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ASSESSMENT OF CROSSING COMPATIBILITY BETWEEN COMMON RYE *SECALE CEREALE SSP. CEREALE* AND A WILD SUBSPECIES *SECALE CEREALE SSP. AFGHANICUM (VAV.) HAMMER*

Summary

Tasks imposed by contemporary agriculture on plant breeding are becoming increasingly complex. In order to meet these requirements breeders are applying various methods to expand genetic variability of stock materials for breeding work. Distant crossing is one of them. Wild subspecies are potential donors of such traits as resistance to diseases, adverse environmental factors or male sterility. Pollen grain germination and penetration of pollen tubes were analyzed in the Secale cereale (L.) species based on pollination between three cultivars of common rye (cv. Dańkowskie Złote, Dańkowskie Nowe, Amilo) and a wild subspecies Secale cereale ssp. afghanicum. Reciprocal cross pollinations were performed and pistils were fixed at 10 time points from pollination from 10' to 4 h. The process of fertilisation was observed by fluorescence staining preparations with aniline blue. In comparison to the control, i.e. open-pollination within cv. Dańkowskie Złote, pollen grain abundance and germination on stigmas in reciprocal crosses with the wild subspecies were lower. Pollen grain germination and penetration of pollen tubes to the ovary were delayed in relation to the control. Symptoms of incompatibility were observed, numerous kernels did not germinate and callose reaction was observed in pollen tubes, along with rupture of tubes and their disorientation, while a lower number of pollen tubes penetrated to ovaries. In all the tested combinations pollen tubes were observed at the micropyles as late as after 4 h from pollination, with pollination efficiency within 0.36 – 7.91 %. In the control experiment penetration of pollen tubes into micropyles was observed as early as after 2^{30}h at pollination efficiency of 91%. It was found that in the three tested rye cultivars the pollen-pistil interaction varied, with cv. Dańkowskie Złote confirmed as the best for crossing with Secale cereale ssp. afghanicum. No marked differences were found between directions of crossing in processes taking place at the stigma. Crossing efficiency was poor, although it was higher when common rye was the maternal plant.

Key words: *Secale cereale, rye, S. c. ssp. afghanicum, intraspecific crosses, pollen grain germination, pollen tube growth, fluorescent microscopy*

OCENA ZGODNOŚCI KRZYŻÓWKOWEJ POMIĘDZY ŻYTEM UPRAWNYM *SECALE CEREALE SSP. CEREALE* A PODGATUNKIEM DZIKIM *SECALE CEREALE SSP. AFGHANICUM (VAV.) HAMMER*

Streszczenie

Współczesne rolnictwo stawia przed hodowlą coraz trudniejsze zadania. Aby sprostać tym wymogom hodowcy starają się różnymi metodami poszerzyć zmienność genetyczną materiałów wyjściowych do hodowli. Jedną z tych metod jest krzyżowanie oddalone. Dzikie podgatunki są potencjalnymi dawcami takich cech jak odporność na choroby, złe czynniki środowiskowe czy męska niepłodność. Przeprowadzono analizy procesu kiełkowania ziaren pyłku i wnikania łagiewek pyłkowych w obrębie gatunku Secale cereale (L.), dokonując zapyleń pomiędzy trzema odmianami żyta uprawnego (cv. Dańkowskie Złote, Dańkowskie Nowe, Amilo) a podgatunkiem dzikim Secale cereale ssp. afghanicum. Wykonano zapylenia wzajemnoprzemienne i utrwalono słupki po 10 czasach od zapylenia od 10' do 4h. Przeprowadzono obserwacje procesu zapłodnienia metodą fluorescencyjną, barwiąc preparaty błękitem anilinowym. W porównaniu do kontroli tj. obcozapylenia w obrębie odmiany Dańkowskie Złote, opylenie i proces kiełkowania ziaren pyłku na znamionach w krzyżówkach wzajemnoprzemiennych z podgatunkiem dzikim były słabsze. Opóźniony w stosunku do kontroli był proces kiełkowania ziaren i wnikania łagiewek do zalążni. Ujawniały się symptomy niezgodności, liczne ziarna pozostawały nieskiełkowane a w łagiewkach obserwowano reakcję kalozową, pękanie łagiewek i dezorientację ich, łagiewki mniej licznie wnikały do zalążni. We wszystkich badanych kombinacjach obserwowano łagiewki przy mikropyle jednak dopiero po 4 godz. od zapylenia a efektywność zapylenia wahała się 0,36-7,91 %. W kontrolnym doświadczeniu wnikanie łagiewek do mikropyle obserwowano już po 2^{30}h a efektywność zapylenia wynosiła 91%. Stwierdzono, że u badanych trzech odmian żyta reakcja ziarno pyłku - znamię była różna. Najlepszą do krzyżowania z Secale cereale ssp. afghanicum okazał się cv. Dańkowskie Złote. Nie zaobserwowano większych różnic w procesach na znamieniu pomiędzy kierunkami krzyżowania. Efektywność krzyżowania była słaba i wyższa gdy matką było żyto uprawne.

