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CROSSABILITY EFFECTS OF SPRING WHEAT (*TRITICUM DURUM* DESF.) WITH RYE (*SECALE CEREALE* L.) GENOTYPES

ABSTRACT

Crossability of tetraploid durum wheat in crosses with rye was investigated in order to identify the best cross-combinations for primary triticale production. Altogether, 365 crosses were made, 65 700 florets were pollinated, 16.4% seed set was obtained, 5 902 embryos were cultured, 1.1% plant set, 12.7% plant viability and 60.8% doubling rate and 16.2 kernels per plant 2n were obtained. 21 cross – combinations with the best crossability were identified. They showed 27.0% of seed set, 56.0% of seeds with embryos, 14.5% of plant viability, 2.2% of plant set., 70.2% of doubling rate, 1.6% of plant fertile set and 50.1 kernels per plant 2n. The results demonstrated the distinct differences in the crossability between the several cross-combinations. Also, the same was observed in a group of 21 cross-combinations identified as having the best crossability.

Key words: primary hexaploids, rye, Secale cereale L. Triticum durum Desf., wheat, wheat – rye crossability

INTRODUCTION

Wheat – rye crosses expand the genetic base of triticale (*X Triticosecale* Witt.). The octoploid primary triticales derived from the crossing hexaploid wheat *Triticum aestivum* L. and rye *Secale cereale* L. are used very often for introgressing the D – wheat genome genes into triticale (Lukaszewski 1986, Wolski 1990, Maćkowiak *et al.* 1993, Kluska and Pilch 1994, Pilch 2001). Also these triticales contribute to the improvement of bread wheat (Zeven 1987).

Creation of new amphiploids by crossing of tetraploid wheat *Triticum durum* Desf. with diploid rye *Secale cereale* L. is one of the ways of introducing new germplasm to the breeding of both spring and winter triticale varieties. So far the breeding of spring triticale was mostly concentrated in the centers in which spring programs dominated, as in CIMMYT (International Maize and Wheat Improvement Center) in Mexico. But recently breeding of varieties of spring type of triticale was

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extended to European centers. Also, the significance of this type of triticale increased in our climatic conditions since the first spring variety Jago was registered in 1987 (Behnke *et al.* 2000). Up to now, the next 6 varieties (Maja, Gabo, Migo, Wanad, Kargo and Mieszko) were developed and introduced to the field cultivation. At the same time, the area of spring triticale in Poland increased and reached 2429 ha in 1999 (Anonymous, 2000).

The increasing of diversity of triticale to the level existing among wheat and rye cultivars has been limited by difficulties in synthesizing new primary hybrids. For a fertile hexaploid hybrid to be obtained, successful pollinations should be followed by (1) culture of embryos on synthetic media and (2) subsequent doubling of the chromosome number of the sterile amphiploid F_1 plants. Some specific parental combinations produce seed only after persistent repetition of the various steps from pollination to seed production.

The objective of this study was to evaluate the crossability effects of tetraploid durum wheat in crosses with rye and identify the best cross-combinations for primary triticale production.

MATERIAL AND METHODS

The experimental material consisted of 365 varieties and lines of spring tetraploid wheat *T. durum* Desf. and 26 of spring diploid rye *S. cereale* L. collected in the wheat programme of the International Maize and Wheat Improvement Center (CIMMYT) – Mexico. The crosses between them were made in the field conditions, the emasculated spikes were bagged to avoid pollination with other plants. After 3 days, the stigmas of emasculated florets were pollinated with fresh rye pollen, then bagged again. Altogether, 365 cross – combinations were made, 5 wheat spikes of each combination were emasculated.

