da. However, their absolute yields were comparatively low compared to the standard Vihren, exceeding it only slightly. Therefore all grown DH lines were discarded. In 2014, both lines tested in PVT gave extremely low absolute yields, lower than 400 kg/da. They were discarded as not promising. In 2015, line 159т/109-1 realized a yield of over 1200 kg/da and exceeded the standard with 160%. It was transferred to CVT for further testing. Line 159т/21-3, due to the high yield it realized, was left for additional investigation in PVT for another growing season.

In harvest year 2016, the lines grown in PVT demonstrated variable reaction. Their yields significantly exceeded the standard with 20-50%. Nevertheless, they were not transferred to the next breeding level but were left for additional observations in the next harvest year. In 2017, out of the three investigated lines, line 159т/74-3 did not confirm the high productivity demonstrated in the previous period; the realized yield was only 18% above the standard, as compared to more than 25% in the other two lines. Therefore this line was discarded. The other two were transferred to PVT for further testing.

Out of the 8 tested lines, which reached the competitive varietal trial, 3 were discarded during the period of investigation. The selection carried out at the CVT level is very strict and is based on all data collected up to this moment on the particular line. At this breeding stage, the yield, which is realized in at least two successive years, is highly important, and in case of high results it is being checked in the next growth seasons, too. The DH lines selected for further testing at the same

Table 4. Absolute and relative yield of DH lines in preliminary variety trial

Harvest year	DH line	Yield, kg/da	Relative yield, %
2013	143т/10-2	899.00	100.67
	143т/16-2	945.00	106.06
	144т/23-1	944.00	105.95
	147т/4-2	981.00	118.34
2014	151т/31-1	328.00	120.59
	148т/44-1	298.00	109.56
2015	159т/109-1	1248.00	262.18
	159т/78-2	572.00	120.17
	159т/71-2	582.00	122.27
	159т/21-3	663.00	157.11
2016	159т/21-3	620.00	137.78
	168т/57-2	676.00	159.06
	159т/74-3	513.00	120.71
2017	159т/21-3	785.00	126.21
	168т/57-2	781.00	138.97
	159т/74-3	666.00	118.51

breeding stage, had high complex score and realized yields which significantly exceeded the mean standard used in the particular trial. The data on the grown lines unequivocally demonstrated that 4 out of the total of 8 lines possessed exceptional properties. This was the reason for their long testing at this breeding level.

The lines tested in CVT can be described as high-yielding regardless of the period of their testing (Table 5). This is so because these lines demonstrated high values at both CVT and at the previous breeding stage (CN or PVT). In 2015, all three tested lines gave yields above 800 kg/da, exceeding the mean standard with more than 15%. The next harvest year, characterized as unfavorable for growing of triticale, differentiated these lines. Line 158т/1-1 realized yield at the level of the mean standard. Its absolute yield outlined it as unpromising and therefore it was discarded. The other two lines, 161т/4-2 and 157т/9-4, confirmed their high productivity and were left for further testing during the next harvest year. In 2017, five of the tested seven lines demonstrated high absolute yields, about and exceeding 650 kg/da, and relative yields with 6% above the mean standard. Lines 157т/9-4, 161т/4-2 and 158т/109-1 gave yields above 680 kg/da. This makes them very promising for further testing as potential candidate-varieties.

DISCUSSION

The breeding process with regard to the DH lines can be clearly determined as efficient. Evidence for this is the data obtained from the testing in CVT. Obtaining of promising lines with high complex score from such amount of initial breeding material (only 1448 progenies) is practically impossible in conventional breeding (Genchev and Vazvazov, 1968). The data of Chahal and Gosal (2000) are a confirmation of this in different plant species using different breeding approaches. The rather high variation in conventional breeding, especially in triticale, does not allow for selection in the early generations, according to Lelley (2006). This is due to the serious variation in F_2 and F_3, regardless of the used triticale cross (Stoyanov and Baychev, 2017). It should be emphasized that the complex selection under contrasting conditions of the environment allowed for more adequate evaluation.