Słowa kluczowe: *Secale cereale, żyto, S. c. ssp. afghanicum, krzyżowanie oddalone, kiełkowanie ziaren pyłku, wzrost łagiewek pyłkowych, mikroskop fluorescencyjny*

1. Introduction

Lower climatic and soil requirements of rye in comparison to wheat in the distant past contributed to the fact that this plant, originally found as a weed in wheat culture, became a bread cereal in Central and Eastern Europe. Thanks to the tradition and properties of rye it is a staple in European cereal production. In Poland, with its predominant light sandy soils, low soil pH and rainfall shortage, rye is an important cereal, with Poland ranking third in grain production worldwide [1, 2]. Breeders are facing increasingly challenging requirements connected with needs of modern agriculture. In order to meet these requirements breeders are trying to produce new cultivars, which are not only more prolific, but also more resistant to disease and environmental factors or exhibiting improved quality traits. As early as the 1970's breeders reported problems caused by the decreased variation within the species *Secale cereale* L. ssp. *cereale,* which has led to interest in related wild species as potential donors of genes for desirable traits [3-10]. Kranz in 1972 [7] indicated that the gene pool of wild and primitive forms of rye should be extensively used despite several problems resulting from the high diversity in biology of fertilization within the genus *Secale*. In that genus we observe all degrees from allogamy through autogamy to cleistogamy. These results in the co-called incompatibility barriers, which may be manifested at two levels: 1) prezygous preventing fertilization, and 2) postzygous manifested after fertilization and causing embryo death or its inappropriate development and as a consequence producing abnormal F_1 progeny. Such problems were met when crossing allogamous forms of rye, e.g. *S.c. ssp cereale*, *S.c. ssp. ancestrale*, *S.c. ssp afghanicum* with self-pollinated forms such as *Secale vavilovii* [8,9]. In distant crosses of rye many valuable agronomic traits were identified, e.g. lodging resistance or resistance to disease such as mildew and brown rust, [3, 9,11-13], short-stemmed genotypes [14] and genotypes with reserve protein were identified [11-13]. Many studies concerned search for male sterility and sterilizing cytoplasmic genes [11, 12, 15], features of great importance in heterosis breeding. Breeding based on distant crossing with primitive species is burdened with numerous problems, requires multiple backcrossing and laborious selection, while additionally being of limited efficiency. Development of heterosis breeding in rye in the 1970's contributed to considerable breeding progress [15] and resulted in a decreased interest of breeders in the generation of distant crosses. However, after a long period of exploitation of the gene pool within *S. c.* ssp. *cereale*, breeding progress is reduced and researchers are reporting high genetic similarity of cultivated rye forms and breeding materials as well as a reduced gene pool [3, 5, 16-18]. All this has contributed to renewed interest in related wild species as potential donors of desirable genes [17, 19-21].

At present many research projects are being conducted to provide better understanding of fertilization processes in plants. Pollen-pistil interactions are highly complicated, as the stigma needs to distinguish among many grains found on the stigma so that the embryo sac is fertilized by appropriate spermatozoids. Next to the interspecies incompatibility in allogamous plants we also observe the self-incompatibility system based on a series of alleles at two loci [22]. The interaction between pollen grain and the stigma, i.e. between the gametophyte and the sporophyte, is highly complicated and needs to be further clarified. Studies indicate a significant role of specific proteins and Ca^{+2} ions in this process. In the past many publications discussed structural and functional aspects of the pollen grain-stigma interaction in species from the family *Poaceae*. Research focused on the identification of processes with a cultivated species or distant crosses.

In previous study conducted on distant crosses of rye we observed variation between rye cultivars in terms of crossing efficiency [17, 21]. A question was raised whether this variation is manifested already at the stigma surface at the prezygous phase or is it only at the postzygous phase. Can we influence crossing efficiency by selecting appropriate rye cultivars for crossing?

