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ANALYSIS AND ASSESSMENT OF AMPHIDIPLOIDS OF TRITICUM-AEGILOPS GROUP AS A SOURCE OF GENETIC DIVERSITY

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Abstract

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Amphidiploids in *Triticum-Aegilops* group are an essential part of breeding programs of wheat species. Difficulties arising from the wide hybridization, suggest further study of amphidiploids and also of their reactions to growing conditions. 10 amphidiploids of *Triticum-Aegilops* group were analyzed by their morphological, physiological and cytological characteristics in order to assess their importance as a source of genetic diversity. Based on the studied biological capabilities of amphidiploids, a genetic diversity evaluation index (GDEI) was calculated. The index demonstrates the general adaptive mechanisms of plant organism from each of the amphidiploid forms, under natural growing conditions. Most of the amphidiploid forms with stable physiological parameters are more close to the GDEI of winter wheat and rye (GDEI = 50–100), but highly productive - to the characteristics of triticale (GDEI > 110). GDEI < 40 exhibit low adaptive potential to the growing conditions. These characteristics define the studied accessions with GDEI > 50 as a good source of genetic diversity and a good initial material for breeding of cultural wheat species.

Key words: biological capabilities, evaluation index, growing conditions

Introduction

Cereals are of greatest economic and food importance worldwide. An ever increasing demand for more and higher quality production is a reason for seeking methods increasing the potential of these crops, which is related to genetic diversity amongst the initial breeding material (Reynolds at al., 1996). On the other hand the need for diversification in agricultural production is reason new crop cultures to be sought combining complex resistance to biotic and abiotic environmental factors (Goncharov et al., 2008). As an instrument of classical breeding, the creation of hybrid plants and amphidiploid forms becomes potential opportunity to increase the genetic diversity.

Amphidiploids are plant forms obtained after artificial duplication of the genome of the hybrids resulting from the wide hybridization. A wide variety of amphidiploid forms was received in *Triticae* (Mujeeb Kazi and Hettel, 1995), and as a result of a number of new conducted studies new accessions are constantly created (Stoyanov, 2013a). The largest number amphidiploids are developed through wide hybridization in the *Triticum-Aegilops* group. The two genera are phylogenetically close and developed in parallel during the evolution of *Poacea* family (Matsuoka, 2011). The presence of natural hybrids and amphidiploid character of cultural and wild tetraploid and hexaploid wheats underlines their phylogenetic proximity (Stoyanov, 2013b). This is the reason intergeneric and interspecific hybrids to be obtained more easily. However, there are many difficulties typical of the wide hybridization and some negative characteristics remain into amphidiploid forms (reduced fertility, immature endosperm, low germination (Mujeeb-Kazi and Hettel, 1995)). Most of the amphidiploid forms in the *Triticum-Aegilops* group are determined by high resistance and high adaptability to the environmental conditions (Stoyanov, 2013a). This is mainly due to the presence of the chromosomes from wild species, but also to the integration of two or more genomes.

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The combination of negative and positive features of amphidiploids in the *Triticum-Aegilops* group is a prerequisite, a certain balance in the characteristics of the accessions, which would participate in breeding programs, to be found. This makes it necessary, certain plant forms to be analyzed in detail in order to clarify all their morphological, physiological and cytological characteristics. Summarizing such data for each of the studied amphidiploids should be carried out using one single indicator that reflects on the adaptive capacity of plants of a given accession to the specific environmental conditions. Thus, the analysis and evaluation would allow quick, accurate and scientifically justifies comparing of studied amphidiploid forms, both between themselves and with the crops and wild species representatives. This would provide an opportunity to assess the genetic diversity of the *Triticum-Aegilops* group.

The purpose of this research is to be analyzed accessions of amiphidploid forms on their morphological, physiological and cytological characteristics and to be developed a method for comparative evaluation between different accessions, cultivated and wild forms which allow they to be classified as a source of genetic diversity.

Materials and Methods

Ten amphidiploid plant accessions were used, whose botanical identity and origin are presented in Table 1. Fifteen seeds of each accession were sown in a scheme with spacing of 30 cm between rows and 5 cm between seeds. Sowing was done on 11–13.11.2012, under field conditions in the area of Stozher, Dobrich region.