The DH lines tested in the separate breeding stages unequivocally confirmed that the use of anther culture is highly important for obtaining of genetic variability. Evidence for this is the great number of investigations in the initial stages of development of DH lines (Tuvesson et al., 2000; Eudes and Chugh, 2009; Lantos, 2009; Slusarkiewicz-Jarzina et al., 2017). The data obtained on the yield, especially in

Table 5. Absolute and relative yield of DH lines in competitive variety trial

Harvest year	DH-line	Yield, kg/da	Relative yield, %
2015	158т/1-1	802.00	115.73
	161т/4-2	914.50	139.41
	157т/9-4	873.75	133.19
2016	158т/1-1	638.50	103.15
	161т/4-2	781.50	125.85
	157т/9-4	828.50	133.41
	159т/109-1	639.25	119.93
2017	157т/9-4	680.00	106.92
	161т/4-2	750.75	121.68
	159т/109-1	681.50	108.17
	172т/19-1	607.00	96.35
	172т/48-3	642.50	105.33
	177т/12-2	645.25	105.78
	173т/52-2	644.75	105.70

the CN, prior to the strict complex selection in the later breeding stages, are an indication that the DH lines are capable of generating sufficient variability from a breeding point of view. The respective long-term testing of the same materials in PVT and CVT confirmed the hypothesis that genotypes with equal score in one period of investigation may differ in another. The early studies on DH triticale lines (Charmet and Branlard, 1985) reached in practice to similar conclusions, namely that the realized variation leads to both highly productive genotypes and to genotypes with insignificant productivity.

The insufficient number of field studies on triticale DH lines does not allow proving in practice that many of the reported problems are not in fact a serious impediment in breeding of triticale. The obtained high-yielding lines did not have high occurrence of aneuploid plants, as pointed out by Oleszczuk et al. (2011). This is due to the extremely strict selection for uniformity carried out as early as the breeding nursery stage. In selection at the breeding nursery, the practical observations reveal that aneuploidy occurred both in the conventional lines and in the DH lines. Therefore it can be assumed that within this investigation aneuploidy should not be considered a big problem which decreases the efficiency of the breeding process.

Concerning the stability of the DH lines, it should be pointed out that in literature results have often been reported, which underlined the phenotypic instability of the triticale DH lines (Wedzony et al., 2015). The results from the three-year testing of promising lines at CVT undoubtedly confirmed their high productivity. The results from the ANOVA performed on the DH lines in CVT emphasized the extreme differences between the genotypes and their different response to the conditions of the environment (Table 6). They also differed in stability. The data from the AMMI analysis highlighted the differences between the tested genotypes and the registered triticale cultivars during the period of investigation.

Figure 1 presents a biplot of AMMI 1 model of the main effects and interactions. The main assumption here is that the genotypes or environments positioned at a greater distance from the center are characterized with higher interaction (Kempton, 1984; Kroonenberg, 1995; Akter et al., 2014; Anisuzzaman et al., 2014; Farshadfar and Farhadi, 2014). According to this model, the genotypes with values of the yield above the average and IPCA values approximating zero are better adapted to all growing conditions. On the other hand, the genotypes with high yield values and also with high IPCA values are adapted to specific growing conditions. Lines 157т/9-4 and 161т/4-2 gave yields exceeding 800 kg/da within a 3-year period. Simultaneously, they demonstrated comparatively high stability as well. The analysis carried out emphasized their considerable difference from the rest of the genotypes. Such results have not been observed in none of the investigated genotypes, either in short-term investigations (Stoyanov and Baychev, 2016b), or in long-term ones (Stoyanov and Baychev, 2016a). Figure 1 shows also a biplot of AMMI II model, which presents the combined interactions of the environment with the genotype. The environmental conditions are presented by vectors from the center of the graph. The longer vectors are usually related to a higher level of interaction (Anisuzzaman et al., 2014). During the investigated periods, the lengths of the three of them were approximately equal, but they were not unidirectional. This shows the rather contrasting conditions of growing. Respectively, the genotypes and the environmental conditions within the same sector indicated positive interaction.