2. Materials and methods

Analyses were conducted on two rye subspecies: the cultivated *Secale cereale ssp. cereale (L.)* and the wild *Secale cereale ssp. afghanicum (Vav.)Hamm.* Three Polish population cultivars: Dańkowskie Złote, Dańkowskie Nowe and Amilo, were selected. Seeds of these cultivars (elite) came from Danko Hodowla Plants sp. z o.o. in Choryń, while seeds of the wild subspecies were obtained from the Gene Bank in Radzików (Pl.). Plants were grown in an experimental plot. At the ear formation phase ears were castrated and isolated, and left until they were ready for fertilization. When stigmas developed, reciprocal pollination was performed, with *S.c. ssp. afghanicum* being pollinated with the cultivar as the paternal form, and next in the reverse order, as the wild subspecies was the pollinator and the cultivars were maternal forms. The control experiment comprised allogamy within cv. Dańkowskie Złote. Pollen grains from pollinating plants was subjected to analyses of viability by staining with the Belling reagent (2% acetocarmine + glycerine at 1:1) [23]. An optic micrometer was used to measure the length and width of grains and the results were averaged. Pistils were collected and fixed at 10 time points after pollination: initially at every 10 min and then at every 30 min up to the 4th hour. From each pollination combination a total of 20 pistils were collected and fixed in Carnoy's fixing solution (95% EOH + CH_3COOH at 3:1) [23]. Pollen grain germination, penetration of pollen tubes and fertilization were observed under an Ergawal Carl Zeiss Jena fluorescent microscope and the specimens were stained with aniline blue [24]. Intensity of processes taking place on stigmas was analyzed applying an original 6-step method, developed by the authors of this study [25]. The following events were included in the observations: a) the number of pollen grains on the stigma, b) the number of germinating pollen grains on the stigma, c) the number of pollen tubes reaching ovaries, d) the number of pollen tubes reaching the embryo, e) length of pollen tubes, f) atypical phenomena such as rupture of pollen tubes, callose plugs, disorientation.

Since it is practically impossible to count all pollen tubes on stigmas, a 6–degree scale was applied to describe the observations:

0 – no pollen grains/pollen tubes on the stigma,
1 – 1-5 pollen grains/pollen tubes on the stigma,
2 – 6-15 pollen grains/pollen tubes on the stigma,
3 – 16-30 pollen grains/pollen tubes on the stigma,
4 – 31-50 pollen grains/pollen tubes on the stigma,
5 – > 51 pollen grains/pollen tubes on the stigma,
>5- number of pollen grains/pollen tubes over 100.

In order to determine crossing efficiency 5 ears were left until maturity for each pollination combination. The percentage share of obtained kernels was calculated in relation to the number of pollinated flowers.

3. Results

Pollen grain of three cultivars and *S.c.ssp. afghanicum* had a typical structure for rye; however, differences were found in grain size. Viability of pollen grain in the three rye cultivars and the wild subspecies *Secale cereale ssp. afghanicum* was very high, exceeding 92% (Tab. 1).

Table 1. Viability and size of pollen grain in three cultivars and a wild subspecies of *Secale cereale L. ssp. afghanicum*
Tab. 1. Żywotność i wielkość ziaren pyłku trzech odmian uprawnych i podgatunku dzikiego żyta Secale cereale L. ssp. afghanicum

Cultivar/subspecies	Mean		
	pollen viability [%]	pollen length [µm]	pollen width [µm]
Secale cereale ssp. cereale L. cv. Dańkowskie Złote	96.7	49.8	44.8
Secale cereale ssp. ssp. cereale L. cv. Dańkowskie Nowe	92.3	45.2	41.2
Secale cereale ssp. ssp. cereale L. cv. Amilo	94.9	46.6	45.8
Secale cereale ssp. afghanicum	96.8	46.8	44.6

Source: own study / *Źródło*: opracowanie własne

Observations of pollen grain germination and penetration of pollen tubes into stigma tissue and into ovaries at open-pollination within cv. Dańkowskie Złote were treated as the control (Tab. 2). In all the examined specimens pollen grains were abundant, on stigmas the number of pollen grains exceeded 50. Grains remained on stigmas to the least time point after pollination, i.e. up to 4 h. Pollen grain germination started already in the first minutes after pollination and it was very intensive. After 30 minutes numerous pollen tubes were observed penetrating towards the main stigma axes. Already after 1^{30} h whole bundles of pollen tubes reached ovaries and after 2^{30} they were observed to penetrate micropyle. The entire process took place with no visible signs of incompatibility, no callose reaction was observed and penetration of pollen tubes was appropriate and in the right direction (Fig. 1). Some pollen grains did not germinate and in these grains the callose reaction was observed, manifested in grain fluorescence. It is a typical incompatibility reaction in a self-incompatible species. Penetration of pollen tubes inside micropyle was considered as fertilization, which was confirmed by the control of pollination efficiency amounting to 91% at open-pollination within cv. Dańkowskie Złote.

In reciprocal crosses of the three rye cultivars with *S.c.ssp ancestrale* several symptoms of incompatibility were observed (Tables 3-5). Already stigmas were covered with a smaller number of pollen grains, ranging from around a dozen to 50 in pollination with cv. Dańkowskie Nowe and Amilo, while in the combination with cv. Dańkowskie Złote the number of pollen grain was over 50, but it was lower than in the control. Since pollination was performed using a highly abundant amount of pollen, the small number of pollen grains on stigmas shows that unattached pollen grain were washed away during fixation.