The field germination (FG) was recorded, as a ratio between the number of seed germinated before winter (NSGW) and the number of seeds sowed before winter (NSSW). The number of plants survived after winter (overwintered plants) (NPSW) are reported as well as the number of plants reached maturity (NP) per accessions. For each accession the total number of spikes (NS) and the average number of tillers per plant (NT) were reported.

Harvesting was done in phase full maturity in the period 12–20.07.2013. Of each sample were randomly selected 20 fully matured spikes, free of infestation by pests. The morphological evaluation of the spikes of each accession was done per six quantitative characteristics: length of spike (LS), length of spike with awns (LSA), weight of spike (WS), number of spikelets per spike (NSS), weight of kernels in a spike (WKS), number of kernels in a spike (NKS). The weight of 1000 grains (M1000) was determined according to standard methodology. Plant height (H) was measured at full maturity simultaneously with spikes collecting.

The phytopathogens attack was carried out under field conditions and natural occurrence of pathogens with 10-level scale: for powdery mildew (EG) the methods of Stoilova and Spetsov (2006) were used, for brown rust (PR) the methodology of Ivanova (2012) was applied; for septoria leaf blight (ST) the methods of Eyal et al. (1987) were used.

The laboratory germination was determined according to standard methodology. The real chromosome number was established by acetocarmine staining according the methods of Georgiev et al. (2008).The expected chromosome number (ECN) was calculated on the basis of parental forms involved into the amphidiploids.

A specific genetic diversity evaluation index (GDEI) was developed and calculated.

To summarize the data, software Microsoft Excel 2003 was used. Cluster analysis via Euclidean distance method was made, to group the accessions according to their GDEI. The correlation between WKS and GDEI was defined. For correlation and cluster analysis software IBM SPSS Statistics 19 was used.

Table 1

Botanical classification and origin of amphidiploid accessions

No	Abbr.	Botanical classification	Origin
1	AE483	*Aegilops ventricosa* x *Triticum dicoccon*	IPK – Gatersleben, Germany
2	TRI17927	*Aegilotriticum erebunii*	IPK – Gatersleben, Germany
3	TRXOAE	*Triticum aestivum* cv. Trakia x *Aegilops ovata*	DAI – General Toshevo, Bulgaria
4	TRI12087	*Aegilops ventricosa* x *Triticum dicoccoides*	IPK – Gatersleben, Germany
5	TRI11943	*Aegilops ventricosa* x *Triticum dicoccon*	IPK – Gatersleben, Germany
6	TRI12090	*Aegilops ventricosa* x *Triticum carthlicum*	IPK – Gatersleben, Germany
7	A1-6	*Triticum durum* cv. Saturn x *Triticum boeoticum*	DAI – General Toshevo, Bulgaria
8	A3-8	*Triticum polonicum* x *Triticum boeoticum*	DAI – General Toshevo, Bulgaria
9	TTTTv	*Triticum turanicum* x *Triticum timopheevii*	DAI – General Toshevo, Bulgaria
10	TTAT	*Triticum timopheevii* x *Aegilops tauschii*	DAI – General Toshevo, Bulgaria

Results and Discussion

The observed wide variation into the analyzed characteristics (Tables 3 and 4) was indicative of a great genetic diversity among the group of studied amphidiploids. Providing of genetic diversity in a plant population is directly related to its size (Ayala and Kiger, 1987) – the larger number of plants – more genetic combinations within populations. This is associated with obtaining of more, resistant to stress factors, genetically stable and healthy, high-yielding plants. Based on this reason, a methodology for comparative assessment of the amphidiploid forms was developed. Index characteristics related to the adaptive capabilities of the plant organism were established (Table 2). By the same method 10 accessions of bread wheat *Triticum aestivum* (TRA), 10 accessions of rye *Secale cereale* (SEC), 5 accessions of triticale ×*Triticosecale* (TRC), 10 accessions of *Aegilops cylindrica* (AEC), 10 accessions of *Aegilops tauschii* (AET), were analyzed and the results were averaged per species. The values of the adaptability index parameters (Table 5) were characterized by the following features:

Number of tillers (NT). Greater number of tillers in TRI12090 and AE483 provided increased offspring, but also correlated with a longer period of formation of spikes and seeds. Some authors (Evers et al., 2005; Kirchev, 2005; Popova and Neykov, 2013) reported analogous results for wheat and barley.

