16th INTERNATIONAL MULTIDISCIPLINARY SCIENTIFIC GEOCONFERENCE

S G E M 2 0 1 6

NANO, BIO AND GREEN – TECHNOLOGIES
FOR A SUSTAINABLE FUTURE
CONFERENCE PROCEEDINGS
VOLUME I

- -

MICRO AND NANO TECHNOLOGIES, ADVANCES IN BIOTECHNOLOGY

- -

30 June – 6 July, 2016
Albena, Bulgaria

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Published by STEF92 Technology Ltd., 51 "Alexander Malinov" Blvd., 1712 Sofia, Bulgaria
Total print: 5000

ISBN 978-619-7105-68-1

ISSN 1314-2704

DOI: 10.5593/sgem2016B61

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MICRO AND NANO TECHNOLOGIES

INTRODUCTION FORMS - APPROACH FOR BIOTECHNOLOGY ADVANCE OF WINTER WHEAT ON ENVIRONMENTAL ADAPTABILITY

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ABSTRACT

A work on creation and test of an introgressive forms for advance biotechnology on environmental adaptability of wheat variety are expedient. The purpose of research – complex test of 12 introgressive forms of winter wheat, F6-F7, studying of their physiological and biochemical, genetic, morphological and productive properties under the environmental factors. Methodology – field and laboratory analyses of breeding material on drought resistance, winter and frost resistance, using the NDVI method for biomass, definition of free proline, biometric indicators, including developments of root system on the WinRHIZO device. Analysis of structure of an yield and general productivity, statistical data processing were applied. Introgressive forms of winter wheat have been received by introduction of a germplasm of wild relatives in a genome of cultivars. Experiments were made on the stands of the Kazakh Research Institute of Agriculture and Plant Growing in 2014-2015.

It is shown that resistance to different abiotic stresses decreases among:

Wild relatives > introgressive forms > wheat cultivars.

Genotypes which exceed average values on stability to each or group of abiotic factors are allocated. Adaptability sources against productivity are determined. It is established to 100% of re-wintering of the introgressive forms. Genotypes with high viability after a low-temperature stress are defined that is confirmed by increase in level of free proline: Eritrospermum 350 x T.kiharae, Steklovidnaya 24 x T.timopheevii (introgressive forms); Ae.cylindrical, T.kiharae (wild); Eritrospermum 350, Progress (wheat cultivars). On biomass accumulation in different growth phases were in the lead Eritrospermum 350 x T.kiharae, Progress x T.timopheevii. Genotypes are determined by the content of free proline in seedlings for the breeding progresson to drought tolerance: Eritrospermum 350 x T.kiharae, (Bezostaya1 x T.militinae) x T.militinae (introgressive forms); T.kiharae, Ae.cylinrica (wild); Eritrospermum 350, Steklovidnaya 24 (wheat varieties). Variability ranges on the power of root growth for wild, cultivars and introgressive forms are established. The detailed analysis of structure of root system the introgressive forms of wheat is submitted and perspective genotypes are revealed. Drought tolerance genotypes against wheat productivity are allocated. The received results on search of diagnostic indicators for the characteristic of resistance to environment prospects of an integrated approach for advance of biotechnology on adaptability of winter wheat are also established.

Keywords: introgressive forms, wheat adaptability, biomass, roots, proline.

INTRODUCTION

Biotechnology advances of winter wheat in Kazakhstan, as well as all over the world, are defined by creation of genotypes which combine resistance to severe winters and a drought during the summer period with productivity and yield of grain. New approaches are focused on using a genetic pool of wild-growing types as introgressive genetic material [1-3].

Originality of researches consists, on the one hand, in an integrated approach to studying of wheat resistance to abiotic stresses (winter hardiness, frost resistance, drought tolerance) with another, in use of wild relatives as recourse of stability. At resent research adaptation ability of wheat determined by key physiology and biochemical indicators of a metabolism – to force of growth processes and accumulation of vegetative biomass and roots, free proline accumulation, and also indicators of grain yield. For an assessment of intensity of growth processes used one of modern methods of an assessment of development of the sheet device and accumulation of green material of Normalized Difference Vegetation Index (NDVI) – the technology allowing defining of Normalized Difference Vegetation Index as the standardized index. Data of NDVI can be used for an estimation of the forecast of yield, accumulation of biomass and growth rates, the characteristic of energy of germination, an assessment of aging and for detection of environmental stress [4].

