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VARIABILITY IN EXPRESSION OF MALE FERTILITY IN TRITICALE (X*TRITICOSECALE* WITTMACK) WITH *TRITICUM TIMOPHEEVI* CYTOPLASM

ABSTRACT

We present a reliable, visual method for evaluation of the level of male fertility during flowering, that is indispensable for breeding of hybrid cultivars of winter triticale based on the cms-*T.timopheevi* system. Detailed observations of anther development were performed on 20 F_2 and BC_1 plants derived from crosses between male-sterile and fertility restoring lines. Variation of anther development within florets, spikelets, spikes, and among spikes of the same plant was examined. Hierarchic analysis showed significant role of these factors in determination of anther development, irrespective of the level of plant fertility. The sterilizing effect of cytoplasm was always better visible in the tip and base spikelets of the spike, in the third floret, and the anther adjacent to the floret axis. Our data indicate that during selection toward male-sterile plants at anthesis, at least 5 spikes should be evaluated. Special attention should be paid to the development of the anthers at the 2nd and 7th spikelet of the spike along with the variability in anther development within spikelet and floret. The anthers in the tip and base spikelets of the spike must be precisely evaluated during selection toward restorer lines.

Key words: anther development, male sterility assessment, triticale, *Triticum timopheevi* cytoplasm

INTRODUCTION

The cms-*T.timopheevi* system is promising for breeding of modern hybrid triticale cultivars (Nalepa 1990, Góral 2002a, Ammar *et al.* 2006). Evaluation of the male fertility restoration (MFR) is necessary at each stage of hybrid breeding during selection of maintainers and restorers. The assessment of the level of anther development and pollen release (ADPR) is routinely performed at the flowering according to arbitrary scale. In triticale with *Triticum timopheevi* cytoplasm, variation of ADPR within and among plants and spikes impedes reliable estimation of male fertility restoration

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during flowering (Góral 2002b, Góral and Jagodziński 2005). The differences in expression of sterility in various florets of the same plant were also observed in rye with C cytoplasm (Łapiński 1977) and in oilseed rape with *polima* cytoplasm (Bartkowiak-Broda *et al.* 1996).

The MFR level in triticale plants with pollen-sterilizing cytoplasm can be scored by counting seeds in bagged spikes, but it can be done when the crossing season is over. Reliable system for evaluation of male fertility during flowering would increase efficiency of breeding efforts. The 9-grade scale was proposed for rye (Geiger and Morgenstern 1975) but no method of determination of MFR for triticale with *T. timopheevi* cytoplasm has been widely approved.

The aim of the present study was: 1) to establish the main sources of variation of MFR in triticale plants with *T. timopheevi* cytoplasm, 2) to develop proper and reliable standards of efficient plant fertility estimation at anthesis.

MATERIALS AND METHODS

Male-sterile *cms* Salvo-15/1 or *cms* Grado-2 lines were crossed with restoring lines to obtain BC_1 and F_2 generations (Table 1). During flowering plant fertility was examined twice by the senior author. ADPR was recorded in 5-grade scale on 2–3 spikes per plant during flowering to obtain initial visual classification (IVC). Plants were marked as fertile (5), fertile-intermediate (4), intermediate (3), sterile-intermediate (2), and sterile (1) (Góral *et al.* 2006). Based on IVC, 20 plants were randomly selected out of hundreds of plants evaluated, to uniformly represent 5 groups according to the IVC scale (Table 1).

Detailed observations of ADPR were performed during flowering on the selected 20 plants according to method described by Góral and Jagodziński (2005). Degeneration of three anthers in each of three florets (of the 1st, 2nd, and 3rd order) in 11 spikelets (counted from the base of ear) from one side of 5 spikes per plant was recorded, to the total number of 9900 anthers. Data from detailed observations were analyzed in hierarchical classification: spikes within plants, spikelets within spikes, florets within spikelet, and anthers within floret. Before flowering, 5 spikes per plant were bagged to control the seed set.

Analysis of variance was performed in mixed model for the whole population of 20 plants, and separately within the five IVC groups. Plants within the groups and spikes within plants were treated as random variables, while remaining factors were regarded as fixed. Components of variation caused by anthers, florets, spikelets, spikes and plants in overall variation were computed with mean squares analysis, and expressed in percentage (Table 2). Standardized mean values of ADPR were arranged in respect to analysed elements of flowering structure and used to calculate a) Euclidean distances

Table 1.