Table 2

Characteristics related to adaptation capabilities of amphidiploids

Characteristics	Abbr.	Biological capability	Formula
Number of tillers	NT	Productive potential	Direct measurement
Weight of 1000 kernels	M1000	Nutrient reserves	Direct measurement
Fertility	F	Reproductive capabilities	NKS/(NSS.2) or NKS/(NSS.3)*
Awns index	AI	Spreading capabilities	LSA/LS
Lodging index	LI	Resistance to lodging	1-(WS/H)
Disease index	DI	Biotic stress adaptability	1/((EG+PR+ST)/3)
Winter index	WI	Winter resistance	NPSW/NSGW
Surviving index	SI	Spring drought tolerance	NP/NPSW
Vitality index	VI	Germination capacity	LG.FG
Chromosome index	ChI	Genetic stability	RCN/ECN
Genetic Diversity Evaluation Index	GDEI	General adaptation capabilities	NT.M1000.F.AI.LI.DI.WI.SI.VI.ChI

* The coefficient 3 or 2 depends on the usual florets formation for maternal and paternal parent participating into the amphidiploids

Table 3

Morphological characteristics of amphidiploids

No	NP	NS	NSS	LS, m.10^{-3}	LSA, m.10^{-3}	WS, kg.10^{-3}	WKS, kg.10^{-3}	NKS	H, m.10^{-2}
AET	8	65	24.00	90.00	115.00	1.40	0.91	25.00	51.00
SEC	14	42	30.00	110.00	120.00	2.00	1.60	45.00	120.00
TRA	14	42	20.00	100.00	125.00	2.75	2.00	48.00	85.00
TRC	15	60	25.00	120.00	150.00	4.30	3.50	56.00	90.00
AEC	10	200	15.00	95.00	145.00	0.60	0.40	20.00	45.00
AE483	5	49	14.05	136.50	171.80	1.76	0.81	21.75	60.00
TRI17927	2	11	15.00	119.90	143.09	0.88	0.30	11.55	58.00
TRXOAE	6	19	6.52	55.00	87.89	0.61	0.19	5.00	40.00
TRI12087	8	42	12.15	118.90	159.50	1.35	0.53	17.05	61.00
TRI11943	11	53	11.65	118.25	154.65	1.40	0.63	18.15	58.00
TRI12090	8	78	12.75	94.25	168.35	1.56	0.97	26.10	63.00
A1-6	2	6	13.33	70.17	204.17	3.26	2.24	37.67	75.00
A3-8	15	63	14.35	90.75	174.15	3.01	2.28	40.70	78.00
TTTTv	8	31	20.00	96.30	192.85	2.70	1.96	38.90	85.00
TTAT	6	43	13.15	80.35	135.55	1.64	0.93	18.70	74.00

Table 4

Physiological and cytological characteristics of amphidiploids

No	NSSW	NSGW	NPSW	LG	FG	EG	PR	ST	ECN	RCN
AET	15	10	8	0.98	0.67	1	5	1	14	14
SEC	15	15	14	0.99	1.00	1	1	1	14	14
TRA	15	14	14	0.99	0.93	2	1	4	42	42
TRC	15	15	15	0.95	1.00	1	1	1	56	56
AEC	15	11	10	0.95	0.73	5	5	1	28	28
AE483	15	8	5	0.99	0.53	1	1	1	42	42
TRI17927	15	2	2	0.95	0.13	1	1	1	28	28
TRXOAE	15	6	6	0.60	0.40	1	1	1	70	70
TRI12087	15	8	8	0.99	0.53	1	4	1	42	42
TRI11943	15	11	11	0.99	0.73	1	4	1	42	42
TRI12090	15	8	8	0.99	0.53	1	4	1	42	42
A1-6	15	6	2	0.95	0.40	1	1	1	42	42
A3-8	15	15	15	0.95	1.00	1	1	1	42	42
TTTTv	15	10	8	0.95	0.67	1	1	1	56	56
TTAT	15	9	6	0.99	0.60	1	1	1	42	42

