



Development of a genetic basis for the comprehensive study of the of inheritance and physiological role of leaf pubescence in bread wheat



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BACKGROUND:

Pubescence of different plant organs formed by trichomes is an important evolutionary adaptation (Johnson, 1975). It plays the role in protection from physical damages, including grains of sand during dust storms and from insects. Additionally, trichome cells may affect stomatal activity and assimilation processes in plants (Schuepp, 1993) including the water stress conditions (Ehleringer, 1982). In wheat leaf pubescence is presented as unicellular epidermal hairs with different length and density. The diversity for leaf hairiness was found both for hairs' length and density among bread wheat cultivars (Genaev et al. 2012). Previously, we studied genetically the pubescence introduced from *Aegilops speltoides* into cv. Rodina (Dobrovolskaya et al., 2007) (Fig1A), as well as the pubescence in line 821 of cv. Saratovskaya 29 (S29), introgressed from *Triticum timopheevii* (Fig. 1B). The lines differed significantly from the parent varieties by the level of photosynthetic processes, the chlorophyll content and the activity of antioxidant enzymes (Pshenichnikova et al., 2016; Pshenichnikova, unpublished)



Aims of the work: a) evaluation of different genotypes – carriers of leaf hairiness for trichome length and density; b) development of isogenic and substitution lines with different types of leaf hairiness; c) physiological study of the lines under different water supply.

Methods

1. Subsequent backcrosses (BC₃-BC₉)
2. Method of high-throughput phenotyping (Genaev et al. 2012)
3. Tests under the conditions of contrasting water supply; measuring of photosynthetic and chlorophyll fluorescence parameters; multivariate statistical data processing

