CELLULAR & MOLECULAR BIOLOGY LETTERS
Volume 7, (2002) pp 483 – 491
http://www.cmbl.org.pl

Received 1 March 2002
Accepted 15 May 2002

THE PRODUCTIVITY CHARACTERISTICS OF SUBSTITUTED BARLEY LINES WITH MARKED CHLOROPLAST AND MITOCHONDRIAL GENOMES

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Abstract: We present the effects of cytoplasm substitution on five productivity traits in an alloplasmic barley collection. 60 lines combining 5 nuclear genomes of cultivated barley varieties and 12 plasmons of two barley species (*H. vulgare*, *H. spontaneum*) displayed various effects depending on definite nuclei-cytoplasm combinations. Only four cytoplasmic genomes (W1, W4, W5, W10) significantly modified the expression of the nuclear genes controlling productivity. RAPD-PCR analysis revealed that both the mitochondrial and chloroplast DNA of the W1, W5, and W10 lines have common molecular characters distinguishing them from the cytoplasmic genomes of the other lines. The cytoplasmic genetic factors influencing the expression of "productivity" genes remain elusive.

Key words: Barley, Alloplasmic Lines, Mitochondrial DNA, Chloroplast DNA, Nuclear-Cytoplasmic Interaction, Productivity Traits

INTRODUCTION

Productivity traits are formed as a result of the cooperative expression of nuclear and organelle genomes [1-3]. Subcellular genomes – both chloroplast and mitochondrial – code for a small number of genes, but these genes are unique and irreplaceable, with an essential role to play in the main energetic processes in plants [4-6]. During the last decade, a great deal of progress in our understanding of the mechanism of nucleus-organelle interaction has been made. Certain organelle signal molecules regulating nuclear gene expression were

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described, as well as nuclear gene products that regulate organelle gene expression and trans-membrane transfer [6-9].

Various models are used to analyse nuclear-cytoplasmic interactions in plants: cytoplasm substitution by repeated backcrossing [10, 11], somatic hybridization and regeneration of cybrid plants [12-14], organelle microinjection [15], and direct transformation of definite organelle genes [16, 17]. The most thoroughly studied example of nuclear-cytoplasmic interaction, one which is also widely used in hybrid seed production, is cytoplasmic male sterility, which is often a result of nuclear-cytoplasmic incompatibility [18, 19].

Alloplasmic lines were first created by Kihara in 1951 by repeated backcrossing of wheat with various ***Aegilops*** and wheat species [11]. Later on, large collections of wheat substituted lines were created in Japan and Bulgaria [12, 20], and a great deal of data were obtained concerning cytoplasm influence on nuclear gene expression [3, 21, 22], pollen fertility [23], and disease resistance [22, 24, 25].

A collection of substituted barley lines was created in the Laboratory of Non-chromosomal Heredity over a twelve year period [26]. It combines 7 nuclear and 12 cytoplasmic genomes (84 unique lines). Here, we present the effects of cytoplasmic substitution on productivity traits in several cultural barley varieties, and we make an attempt to discuss these data with a view to establishing relationships between nuclear genome expression and chloroplast and/or mitochondrial genome organization.

MATERIALS AND METHODS

60 substituted lines, combining 5 nuclear and 12 cytoplasmic genotypes (Tab. 1), were selected from a collection of 84 allo- and isoplasmic lines created in our laboratory. All the lines and their parent cultivars were grown randomly in 3 repeats. Each group having the same nuclei and varying cytoplasms was compared with its euplasmic form. Agronomic traits were measured following standard procedures. Mitochondrial and chloroplast DNA was extracted as per our method for the isolation of chloroplast DNA and mitochondrial DNA [27]. RAPD-PCR was carried out with 22 primers, and the amplified products were resolved by electrophoresis on a 2% agarose gel [28].

RESULTS AND DISCUSSION

Table 2 contains the results of analyses on the effect of cytoplasm substitution on productivity traits (data obtained in 1998). The majority of nuclear-cytoplasmic combinations did not substantially change the productivity traits that are typical for intra-specific hybrids [29]. A significant decrease in the expression of the productivity traits was noted for both the 'Vezha' variety and those alloplasmic lines with a 'Vezha' nucleus (Tab. 2).

Tab. 1. Source of barley nuclear and cytoplasmic genotypes combined with each other in a collection of 30 variants.

Cytoplasmic source						
Species	Samples	CPI** No	Origin		Nuclear source	
H.vulgare	Atlas	77151	California		Zazersky-85	26965
«	Himalaya	94435	Nepal	×	Vezha	29912
«	W_1	77133	Israel, Hermon-9		Gonar	29405
«	W_3	77129	Israel, Atlit-37		Gostinets	29915
«	W_4	77129	Israel, Atlit- 55		Roland	26897
«	W_5	77144	Israel, Talpiyyot-4			
«	W_7	77137	Israel, Mehola-7			
«	W_8	77135	Israel, Wadi Quilt-23			
«	W_9	77141	Israel, Sede-Boker-21			
	W_{10}	77154	Iran, Andimesk			

K* - VIR catalogue No CPI** - Commonwealth Plant Introduction

In contrast to these results, in the substituted lines combining the 'Gostinets' nuclear genome with the majority of the cytoplasmic genomes (5 out of the 6 tested), expression of all the productivity traits was significantly increased relative to the euplasmic variety. The alloplasmic lines with 'Gostinets' nuclei were analysed again in 2001 with all 12 cytoplasmic genomes in our collection.