The 12 introgressive forms of winter wheat have been served as object of research. Introgressive forms, F6-F7, are identified by cytology, breeding methods and selected for homogeneity, evaluated in terms of yield and grain quality, adaptability to local growing conditions [5]. They were formed from introgression of wild wheat germplasm to winter common wheat cultivars (Triticum aestivum L.). Wild types of wheat – Triticum timopheevii, Triticum militinae, Triticum kiharae, Aegilops cylindrical, Aegilops triaristata and winter wheat varieties of the Kazakhstan breeding - Karlygash, Eritrospermum 350, Zhetysu, Steklovidnaya 24, Komsomolskaya 1 are involved in researches. Bezostaya 1 is used as the grade (standard) in Kazakhstan for a long period.

Grain was grown up in identical conditions on the field sets of the Kazakh Research Institute of Agriculture and Plant Growing. Field and laboratory researches are carried out. Researches were conducted on plants of one reproduction, the yield of 2014 and 2015.

1. Winter hardiness and frost resistance.

Unique results on winter hardiness are received; all 12 forms of investigated introgressive material of wheat have shown to 100% survival in field conditions of Almaty region in 2015. Cultivars of winter wheat have wintered only for 75%, and have shown the best result - Eritrospermum 350 and Bezostaya 1. Wild relatives have wintered on average for 96%.

For the estimation of frost-resistance in laboratory terms was applied the method based on free proline determination of 5-7 - daily seedlings at the step cooling of wheat in fosters to -2 ^{0} C [6]. Conclusion of frost-resistance plants could be fined by the coefficient of proline accumulation in correlation experience/control (fig.1).

It is shown that under the cooling there is an accumulation of free proline in the seedlings of all studied genotypes of wheat. Wild relatives reacted on low temperature stress in a greater degree; a coefficient of accumulation of proline was in a range 1,84-2,23.

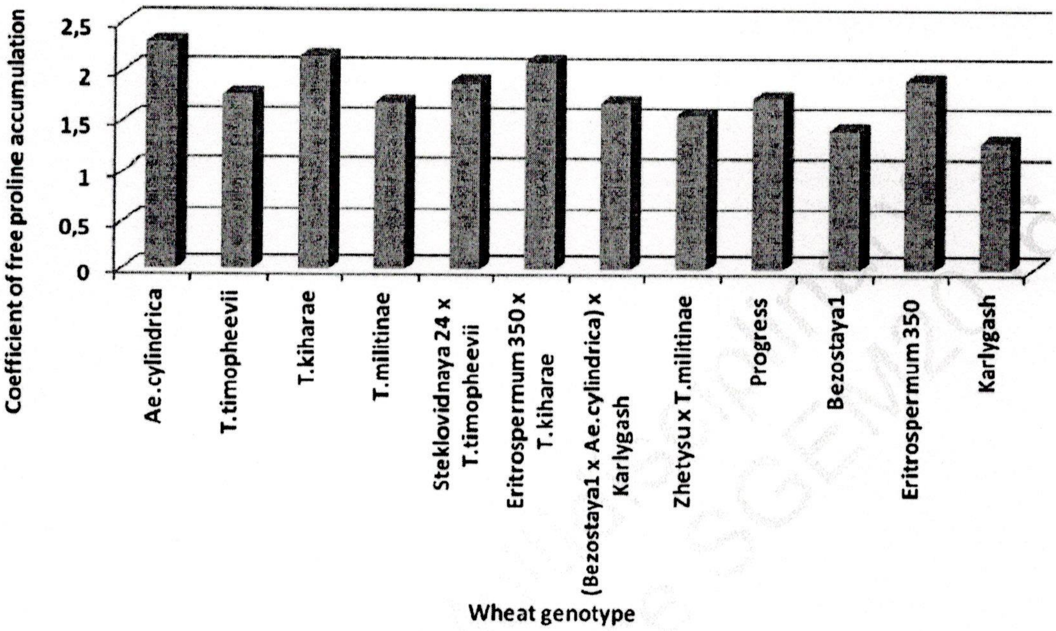

Figure 1- Free proline accumulation of seedlings in wild, cultural and introgressive forms of wheat under the cooling

Introgressive forms fell behind from wild relatives insignificantly with the coefficient of proline accumulation within the limits of 1,73-2,15, cultivars were characterized the most low level of proline increase in a range 1,38-1,83. Samples exceeding other on the degree of reacting on low temperature stress exuded between each of groups, for example Ae.cylindrica, Eritrospermum 350 x T.kiharae, Progress.