Initial genotypes

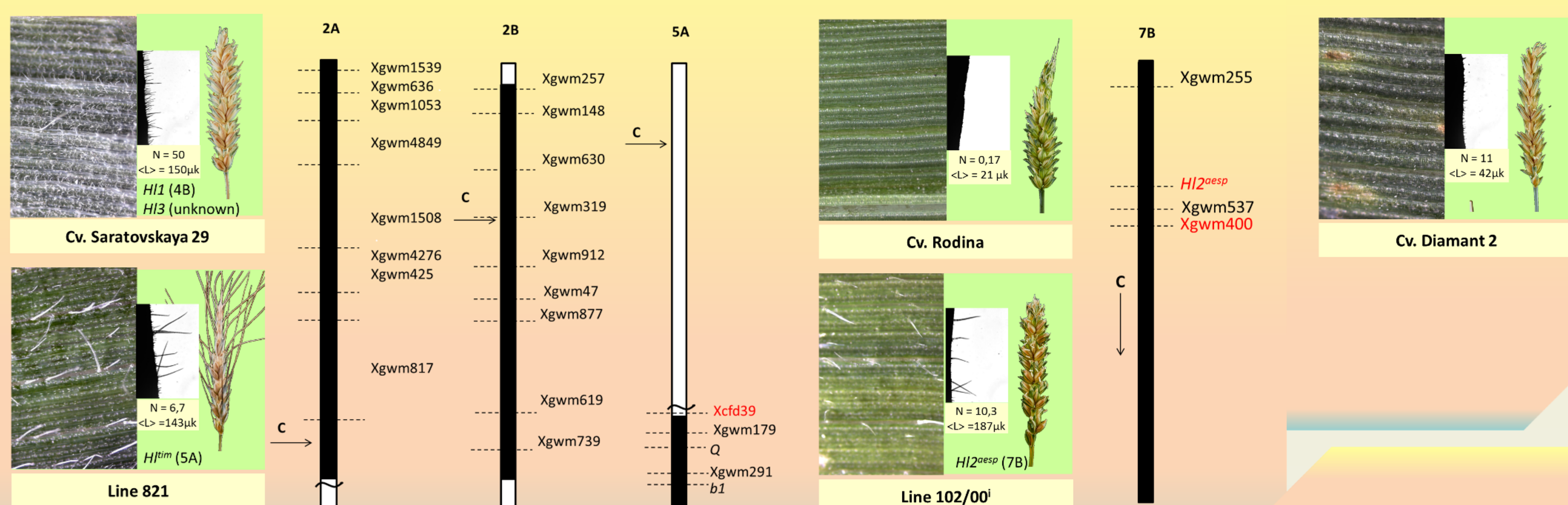
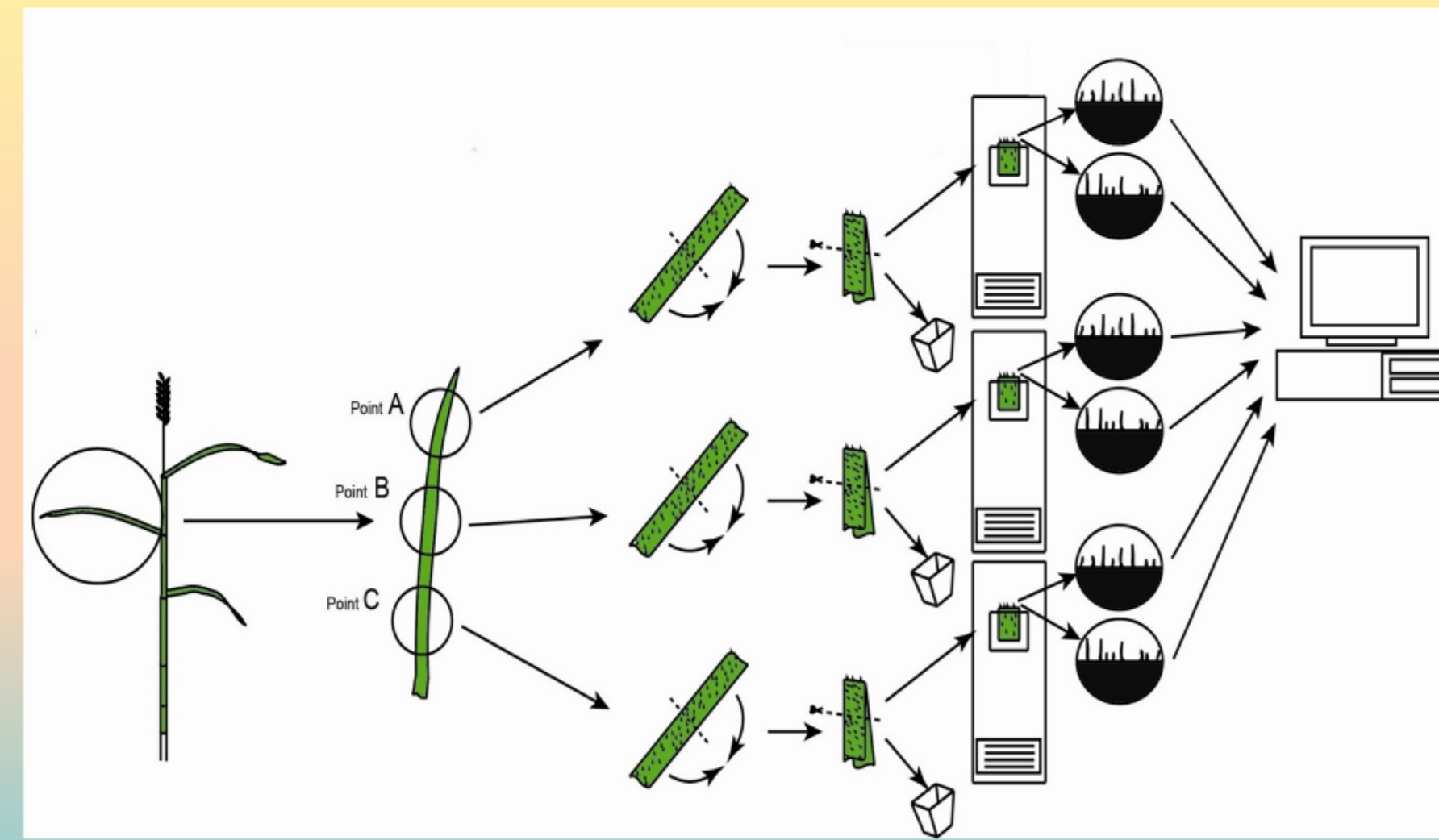


Figure 1. Leaf blades, folds and spikes of parental cultivars Saratovskaya 29, Rodina and Diamant 2 and introgression lines. On the right: molecular maps of introgressions (partially from Dobrovolskaya et al., 2007 and Leonova et al. 2001); the marker *Xcfd39* associated with leaf hairiness of *Triticum monococcum* is taken from Jung et al., 2007

Obtaining and computer processing of microphotographs of leaf folds (Genaev et al. 2012)



Spring cultivar S29 is densely haired and carries two genes for leaf hairiness, *H11* and *H13*. The line 821 with introgression from *Triticum timopheevii* obtained on the genetic background of S29, carries long and rare hairiness. Rodina is practically lacks hairs. The line with introgression from *Ae. speltoides* into Rodina has rare but long pubescence controlling by the gene *H12^{esp}* on 7B chromosome (Dobrovolskaya et al, 2007). Cv. Diamant 2 (Dm2) is weakly haired (Fig.1).

