Genetic analysis of introgressive common wheat lines for the character awned spike

Laboratory of genetics and cell biolgy
National University of Kyiv-Mohyla Academy

Prokopyk Daria, Antonyuk Maksym, Ternovska Tamara

04070 Skovorody str. 2, build. 2-303, Kyiv, Ukraine
Author for correspondence: prokopyk.d@gmail.com

Introduction

Development of awned spike in common wheat is controlled by three dominant inhibitors, *Hd* (4AS), *B1* (5AL), and *B2* (6BL). Although existence of some awning promoters has been communicated, none of these genes is yet mapped and their action is not discovered. In this research, a number of hexaploid introgressive common wheat lines, which differ in a degree of awn development, were used. These lines were developed on the basis of Aurora variety genotype and origin from genome-substituted forms Aurolata (AABBUU, UU is a genome of *Ae. umbullulata),* Aurosis (AABBS^{l}S^{l}, SlSl is a genome of *Ae. sharonensis*), and Aurodes (AABBSS, SS is a genome of *Ae. speltoides*). Aurora variety is awnless and is thought to possess awning inhibitor *B1* on the 5AL chromosome, and a suggestive weak awn promoter *bn* on the 6D. Though Aurora variety is awnless and all three genome-substituted forms are weakly awned, some introgressive lines have a completely awned spike. The object of this study was to investigate the genetic control of awned spike character in these introgressive lines. In this purpose, introgressive lines with identical and contrasting phenotypes were crossed with each other and with Aurora variety, and the segregation in the F_2 and F_3 was studied. On the basis of segregation observed, and microsatellite analyses of introgressive lines a way of awned spike character genetic control in these lines was proposed.

The development of introgressive common wheat lines used in this study


Aurosis AABBS^{l}S^{l}


Aurodes AABBSS


Aurolata AABBUU


Aurora AABBDD

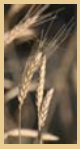
AABB x XX

Tetra-component of Aurora variety

XX is a genome of diploid of *Aegilops* species:

UU of *Ae. umbullulata*
SS of *Ae. speltoides*
S^{l}S^{l} of *Ae.sharonensis*


Ae.umbellulata


Ae.speltoides


Ae.sharonensis

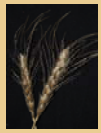
Aurolata AABBUU
Aurodes AABBSS
Aurosis AABBS^{l}S^{l}

AABBXX x AABBDD

Amphidiploid — Aurora variety

(AABBDX)

Self-pollination during several years


Awnedintrogression line


Terminally awned spike

A number of hexaploid common wheat lines that include alien genetic material at a different extent: as a translocation, chromosome or arm substitution is developed. Up to several chromosomes could be alien. Some lines are awned or have incompletely developed awns (awn-like sprouts), though neither Aurora nor Aurosis is awned. The presumption about inhibitor gene loss due to deletion arises.


Awnless introgressio line


Awn-like sprouts

Table 1
F_1 and F_2 plants phenotypes and F_2 segregation in the crosses of lines, which origin from Aurosis, with each other and with Aurora variety

Crossing combination	F_1 phenotype	Segregation in the F_2			F_2 volume
		Awnless	Awned	Awn-like sprouts	
awned x awned two obligatory univalents in M1 of meiosis in F_1 plant	Awned		1471	16	1487
awned x awned four obligatory univalents in M1 of meiosis in F_1 plant	Awned		842	24	866
awned x awnless	Awn-like sprouts	83	144	262	489
Awn-like sprouts x Aurora: 1)res788 x Aurora 2)res937 x Aurora 3)res890 x Aurora	 Awn-like sprouts Awnless Awnless	23 - -	- -	72 - -	95 - -
res 937 x res936 awn-like sprouts x awnless	Awnless	54		19	73
res788 x res 936 awn-like sprouts x awnless	Awn-like sprouts	19		48	67

We presume that awned lines occurance from weekly awned Aurosis could be due to *B1* gene loss. This gene is localized distally on the 5A chromosome, and could be deleted during cytogenetic rearragements that had occurred during lines' development. The cross between awnless and awned Aurosis derivative lines could be written in a following way:

B1B1b$_n$b$_n$ x del del awn^{P}awn^P

1*del del awn^{P}awn^P* awned
2*del del awn^{P}b$_n$* awned
1*del del b$_n$b$_n$* awn-like sprouts
1 *B1B1 awn^{P}awn^P* awn-like sprouts
2 *B1B1 awn^{P}b$_n$* awnless
1 *B1B1 b$_n$b$_n$* awnless
2 *del B1 awn^{P}awn^P* awned
4 *del B1 awn^{P}b$_n$* awn-like sprouts
2 *del B1b$_n$b$_n$* awn-like sprouts

In the F_2 the ratio **8 (awn-like sprouts) : 5(awned) : 3(awnless)** should be expected.
The empirical classes volumes match the theoretic ones:

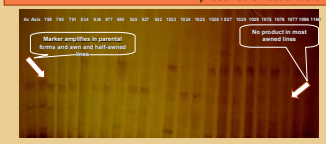
246 (awn-like sprouts) + 154 (awned) + 92 (awnless), χ^2=3,01< st d.f.=2

To verify our assumption and to estimate possible deletion extent we studied these lines by means of SSR-markers, specific to 5AL chromosome: Xgwm291, Xwmc524, Xwmc577, Xwmc110, та Xcfa2155. The more proximate is marker to *B1* gene, the more possible it would be absent on the awned lines' spectra was our hypothesis. In order to find 6S chromosome that could *awn^P* possess we used Xcfd76-6D marker.

