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NEW AMPHIDIPLOID WHEAT SPECIES (NOTHOSP. NOV.) AS A RESULT OF ARTIFICIAL HYBRIDISATION

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Abstract

The continuous growth of the demand of cereals and diversification of agricultural production is reason to seek new and high-yielding plant species. Remote hybridization and artificial polyploidisation are methods creating new species which combine the valuable properties of their parental forms and in some cases outmatch them. A large number of hybrids and amphidiploid forms in the genus Triticum are created and characterized with resistance to diseases, high yield potential and high adaptability under different growing conditions. Some of them are genetically stable in subsequent generations without sharp variation to the specific characteristics. Similar plant accessions exhibiting significant differences from their parental components as well as from other plant species in the same genus, furthermore in combination with their high genetic stability, should be separated as a new species within the genus Triticum. In studies on the morphology and physiology of three amphidiploid accessions (Triticum polonicum × Triticum boeoticum, Triticum durum × Triticum boeoticum, Triticum turanicum × Triticum timopheevii), for a period of three years (2011-2013) their stability, lack of sharp variations, high adaptability to growing conditions, high yield performance, resistance to diseases and tolerance to pests are established. As a result of their complex evaluation, the accessions are differentiated and described as a new species in the genus Triticum as follows: Triticum ×savovii H.P. Stoyanov nothosp. nov. (Triticum polonicum × Triticum boeoticum); Triticum ×spetsovii H.P. Stoyanov nothosp. nov. (Triticum durum × Triticum boeoticum); Triticum ×toschevii H.P. Stoyanov nothosp. nov. (Triticum turanicum × Triticum timopheevii). The described new species could be successfully introduced as grain cereals and used as initial material in winter wheat breeding programs.

Key words: *new species, polyploidisation, Triticum sp., wide hybrids.*

INTRODUCTION

Modern agricultural production is based on high yields of crops as well as the ever increasing demand for quality agricultural production. This requires more high-yield varieties of certain plant species to be created which could meet food needs of the population and the need for livestock feed. Diversification of production enables plant resources to be used in accordance with good agricultural and plant protection practices, but also to provide diversity as regards food production. Introduction of new crops into the grain production is done by introduction of plant species from other geographical regions, but also by artificially creating of cultural hybrid and amphidiploid species (Ayala and Kiger, 1987).

Amphidiploids are plant organisms created by the methods of wide hybridization and combining the genomes (or specified parts of them) of the parental species involved in the cross (Stoyanov, 2013a). Such organisms combine valuable breeding characteristics such as resistance to biotic and abiotic stress, which also determines the high yield potential. Despite these properties amphidiploids are characterized by a number of negative others caused by interspecies incompatibility of organisms participating into the cross (Stoyanov 2013b, Spetsov et al., 2008). The biotechnological methods accepted in modern breeding programs enable to overcome such difficulties, but the creation of cultural amphidiploids is associated with large-scale study of the applicability of the new plant organism as a potential food product.

A large number amphidiploids are created into the tribus *Triticae*. These plant organisms possess a number of valuable qualities and combine high variability because of the high degree of polymorphism and possibilities for recombination due to the integration of diverse genomes, which depends on the species participating into the initial crosses (Stoyanov

et al., 2012; Kolev, 1984). The largest number of created amphidiploids are synthetic hexaploid wheats (2n=6x=42, AABBDD), because of the identity of their genomic constitution with that of bread wheat *Triticum aestivum* (2n=6x=42, AABBDD), allows for the introduction of various genes from wild species *Aegilops tauschii* (2n=2x=14, DD) (Spetsov et al., 2008; Spetsov et al., 2009; Stoyanov et al., 2010). Synthetic forms also could combine the genomes of various forms tetraploid wheats (*Triticum durum*, *Triticum polonicum*, *Triticum dicoccon*, etc.) and diploid wild species (*Aegilops* sp., *Hordeum* sp., *Secale* sp., *Dasypyrum* sp., *Triticum* sp.), allowing the transfer of valuable breeding characteristics, and also leads to the creation of high-yield plant forms. These are amphidiploid grain cereals triticale (×*Triticosecale*) and tritordeum (×*Tritordeum*) (Stoyanov, 2013a; Knuepffer, 2009; Martin et al., 2000; Kolev, 1978).