Development of substitution and isogenic lines

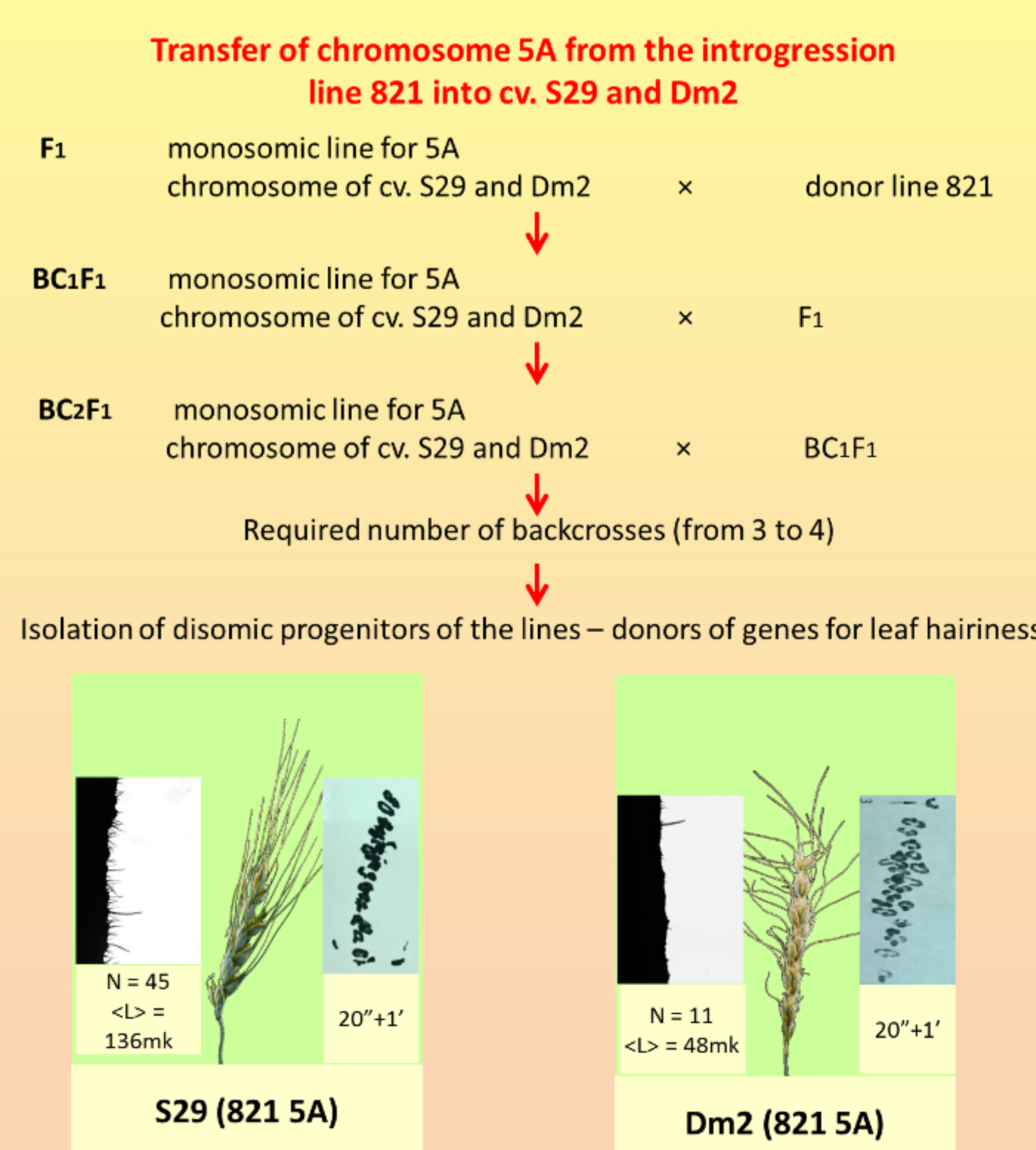


Fig. 2. Spikes, leaf folds and pictures in metaphase I of meiosis of substitution lines S29 (821 5A) (on the left) and Dm2 (821 5A) (on the right).