Pedigree, initial visual classification (IVC) during flowering, groups based on Euclidean distances, and seed set in bagged spikes of the 20 selected plants

Number of plant and combination	Pedigree	IVC	Euclidean group	Seed set, [%]
K1-1	Cms Salvo 15/1 × Lasko 7/1/1, BC1	5	4	41.8
K2-1	Cms Salvo 15/1 × Nemo 4/1, BC1	5	4	54.4
K3-1	Cms Grado 2 × Lasko 7/1/1, BC1	5	4	61.2
K4-1	Cms Salvo 15/1 × Tewo 1/1, F2	5	4	85.2
K1-2	Cms Salvo 15/1 × Lasko 7/1/1, BC1	4	3	35.0
K3-2	Cms Grado 2 × Lasko 7/1/1, BC1	4	3	24.2
K4-2	Cms Salvo 15/1 × Tewo 1/1, F2	4	4	46.0
K5-1	Cms Grado × Bogo 5/3, BC1	4	3	73.5
K2-2	Cms Salvo × Nemo 4/1, BC1	3	2	13.8
K4-3	Cms Salvo 15/1 × Tewo 1/1, F2	3	3	3.8
K6-1	Cms Salvo 15/1 × Ugo 1/1, BC1	3	2	29.8
K7-1	Cms Grado 2 × Moreno 2/4, BC1	3	2	11.0
K4-4	Cms Salvo 15/1 × Tewo 1/1, F2	2	2	6.7
K5-2	Cms Grado × Bogo 5/3, BC1	2	2	3.6
K8-1	Cms Salvo 15/1 × Moreno 2/4, BC1	2	1	0.0
K9-1	Cms Grado 2 × Ugo 1/1, BC1	2	2	3.4
K4-5	Cms Salvo 15/1 × Tewo 1/1, F2	1	1	1.8
K6-2	Cms Salvo 15/1 × Ugo 1/1, BC1	1	1	0.6
K10-1	Cms Grado 2 × Bogo 5/3, BC1	1	1	1.3
K11-1	Cms Salvo 15/1 × LAD 593, BC1	1	1	0.0

Table 2.

Expected mean square values and proportion of variance components in total variation of anther development

Source of variation	Expected values of mean squares
Plants [pl]	$\sigma_{an}^2 + an \times fl \times \sigma st^2 + an \times fl \times st \times \sigma_{sk}^2 + an \times fl \times st \times sk \times \sigma_{pl}^2$
Spikes [sk]	$\sigma_{an}^2 + an \times fl \times \sigma_{st}^2 + an \times fl \times st \times \sigma_{sk}^2$
Spikelets [st]	$\sigma_{an}^2 + an \times fl \times \sigma_{st}^2$
Florets [fl]	$\sigma_{an}^2 + an \times \sigma_{fl}^2$
Anthers [an]	σ_{an}^2

between 20 plants, and b) simple regression explaining seed set. Euclidean distances were applied for grouping plants using an UPGMA (un-weighted pair-group method with arithmetic averages) method performed with the NT-SYS 2.10q (Rohlf 2001).

RESULTS

Detailed analyses suggest that anther development depends significantly on sampled plant, and anther position in spike, spikelet, and floret (Table 3). The highest influence on overall ADPR variation was caused by plants (59.1%). In analyses within IVC groups, variation attributed to plants was low and varied from 3.1% (group IVC-2) to 12.7% (group IVC-3). The highest variation within the groups 5, 4, and 3 was caused by position of spikelet in spike (from 38.9 to 41.2%). In groups 2 and 1 the highest variation of ADPR was connected with position of anthers in floret (46.9% and 42.1%, respectively). Variability between spikes was low in groups IVC-5, IVC-4, and IVC-3 (2.9–4.9%), but in groups IVC-2 and IVC-1 increased to 9.4 and 15.8%, respectively (Table 3).