Amphidiploid species in the genus *Triticum*, which combine the genomes of various wheat forms, are distinguished by simple crossability of parental forms and a variety of properties inherited from wild species – high protein content, resistance to pathogens and insect pests, tolerance to drought. These include species such as *Triticum* ×*petropavlovskyi*, *Triticum* ×*timococcum*, *Triticum* ×*fungicicum* (Mico et al., 2013; Goncharov et al., 2007; Masum-Akond and Watanabe, 2005; Badaeva et al., 1990; Scharen and Eyal, 1980; Krupinsky et al., 1972). Research and collection of detailed information about the hybrid plants in the genus *Triticum* is essential for the development, description and release of new cultural amphidiploids. New wheat species that are genetically stable in their morphology, physiology are key factor to the diversification of grain cereals (Matsuoka et al., 2011).

The purpose of this research is to be studied and described high-yield amphidiploid wheat forms that could be differentiated as separate species in botanical terms, and to delineate as different crop or to take part in the breeding programs of wheat species.

MATERIALS AND METHODS

3 samples of amphidiploid wheat forms (*Triticum polonicum* × *Triticum boeoticum*, *Triticum durum* × *Triticum boeoticum*, *Triticum turanicum* × *Triticum timopheevii*) are used, which are derived from Dobrudja Agricultural Institute – General Toshevo. 15 seeds of each amphidiploid accession are sown in a scheme with a spacing 30 cm between rows and 5 cm inside rows. The sowing was carried out respectively on 06.11.2011 and on 08.11.2012, under field conditions in the area of Stozher, Dobrich region. Harvesting is done in phase full maturity in the period 12-20.07.2013. Field germination (FG) is recorded. 10 fully matured spikes, free of infestation by pests were randomly selected of each accession. In 2010, 2011 and 2012 under laboratory conditions, between 15 and 20 October amphidiploid seeds are placed for germination. After they germinated, laboratory germination (LG) is reported. In 2010 and 2011 plants were transferred into pots and acclimatized in an unheated plastic greenhouse where they are cultivated to maturity. Spikes are harvested at full maturity. In order to determine the morphological and genetic stability of the studied accessions, indices fertility (F) and weight of 1000 grains (M1000) were reported and averaged for three years period. It is also reported the variation into characteristics LG, FG, F and M1000.

The standard varieties are sown into the same scheme: for susceptibility to powdery mildew (*Erysiphe graminis*) - Sadovska ranozreyka; brown rust (*Puccinia recondita*) - Michigan Amber; septoria leaf blight (*Septoria tritici*) - Enola. A comparative analysis of susceptibility to phytopathogenic infection of amphidiploid accessions compared to those varieties of wheat is done.

The determination of the phytopathogens attack aiming determining of the physiological stability is carried out under field and greenhouse conditions with naturally presented pathogens races: for powdery mildew (EG) using the methodology of Stoilova and Spetsov (2006); for brown rust (PR) using methodology of Ivanova (2012) and Roelfs et al. (1992); for septoria leaf blight (ST) using methodology of Eyal et al. (1987). Reporting is done in a 10-point scale and resistant (0-2), medium resistant (3-6) and susceptible (7-9) accessions are established. It is reported the attack of cereal leaf beetle and adult insects found on plants

before oviposition are counted. The number is averaged per plant.

To summarize the data and for the analysis of variance software Microsoft Excel 2007 is used.

For each year anatomical features of plants are described in order to trace some differences and similarities of consecutive generations.

Based on the analysis aiming establishing a morphological, physiological and genetic stability of the studied amphidiploids and their anatomical features, the accessions are separated as new hybrid botanical species and described in English and Latin in accordance with the International Code for Nomenclature for Algae, Fungi and Plants (2012) (ICNAFP) (Melbourne Code). When compiling crosses, the formulas were based on the classification of the genus *Triticum* by Goncharov et al. (2009). Latin description is based on Stearn (2004).

RESULTS AND DISCUSSIONS

The data of the morphological and physiological characteristics of the studied accessions (Tables 1-3) demonstrate the presence of certain genetic stability. Low variation in indices F and M1000 proved the lack of hybrid dissociation in consecutive generations. The values of the coefficient of variation within 1-3% are indicative of the modification and the recombinant variation that occurs in plant organisms, but also emphasizes the high level of homozygosity into the studied amphidiploids. Similar are the values reported for LG. The slight variation in this indicator and its high values suggest the lack of physiological disturbances due to the hybrid nature of the plants. However, in the FG significant variation of the indicator is observed, which is indicative of the changing conditions of the environment where the experiment was carried out. Such data reported Stoaynov (2012) in a similar conducted experiment on physiological characteristics of the studied amphidiploids.

As regards resistance to diseases, all studied amphidiploid accessions exhibit no symptoms of powdery mildew, brown rust and septoria leaf blight. In 2011, because of the intensity of precipitation and the presence of high infective background, on studied standard varieties, pathogenic expression is too high, while there are no symptoms on the amphidiploid accessions.