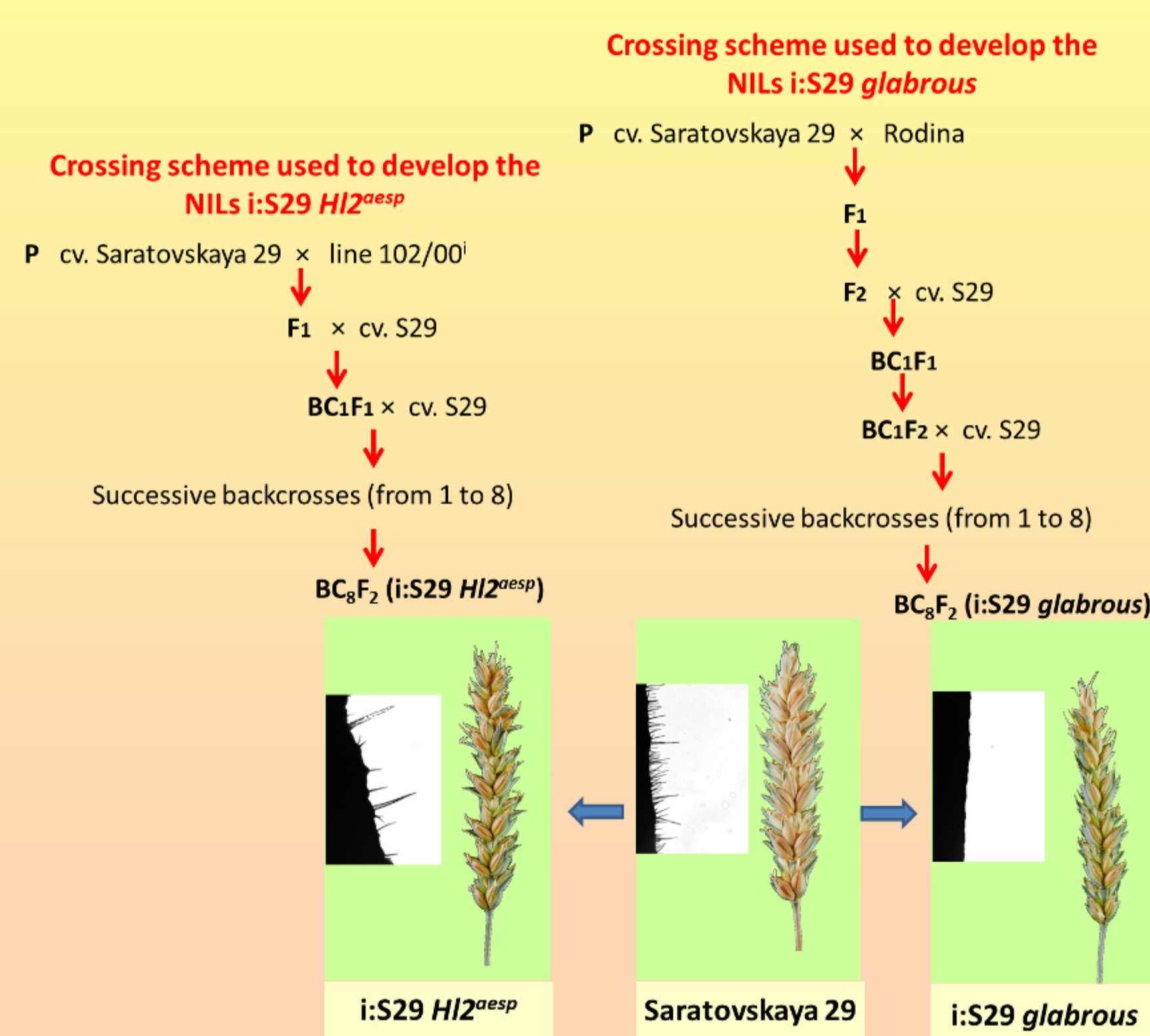


Fig.3. Leaf folds and spikes of parental cultivar and isogenic lines with contrasting leaf pubescence.