The climatic conditions of the 2001 season were quite different from those of 1998. The dry and cold late spring without precipitation and cold rainy summer in 1998 resulted in depressed productivity in the euplasmic variety 'Gostinets', as well as in the other varieties tested (Tab. 3).

The 2001 season was more favourable, and 'Gostinets' was almost twice as productive as it had been in 1998. The effects of the cytoplasm substitution in 2001 were also quite different, and depended on the definite cytoplasmic type. While in 1998 all the cytoplasmic genomes had significantly increased the expression of all the productivity traits (except spike number/plant), in 2001, we observed quite variable types of reaction depending on the cytoplasm.

In 2001, the expression of the main productivity trait (grain weight/plant) was significantly decreased in alloplasmic lines with W1, W4 or W5 cytoplasms. Other productivity traits was also depressed in certain alloplasmic lines (Tab. 3, marked *). Note that in 2001, we did not observe any increase in the expression of either trait value in the 12 substituted lines with ‘Gostinets’ nuclei.

Furthermore, in some lines the cytoplasm substitution resulted in a slight but statistically significant depression of most productivity traits (Tab. 3).

Tab. 2. Comparative agronomical analysis of cultivars and their analogues – lines with substituted cytoplasms.

Cultivars	Lines	Characters					
Nucleus	Cytoplasm	Plant height	Spike number /plant	Spike length	Grain number /spike	Grain number /plant	Grain weight /plant
Zazersky-85	Zazersky-85	54.181	3.724	7.095	21.886	70.057	3.238
	A	**57.886****	3.800	6.733	20.943	67.514	3.130
	L1	53.333	4.248	7.257	22.190	78.495	3.346
	W3	**58.486****	**4.467***	**7.543***	22.371	**83.429***	3.696
	W4	53.762	4.295	7.010	21.724	78.648	3.603
	W5	**56.467****	4.210	7.276	22.895	78.114	3.520
	W8	54.952	3.838	**6.619****	20.743	65.143	2.944
Roland	Roland	62.02	4.43	7.40	22.10	83.84	3.42
	A	61.51	4.69	7.05	21.61	86.56	3.42
	L1	**67.03***	4.78	7.50	22.41	92.70	4.01
	W3	63.66	4.51	7.78	23.09	89.78	3.59
	W4	**65.72****	4.51	7.61	22.31	89.71	3.69
	W5	63.57	4.65	7.31	22.14	87.04	3.88
	W8	**67.98****	4.87	7.58	23.33	92.49	**4.11***
cv.Visit	Visit	63.152	4.438	7.267	23.133	89.238	4.253
	A	63.124	**3.810***	7.543	22.619	78.638	3.904
	L1	**70.295****	4.219	7.476	22.076	78.552	3.987
	W3	62.543	4.048	7.390	22.257	79.019	3.724
	W4	62.943	4.324	7.210	22.400	84.038	3.890
	W5	62.619	**3.781***	6.895	22.810	**71.314****	**3.532***
	W8	63.257	4.571	**7.610***	22.733	86.600	4.089
cv.Vezha	Vezha	52.914	2.876	5.276	33.514	81.410	3.167
	A	52.505	2.629	**4.895***	31.086	**68.048***	2.791
	L1	**49.343****	**2.533***	**4.971***	31.476	**65.181****	2.666*
	W3	**50.829****	2.686	5.086	33.448	72.990	3.087
	W4	**50.314****	2.733	5.086	32.886	76.457	3.252
	W5	52.448	2.876	**4.867***	**30.238***	**69.381***	**3.442***
	W8	52.057	2.905	5.057	34.581	82.819	3.213

Differences to the euplasmic form were significant at: * – P<0.05; ** – P<0.01.

Tab. 3. Comparative morphological analysis of substituted lines combining the cv.Gostinets nuclear genome with different cytoplasms

Cultivars	Lines	Characters				
Nucleus	Cytoplasm	Plant height	Spike number /plant	Spike length (cm)	Grain number /spike	Grain weight /plant (g)
cv.Gostinets	Gostinets	**52.124**	**4.733**	**6.610**	**18.438**	**2.767**
1998	A	56.800**	6.410**	7.467**	21.362**	4.149**
	L1	60.829**	5.057	7.048*	19.924*	3.521**
	W3	55.705**	5.429	7.524**	21.952**	3.715**
	W4	57.905**	4.371	7.381**	19.962*	3.568**
	W5	60.943**	4.524	7.286**	21.181**	3.470**
cv.Gostinets	Gostinets	**84.412**	**7.017**	**8.854**	**23.422**	**5.445**
2001г.	A	83.071**	8.058	9.446	22.425**	5.265
	W3	80.137	6.412**	9.029	23.542	5.039
	W8	82.104	7.108	8.667	24.218	5.278
	L2	82.483	6.508	8.304**	22.689**	4.942
	H	83.225	7.608	8.771	23.183	5.455
	W1	75.300**	**8.708****	8.421**	21.475**	4.537**
	W4	77.125	6.403**	8.334**	21.017**	4.565**
	W5	79.071	6.933**	8.288**	22.630**	4.719*
	W7	84.588	7.125	8.842	**24.492****	5.842
	W10	81.569**	9.052	8.492**	22.422**	5.586
	L1	89.471	6.667**	8.887	23.992	5.093
	W9	81.934	7.125	8.454**	23.000	5.144