In 2012 the possibility of occurrence of phytopathogenic attack is very limited in the largest part of the vegetation of plants, due to relatively low temperatures and lack of moisture, which does not allowing initial inoculation. Therefore, before flowering, at all observed accessions, including standards for the susceptibility of the pathogens no signs of the disease are occurred. After flowering in May-June period 2012, due to the intense rainfall and high temperatures, on stadart wheat varieties was observed only slight attack by powdery mildew, brown rust and septoria leaf blight. Too high temperatures, however, inhibit the development of pathogens which caused the lack of significance in some accessions. In the majority of the accessions during the tillering phase and after flowering, they show full resistance to pathogens of powdery mildew, brown rust and septoria leaf blight.

The lack of an infectious process in 2013 emphasizes sustainability of the three amphidiploid accessions to pathogens of powdery mildew, brown rust and septoria leaf blight.

Studies on the reaction of amphidiploids to phytopathogens, indicate that they largely show resistance to powdery mildew and brown rust. Bred in DAI-GT, amfidiploids, in the period 1950-1990, possess a high degree of resistance to the listed pathogens (Spetsov and Savov, 1992). Similar results are reported by Sharma and Gill in studies of other amfidiploids in genus *Triticum*.

In relation to insect pests, during the vegetation, under field conditions in 2012 and 2013, adults and larvae of *Lema melanopa* and *Lema lichensis* (cereal leaf beetle) are found, with numbers below the economic threshold for bread wheat (Lecheva et al., 2003). In amphidiploid *Triticum turanicum* x *Triticum timopheevii* no damage of these insect pests was observed. The lack of attack could be attributed to a horizontal or vertical resistance (Shulembaeva, 2012; Gallun et al., 1966). On the remaining two accessions, attack of both adults and limited by larval forms of the two insects is reported.

Based on the reported genetic stability in relation to morphological and physiological charachteristics of accessions and also on the lack of sharp variation in the anatomy of plants of the three studied amphidiploids, for three years period, they are separated into independent amphidiploid species as follows: *Triticum* ×*savovii* HP Stoyanov nothosp. nov. (*Triticum polonicum* × *Triticum boeoticum*); *Triticum* ×*spetsovii* HP Stoyanov nothosp. nov. (*Triticum durum* × *Triticum boeoticum*); *Triticum* ×*toschevii* HP Stoyanov nothosp. nov. (*Triticum turanicum* × *Triticum timopheevii*). Spikes and seeds from the accessions are shown on Figures 1-6. The compilation of the names is based on the requirements and recommendations of ICNAFP. Descriptions of new species are formulated:

Figure 1. Spikes of *Triticum* ×*savovii*

Table 1. Characteristics of *Triticum* ×*savovii*

Species	***Triticum* ×*savovii***				
Year	**2011**	**2012**	**2013**	**AV**	**VC**
F	89.00	92.00	95.00	92.00	3.26%
LG	94.00	93.00	95.00	94.00	1.06%
FG	-	85.00	100.00	92.50	11.47%
M1000	53.12	54.68	56.02	54.61	2.66%
EG	0	0	0	0	-
PR	0	0	0	0	-
ST	0	0	0	0	-
LM	0	5	10	5	-

F – fertility; LG – laboratory germination; FG – field germination; M1000 – weight of 1000 grains; EG – powdery mildew; PR – leaf rust; ST – septoria leaf blotch; LM – cereal leaf beetle.

Vernacular description of *Triticum* ×*savovii* H.P. Stoyanov nothosp. nov. (*Triticum polonicum* × *Triticum boeoticum*).

Annual, tufted grass up to 100 cm tall, with 2-5(-10) tillers; stem (culm) cylindrical, smooth, hollow except at nodes. Leaves distichously alternate, simple and entire; leaf sheath rounded, auricled; ligule membranous; blade linear, 20-40 cm × 1-2 cm, parallel-veined, flat, glabrous. Inflorescence a terminal, distichous spike 7-12 cm long, with sessile spikelets borne solitary on zigzag rachis. Spikelet 15-20 mm long, laterally compressed, 3-6-flowered with bisexual florets, but 2-3 uppermost ones usually rudimentary, sometimes only 2 of the florets bisexual; glumes almost equal, oblong, exceeding spikelet apex, thinly leathery, acuminate, veined; lemma rounded on back but keeled towards the tip, leathery, with an awn 10-20 cm long; palea 2-keeled, not hairy on the keels; lodicules 2, ciliate; stamens 3; ovary superior, tipped by a small fleshy hairy appendage and with 2 plumose stigmas. Fruit an ellipsoid caryopsis (grain) at one side with a central groove, reddish brown to yellow brown.