For embryo – culture, the TAIRA and MS mediums were used according to Taira and Larter (1978) and Murashige and Skoog (1962). The 12 – 21 day embryos were extracted and transplanted on the sterile TAIRA medium and grew in the darkness at temperature from 0 to 5°C during 21 days. Then, the germinated embryos were transferred into the MS medium and developed until 1 Feekes" s stage (Fig.1). At this stage the seedlings were potted in small pots with the controled soil pH 7.0 in the grow–chamber for 3 weeks at the temperature 5°C and day/nights 12:12 hours. At the same time the leaves were cut off. In the greenhouse the seedlings grew at 15°C night and 25°C day with the day/night 10:14 hours with high humidity (Fig. 2). At the 4 Feekes' s stages the seedlings were treated with colchicine using the root – technique according to Kluska and Pilch (1995). The F₁ plants grew and were harvested in the greenhouse (Fig. 3, 4), but their F₂ generation was grown in the field.

The crossability was judged by: seed set (number of F_1 seeds per florets pollinated), seeds with embryo (F_1 seeds per seeds in embryos cultured),

plant viability (growing F_1 amphihaploid plants per embryos), plant set (amphihaploid plants per florets pollinated), doubling rate % (amphidiploids per amphihaploids), plant fertile set (doubled plants per florets pollinated) and number of kernels per plant 2n.

The percentages were transformed to arcsine I/x (sine -1 I/x) according to Bartlett (1947) and these values were subjected to analysis of variance (Little and Hills, 1975) and Tukey" s HSD test (Tukey, 1949).

The F_1 -hybrids (2n=21) and the hexaploids (2n=42) were identified with the chromosome metaphase plates according to Kluska and Pilch (1995).

RESULTS AND DISCUSSION

In the triticale breeding programme, the progenitors of primary triticales are often chosen according to their agronomic qualities. The crossability of the parents varies and is often unpredictable. The crossability could be improved prior to making the interspecific cross if lines with high crossability could be identified.

Table 1 Crossability effects of 365 crosses of tetraploid wheat *Triticum durum* Desf. with diploid rye *Secale cereale* L.

No	Crossability effects	Total	21 best crosses
1	No. of florets pollinated	65700	3780
2	Seed set (%)	16.4	27.0
3	Seeds with embryo (%)	54.8	56.0
4	Plant viability (%)	12.7	14.5
5	Plant set (%)	1.1	2.2
6	Doubling rate (%)	60.8	70.2
7	Plant fertileset (%)	0.3	1.6
8	No. of kernels per $2n$ plant	16.2	50.1

In this study, 365 cross-combinations of various lines and varieties of durum wheat with rye genotypes were made. The summary analysis was presented in Table 1. As can be seen, the durum wheats had higher seed set (16.4%) than hexaploid wheats presented by Kluska and Pilch (1995) – 5.3%, Pilch (2001) – 5.6% and by Sirkka *et al.* (1993) – 4.5%. Also, they exhibited higher seed set in comparison to those presented by Oettler (1984) and Sirkka *et al.* (1993). According to the personal information in Germany two durum lines D1 and D4 with high seed set were selected – their average percentage was 23.5% and a high percentage of plant survival (Oettler 1982). The durum wheat – rye crosses usually show both endosperm failure and poor embryo differentiation leading to low embryo viability. Our data corroborated these results. In 365 crosses presented, the percentages of seeds with embryos were similar to those

provided by Sirkka *et al.* (1993) but showed higher plant viability (12.7%) and plant set (1.1%) were observed. The distinct differences were found in the chromosome doubling rate which was much higher than those obtained by Oettler (1982) and Sirkka *et al.* (1993). Probably these differences resulted from different colchicine techniques used and may be to due to sensitivity of amphihaploids to colchicine treatment which results in high mortality found by Kaltsikes (1974) and Oettler (1982). As a consequence was the formation of F_2 kernels in the spikes of fertile plants. In our experiment, the average kernels per 2n plant number of approximated 16.2. Oettler (1982) recorded only 6.5 kernels per 2n plant in the crosses of durum wheat D1 and D4 showing high crossability with rye.