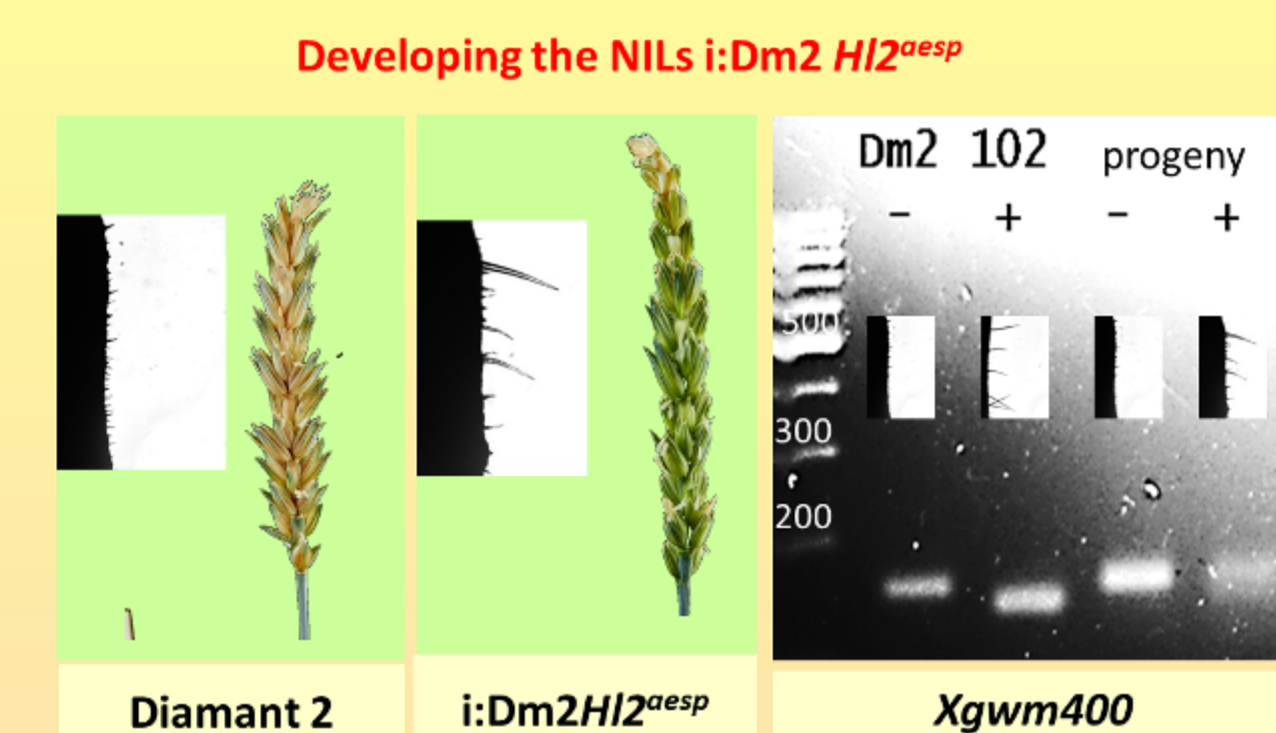


Fig.4. Leaf folds, spikes and microsatellite genotyping of parental cultivars, isogenic lines and segregating progeny in backcrosses.

Earlier, the locus for leaf pubescence was identified in chromosome 5A of *T. monococcum* near the marker *Xcfd39* (Fig.1). Monosomic lines for 5A chromosomes of cvs. S29 and Dm2 were used to transfer 5A chromosome from line 821 into both genotypes (Fig.2). The lines will be used for precise mapping of the gene responsible for exotic type of leaf pubescence.

S29 is a drought tolerant cultivar. Dense leaf pubescence is an important part of its whole adaptation mechanism. The isogenic lines of S29, one of which was completely devoid of pubescence while another contained additional gene for leaf pubescence (Fig.3), were obtained to study the physiological reactions of leaf and whole plant under different water regimes.

To introduce the *H12^{esp}* gene into genotypes of cvs. Dm2 and Rodina, a microsatellite marker *Xgwm400* was used. With its help, this gene was mapped in chromosome 7B of line 102/00ⁱ. The line and cultivars showed allelism for the marker *Xgwm400* (Fig. 4). The allelic variant of line 102/00ⁱ correlated with the presence of the pubescence characteristic of this line and of *Ae. speltoides*.

Physiological study of isogenic and substitution lines with contrasting leaf pubescence under normal and water deficit conditions

Table 1. The comparative values of morphological characteristics of cultivars and their derived forms - isogenic and substitution lines under contrasting water regimes. Comparisons are made with the corresponding recipient, YP was compared with S29

Cultivars and lines	Trichomes				Stomata				Leaf size			
	Number on upper side		Number on bottom side		Number on upper side		Number on bottom side		Length		Width	
	control	drought	control	drought	control	drought	control	drought	control	drought	control	drought
S29	39,7	55,4	48,2	66,4	140,9	138,8	133,8	115,8	56,8	79,0	42,4	278,0
i:S29 glabrous	5,3	9,4	3,3	18,1	45,0	37,5	67,8	42,7	60	101,7	44,9	293,4
i:S29 H12	41,9	61,4	56,4*	72,2*	213,9	191,9	188,2	165,3	62,5	93,1*	45,6	267,9
S29 (YP 4B)	19,5	33,4	21,9*	35,4*	70,7	62,8	64,7	61,7	62,0	105,9	41,8	231,1
Rodina	0,56	0,4	0,2	0,2	18,9	11,1	11,2	17,3	66,5	94,4	38,6	176,1
102/00 ⁱ	14,6	23,8	10,5*	14,4*	281,4	141,4	223,1	125,0	57,3	80,3	51,3	284,5
Yanetski s Probab	4,0	15,5	2,7	10,3	31,4	38,8	42,9	41,8	46,7	73,2	34,0	132,3

*** - P<0,001; ** - P<0,01; * - P<0,05

Table 2. The comparative values of gas exchange parameters of cultivars and their derived forms - isogenic and substitution lines under contrasting water regimes. Comparisons are made with the recipient, YP was compared with S29