Figure 2. Seeds of *Triticum* ×*savovii*

Latin description of *Triticum* ×*savovii* H.P. Stoyanov nothosp. nov. (*Triticum polonicum* × *Triticum boeoticum*).

Gramen annuum, caespitosum, usque 100 cm altum. Culmi (2-5(-10)) cylindrici, leaves, praeter nodis cavi. Folia distiche alterna, simplicia et integra; folii vagina rotunda, auriculata; ligula membranacea; lamina linearis, 20-40 cm × 1-2 cm, parallelinervia, plana, glabrata. Inflorescenia spica, terminalis, disticha 7-12 cm longa, sessilispiculae; Spicula solitare portata, rachidi valde flexuosae insidens, 15-20 mm longa, laterale compressa,

3-6-flora bisexualibus flosculis; Flosculi 2-3 summi plerumque rudimentalis; flosculi due interdum bisexuales; glumae paene aequialae, oblongae, apicem spiculae superantem, tenuiter coriaceae, acuminatae, nerviae; lemma postice rotundata sed apice carinata, coriacea, arista 10-20 cm longa; Palea 2-carinata, glabricarinata; Lodiculae duae, ciliatae; Stamina 3; Ovarium superum, ad apicem carnose ciliate apendiculatum, bistigmatibus plumosis. Fructus caryopsis ellipsoidea, a ventre sulco laterali, spadicea vel testacea.

Figure 3. Spikes of *Triticum ×spetsovii*

Table 2. Characteristics of *Triticum ×spetsovii*

Species	***Triticum ×spetsovii***				
Year	**2011**	**2012**	**2013**	**AV**	**VC**
F	84.00	91.00	94.00	89.67	5.72%
LG	99.00	93.00	95.00	95.67	3.19%
FG	-	65.00	40.00	52.50	33.67%
M1000	58.36	56.18	59.46	58.00	2.88%
EG	0	0	0	0	-
PR	0	0	0	0	-
ST	0	0	0	0	-
LM	0	6	8	4.67	-

F – fertility; LG – laboratory germination; FG – field germination; M1000 – weight of 1000 grains; EG – powdery mildew; PR – leaf rust; ST – septoria leaf blotch; LM – cereal leaf beetle.

Vernacular description of *Triticum ×spetsovii* H.P. Stoyanov nothosp. nov. (*Triticum durum* × *Triticum boeoticum*).

Annual often tufted grass up to 90-100 cm tall with 2-5(-8) tillers; stem (culm) cylindrical, smooth, hollow except at nodes. Leaves distichously alternate simple and entire; leaf sheath rounded, auricled: ligule membranous; blade linear, 25-35 cm x 1-2 cm parallel-veined, flat, glabrous. Inflorescence a terminal, dense distichous spike 6-8 cm long, with sessile spikelets borne solitary on zigzag, brittle rachis. Spikelet 10-15 mm long, laterally compressed, 3-5-flowered, with bisexual florets, but the 1-2 uppermost ones usually rudimentary; glumes almost equal, oblong, shorter than to almost as long as spikelet, thinly leathery, veined; lemma rounded on back but keeled towards the tip, leathery, with an awn 15-20 cm long; palea 2-keeled, not hairy on the keels; lodicules 2 ciliate; stamens 3; ovary superior tipped by a small fleshy hairy appendage and with 2 plumose stigmas. Fruit an ellipsoid caryopsis (grain) at one side with a central groove, gray to brown.

Figure 4. Seeds of *Triticum ×spetsovii*

Latin description of *Triticum ×spetsovii* H.P. Stoyanov nothosp. nov. (*Triticum durum* × *Triticum boeoticum*).

Gramen annuum, caespitosum, usque 90-100 cm altum. Culmi (2-5(-8)) cylindrici, leaves, praeter nodis cavi. Folia distiche alterna, simplicia et integra; folii vagina rotunda, auriculata; ligula membranacea; lamina linearis, 25-35 cm × 1-2 cm, parallelinervia, plana, glabrata. Inflorescenia spica, terminalis, disticha 7-12 cm longa, sessilispiculae; Spicula solitare portata, rachidi fragili valde flexuosae insidens, 10-15 mm longa, laterale compressa, 3-5-flora bisexualibus flosculis; Flosculi 1-2 summi plerumque rudimentalis; glumae paene aequialae, oblongae, apice spiculae breviores vel paene aequilongi, tenuiter coriaceae, nerviae; lemma postice rotundata sed apice carinata, coriacea, arista 15-20 cm longa; Palea 2-carinata, glabricarinata; Lodiculae duae,

ciliatae; Stamina 3; Ovarium superum, ad apicem carnose ciliate apendiculatum, bistigmatibus plumosis. Fructus caryopsis ellipsoidea, a ventre sulco laterali, fulvia vel cinerea.