Table 2
The verification of hypothesis about awned spike character control in some introgressive lines.
The F_1 phenotypes and segregation in F_2 are taken into account

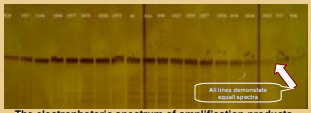
№	Cross combination	F_1 pheno-type	Phenotypic classes				$\Sigma\chi^2$	The explanation
			Class	Volume Observed	Theoretical background of class formation	Theoretical class volume		
1	res2 awnless x res16 awned		awnless	46	3*B1_b$_n$b$_n$*	41	0,97	The presence of dominant awn inhibitor gene in the absence of strong promoting allele causes awnless phenotype. The simultaneous presence of the promoter *awn^P* and inhibitor *B1* form phenotype with awn-like sprouts, and promoter *awn^P* forms awned phenotype in the absence of the inhibitor *B1*.
			awned	51	*4deldel awn^P_+ del de b$_n$b$_n$*	54		
			Terminal-ly awned	29	9*B1_awn^P_*	122		
			Awn-like sprouts	90				
2	res15 awnless x res16 weakly awned		awnless	25	3*B1_b$_n$b$_n$*	21	1,48	The explanation is the same as previous
			awned	30	*4deldelawn^P_+ 1deldelb$_n$b$_n$*	28		
			Terminal-ly awned	19	9 *B1_awn^P_*	63		
			Awn-like sprouts	38				
3	res105 awnless x res79 awned		awnless	11	3*B1_b$_n$b$_n$*	17	3,40	The explanation is the same as previous
			awned	28	*4deldelawn^P_+ 1deldelb$_n$b$_n$*	23		
			Terminal-ly awned	27	9 *B1_awn^P_*	52		
			Awn-like sprouts	27				
4	res5 awned x res105 awnless		awnless	27	3*B1_b$_n$b$_n$*	20	5,77	The deletion of the awn inhibitor B1 gene is also supposed for the awned parental form. Its awn promoter *awn^P, weak promoter bn* of Aurora variety, and *B1* gene together could form different phenotypes depending on their dosage.
			awned	37	5 *3deldelawn^P_+2del B1awn^{P}awn^P*	33		
			Terminal-ly awned	5	8=1*B1B1awn^{P}awn^P +deldelb$_n$bn+ 4delB1awn^{P}b$_n$+2del B1b$_n$b$_n$*	52		
			Awn-like sprouts	36				

The electrophoretic spectra of introgressive common wheat lines. The number means line; lines 936, 1166 are awnless, lines 788, 937, 890 have awn-like sprouts, and the rest of lines is completely awned. Arrow indicates presence or absence of fragment of interest.

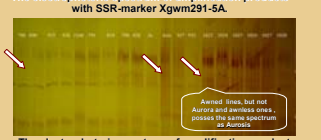


The electrophoteric spectrum of amplification products with SSR-marker Xwmc524-5AL.

The electrophoteric spectrum of amplification products with SSR-marker Xgwm291-5A.



The electrophoteric spectrum of amplification products with SSR-marker Xcfa2155-5AL.



The electrophoteric spectrum of amplification products with SSR-marker Xcfa76-6D.

Conclusions

The introgression common wheat lines that origin from common wheat variety Aurora and amphidiplods Aurosis, Aurolata, and Aurodes differ significantly in a degree of awn development from their parental forms.

On the basis of studied lines phenotypes, phenotypes and their ratios observed in F1 and F2 from introgression lines crossing with each other and Aurora variety we could draw a conclusion that awned lines should have lost *B1* awn inhibitor during their development. Also, they possess an awn promoting gene *awnP* which was introgressed from *Aegilops* species. The promoter and inhibitor act in incomplete epistatic way.

Additionally, the microsatellite analysis shows that awned lines did not amplify some markers (Xgwm291, Xwmc524) that are located proximately to *B1* gene. The more distant from it markers (Xcfa2155, Xwmc110, Xwmc577, not shown) gave equal amplification patterns in all lines studied. This allows us to estimate a deletion extent in different lines.

The presence of 6S chromosome in awned lines-derivatives of Aurosis was shown by means of microsatellite marker.