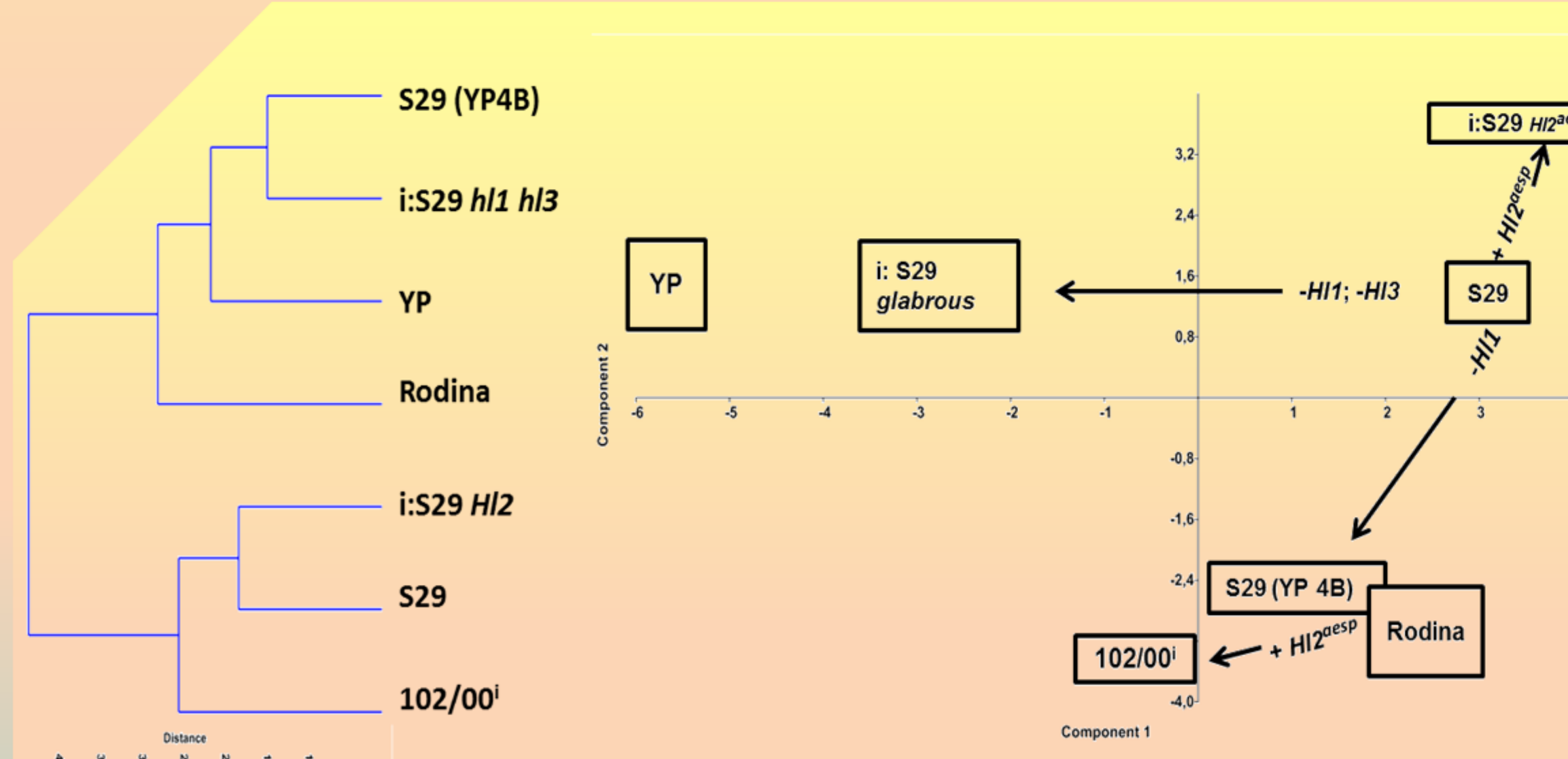
Cultivars and lines	Transpiration rate, mmol m-2 s-1		Stomatal conductance, mmol m-2 s-1		Photosynthetic rate, μmol m-2 s-1		WUE		Biomass, g		IT*
	control	drought	control	drought	control	drought	control	drought	control	drought	
S29	0,38	0,40	26,9	28,5	2,36	2,40	7,1	8,3	3,9	1,3	33,4
i:S29 H11 H13	0,92***	0,50*	67,4***	35,2*	3,25***	2,36	3,8***	5,4***	3,4*	0,98**	30,4
i:S29 H12	0,27*	0,29*	19,2*	20,8*	1,54***	1,93***	6,1	8,6	4,1	1,1	27,1
S29 (YP 4B)	0,63***	0,53**	45,4***	38,2**	2,4	2,08*	5,5**	4,3***	3,4*	1,2	36,5
Rodina	0,75	0,47	55,8	34,1	2,9	2,82	5,5	7,1	4,5	1,1	24,8**
102/00 ⁱ	0,70	0,38*	52,0	26,3*	2,8	1,85***	5,0	6,1	4,9	1,1	23,5**
YP	0,92***	0,24***	68,3***	16,7***	3,3***	2,02***	4,1***	9,4	5,4***	1,2	23,1**