The 365 cross - combinations showed wide variability of the crossability. The maximum values for seed set was 60.0%; seeds with embryo – 87.5%; plant viability – 36.4%; plant set – 5.0%; doubling rate -100.0%; plant fertile set -8.3% and for the number of kernels per 2n plant – 239 kernels. These results indicate that both Kr – alleles not only influence the crossability determined by seed set in lines of hexaploid wheat with rye but also with other species (Falk and Kasha 1981, 1983, Sitch et al. 1985, Sitch and Snape 1986). Thus publication of the crossability percentages of hexaploid wheat with rye is not only helpful for those who are looking for hexaploid wheats with high level of crossability with rye, but also for those who cross wheat with other species. The crossability of hexaploid wheat with other species is controlled by the dominant or recessive Kr – alleles representing all genomes and are located to the chromosomes: 5B - Kr1, 5A - Kr2, 5D - Kr3 and 1A - Kr2Kr4 (Riley and Chapman 1967, Krolow 1970, Fedak and Jui 1982, Luo *et al.* 1992). Therefore, the crossability of durum wheat is limited to the Kr – alleles of the genomes A and B and regulated only by three al– leles: Kr1, Kr2 and Kr4. For hexaploid wheat Lein (1943) suggested the occurence of four crossability classes basing on the percentages of seed set: 1% - 10% Kr1 Kr1 Kr2 Kr2, 10% - 30% Kr1 Kr1 kr2 kr2, 30% - 50% *kr1 kr1 Kr2 Kr2* and > 50% *kr1 kr1 kr2 kr2*. Falk and Kasha (1981) pro– posed lower limits for these classes. Since this classification has been based upon the expression of two alleles Kr1 and Kr2 of the A, B genomes, consequently it is adequate for durum wheat, as well. Basing on this proposal, the durum varieties and the lines used in our study can be classified to the following classes: 94.7% in the class of the dominant genotype Kr1 Kr1 Kr2 Kr2; 3.0% in the class of the genotype Kr1 Kr1 kr2 kr2; 1.6% – in the class of the genotype kr1 kr1 Kr2 Kr2; and 0.5% represented the class of the highest crossability – the recessive genotype kr1kr1 kr2 kr2.

The crossability could be also modified by gene or genes of the rye genome. Tanner and Falk (1981) recognized one gene in rye controlling the crossability with wheat, its dominant character being responsible for

P	erfor	mances of 21 durum – rye combinations having the best crossability among 36: (<i>Triticum durum</i> Desf.) x diploid rye (<i>Secale cereale</i>	crosses L.)	of tet	raploi	id du	urum w	Ta heat	ble 2
Entry no.	Cross no.	Cross-combination	2^1	3^1	41	5^1	6^1	71	81
1.	265	(Mexicali 75 "s" - Flamingo "s") × Turkey rye	60.0	37.5 1	2.5 2	2.7	40.0 1		75.0
2.	271	(Mexicali 75 "s" – Flamingo "s") × Australian rye	50.1	50.0	2.2 0	.5]	100.0 0	.51	52.0
ŝ	14	(Cambridge 03 – Grulla "s") × Snoopy rye	40.5	36.9 1	4.8 2	2.2 1	0.00	5	14.0
4.	32	[/ (Leeds Mut.) × Petrel "s" / × (Coot "s"– Mexicali 75 "s")] × Snoopy rye	36.1	66.1 2	0.9 5	2.0	22.2 1	Ę	13.0
5.	62	[/ (Gerardo VZ466 – Crane "s") x Hercules/ × Pinguino"s"] × Athens Abruzzi rye	33.3	$55.0 \ 1$	8.2 3	3.3	33.3 1	L.	25.5
6.	149	(/ Anhinga''s'' × Gaviota''s'' / × Flamingo''s'') x Australian rye	33.3	73.0 3	6.4 8	8.8	33.7 8		36.7
7.	271	(Mexicali 75 "s" \times Flamingo "s") \times Everest rye	33.3	48.3 (3.9 1	1.1	100.0 1	L.	23.5
ò	193	[/ (Moa – Merganser) × Mexicali 75 "s" / x Gediz "s"] × Athens Abruzzi rye	30.5	41.8 1	3.0 1	1.6	100.0 1	9	26.6
9.	173	[(Jori C69 – Anhinga "s") × / (Maghrebi 72 "s" – Ganso "s") × Anhinga "s" / Rabicorno "s") × Anhinga "s"] × Mikulickie Wczesne rye	30.0	61.1 1	2.1 2	2.2	75.0 1	9	16.3
10.	203	Goose "s" – Bilicer rye	30.0	24.1 '	7.7 0	.5]	100.0 0	.52	39.0
11.	96	(Grulla "s" – Boyeros) × Mc Nair rye	27.7	52.0 1	1.5 1	1.6	100.0 1	. 9.	0.07
12.	194	/ (Flamingo "s" – Booby "s") × Huevos de Oro / × Forex rye	26.6	70.8	3.8 1	1.6	100.0 1	9	15.0
13.	27	(Flamingo – Grulla "s") × Snoopy rye	23.8	69.7 1	3.3	2.2	50.0 1	L.	21.5
14.	278	Kingfisher "s" – Turkey rye	23.3	59.5 1	6.0 2	2.2	75.0 1	9.	19.3
15.	195	[[/ (Gaviota "s" – Anhinga"s") × Mexicali 75 "s" / ×[/ (Maghrebi 72 "s"– Ganso"s") × Anhinga"s" / × Rabicorno"s"] × Anhinga"s"] × Cinquesento rye	22.2	87.5 1	4.3 2	2.7	60.0 1	9	14.0
¹ Desig1	natior	is of crossability effects as in the first column of Table 1							