Components of variation are significant at: *: $P<0.05$; **: $P<0.01$

In our opinion, the potential productivity traits of 'Gostinets' were not completely expressed under the stress conditions of 1998 – the plants were suppressed, while the cytoplasm substitution resulted in the more successful expression of the ontogenetic program and more productive plants. The favourable year 2001 permitted a complete expression of the potential qualities of the variety itself, under such conditions that the cytoplasm substitution did not result in any further increase in productivity. In fact, dispersion analysis revealed a small but statistically significant influence of the cytoplasmic genome on all the productivity traits tested, while the influence of the environment was much more pronounced (Tab. 4).

Among the 12 cytoplasmic genomes studied, W1, W4, W5 and W10 can be highlighted. These genomes significantly modified several (3-5) productivity traits of 'Gostinets', while W8 and H cytoplasms did not change any trait, and W3, W7 and W9 induced the alteration of one trait out of the five tested.

Tab. 4. Fraction of effect of cytoplasm and environment on productivity traits in substitution barley lines

	F-criterion			Fraction of effect (%)			
Character	Cyto-plasm	Environ-ment	Inter-action	Cyto-plasm	Environ-ment	Inter-action	Residual
Plant height	22.3**	3615.1**	20.8**	2.46	66.5	2.29	28.72
Spike number/plant	11.2**	216.3**	2.7*	3.61	11.6	0.88	83.88
Spike length	3.1**	172.7**	2.3*	1.06	9.8	0.78	88.38
Grain number /spike	3.7**	245.7**	5.1**	1.25	13.8	1.73	83.21
Grain weight/plant	9.2**	209.2**	13.2**	2.90	11.04	4.19	81.87

Components of variation are significant at: *: $P<0.05$; **: $P<0.01$

In order to detect particular differences between those genomes that influenced the nucleus and those that did not, we analysed the restriction patterns of their mitochondrial DNA with 9 endonucleases and defined several cytotypes [30]. Unfortunately, we did not find correlations between the cytotype and its "modification force" on the productivity trait controlling nuclear genes of 'Gostinets'. More precise methods of organelle genome analysis (comparison of organelle genome sequences and gene localisation in various cytoplasmic types) certainly need to be used to elaborate further on this phenomenon.

The PCR-analysis of organellar DNA with random primers was originally carried out to investigate the mitochondrial and chloroplast genomes of rye [31]. Here, we have reported on the first results of the application of RAPD-PCR to a collection of barley organellar DNA. We carried out RAPD-PCR with several primers and constructed dendrograms of mitochondrial and chloroplast genomes (Shymkevich, Ignatov, 2002, unpublished). Three cytoplasms that substantially modify many productivity traits (W1, W5, W10) were located in the same cluster, both in the plastid and mitochondrial dendrograms, while W4 cytoplasm was somewhat distant in both trees. Eight "neutral" cytoplasmic genotypes were located in various regions of the dendrogram, surrounding the group consisting of W1, W5 and W10. These data are preliminary and need more thorough investigations of both organellar and nuclear genomes to reveal their coadaptation.

Several investigations directly prove the existence of such a nuclear-cytoplasmic interaction. A thorough analysis of nuclear-cytoplasmic coadaptation was performed for 247 accessions of wild barley [32]. Statistically significant associations were detected between six nuclear loci and three chloroplast DNA genotypes. The observed cyto-nuclear associations form as a result of the adaptation of barley genotypes to specific environmental conditions [32]. The analysis of 46 wheat alloplasmic lines with *Triticum* and *Aegilops* plasmons (31 species of the two genera) revealed that chloroplast genomes play a more important role in the vegetative phase of the life cycle while the mitochondrial genomes are more important during the reproductive phase [33]. In distant nuclei-cytoplasmic hybrids, various reorganisations overcoming the depressive effects of an alien cytoplasmic genome were found. These reorganisations are: 1) addition of defined chromosomal fragments (arms) from cytoplasmic donors to the caryotype of a nuclear donor [34, 35]; and 2) formation of recombinant organelle genomes combining mitochondrial DNA fragments of both parents [36, 37]. Our data show that under unusual stress coming from environmental conditions, a certain alien nuclei-cytoplasmic combination can be more adaptable than a euplasmic form. Cytoplasmic genetic factors influencing the expression of "productivity" genes are still elusive.

Acknowledgment. The work was partly supported by Grant B99R-130 of the Fund of Fundamental Research of Belarus.

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