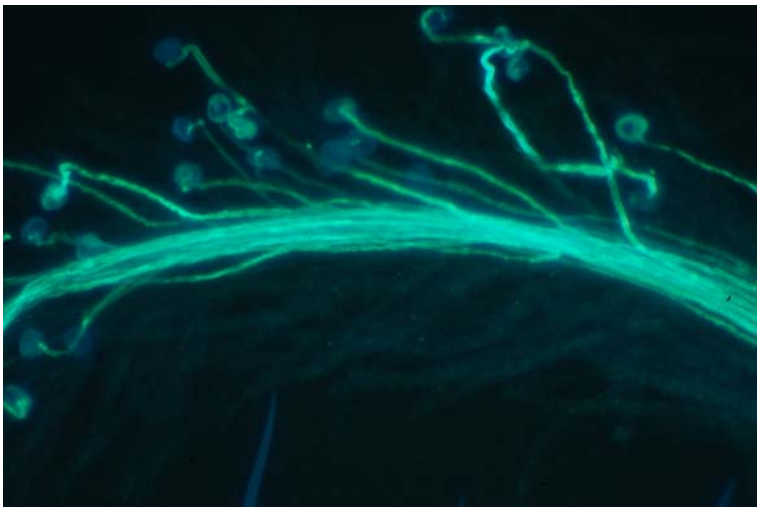
Observations of pollen grain germination and penetration of pollen tubes showed differences in these processes between the investigated cultivars. Germination rate and penetration rate of pollen tubes in cv. Dańkowskie Złote (Tab. 3) were similar to the course of fertilization in the control. Although the number of pollen tubes did not exceed 50 at all the investigated time points, germination started immediately after pollen grains fell on the stigma and after 20 minutes most of them were germinated. The speed at which pollen tubes reached the micropyle was slightly slower in comparison to the control and single pollen tubes were observed in the area of micropyle after 3 h. However, crossing efficiency was low, amounting to 7.91% when cv. Dańkowskie Złote was the maternal plant and 2.05% when *S.c.ssp ancestrale* was the maternal plant (Tab. 6). Such results indicate that despite penetration of pollen tubes into the micropyles, fertilization does not occur or embryos die.

Table 2. Germination of pollen grain and penetration of pollen tubes into the ovary in the case of open-pollination in *Secale cereale* ssp. *cereale* L. *cv* Dańkowskie Złote. (Control)
Tab. 2. Kiełkowanie ziaren pyłku i wnikanie łagiewek pyłkowych do zalążni w przypadku obcozapylenia w obrębie odmiany żyta Secale cereale ssp. cereale L. cv. Dańkowskie Złote.(Kontrola)

♀ *Secale cereale ssp. cereale L.* x ♂*Secale cereale ssp. cereale L.*						
time from pollination	pollen grain abundance*	the number of pollen tubes				Comments
		Stigma	style	ovary	Micropyle	
10 min	4	4	0	0	0	onset of germination
20 min	.>5	4	0	0	0	intensive germination
30 min	>5	4	0	0	0	numerous pollen tubes on the stigma
1 h	>5	4	1	0	0	numerous pollen tubes on the stigma
1^{30}	>5	4	3	1	0	pollen tubes at ovaries
2 h	5	4	3	1	0	pollen tubes inside ovaries
2^{30}h	>5	5	3	2	1	Fertilization
3 h	>5	>5	3	2	1	Fertilization
3^{30} h	>5	>5	4	2	2	Fertilization
4 h	>5	>5	5	3	2	Fertilization

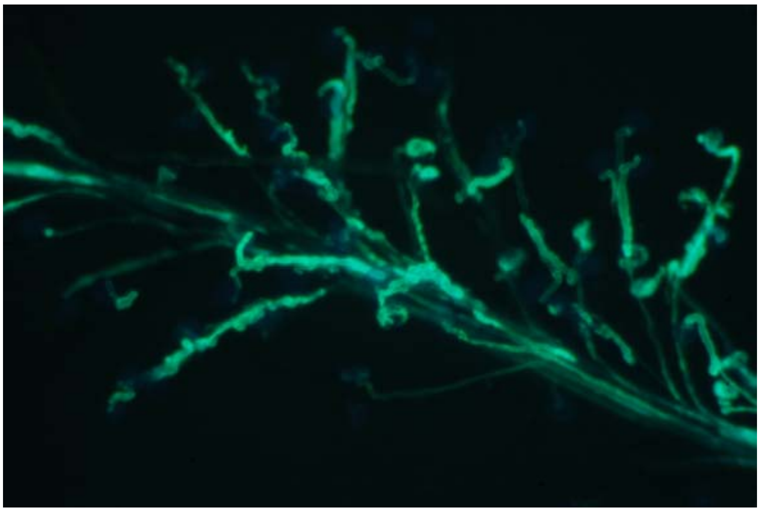
*a 6-point scale was applied (as described in the text)
**Zastosowano skalę 6-stopniową (opis w tekście)*