2. Biomass accumulation.

Skinning of resource material allowed to obtained a variableness the method of NDVI in the accumulation of vegetative mass: from 0,174 to 0,239 in the stage of tillering" (bushing up); from 0,184 to 0,437 in the stage of "stem elongation" (stem jointing) in the yield of 2014; from 0,23 to 0,76 in the stage of "tillering" and from 0,63 to 0,82 in the stage of "stem elongation" in 2015 crop yield.

The row of decrease is set on the accumulation of productive biomass in the stage of "flowering": T.kiharae (0,75) > T.timopheevii (0,66) > T.militinae (0,58) > T.aestivum (0,58-0,43). Between introgressive forms a genotype exuded Progress x T.timopheevii (0,72).

Among wheat cultivars on the index of green mass in the stage of "tillering" and "stem elongation" stage led in 2014: Komsomolskaya 1 (0,22 and 0,39) and Karlygash (0, 21 and 0,40), accordingly. In the following 2015 on a measureable index the wheat cultivar Eritrospermum 350 (0,62 and 0,79) attained the maximal result.

The analysis of constant forms in the stage of " tillering" allowed to obtained a changeability of green mass index from 0,150 to 0,238 and from 0,106 to 0,280 at minimum values of foreign variety – standard Karahan (Turkey) and maximal for the genotype of Eritrospermum 350 x T.kiharae (2015 crop yield).

It is known that the best using of climatic, soil resources, and also receptions of agro technical approaches takes place in sowing with an optimal green mass and leaves surface. The study of biomass accumulation of wheat genotypes in the process of vegetation has an important value both for understanding of conformities to understanding of yield formation process and for development of breeding criteria on the high productivity and models of drought tolerance forecast in agro ecosystem [7].

3. Accumulation of proline and drought resistance.

It is established that the accumulation of free proline in a stage of "flowering" for wheat varieties fluctuated from 29 to 38 mg of %, and the greatest number of proline was fixed at cultivars: Steklovidnaya 24 and Zhetysu, which are parental forms for the investigated introgressive ones. Content of free proline in wild relatives considerably exceeded varieties and was in limits of 43-58 mg of % that surpassed cultivars by 1,8-2 times. Were distinguished from wild forms Triticum kiharae and Triticum militinae on the level of free proline. Introgressive forms have shown variability on the accumulation of proline , the amount of amino acid in leaves contained in limits of 35-55 mg of %, the greatest number has been noted at forms: Steklovidnaya 24 x T.timopheevii (50 mg of %), Eritrospermum 350 x T.kiharae (52 mg of %) and Zhetysu x T. militinae (55 mg of %). Results of this series of experiences are summarized in table 1.

Table 1 – Variability in accumulation of free proline in leaves of wild, wheat cultivars and the introgressive forms in various phonological stages of plant development

Wheat genotype	Variability range on accumulation of free proline, mg %	The sample which has shown the best result
Phase of "flowering"		
Wild	43-58	T.kiharae, T.militinae
Introgressive forms	35-55	Steklovidnaya 24 x T.timopheevii, Eritrospermum 350 x T.kiharae, Zhetysu x T.militinae
Cultivars	29-38	Steklovidnaya 24, Zhetysu
Phase of "filling grain"		
Wild	51-68	Ae.cylindrica, T.kiharae
Introgressive forms	76-85	Eritrospermum 350 x T.kiharae, (Bezostaya 1 x T.militinae) x T.militinae
Cultivars	46-60	Eritrospermum 350, Steklovidnaya 24

In process of temperature increase in July and in the following drought critical phase by the end of month the distinct tendency on increase in amount of free proline in leaves of the studied wheat varieties was observed. The level of proline has increased on average for 28-35% at all forms of wheat. However, the expected growth of amount of proline at wild relatives has stopped a little, cultivars - Eritrospermum 350, Steklovidnaya 24 and introgressive forms have shown increase in the content of free amino acid by 1,4-1,6 times, respectively. Perhaps, the mechanisms of adaptation of wheat cultivars connected with water-retaining ability of proline are more developed.