Table 3

Mean squares (MS) from ANOVA analysis of the anther development and pollen release (ADPR) variation and distribution of variation (% VAR) in respect to sources within five initial visual classification (IVC) groups, and for the all 20 plants studied

IVC group		Source of variation				
		Plant	Spike	Spikelet	Floret	Anther
5	MS	52.24**	11.97*	5.27**	1.82**	0.19
	% VAR	5.6	4.9	38.9	37.5	13.1
4	MS	81.64**	8.94	5.39**	1.18**	0.32
	% VAR	11.0	2.9	41.2	21.3	23.5
3	MS	52.38**	6.02*	2.81**	0.49*	0.21
	% VAR	12.7	4.2	40.8	12.7	29.6
2	MS	8.58*	3.72**	0.99**	0.28	0.15
	% VAR	3.1	9.4	28.1	12.5	46.9
1	MS	8.27*	3.36**	0.35**	0.21**	0.08
	% VAR	5.3	15.8	15.8	21.1	42.1
Total	MS	534.99**	6.80*	2.96**	0.80**	0.19
	% VAR	59.1	2.2	17.1	11.0	10.6

*, ** variation significant at level $\alpha = 0.05$ and 0.01, respectively

Mean values of ADPR from subsequent spikes, spikelets, florets and anthers were used for grouping of the 20 plants studied into phenotypicaly uniform groups. UPGMA dendrogram based on Euclidean distances revealed 4 phenotypic groups only (Fig. 1). Mean values of ADPR estimates in the first group of plants (EUC-1) varied from 1.0 to 1.4, and in the second group (EUC-2) from 1.5 to 1.8. Group EUC-3 was subtracted based on mean ADPR from 2.3 to 3.2, while an average level of ADPR in the forth group varied from 3.5 to 4.1.

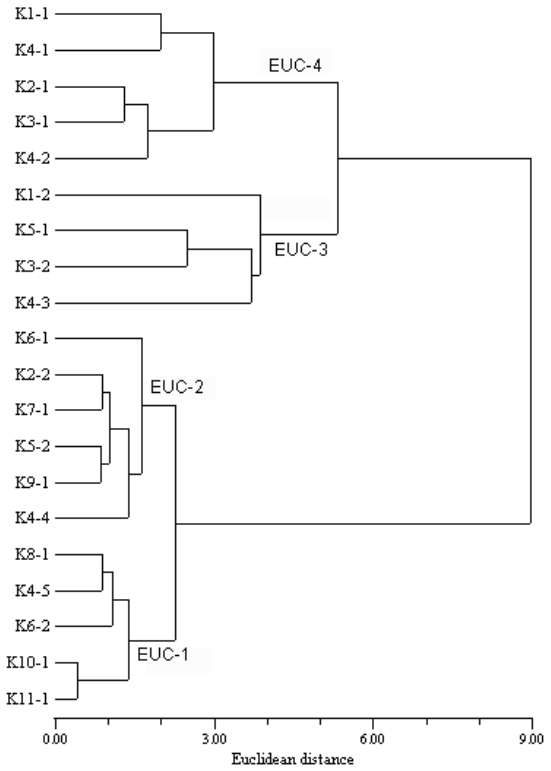

Fig. 1. Clustering of 20 triticale plants with the UPGMA method based on Euclidean distances for the anther development and pollen release (ADPR)

Based on Euclidean distances 4 groups of plants with different ADPR were identified (Table 1). Correlation coefficients between rank estimates of ADPR both for initial classification (IVC) and Euclidean groups with seed set were the same and equal to 0.86. However, in a group of sterile and intermediate plants (IVC-1 and IVC-2) some discrepancies were found. Three out of 4 plants from IVC-1 group (assessed as sterile) set single seeds, and one plant from IVC-2 group (assessed as intermediate-sterile) set no seeds in bagged spikes. Predictions based on Euclidean clusters were more precise and 2 out of 5 plants from EUC-1 group were totally sterile.

Mean values of ADPR were used in regression analysis to explain seed set in bagged spikes (Table 4). Observations of anthers from 2nd and 7th spikelet explained the highest range (77.2% and 79.4%) of seed set variation. This may come out from the fact that spikelets in a middle part of spike had the highest fertility within analysed groups IVC (Fig. 2). Observations of ADPR in 1st- and 2nd-row florets explain about 80% of variation in seed set (80.1%, and 81.5%, respectively). Similarly, development of 1st and 2nd

anther within floret explained respectively 80.7% and 80.5% of total variation of seed set.

Table 4.