Source: own study / *Źródło*: opracowanie własne



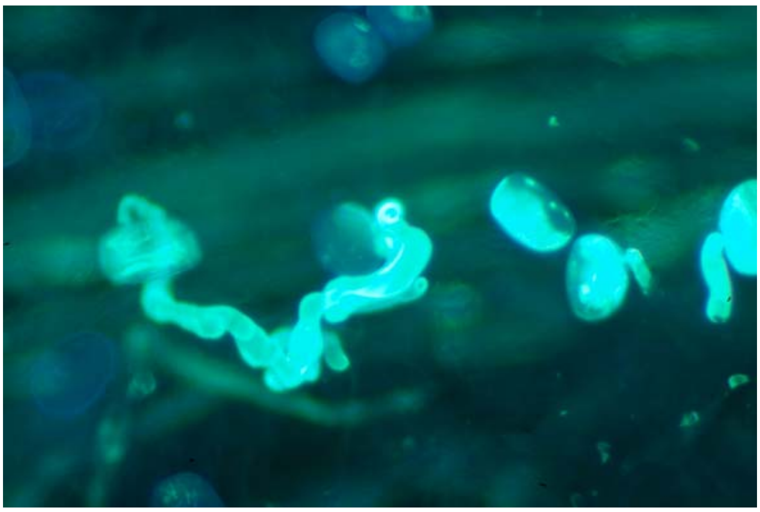
Source: own study / Źródło: opracowanie własne

Fig. 1. Pollen tube bundle on the stigma of *S. c. ssp. cereale* cv. Dańkowskie Złote 1.3 h after open-pollination (control)
Rys. 1. Wiązka łagiewek na znamieniu u S. c. ssp. cereale cv. Dańkowskie Złote po 1,3 h od obcozapylenia w obrębie odmiany (kontrola)



Source: own study / Źródło: opracowanie własne

Fig. 2. Abnormal growth of pollen tubes on the stigma 2 h after pollination of *S. c. ssp. afghanicum* with pollen of *S. c. ssp. cereale* cv. Dańkowskie Nowe
Rys. 2. Nieprawidłowy wzrost łagiewek na znamieniu po 2h od zapylenia S. c. ssp. afghanicum pyłkiem S. c. ssp. cereale odmiany Dańkowskie Nowe



Source: own study / Źródło: opracowanie własne

Fig. 3. Twisted pollen tubes and ungerminated pollen grains with manifested callose reaction 3 h after pollination *S.c. ssp. cereale cv.* Amilo with pollen of *S. c. ssp. afghanicum*
Rys. 3. Splątane łagiewki i nieskiełkowane ziarna pyłku z ujawniającą się reakcją kalozową po 3h od zapylenia S.c. ssp. cereale cv. Amilo pyłkiem S. c. ssp. afghanicum

Table 3. Germination of pollen grain and penetration of pollen tubes into the ovary in reciprocal pollination between *Secale cereale* ssp. *cereale* L. *cv.* Dańkowskie Złote and *Secale cereale ssp. afghanicum*

Tab. 3. Kiełkowanie ziaren pyłku i wnikanie łagiewek pyłkowych do zalążni w zapylaniu wzajemno-przemiennym między Secale cereale ssp. cereale L. cv. Dańkowskie Złote a Secale cereale ssp. afghanicum

♀ *Secale cereale ssp. cereale L. cv. Dańkowskie Złote* x ♂ *Secale cereale ssp. afghanicum*						
time from pollination	pollen grain abundance*	the number of pollen tubes				Comments
		Sigma	style	ovary	micropyle	
10 min	4	2	0	0	0	poor germination, short pollen tubes
20 min	5	4	0	0	0	non-uniform germination
30 min	5	4	0	0	0	callose plugs in pollen tubes
1 h	5	4	0	0	0	twisted pollen tubes with callose
1^{30}	5	3	0	0	0	twisted pollen tubes with callose
2 h	5	4	1	0	0	single pollen tubes at ovaries
2^{30}h	5	4	2	1	0	single pollen tubes at ovaries
3 h	4	4	3	1	1	pollen tubes at micropyle
3^{30} h	5	4	3	2	1	pollen tubes at micropyle
4 h	5	4	3	2	1	pollen tubes at micropyle
♀ *Secale cereale ssp. afghanicum* x ♂ *Secale cereale ssp. cereale L. cv. Dańkowskie Złote*						
10 min	5	4	0	0	0	no germination
20 min	5	4	0	0	0	no germination
30 min	5	4	0	0	0	no germination
1 h	5	4	2	0	0	non-uniform germination
1^{30}	5	4	3	0	0	non-uniform germination
2 h	5	4	3	1	0	twisted pollen tubes, callose plugs
2^{30}h	5	4	3	2	0	twisted pollen tubes, callose plugs
3 h	5	4	3	2	1	twisted pollen tubes, callose plugs
3^{30} h	5	4	3	3	1	twisted pollen tubes, callose plugs
4 h	5	4	3	3	1	fertilization