It is worth mentioning that lines 157т/9-4 and 161т/4-2 were positioned in different sectors ac-

Table 6. ANOVA of the studied DH lines in CVT along with registered triticale cultivars

Source of variation	Sum of Squares	df	Mean square	F	Sig.
Genotype	1166017,083	19	61369,320	12,872	,000
Environment	1614152,125	2	807076,062	169,287	,000
Genotype x environment	815120,750	36	22642,243	4,749	,000
Error	829543,875	174	4767,494		
Total	4397690,155	231			

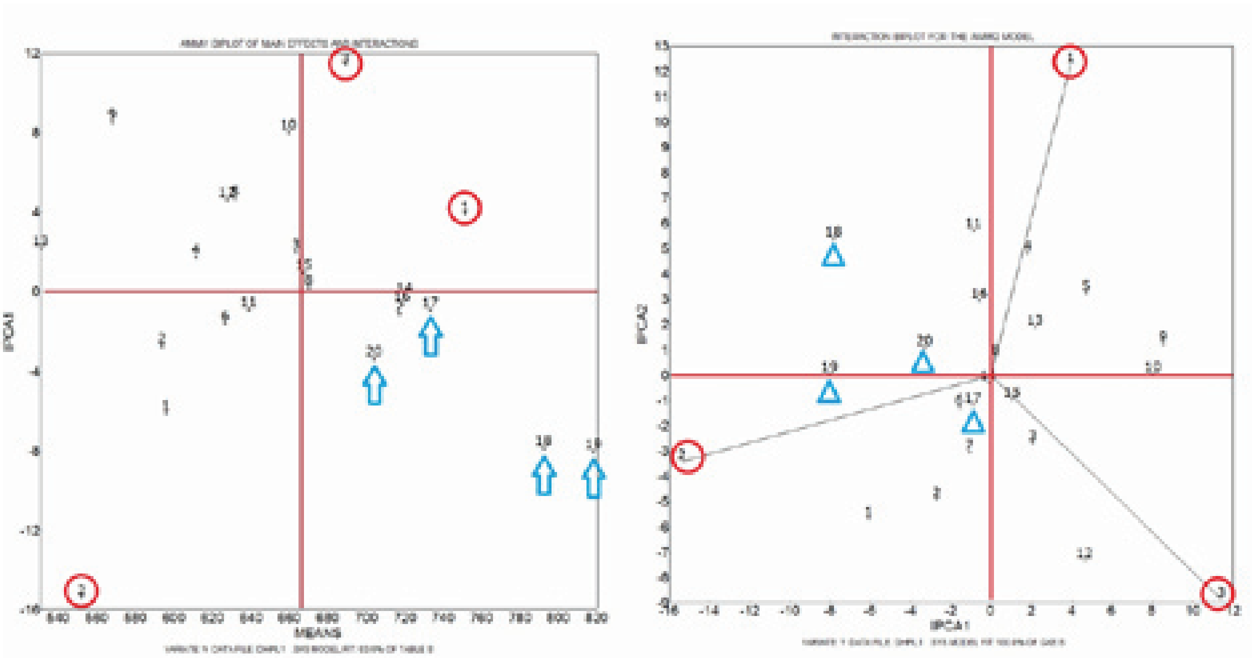

Figure 1. AMMI1 and AMMI2 biplots of studied DH lines and varieties
1) AD-7291, 2) Vihren, 3) Rakita, 4) Lasko, 5) Presto, 6) Kolorit, 7) Atila, 8) Akord, 9) Respekt, 10) Bumerang, 11) Irnik, 12) Dobrudzhanets, 13) Lovchanets, 14) Doni 52, 15) Blagovest, 16) Borislav, 17) 158т/1-1, 18) 157т/9-4, 19) 161т/4-2, 20) 159т/109-1

cording to each period of growing. This is related to highly complex interactions of the genotypes with the growing environment. Similar complex interactions are shown in the investigation of Lozano del Rio et al. (2009) and Goyal et al. (2011). Such characteristics make the investigated DH lines extremely valuable genotypes which can be successfully tested as candidate-varieties under the various soil and climatic conditions of Bulgaria. This shows that the DH lines also possess high productivity and their use in the breeding of triticale is highly efficient as a technological process.

CONCLUSIONS

Based on the above results, the following conclusions can be drawn:

1. Out of the 1448 DH lines investigated in the breeding nursery, only 8 reached CVT level, which indicated the comparatively low efficiency of the breeding process, regardless of their theoretical uniformity.

2. The data from the testing of the DH lines in CN, PVT and CVT pointed out that the DH lines realized diverse variation according to their properties and yield dependent both on the investigated genotype and on the year.