Simple regression coefficients of the anther development and pollen release (ADPR) in respective parts of spike explaining variation of seed set

Element of spike	Regression	
	b	R^2 [%]
Spikelet 1	20.4	45.5
Spikelet 2	28.5	77.2
Spikelet 3	23.2	73.5
Spikelet 4	20.3	75.2
Spikelet 5	19.0	75.2
Spikelet 6	18.3	76.4
Spikelet 7	18.3	79.4
Spikelet 8	18.1	67.8
Spikelet 9	20.2	72.8
Spikelet 10	23.9	76.4
Spikelet 11	24.0	57.1
Floret 1	20.7	81.0
Floret 2	21.7	81.5
Floret 3	28.1	72.4
Anther 1	23.2	80.7
Anther 2	23.1	80.5
Anther 3	23.4	76.8

DISCUSSION

Determination of plant sterility level during anthesis is difficult when plants show variable level of fertility. Male sterility/fertility expression is often influenced by environment (Góral *et al.* 2006), and varies on spikes of the same plant, specially between main and remaining spikes, among florets within spikes, and among anthers of the same floret (Geiger and Morgenstern 1975). Our results show that variation of fertility within spikes plays a major role in all groups of plants, and that between spikes particularly in groups classified as male-sterile and intermediate-male sterile. Thus, the number of spikes for evaluation can be reduced only in the process of selection of good restorers.

Anthers in florets from upper part of spikes were on average more degenerated than those in florets from a middle or base part of spike (Fig. 2). This observation was specially valid in groups of male-fertile and intermediate

plants. Similar variation of pollen sterility in respect to position of spikelet in the spike was found in rye with C cytoplasm (Łapiński 1977). Reduced fertility of florets located at spike tip was found in common wheat with *T. timopheevi* cytoplasm (Sage 1972). Usually, intermediate plants had normally developed anthers in central part of spike, whereas florets in top part were male-sterile. This was explained by gradient concentration of undefined substance necessary for fertility restoration that was transported from stem. Sufficient concentration is more easily achieved in florets in the base of spike (Sage 1972). A question, why do florets of a partially male sterile plant develop fertile and some sterile anthers remains still an open problem in male sterility (Kaul 1998).

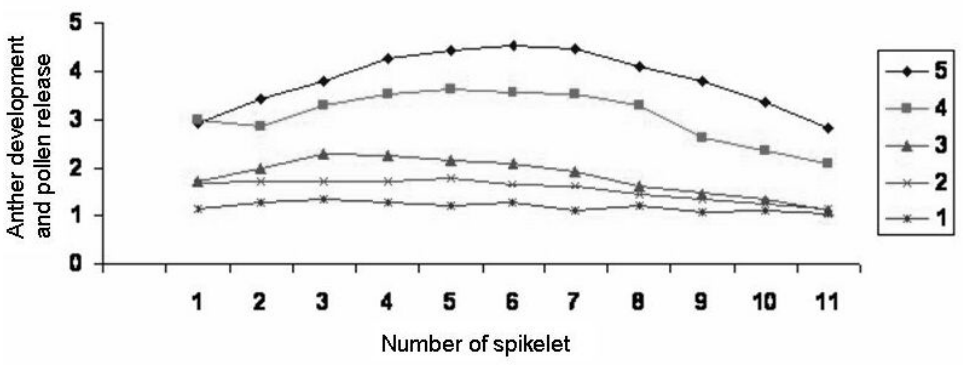

Fig. 2. Development of anthers in spikes (ADPR) in the fife initial visual classification (IVC) groups of triticale plants

Evaluation of MFR of triticale plants during flowering in 72.9% corresponded with seed set in bagged spikes (Góral 2002b). Differences between both methods (Table 1) possibly result from variation in fertility restoration within plant. Our studies show, that predictability of seed set based on observations during flowering may be increased to 80% (Table 4). Therefore IVC should be verified with seed set in bagged spikes. In order to improve IVC in selecting male sterile lines special attention should be paid to development of anthers in 2nd and 7th spikelet of the spike, and on variation of anthers within spikelet and floret. Selection of plants based on evaluation of anthers in the middle part of spike is not sufficient and may lead to misclassification, as spikelets located in top part of spike are usually more degenerated.

CONCLUSIONS

The variation of anther development within florets and among florets, among spikelets and spikes depends on the sterility groups. In sterile and intermediate plants variation among anthers within florets, and within florets and spikelets is the highest.

Detailed analysis of anther development only slightly improved present classification standards in respect to predictability of seed set on bagged spikes. Observations of anthers in 2nd and 7th spikelet, 1st and 2nd floret, and the two anthers adjacent to the floret axis provide the best indices for ascribing plants into adequate male fertility restoring groups.

Euclidean distances suggest, that in procedure of determination of plant sterility level during anthesis it may be sufficient to classify plants into 4 phenotypically distinct groups instead of 5. Predictability of seed set based on observation during anthesis is limited to 80%, thus data on seed set in bagged spikes should be used for final scoring of male fertility restoration.

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