Source: own study / *Źródło*: opracowanie własne

Table 4. Germination of pollen grain and penetration of pollen tubes into the ovary in reciprocal pollination between *Secale cereale* ssp. *cereale* L. *cv.* Dańkowskie Nowe and *Secale cereale ssp. afghanicum*

Tab. 4. Kiełkowanie ziaren pyłku i wnikanie łagiewek pyłkowych do zalążni w zapylaniu wzajemno-przemiennym między Secale cereale ssp. cereale L. cv. Dańkowskie Nowe a Secale cereale ssp. Afghanicum

♀ *Secale cereale ssp. cereale L cv Dańkowskie Nowe* x ♂ *Secale cereale ssp. afghanicum*						
time from pollination	pollen grain abundance*	the number of pollen tubes				comments
		Stigma	style	ovary	micropyle	
10 min	3	0	0	0	0	no germination
20 min	3	0	0	0	0	no germination
30 min	3	0	0	0	0	no germination
1 h	3	2	0	0	0	non-uniform germination
1^{30}	4	2	0	0	0	short pollen tubes
2 h	4	3	0	0	0	short pollen tubes, callose appears
2^{30}h	4	3	0	0	0	short pollen tubes with callose
3 h	4	3	1	0	0	single pollen tubes at ovaries
3^{30} h	4	3	2	1	0	single pollen tubes inside ovaries
4 h	4	3	2	1	1	pollen tubes at micropyle
♀ *Secale cereale ssp. afghanicum* x ♂ *Secale cereale ssp. cereale L. cv Dańkowskie Nowe*						
10 min	3	0	0	0	0	no germination
20 min	3	0	0	0	0	no germination
30 min	3	0	0	0	0	no germination
1 h	3	2	0	0	0	non-uniform germination
1^{30}	4	2	0	0	0	non-uniform germination
2 h	4	3	0	0	0	twisted pollen tubes, callose plugs
2^{30}h	4	3	0	0	0	twisted pollen tubes, callose plugs
3 h	4	3	2	0	0	single pollen tubes at ovaries, callose
3^{30} h	4	3	2	1	0	single pollen tubes inside ovaries
4 h	4	3	2	1	1	pollen tubes at micropyle

Source: own study / *Źródło*: opracowanie własne

Table 5. Germination of pollen grain and penetration of pollen tubes into the ovary in reciprocal pollination between *Secale cereale* ssp. *cereale* L. *cv.* Amilo and *Secale cereale ssp. afghanicum*
Tab. 5. Kiełkowanie ziaren pyłku i wnikanie łagiewek pyłkowych do zalążni w zapylaniu wzajemno-przemiennym między Secale cereale ssp. cereale L. cv. Amilo a Secale cereale ssp. Afghanicum

♀ *Secale cereale ssp. cereale L cv. Amilo* x ♂ *Secale cereale ssp. afghanicum*						
time from pollination	pollen grain abundance*	the number of pollen tubes				comments
		stigma	base of stigma	ovary	micropyle	
10 min	3	0	0	0	0	no germination
20 min	3	0	0	0	0	no germination
30 min	3	0	0	0	0	no germination
1 h	3	2	0	0	0	non-uniform germination
1^{30}	4	2	0	0	0	short pollen tubes
2 h	4	3	0	0	0	short pollen tubes, callose appears
2^{30}h	4	3	0	0	0	short pollen tubes with callose
3 h	4	3	1	0	0	single pollen tubes at ovaries
3^{30} h	4	3	2	1	0	single pollen tubes inside ovaries
4 h	4	3	2	1	1	pollen tubes at micropyle
♀ *Secale cereale ssp. afghanicum* x ♂ *Secale cereale ssp. cereale L. cv Amilo*						
10 min	3	0	0	0	0	no germination
20 min	3	0	0	0	0	no germination
30 min	3	0	0	0	0	no germination
1 h	3	2	0	0	0	non-uniform germination
1^{30}	4	2	0	0	0	non-uniform germination
2 h	4	3	0	0	0	twisted pollen tubes, callose plugs
2^{30}h	4	3	0	0	0	twisted pollen tubes, callose plugs
3 h	4	3	2	0	0	single pollen tubes at ovaries, callose
3^{30} h	4	3	2	1	0	single pollen tubes inside ovaries
4 h	4	3	2	1	1	pollen tubes at micropyle