*** - P<0,001; ** - P<0,01; * - P<0,05; Abbreviations see Table 1. # - tolerance index; *italics* – difference is significant comparing to S29 and other related to S29 genotypes

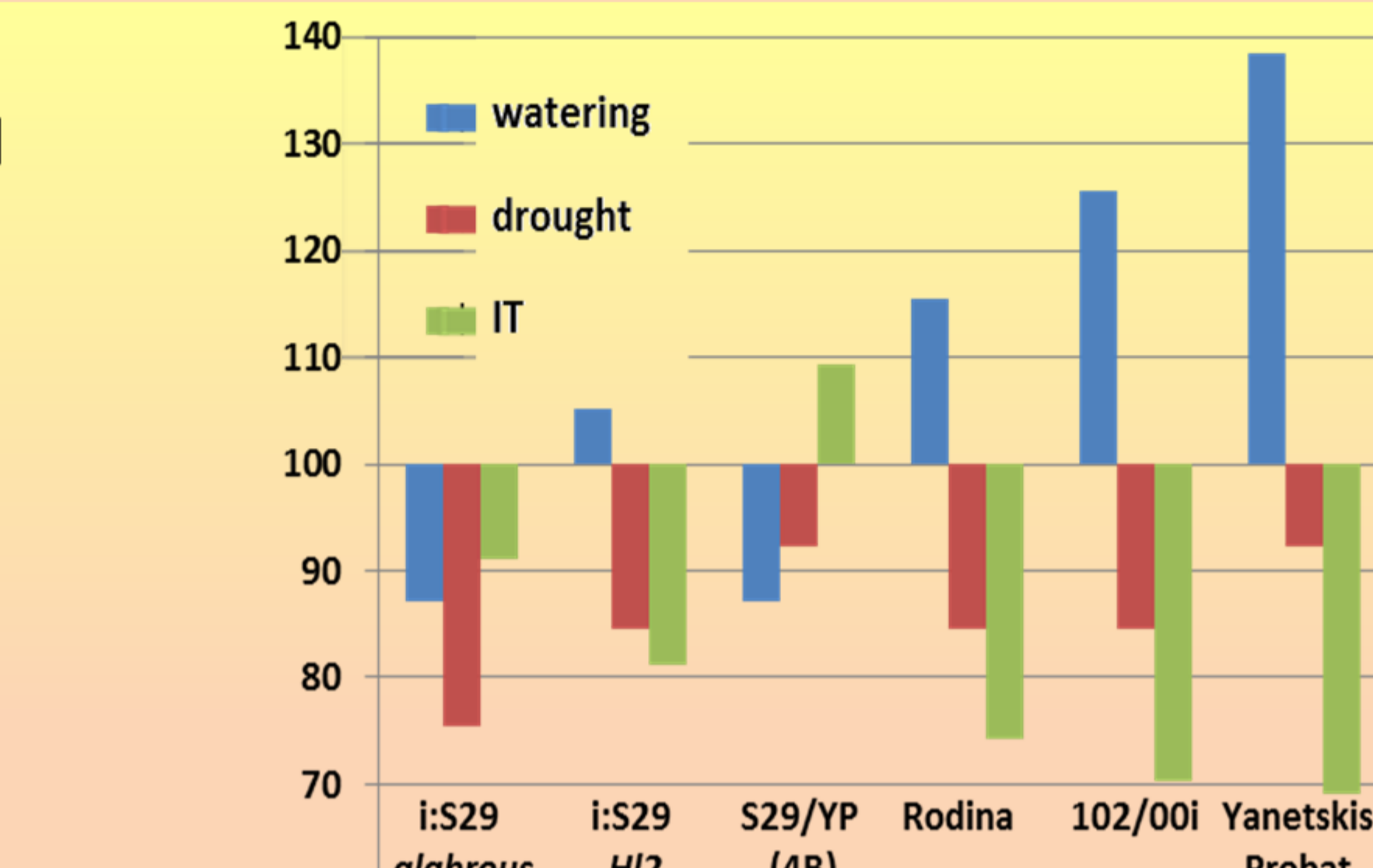
Table 3. The comparative values of chlorophyll fluorescence parameters of cultivars and their derived forms - isogenic and substitution lines under contrasting water regimes. Comparisons are made with the recipient, YP was compared with S29