3. A part of the investigated lines realized yields exceeding the mean standard with 20-100% under the conditions of the different breeding stages, which is an exceptional achievement in the breeding of triticale.

4. Lines 157т/9-4 and 161т/4-2 realized yields of over 800 kg/da within a three-year testing, which make them highly suitable for testing within the system of the national Executive Agency of Variety Testing, Field Inspection and Seed Control.

5. The results from the DH lines investigated under filed conditions undoubtedly show that dihaploidy is an important tool of contemporary breeding for production of high-yielding triticale genotypes.

ACKNOWLEDGEMENTS

This paper was presented in the 4th Balkan Scientific Conference on Biology, Plovdiv, Nov. 1-3, 2017, organized by Faculty of Biology, Plovdiv University, Bulgaria.

REFERENCES

Akter, A., Jamil, H. M., Umma, K. M., Islam, M. R., Hossain, K., & Mamunur, R. M. (2014). AMMI biplot analysis for stability of grain yield in hybrid rice (Oryza sativa L.). *J. Rice Res*, *2*(2), 126.

Anisuzzaman, M., Islam, M. R., Khatun, H., Miah, M. S. & Biswas, P. S. (2014). Analysis of G x E interaction by using the AMMI in advanced rice genotypes. *Eco-friendly Agril. J.*, 7(10), 119-123.

Baychev, V. (1990). Creation and investigation of primary and secondary triticales, PhD Thesis, General Toshevo (Bg).

Baychev, V. (1996). Research on hybrid forms triticale in F_1 and F_2. *Rastenievadni nauki*, 33(2), 51-55 (Bg).

Belchev, I., Tchorbadjieva, M. & Panchev, I. (2004). Effect of 5-azacytidine on callus induction and plant regeneration potential in anther culture of wheat (*Triticum aestivum* L.). *Bulg. J. Plant Physiol.*, 30(1-2), 45-50.

Bos, I. & Caligari, P. (2008). Population Genetic Effects of Inbreeding. In: *Selection Methods in Plant Breeding.* Springer, Dordrecht.

Chahal, G. S. & Gosal, S. S. (2000). Principles and procedures of plant breeding: Biotechnological and conventional approaches. CRC Press, New York.

Charmet, G., & Branlard, G. (1985). A comparison of androgenetic doubled-haploid, and single seed descent lines in Triticale. *Theoretical and Applied Genetics*, *71*(2), 193-200.

Eudes, F. & Chugh, A. (2009). An overview of triticale doubled haploids. In: Touraev A, Forster BP, Jain SM (eds), *Advances in Haploid Production in Higher Plants*. Springer, Dordrecht

Farshadfar, E., & Farhadi, M. (2014). AMMI and AMMI based analysis of phenotypic stability in wheat-Agropyron disomic addition lines. *Journal of Biodiversity and Environmental Sciences*, *5*(4), 548-557.

Genchev, G. & Vazvazov, I. (1968). Breeding and seed production of field crops. Hr. G. Danov (Bg).

Genchev, G., Marinkov, E., Yovcheva, V. & Ognyanova, A. (1975). Biometrical methods in plant production, genetics and breeding. Zemizdat.

Goyal, A., Beres, B. L., Randhawa, H. S., Navabi, A., Salmon, D. F., & Eudes, F. (2011). Yield stability analysis of broadly adaptive triticale germplasm in southern and central Alberta, Canada, for industrial end-use suitability. *Canadian Journal of Plant Science*, *91*(1), 125-135.

Grauda, D., Lepse, N., Strazdiņa, V., Kokina, I., Lapiņa, L., Miķelsone, A., Ļubinskis, L. & Rashal, I. (2010). Obtaining of doubled haploid lines by anther culture method for the Latvian wheat breeding. *Agronomy Research*, *8*(Special Issue III), 545-552.

Hussain, B., Khan, M. A., Ali, Q., & Shaukat, S. (2013). Double haploid production in wheat through microspore culture and wheat x maize crossing system: an overview. *IJAVMS*, *6*(5), 332-344.

Kempton, R. A. (1984). The use of biplots in interpreting variety by environment interactions. *The Journal of Agricultural Science*, *103*(1), 123-135.

Kroonenberg, P. M. (1995). Introduction to biplots for G.E tables. *Dep. of Mathematics Research. Report. No. 51*, University of Queensland Australia.