Source: own study / *Źródło*: opracowanie własne

Table 6. Crossing efficiency of three rye cultivars with the wild subspecies Secale cereale L. ssp. afghanicum
Tab. 6. Efektywność krzyżowania trzech odmian uprawnych żyta z podgatunkiem dzikim Secale cereale L. ssp. Afghanicum

Cross combination		Number of		Efficiency of crossing [%]
maternal plant	paternal plant	pollinated flowers	grains	
S. c. ssp. cereale L. cv. Dańkowskie Złote	*S. c. ssp. afghanicum*	240	19	7.91
S. c. ssp. afghanicum	*S. c. ssp. cereale L. cv.* Dańkowskie Złote	293	6	2.05
S. c. ssp. cereale L. cv. Dańkowskie Nowe	*S. c. ssp. afghanicum*	265	4	1.51
S. c. ssp. afghanicum	*S. c. ssp. cereale L. cv.* Dańkowskie Nowe	280	1	0.36
S. c. ssp. cereale L. cv. Amilo	*S. c. ssp. afghanicum*	251	7	2.79
S. c. ssp. afghanicum	*S. c. ssp. cereale L. cv.* Amilo	277	4	1.44

Source: own study / *Źródło*: opracowanie własne

Pollination processes in combinations with cv. Dańkowskie Nowe (Tab. 4) and Amilo (Tab. 5) were similar and the results were marked inferior to those with cv. Dańkowskie Złote. The onset of pollen grain germination was markedly delayed, as it started as late as 1 h after pollination in both directions of crossing and germination was not uniform and extended up to 4 h. Many pollen grains did not germinate and callose reaction developed (Fig. 2). The style was reached by very few pollen tubes and it was only after 2^{30} h and after 4 h single pollen grains were observed at the micropyle. A vast majority of pollen tubes were too short, frequently strongly twisted or pollen grains had marked symptoms of the callose reaction (Fig. 3). Ruptured or branched pollen tubes were also observed. Crossing efficiency was very poor, for the combination with cv. Dańkowskie Nowe it was 1.51% and 0.36%, while for that with cv. Amilo it was 2.79% and 1.44%, respectively (Tab. 6).

4. Discussion

Common rye *Secale cereale ssp cereale* and *Secale cereale ssp. afghanicum* are two subspecies of *Secale cereale* – an allogamous, wind-pollinated species. The dry stigma is adapted in its structure to capture from the air and retain large amounts of pollen grains [26, 27]. Similarly as in most grasses, the stigma is composed of a two-branched feathery stigma, a very short style and a unilocular ovary.

The feathery stigma is composed of two primary branches (stylodia), with secondary papillate branches (papilles). Mature pollen grains are composed of three cells, a large vegetative cell surrounding two small sperm cells. The grain is surrounded by the sporoderm wall composed of the outer exine and the inner intine with one pore [27].

In the presented study we used three rye cultivars produced at the same breeding station Danko Hodowla Plants Sp. z o.o. Observations showed differences between the cultivars in the pollen-pistil interaction at crossing with the wild subspecies *Secale cereale ssp. afghanicum*. Winiarczyk et al. [27] conducted studies of several self-compatible and self-incompatible rye lines coming from the same station, including cv. Amilo and Kier. Those authors found no differences between the tested lines in the process of pollen tube germination in the self-pollination in cv. Amilo and Kier, which were similar and typical to plants with genotypic self-incompatibility. The rejection reaction was manifested and only very few pollen grains germinated on stigmas and the callose reaction was observed. However, studies of sporoderm ultrastructure in mature pollen grains showed that the exine thickness in the investigated lines and cultivars varied and ranged from 193 to 363 nm, while it was thickest in cv. Amilo. Sporoderm in rye is characteristic to *Poaceae*, it is composed of an outer thin exine with radially arranged channels and the inner intine. Exine plays a key role at the moment when pollen grains fall onto the stigma. It is thanks to exine that the process of vegetative cell hydration is initiated, constituting the beginning of the reaction of identification and further expansion. Studies by Lord et al. [30, 31] showed that attachment of pollen grain to the stigma is caused by intercellular adhesion. This process is controlled genetically and it is dependent on the structure of cell walls in both partners, particularly the composition of lipids and glycoproteids in the exine [3]. Winiarczyk et al. [27] applied two-directional electrophoresis to compare the composition of peptides and surface proteins detected in the pollen wall and in pollen protoplasts. Also here variation was found between the tested lines in terms of the composition of proteins and peptides of various molecular masses (kDa). Forms investigated by the authors of this study differed in the structure of pollen grain in terms of size [Tab.1], thus it may be assumed that the molecular structure was also different, which may have influenced the pollen-pistil interaction.