Table 5

Data of characteristics related to adaptation capabilities of amphidiploids

No	NT	M1000	F	AI	LI	DI	WI	SI	VI	ChI	GDEI
AET	8.13	36.40	0.52	1.28	0.97	0.43	0.80	1.00	0.65	1.00	**42.88**
SEC	3.00	35.56	0.75	1.09	0.98	1.00	0.93	1.00	0.99	1.00	**79.30**
TRA	3.00	41.67	0.80	1.25	0.97	0.43	1.00	1.00	0.92	1.00	**47.90**
TRC	4.00	62.50	0.75	1.25	0.95	1.00	1.00	1.00	0.95	1.00	**211.08**
AEC	20.00	20.00	0.44	1.53	0.99	0.27	0.91	1.00	0.70	1.00	46.24
AE483	9.80	37.08	0.77	1.26	0.97	1.00	0.63	1.00	0.53	1.00	**113.39**
TRI17927	5.50	26.14	0.38	1.19	0.98	1.00	1.00	1.00	0.13	1.00	**8.24**
TRXOAE	3.17	38.00	0.38	1.60	0.98	1.00	1.00	1.00	0.24	1.00	**17.43**
TRI12087	5.25	31.20	0.70	1.34	0.98	0.50	1.00	1.00	0.53	1.00	**40.00**
TRI11943	4.82	34.52	0.78	1.31	0.98	0.50	1.00	1.00	0.73	1.00	60.02
TRI12090	9.75	37.15	0.68	1.79	0.98	0.50	1.00	1.00	0.53	1.00	**113.66**
A1-6	3.00	59.46	0.94	2.91	0.96	1.00	0.33	1.00	0.38	1.00	**59.24**
A3-8	4.20	56.02	0.95	1.92	0.96	1.00	1.00	1.00	0.95	1.00	**389.87**
TTTTv	3.88	50.31	0.65	2.00	0.97	1.00	0.80	1.00	0.63	1.00	**124.17**
TTAT	7.17	49.89	0.47	1.69	0.98	1.00	0.67	1.00	0.59	1.00	**110.71**

Weight of 1000 kernels (M1000). Values of M1000>50 (amphidiploids A1-6, A3-8, TTTTv), determined higher germination energy and healthy, well-rooted plants before the winter influences. Similar results were obtained for wheat (Dochev, 2011; Mian and Nafziger, 2013).

Fertility (F). All studied amphidiploids were fertile, but the values vary according to the genotype. Higher seedset was obtained in A1-6 and A3-8, which demonstrate their ability to reproduce properly. Stoyanov (2013a) and Mujeeb-Kazi and Hettel (1995) reported similar results for different amphidiploid forms.

Awns index (AI). The presence of long awns in some of the studied amphidiploids (A1-6, A3-8 and TTTTv) allows easier distribution of spikes and digging of the spikelets into the soil when they are in contact with soil moisture. This is a mechanism, specific to the wheat species (Elbaum et al., 2007; Li et al., 2009).

Lodging index (LI). Larger spikes, located at a greater height, defined a higher risk of lodging in amphidiploids A1-6 and A3-8. Obtained results for other cereals (Baker et al., 1998; Berry et al., 2004; Zhu et al., 2006) underlined the importance of plant height during the vegetation.

Disease index (DI). Winter index (WI); Surviving index (SI). All observed plants were resistant to pathogens of powdery mildew, brown rust (excluding TRI11943, TRI12087,

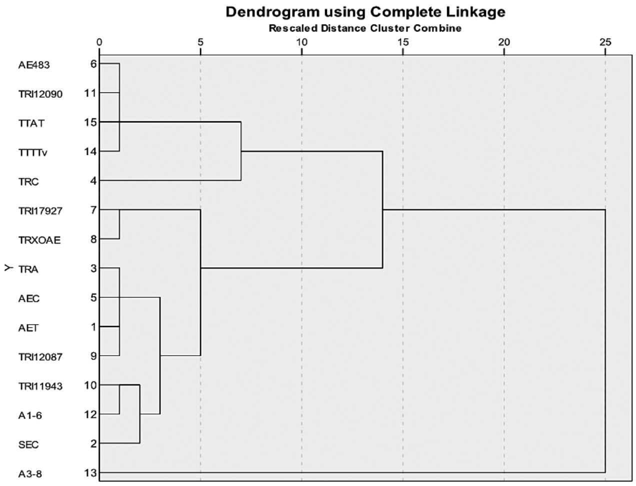

Fig. 1. Dendrogram of cluster analysis of amphidiploids by their GDEI

TRI12090), septoria leaf blotch and spring drought. The resistance to winter conditions in studied amphidiploids varied from very low in accession A1-6, to high in accession A3-8. Similar results were obtained in previous research with studied amphidiplods (Spetsov and Savov, 1992; Plamenov et al., 2009; Stoyanov, 2012). This resistance is indicative to high productivity and adaptability of studied amphidiploids.