4. Root system.

Characteristics of the root system with the use of climate-controlled green house and the WinRHIZO device equipment (Canada) implemented in the CIMMYT laboratory (Turkey) on at the different stages of wheat seedling development. Specifications were determined by following aspects: length of roots, area, PA, volume, average diameter, root tips, length of secondary roots, number of branches (cm), while mass of fresh leaves and mass of roots were determined by weighting, mass of dry leaves and mass of dry roots after drying in a heat chamber until absolutely dry weight (g) [8].

Analysis of the root system condition of wild, cultural and introgressive forms showed that there was significant variability in the analyzed plants by studied indicators. Genotypes were distinguished among introgressive forms and their parents, surpassing the level of the root system development of compared samples within each group (table 2).

Table 2 – Total length of root system of wheat genotypes, cm

Plant development stage	min-max	average	Wheat genotype
8-10 day seedlings	159,9-306,2	254,3	(Bezostaya 1 x Ae.triaristata) x Karlygash; Zhetysu x T.timopheevii; Karlygash; Komsomolskaya 1; T.timopheevii
Above-ground part is cut, determination in 21 day roots ("tillering")	257,1-2263,8	1212,7	Eritrospermum 350 x T.kiharae; Zhetysu x T.militinae; Eritrospermum 350; T.kiharae; Ae.triaristata
Above –ground part is not cut, 21 day seedlings ("tillering")	897,1-1731,4	1348,1	Eritrospermum 350 x T.militinae; (Bezostaya 1 x T.militinae) x T.militinae; Steklovidnaya 24; Bezostaya 1; T.timopheevii; T.militinae

'It is known that cutting the above-ground part and analysis of root system can be indirectly connected with drought tolerance and is an additional characteristic of germination rate [9]. It is believed that indicator of root-sufficiency of plants can be also represented in a simple way by ratio of root mass and above-ground parts of plants [10]. Cutting of above-ground part is considered as a stress that induces the development of root system. It should be noted that the length of secondary roots and branches characterize the intensity of root growth, point on the power of root mass of wheat, that is confirmed in the works [2, 10]. In the given series of experiments there have been clearly distinguished genotypes: (Bezostaya 1x T.militinae) x T. militinae-4; Bezostaya 1x T.militinae) x T. militinae-9 with the most developed secondary root system. On all aspects, there have been distinguished 2 genotypes with the increased rate of reaction to stress: Eritrospermum 350 x T.kiharae; Zhetysu x T.militinae. They significantly have surpassed their parents and Turkey wheat standard Karahan by mass of roots, branching and the mass of root system.

5. Screening of drought tolerance and indicators of wheat yield.

In one reproduction of the 2015 crop yield drought tolerance sources against productivity have been allocated. Based on researches [7, 11], for the characteristic of drought tolerance on the productivity indicators were used several ones, such as "1000 grain weight" (thousand kernel weight), "mass of grain in the main spike" (main stem grain yield) and "productive tiller number".

According to an indicator "1000 grain weight" 6 introgressive forms exceeded standard Almaty (47.2 g), of which 2 introgressive forms were higher than the other standard Karahan (52.1 g). In ascending order of this indicator they are located in sequence: (Bezostaya 1 x T.militinae) x T.militinae-4, Zhetysu x T.timopheevii, Eritropermum 350 x T.kihrae, Bezostaya 1 x Ae.cylindrica, Zhetysu x T.militinae, Vitreous 24 x Ae.cylindrica.

On an indicator of "mass in the main spike" among the leaders was Vitreous 24 x Ae.cylindrica (7.9 g), which exceeded given varieties standard Almaly up to 1.3 times. Introgressive forms: Zhetysu x T.timopheevii, Eritropermum 350 x T.kihrae, Zhetysu x T.militinae on the same indicator reached 7,0 g or more.