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Entry no.	Cross no.	Cross – combination	2^1	3^1	4^1	5^1	6^1	7^1	81
16.	139	[/Kingfisher "s" × (Langdon 372 – Yuma/ Coot"s")/ x/ Mario"s" × (Coot" Mexicali 75"s")/] × Bilecir rye	- 17.2	67.7	19.1	2.2	25.0	0.51	42.0
17.	300	(Balcarce – Memo "s") × Techermaks Veredetter Marchfeld rye	16.6	86.6	11.5	1.6	100.0	1.6	28.3
18.	250	Patric – Harlam IR 4096 rye	11.1	80.0	18.7	1.6	66.0	1.1	16.5
19.	140	[/ (S.15 – Grulla "s") × Pelicano "s" / ×(Mexicali 75 "s" – Ruff "s")] × Tagutlo rye	10.5	73.6	14.3	1.1	100.0	1.1	11.5
20.	76	Cocorit 71 – Sarden rye	8.3	66.6	10.0	0.5	100.0	0.5	33.0
21.	178	/ (Rabicorno "s" – Intrat 69) × (Gaviota "s" Flamingo "s") / × Weser rye \cdot	8.3	73.3	9.1	0.5	100.0	0.5	29.0
		Mean	27.3	65.0	14.5	2.2	70.2	1.6	50.1
		S. deviatio	8.8	10.7	3.3	3.9	2.9	3.5	58.5
		Variance	77.8	115.9	11.2	15.3	8.6	122	3429.0
		CV	28.0	39.1	83.5	52.6	104.3	59.0	116.8
		TSD	8.4	7.9	3.4	0.3	6.1	0.3	5.1

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Table 2

Continued

the high degree of crossability. According to this analyses this gene suppresses the dominant Kr – alleles in wheat.

Apart of this, crossability could be modified by the gene Ph1 (homoeologous pairing) in wheat located on the chromosome 5BL (Riley and Chapman 1958, Segal *et al.* 1997) and its recessive allel *ph1c* which was identified in tetraploid wheat (Dhaliwal 1977, Giorgi, 1978). These Ph – alleles controlling chromosome pairing at meiosis could affect embryo formation, and viability of embryos and amphihaploids.