Vitality index (VI). LG in all accessions was high (95-99%), while the FG varied according to the examined genotype (13-100%). Summarizing of FG and LG determined the adaptability of the amphidiploids to different conditions which was confirmed by our previous investigations (Stoyanov, 2012).

Chromosome index (ChI). All plants were genetically stable and severe physiological disturbances are not observed. Analogous results reported Mujeeb-Kazi and Hettel (1995).

Genetic diversity evaluation index (GDEI). Accumulation of indicators characterizing the formation of the yield, reaction to stress and genetic stability, allowed amphidiploids to be evaluated as a source of genetic diversity, but also allows proper selection of initial breeding material. The considerable (61.3%) and significant ($\alpha < 0.05$) correlation of this index with WKS (as a structural element of the yield), underlines the relation of wide genetic diversity with higher levels of yield and greater resistance to biotic and abiotic stress factors.

Based on the conducted correlation analysis by GDEI indicator, studied amphidiploids were grouped according to their general adaptation abilities. The studied accessions could be separated into four groups by their GDEI: Group A – amphidiploids with GDEI<40 (accessions TRI17927 and TRXOAE), which were unable to respond properly to the specific environmental conditions, do not possess ecologic plasticity and they could not be classified as a good source of genetic diversity; Group B – amphidiploids with GDEI>50 and GDEI<110 (amphidiploids close to wheat, rye and wild forms – TRI11943, TRI12087, A1-6); Group C – amphidiploids with GDEI>110 (accessions with values close to those of TRC – AE483, TRI12090, TTAT, TTTTv); Group D – accessions with very high values of GDEI (GDEI>300-400) (accession A3-8). Assembled dendrogram (Figure 1) illustrates the ability of some amphidiploids to remain with similar characteristics to the cultural and wild species. Falling of *Aegilops* accessions and winter wheat accessions in the same cluster emphasizes their similar adaptation capabilities as evidenced by their parallel evolution and possessing the D-genome. A3-8 forms separate cluster, which highlights its high adaptability and ecological plasticity compared with other amphidiploids. Similar features exhibit highly productive amphidiploids *Triticum ×fungicidum* (Scharen and Eyal, 1980), *Triticum ×timococcum* (Goncharov et al., 2007), *Triticum ×petropavlovskyi* (Masum-Akond and Watanabe, 2005). Four accessions of the studied amphidiploids by their values of GDEI were close to the characteristics of TRC (Table 5), which is due to its amphidiploid character. Most of the amphidiploids obtained from wide hybridization possessed characteristics similar to those of triticale (high productivity, disease resistance, genetic stability (Tsitsin, 1978; Spetsov and Savov, 1992; Martin et al., 2000; Plamenov et al., 2009; Stoyanov, 2013a)). These were also synthetic hexaploid wheats after their second generation (Stoyanov et al., 2010; Plamenov and Spetsov, 2011). Despite some of their positive characteristics, amphidiploids TRI17927 and TRXOAE had values for GDEI, lower than those of cultivated and wild species of the group *Triticum-Aegilops*. Their weak adaptation capabilities to environmental conditions were due to low fertility and poor germination. Similar results have been reported in many studies of amphidiploids where the genome of genus *Aegilops* was included (Mujeeb-Kazi and Hettel, 1995).

Conclusions

A methodology, based on morphological, physiological and cytological characteristics of amphidiploid accessions, that allows the implementation of comparative assessment, both between samples and to similar cultural and wild forms, using aggregative indicator – GDEI, has been developed. The relationship between genetic diversity and adaptive capabilities of amphidiploids was demonstrated. Greatest genetic diversity evaluated by their GDEI was able to realize accessions with GDEI>110 (Group C and D), but the smallest – accessions with

GDEI<40 (Group A). Despite some of their negative characteristics, all accessions with GDEI>50 are suitable to be included into the breeding program of different wheat species.

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