According to another characteristic of the yield structure "height of the plants" allocated Zhetysu x T.timopheevii (127, 8 cm) and Bezostaya 1 x Ae.cylindrica (129,3 cm), were on the level of Karahan (128.4 cm) and above. All introgressive forms showed good growth and superior of standard Almaly (104.6 cm), while within the 105,7-129,3 cm. An indicator "productive tiller number" had minor changes in the range 3,7-4,2 for all studied genotypes, without isolation of any form.

CONCLUSION

The differences in the response to the conditions of 2015 year wintering of wild, cultivars and introgressive forms of wheat have been set. The range of plants survival in winter field conditions was 62-87% for cultivars, 88-100% for wild relatives. Unique results on 100% wintering of introgressive forms are obtained.

Diagnosis of frost- resistance on the accumulation of free proline levels in the leaves of seedlings showed that the pattern detected in field trials is generally confirmed and consistent with the works [1, 5, 6]. There have been identified the genotypes that showed the best result for the preservation of the viability and the increase of free proline level in response to cold stress: Ae.cylindrical, T.kiharae (wild); Eritrospermum 350 x T.kiharae, Steklovidnaya 24 x T.timopheevii (introgressive forms); Progress, Eritrospermum 350 (cultivars).

Different quality has been found in the accumulation of vegetative mass of studied forms of wheat by development stages that are confirmed in works [4, 5]. Among cultivars leading in 2014 crop yield were Komsomolskaya 1 and Karlygash, in 2015 - Eritrospermum 350. The maximum value of green mass index had Eritrospermum 350 x T.kiharae ("tillering" stage, 2015 crop year). In the flowering phase for wild relatives a series of descending on the accumulation of productive biomass has been established. Genotype Progress x T.timopheevii has been distinguished among introgressive forms.

The variability is registered in accumulation of proline in the leaves of studied wheat in critical drought periods (June-July) for the development of plants, conceding with the stage of "flowering" in reproduction of 2015 crop yield, what is confirmed in works [6, 7, 12]. Identified the ranges of variability in the accumulation of free proline for wild, cultural and introgressive forms, and also promising genotypes in breeding for drought tolerance. These include among wild forms - T.kiharae, Ae.cylinrica, introgressive forms - Eritrospermum 350 x T.kiharae, (Bezostaya 1 x T.militinae) x T.militinae, cultivars - Eritrospermum 350, Vitreous 24.

There were established the ranges of variability by power of root growth for wild, cultural and introgressive forms and identified promising samples: (Bezostaya 1 x Ae.triaristata) x Karlygash; Zhetysu x T.timopheevii; Karlygash; Komsomolskaya 1; T.timopheevii (8-10 days seedlings stage); Eritrospermum 350 x T.kiharae; Zhetysu x T.militinae; Eritrospermum 350; T.kiharae; Ae.triaristata (21 days roots, above-ground part is cut). Perspective samples at various stages of development of seedlings are revealed. Introgressive forms and wheat varieties have been compared by productivity, promising samples on considered indicators and general productivity have been selected. Sources of drought tolerance on the base of wheat productivity have been identified, what is coincides with the approaches [1, 2, 7, 11]. The obtained information supports the opinion [11], that the main indicator to estimate of wheat yield, paired with drought tolerance, is «1000 grain weight» (thousand kernel weight). On this indicator 6 introgressive forms, surpassing variety standard have been identified.

It was obtained, that resistance to different abiotic stresses decreased among: wild relatives > introgressive forms > wheat cultivars.

Considering the proline is potential reserve metabolite, performing multifunctional biological role in plant metabolism, level and variability in its accumulation can be quite informative criterion of the adaptive capacity of plants [12, 13].

Obtained results indicate different quality of introgressive forms of winter wheat in response to stress factors (overwintering, frost-resistance, drought tolerance), what is due to differences of accumulation level of free proline, biomass accumulation of seedling and roots by stages of vegetation, to indicators of productivity [14, 15]. Therefore, the studied introgressive forms are promising to be an advanced biotechnological resource for breeding on improving of environmental sustainability and biological variety of wheat.

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