Lantos, C. (2009). In vitro androgenezis induction in wheat (*Triticum aestivum* L.), triticale (x*Triticosecale* Wittmack), spice pepper (*Capsicum annum* L.) and integration of the results into breeding. Thesis of the Ph.D dissertation. Gödöll.

Lelley, T. (2006). A low-input cereal with untapped potential. In: Singh RJ, Jauhar P (eds) *Genetic resources, chromosome engineering, and crop improvement cereals* (Chap. 13), vol 2. CRC Press, Boca Raton, pp. 395-430.

Lozano-del Río, A. J., Zamora-Villa, V. M., Ibarra-Jiménez, L., Rodríguez-Herrera, S. A., de la Cruz-Lázaro, E., & de la Rosa-Ibarra, M. (2009). AMMI analysis of genotype-environment interaction and production potential of forage triticale (x Triticosecale Wittm.). *Universidad y Ciencia Tropico Humedo*, *25*(31), 81-92.

Oleszczuk, S., Rabiza-Swider, J., Zimny, J., & Lukaszewski, A. J. (2011). Aneuploidy among androgenic progeny of hexaploid triticale (XTriticosecale Wittmack). *Plant Cell Reports*, *30*(4), 575-586.

Plamenov, D., Belchev, I., Daskalova, N., Spetsov, P., & Moraliyski, T. (2013). Application of a low dose of gamma rays in wheat androgenesis. *Archives of Biological Sciences*, *65*(1), 291-296.

Randhawa, H. S., Bona, L., & Graf, R. J. (2015). Triticale breeding: progress and prospect. In *Triticale* (pp. 15-32). Springer, Cham.

Ślusarkiewicz-Jarzina, A., Pudelska, H., Woźna, J., & Pniewski, T. (2017). Improved production of doubled haploids of winter and spring triticale hybrids via combination of colchicine treatments on anthers and regenerated plants. *Journal of Applied Genetics*, *58*(3), 287-295.

Stoyanov, H. & Baychev, V. (2016a). Achievements and trends in the breeding of triticale in Bulgaria. In: *9th International Triticale Symposium, Szeged, Hungary,* May 23-27, 2016, Book of Abstracts, 20.

Stoyanov, H. & Baychev, V. (2016b). Analysis on "genotype x environment" interaction in Bulgarian triticale (×*Triticosecale* Wittm.) cultivars. *Scientific Works of Institute of Agriculture – Karnobat*, (in press).

Stoyanov, H. & Baychev, V. (2017). Research on the variability in triticale (×*Triticosecale* Wittm.) crosses as a source of genetic diversity. Youth Scientific Conference "Kliment's Days", Sofia 2016, Annuaire de l'Université de Sofia "St. Kliment Ohridski", Faculte de Biologie, 2017, 102(4), 105-126.

Stoyanov, H., Baychev, V. & Mihova, G. (2017). Analysis and assessment of yield ranking models in triticale (x*Triticosecale* Wittm.) in contrasting environmental conditions. *Journal of Tekirdag Agricultural Faculty,* *The Special Issue of 2nd International Balkan Agriculture Congress, May 16-18, 2017*, 83-90.

Thiemt, E. M., & Oettler, G. (2008). Agronomic performance of anther-derived doubled haploid and single seed descent lines in crosses between primary and secondary winter triticale. *Plant Breeding*, *127*(5), 476-479.

Tsenov, N., Atanasova, D., Nankova, M., Ivanova, A., Tsenova, E., Chamurliiski, P. & Raykov, G. (2014). Approaches for grading breeding evaluation of winter wheat varieties for grain yield. *Scientific Works of Instiute of Agriculture – Karnobat*, 3(1), 21-35.

Tuvesson, S., Ljungberg, A., Johansson, N., Karlsson, K. E., Suijs, L. W., & Josset, J. P. (2000). Large-scale production of wheat and triticale double haploids through the use of a single-anther culture method. *Plant Breeding*, *119*(6), 455-459.

Wędzony, M., Żur, I., Krzewska, M., Dubas, E., Szechyńska-Hebda, M. & Wąsek, I. (2015). Doubled haploids in triticale. In: Eudes F. (eds) *Triticale*. Springer, Cham.