Table 2 listed 21 cross combinations which showed the highest effects of crossability, including those with seed set exceeded 8%. Seed set, seeds with embryo, plant viability, plant set, doubling rate, fertile plant set and number of kernels per 2n plant averaged for all crosses made with these varieties and lines, illustrated overall performance. Nineteen of them were likely to have Kr1, Kr2 alleles in the recessive form and represented the crossability classes 2 - 4 in the Lein's classification (Lein, 1943). Also, 21 best cross-combinations varied considerably and were grouped in different classes. But these groups did not coincid with the Kr – alleles classes which may indicate that the effect of Kr – alleles on crossability is not exclusive. It might involve the environmental influence, as well. Inagaki (1986) reported a positive correlation between seed set and temperature one day after pollination in the crosses of hexaploid wheat with rye. The minimum temperature had no effect on seed set. With durum wheat, the temperature after pollination was not correlated with seed set. It seemed that the environmental effects on fertilization and embryo development may be different for tetraploid wheat x rye and hexaploid wheat \times rye crosses. Seed set ranged from 8.3% to 60.0%. The average 27.0% was much higher than the average for all crosses. The highest seed seting was noticed in the two cross-combinations (entries 1, 2) in which the line Mexicali 75 "s" or Flamingo "s" was crossed with rye. The varieties Mexicali 75 "s" and Cocorit 71 were very well appraised in the breeding program at CIMMYT because of their good crossability with rye and were used frequently in the intergeneric crosses (Sirkka et. al. 1993). Regarding seed set, the best cross – combinations could be grouped into six groups. The best group with the highest seed set was represented by one cross - combination, only (entry 1). Also, the second group was represented by one cross - combination, (entry 2). The next one with a lower percentage of seed set included two cross-combination, entries 3 and 4 (40.5% and 36.1%, respectively). The next groups with seed set between 33.3% and 26.6% was represented by eight cross-combinations (entries 5 (-12); 24.8% (-17.1)% (-1000) four cross (-1000) combinations (entries (13-16)); and the last group with the lowest seed set included six cross-combinations (entries 17 - 21).

Distribution of the 21 best cross-combinations based on their crossability in terms of seeds with embryo, plant viability, plant set, doubling rate, plant fertile set and the number of kernels per 2n plant did not coincide with classification according to seed set. The effect of the

crossability genes on embryo and endosperm development is still not known (Lange and Wojciechowska, 1976). The best cross – combinations exhibited the number of seeds with embryo within the range 24.1% – 87.5%. Their average was higher (56.0%) than in all cross–combinations. Accordingly groups of combinations could be distinguished. The first one which had the highest level of seeds with embryo included three cross – combinations (entries 15, 17 and 18). The next groups with lower values comprised five cross–combinations (entries 19, 21, 6, 12 and 13); five cross–combinations (entries 16, 20, 4, 9 and 14); four cross–combinations (entries 5, 11, 2, 7) and three cross–combinations (entries 8, 1, 3). The last group showing the lowest seeds with embryo was represented only by one cross–combination (entry 10).

In terms of plant viability, variation of twenty-one best cross-combinations ranged from 36.4% to 2.2%. The average was much higher (14.5%) as compared to the total cross-combinations made. Six different groups could be distinguished, but the highest value showed only one cross-combination (entry 6). The next groups contained four cross- combinations (entries 4, 16, 18 and 5), six cross-combinations (entries 14, 3, 15, 19, 13 and 8); four cross-combinations (entries 1, 9, 11 and 17) and five cross-combinations (entries 20, 21, 12, 10 and 7). The lowest plant viability exhibited only one cross-combination (entry 2).

Plant set of 21 cross – combinations with the highest crossability ranged from 8.8% to 0.5%. The average was twice as high (2.2%) as the average of all 365 cross – combinations made. The variation was high and eight distinct groups could be distinguished. The highest (8.8%) plant set presented one cross – combination (entry 6), only. The second group comprised one cross – combination, with 5.0 % plant setting (entry 4), the group showing 3.3% plant set was represented by one cross – combination (entry 5), and that with 2.7% of plant set by two cross – combinations (entries 1, 15). Five cross-combinations (entries 3, 9, 13, 14, and 16) formed a separate group with 2.2% of plant set. Five other cross-combinations (entries 8, 11, 12, 17, 18) showed the same 1.6% of plant setting. Two other cross-combinations (entries 7, 19) with 1.1% plant settingwere included in the next group and the group having the lowest plant set (0.5%) included four cross-combinations (entries 2, 10, 20, and 21).