Cultivars and lines	Basic chlorophyll fluorescence yield (F ₀)		Potential efficiency of photosynthesis (F _v /F _m)		Actual effectiveness of photosynthesis (Yield)		Rate of electron transport in photosystem II (ETR)		Non-photochemical quenching of fluorescence (NPQ)	
	control		control		control		control		control	
	control	drought	control	drought	control	drought	control	drought	control	drought
S29	117,3	70,8	0,785	0,760	0,452	0,525	30,3	35,1	0,38	0,41
i:S29 H11 H13	107,4	61,4	0,752**	0,750	0,484*	0,513	32,5*	34,4	0,63***	0,45
i:S29 H12	110,9	58,7*	0,696**	0,749	0,477	0,538	31,9	36,0	0,26***	0,35**
S29 (YP 4B)	112,3	77,3	0,722**	0,742	0,478	0,476**	32,0	31,9**	0,39	0,32*
Rodina	102,3	79,6	0,732	0,742	0,502	0,500	33,7	33,5	0,44	0,43
102/00 ⁱ	95,7	61,8***	0,776**	0,799**	0,464*	0,480*	29,4**	30,5***	0,63**	0,66***
YP	92,6***	65,7	0,779	0,751	0,537**	0,523	36,0***	35,1	0,36	0,32**

*** - P<0,001; ** - P<0,01; * - P<0,05; Abbreviations see Table 1



Clustering of genotypes (A) and Principal Component analysis (B) based on normalized differences of gas exchange and chlorophyll fluorescence values between two watering regimes



Relative weight (%) of the main shoot on watering and drought (blue and brown, correspondingly) and index of tolerance (IT) of this trait. Cv. S29 was taken as 100%

1. It was found that gas exchange parameters are inversely proportional to the density and length of trichomes. The boundary layer provided by the presence of trichomes reduces transpiration, both on watering and on drought.
2. In drought conditions, the density of trichomes increased and their length decreased, with the observed decreased in the level of gas exchange. A similar dependence was observed in the level of non-photochemical quenching of chlorophyll fluorescence.
3. The relationship between the presence of pubescence and shoot biomass was studied. Under optimal conditions, the glabrous cultivars exhibited a greater biomass than pubescent cultivar S29, but under the conditions of water deficiency biomass was significantly reduced in glabrous cultivars and they showed a low tolerance index.

- Literature
- Genaev M.A., Doroshkov A.V., Pshenichnikova T.A., Kolchanov N.A., Afonnikov D.A. (2012) Extraction of quantitative characteristics describing wheat leaf pubescence with a novel image-processing technique. *Planta* 236(6):1943-1954. doi: 10.1007/s00425-012-1751-6
- Genaev M. A., Doroshkov A. V., Morozova E. V., Pshenichnikova T. A., Afonnikov D. A. WheatPGE: A system for analysis of relationships among the phenotype, genotype, and environment in wheat. *Russian Journal of Genetics: Applied Research*, 2012, Vol. 2, No. 3, pp. 262–269.
- Ehleringer J. (1982) The influence of water stress and temperature on leaf pubescence development in *Encelia farinosa*. *Amer J Bot* 69: 670-675.
- Schuepp P. H. (1993) Leaf boundary layers. *New Phytologist* 125: 477-507.
- Johnson H.B. (1975) Plant pubescence: an ecological perspective. *Bot Rev* 41(3):233–258.
- Dobrovolskaya O., Pshenichnikova T.A., Arbuzova V.S., Lohwasser U., Röder M.S., Börner A. (2007) Molecular mapping of genes determining hairy leaf character in common wheat with respect to other species of the Triticeae. *Euphytica* 155(3):285–293.
- Pshenichnikova T. A., Permyakov A. V., Osipova S. V., Permyakova M. D., E. G. Rudikovskaya, V. V. Verchuturov. Effects of limited introgressions from *Triticum timopheevii* Tausch. into the genome of bread wheat (*Triticum aestivum* L.) on physiological and biochemical traits under normal watering and drought. *Russian Journal of Genetics: Applied Research*, 2016, Vol. 6, No. 5, pp. 553–559.
- Leonova, I.N., Kalinina, N.P., Budashkina, E.B., Röder, M.S., Salina, E.A. (2001) Comparative molecular and genetic analysis of *Triticum aestivum* × *Triticum timopheevii* hybrid lines resistant to leaf rust, Proc. 11th EWAC Conf., Novosibirsk.
- Jing H-Ch, Kornychuk D., Kanyuka K., Orford S., Zlatka S., Mitrofanova O.P., Koeber R., Hammond-Kosack K. Identification of variation in adaptively important traits and genome-wide analysis of trait-marker associations in *Triticum monococcum*. *Journal of Experimental Botany*, Vol.58, No 13, pp 3749-3764, 2007