Figure 5. Spikes of *Triticum ×toschevii*

Table 3. Characteristics of *Triticum ×toschevii*

Species	***Triticum ×toschevii***				
Year	**2011**	**2012**	**2013**	**AV**	**VC**
F	-	59.00	65.00	62.00	6.84%
LG	-	96.00	95.00	95.50	0.74%
FG	-	100.00	67.00	83.50	27.95%
M1000	-	48.25	50.30	49.28	2.94%
EG	0	0	0	0	-
PR	0	0	0	0	-
ST	0	0	0	0	-
LM	0	0	0	0	-

F – fertility; LG – laboratory germination; FG – field germination; M1000 – weight of 1000 grains; EG – powdery mildew; PR – leaf rust; ST – septoria leaf blotch; LM – cereal leaf beetle.

Vernacular description of *Triticum ×toschevii* H.P. Stoyanov nothosp. nov. (*Triticum turanicum* × *Triticum timopheevii*).
Annual, tufted grass up to 150-160 cm tall, with 3-5(-12) tillers; stem (culm) cylindrical, smooth, hollow except at nodes. Leaves distichously alternate, simple and entire; leaf sheath rounded, auricled; ligule membranous; blade linear, 15-40 cm × 1-1.5 cm, parallel-veined, flat, glabrous or slightly pubescent. Inflorescence a terminal, distichous spike 7-12 cm long, with sessile spikelets borne solitary on zigzag rachis. Spikelet 10-15 mm long, laterally compressed, 3-6-flowered with bisexual florets, but 1-2 uppermost ones usually rudimentary; glumes almost equal, oblong, shorter than to almost as long as spikelet, thinly leathery; lemma rounded on back but keeled towards the tip, leathery, with an awn 10-15 cm long; palea 2-keeled, not hairy on the keels; lodicules 2, ciliate; stamens 3; ovary superior, tipped by a small fleshy hairy appendage and with 2 plumose stigmas. Fruit an ellipsoid caryopsis (grain) at one side with a central groove, moderate shriveled, reddish brown to yellow brown.

Figure 6. Seeds of *Triticum ×toschevii*

Latin description of *Triticum ×toschevii* H.P. Stoyanov nothosp. nov. (*Triticum turanicum* × *Triticum timopheevii*).
Gramen annuum, caespitosum, usque 150-160 cm altum. Culmi (3-5(-12)) cylindrici, leaves, praeter nodis cavi. Folia distiche alterna, simplicia et integra; folii vagina rotunda, auriculata; ligula membranacea; lamina linearis, 15-40 cm × 1-1.5 cm, parallelinervia, plana, puberule nervia. Inflorescenia spica, terminalis, disticha 7-12cm longa, sessilispiculae; Spicula solitare portata, rachidi valde flexuosae insidens, 15-15 mm longa, laterale compressa, 3-5-flora bisexualibus flosculis; Flosculi 1-2 summi plerumque rudimentalis; glumae paene aequialae, oblongae, apice spiculae breviores vel paene aequilongi, tenuiter coriaceae, acuminatae, nerviae; lemma postice rotundata sed apice carinata, coriacea, arista 10-15 cm longa; Palea 2-carinata, glabricarinata; Lodiculae duae, ciliatae; Stamina 3; Ovarium superum, ad apicem carnose ciliate apendiculatum, bistigmatibus plumosis. Fructus caryopsis ellipsoidea, a ventre sulco laterali, spadicea vel testacea.

Thus, described and characterized by a number of valuable properties including resistance to economically important diseases, the new species in the genus *Triticum*, could be introduced as a crop or could participate as a starting material in the breeding programs of wheat species.

CONCLUSIONS

Considering those results, the following conclusions could be drawn:

1. Studied amphidiploids features slight variations in the reported morphological and physiological indicators, which highlights their genetic stability and high level of homozygosity.
2. All studied accessions exhibit resistance to pathogens of powdery mildew, brown rust and septoria leaf blight under field and greenhouse conditions, during the three-year period of observation.
3. Based on its valuable properties and stable anatomy, studied amphidiploid wheat accessions are separated and described as a new species in the genus *Triticum* as *Triticum* ×*savovii*, *Triticum* ×*spetsovii* and *Triticum* ×*toschevii*.

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