The average of doubling rate (70.2%) of 21 best cross – combinations was much higher than that of all cross – combinations. The doubling rate ranged from 100% to 22.2% and might be separated into seven distinct groups including different number of the cross–combinations. The high–est doubling rate (100%) exhibited 11 cross–combinations (entries 2, 3, 7, 8, 10 – 12, 17, 19 – 21). The next group with 75% doubling rate was represented by two cross – combinations (entries 9, 14). The remaining groups included: two cross – combinations, entries 18 and 15; one cross–combination, entry 13; one cross – combination, entry 1; two cross – combinations, entries 6 and 5; and the lowest doubling rate showed two cross –combinations, entries 16 and 4.

The of plant fertile set of 21 best cross-combinations was very similar to that of plant setting, but ranking of several cross-combinations was different. It ranged from 8.3% to 0.5%. The average (1.6%) exceeded many times the average of all cross-combinations. The highest value (8.3%) was exhibited by entry 8, the same which had the highest plant set. The next group with much lower value (2.2%) was represented only by one cross-combination (entry 3). Plant fertility of seven cross-combinations (entries 8, 9, 11, 12, 14, 15 and 17) approximated 1.6%. The next group with 1.1% of fertile plant included seven cross-combinations (entries 1, 4, 5, 7, 13, 18 and 19). The lowest plant fertility (0.5%) was noted by five cross-combinations (entries 2, 10, 16, 20 and 21).

The results included also the number of kernels per 2n plant representing the F₂ generation obtained from the plants doubled following colchicine treatment. The average for 21 best cross-combinations approximated 50.1 kernels per 2n plant and was much higher than in 365 cross-combinations (16.2%). The variation between the cross-combinations was large and ranged from 239 kernels (entry 10) to 11.5 kernels (entry 19). The highest number of kernels per 2n plant had one cross-combination (entry 10). The next groups with lower values were represented also by single cross-combinations: 152 kernels (entry 2) and 142 kernels (entry 16). The remaining groups included: two cross-combinations (entries 1, 11); one cross-combination (entry 14); two cross-combinations (entries 6, 20); two cross-combinations (entries 17, 21); four cross - combinations (entries 5, 7, 8, 13); and the last group represented by seven cross-combinations (entries 18, 9, 12, 3, 15, 4 and 19).

Twenty one cross-combinations were found to have the highest crossability out of 365 crosses included 33 durum lines and varieties in their pedigrees. They were frequently used in both durum and triticale programs at CIMMYT. Among them, two varieties Mexicali 75 "s" and Flamingo "s" participated most frequently in 21 the best cross-combinations: Mexicali 75 "s" – in 8 (entries 1, 2, 4, 7, 8, 15, 16 and 19) and Flamingo "s" – in 7 cross – combinations (entries 1, 2, 6, 7, 12, 13 and 21). Eight varieties and lines were identified in 2 – 4 cross-combinations: Grulla "s" – 4 (entries 3, 11, 13, 19), Anhinga "s" – 3 (entries 6, 9, 15), Cocorit 71 – 3 (entries 9, 15, 21), Maghrebi 72 "s" – 2 (entries 9, 15), Ganso "s" – 2 (entries 9, 15) and Kingfisher "s" – 2 (entries 14, 16). The remaining 23 varieties and lines participated only once in the cross-combinations.

CONCLUSIONS

1. Out of 365 intergeneric crosses in combinations between *T. durum* Desf. and diploid rye *S. cereale* L. a group of 21 cross–combinations with high crossability has been found. They can be successfuly used

in the triticale breeding programs for production of primary hexaploid forms.

- 2. Among the durum wheat components, two lines Mexicali 75 "s" and Flamingo "s" seemed to assure the highest crossability with rye.
- 3. Breeding for improved crossability in wheat using medium crossable lines might not be easy due to dominance of low crossability. Selection of wheat genotypes and the obtained cross-combinations with high crossability with rye provides successful production of primary triticale.

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