In wind-pollinated plants in order to increase their chance for fertilization, huge amounts of pollen grains are produced and stigmas have a structure promoting capture and retention of pollen grain. In common rye after cross-pollination numerous pollen grains are deposited on stigmas and pollen grains are retained for many hours after pollination [27]. Also in the presented control experiment abundant pollen grain cover was maintained up to 4 h, while after distant pollination the number of pollen grains remaining on the stigma was below 50 and decreased with time after pollination. This is a similar reaction to that observed by Winiarczyk et al. [27] in the case of self-pollination of self-incompatible cv. Amilo and Kier. The loss of grains on the stigma is an effect of the rejection reaction of incompatible grains. Ikeda [32] when conducting studies on Brassicaceae stated that the rejection reaction of self-incompatible pollen grains consists in the prevention of their hydration. In rye, tubes emerge strictly at the apertures, by dissolving apertural intine layers, rupturing the thin sporopollenin wall, and displacing the opercula that guard these sites. The reaction of pollen grain recognition and hydration involves lipids found in the exine [29, 33]. Many studies discussed variation in the lipid-protein composition of pollen grains, resulting from genetic polymorphism, for example [34-36]. Bolibok-Brągoszewska et al. [36] using DArT markers showed similarity of cv. Dańkowskie Złote and Dańkowskie Nowe to amount to 98%. Similarity between other investigated rye cultivars varied. It may be assumed that the variability in the structure and protein-lipid composition of the exine, while slight, results in the varied pollen-pistil interaction, which was observed in this study.

The further process of pollen tube elongation is also affected by adhesion and it involves pectins and proteins [32]. Adhesion of the pollen tube to these specialized extracellular matrices might be a mechanism of guidance and tube cell movement in the style. In lily investigated by Lord [30], the stylar adhesion molecules are a pectin and a small, basic cysteine-rich protein, both of which are necessary to induce tube cell adhesion to an artificial, in vitro style matrix. A key role is also found for Ca^{+2} ions in the reception of signals sent from the sporophyte [37- 40] and targeting of pollen tubes to the embryo sac. In the case of an incompatible gametophyte the callose reaction appears, callose plugs are formed at the apex of pollen tube and inhibit its growth [41]. In the compatible gametophyte we observed an active participation of the sporophyte and synergids in the process of pollen tube targeting. As a result of a lack of targeting signals, pollen tubes penetrate in the wrong direction and most frequently twist around pollen grains or fuse with one another [Fig.3]. Such a disturbed process observed in the presented study was not surprising, as crossing was performed between two subspecies and incompatibility reactions are typical in such pollinations. However, differences were recorded between rye cultivars in processes taking place on the stigma in the rate of pollen grain germination and penetration into the embryo sac as well as the number of pollen tubes.

According to Khush [6], all wild subspecies of *Secale cereale* easily cross with common rye, with the hybrids being viable and fertile. The close affinity of *Sc. Ssp cereale* and *S.c.ssp afghanic*um is confirmed by molecular studies [42-45]. In the 20th century many authors produced inter-species crosses in the genus *Secale*. Singh [8] reported that crossing efficiency between *Secale cereale* (German cultivars) and wild *S. vavilivi*, *S. africanum*, *S. montanum* and *S. silvestre* varied greatly, ranging from 0 to 27.6%. The authors of presented studies produced crosses within one species - *Secale cereale* L., although crossing efficiency was very poor and additionally varied between cultivars. Dańkowskie Złote proved to be the best cultivar for crossing with *Secale cereale ssp. afghanicum* [Tab.6]. The process of fertilization diverged the least from the control and crossing efficiency was the highest. Presented results are also consistent with observations reported by other researchers, indicating that crossing between *Secale cereale* ssp. *cereale* and wild forms is more effective when common rye is the maternal form [10, 19, 21].

The various cited publications show that within the gene pool of common rye variation is found in the composition of the exine, which obviously influences the pollen-pistil interaction. Based on the presented observations it may be stated that even closely related cultivars exhibit different predispositions for overcoming incompatibility barriers manifested on stigmas of another subspecies. Diversification between rye cultivars in the course of pollen grain germination and penetration of pollen tubes into ovaries influences fertilization efficiency and thus the outcome of crossing between *Secale cereale ssp cereale* and *Secale cereale ssp. afghanicum.* Thus it is of importance what cultivar is selected for distant crossing.

5. References

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