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A winter rye apomict.

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Zea mays L. ssp. *mays* and *Tripsacum dactyloides* L. hybrids are known among the cultivated cereals for regular apomixis (Sokolov et al. 2007). Hybrid lines of *Z. mays* with nine additional *Tr. dactyloides* chromosomes reproduce by apomixis. Nine is the minimum number of *Tr. dactyloides* chromosomes required for apomixis in the phenotype. The apomict was patented in the U.S. No other examples of apomictic reproduction among the cultivated cereals have been described in the literature. A high seed-set rate of plump seeds was discovered during experiments on the fertilization of an R-2 diploid spring rye with pollen of a A-1 soft spring wheat (Kyzlasov 2008). All offspring were found to be diploid (2n = 14) rye plants. The 2n = 42 pollen of soft wheat induced apomixis in rye. Becasuse of this fact, diploid rye can be reproduced by fertilization with soft wheat pollen. Polyhaploid offspring with partially fertile pollen arose in the reciprocal combination (wheat/rye, 2n = 28).

After long-term research, we have succeeded in creating an apomict of winter rye. The parent plant, R-1 rye, was found in a soft winter wheat crop as a species admixture (Kyzlasov 2005). Flowers were fertilized by the pollen of Nemchinovskaya 24 soft winter wheat. Seed set rate was ~20% of the number of fertilized flowers in the fertilization year. The formed caryopses were all plump and had a high germination rate. All offspring in the first generation contained fertile pollen, and they were completely identical with the diploid maternal plant. There were no hybrid plants. After refertilizing the maternal plants with soft wheat pollen, the offspring also was identical to the maternal plant. The results of the previous experiment have been repeated. The apomixis induced by soft wheat pollen in R-1 rye was manifested in both first and second generations. The maternal offspring varied in quantitative characteristics (stem length, spike productivity). The R-1 rye plant was heterozygous. This population was reproduced on an isolated plot under wind pollination. Apomictic embryo development in the flowers of soft wheat and triticale induced by R-1 winter rye pollen was known from previous experiments (Kyzlasov 2005, 2007). The technique for creating plants showing no segregation in the offspring has a practical application in breeding in the creation of lines of Nemchinovskaya 24 soft winter wheat with similar stem lengths. Initially, this cultivar was segregating for stem length for some obscure reasons. However, maternal families of Nemchinovskaya 24 obtained after fertilization with R-1 winter rye pollen were similar for stem length.

Plants with sterile pollen and abortive anthers were found in the next generation within the studied rye population offspring. Plants with sterile pollen showed involution in the development of quantitative characters; approximately 6% of the total number of plants in the population. Abortive anthers were removed to eliminate the possibility of self-pollination and emasculated spikes covered with paper cages. Isolated caryopses were formed in unpollinated flowers without the participation of a paternal parent. The caryopses from the paper cages without pollination were sown in a glass house and their offspring produced no seeds unless pollinated with a paternal plant. Their pollen was sterile. Plants with fertile pollen were allowed to wind pollinate on an insolated plot with the pollen of their own population. The derived seeds were sown in the field. Approximately 25% of offspring had sterile pollen at anthesis. Plants with fertile pollen and plants with sterile pollen were grown in wheat crops free from wind pollination. Seeds from the plants with sterile pollen were used for further research. Making genetic analysis of self-sterile plants with wind pollination presents great difficulties.

Seeds taken from plants without pollen were sown in the field. Among the derived offspring, approximately 67% formed normal pollen and 33% formed sterile pollen. At the beginning of anthesis, plants with fertile pollen were removed. The plants with sterile pollen were reproduced in a winter wheat crop. However, the fact that rye pollen brough in by wind from remote parts of the field on to pistils of the flowers with sterile pollen could not be absolutely excluded. Mean seed set of the plants without pollen under free wind pollination was 55.4%. The number of flowers in a spike was 60.9, grains/spike was 33.8, 1,000-kernel weight was 28.9, and spike productivity was 0.98. The indices of the five best plants are presented in Table 1.

Table 1. Seed set and the development of spike productivity features of rye plants with sterile pollen under free wind pollination in a wheat crop.

Plant number	Seed set (%)	Flowers/spike	Grains/spike	1,000-kernel weight (g)	Spike productivity (g)
1	75.7	75.6	57.2	29.2	1.67
2	73.0	63.8	46.6	29.9	1.39
3	72.7	60.0	43.6	37.4	1.63
4	72.5	76.4	55.4	28.2	1.56
5	70.6	52.4	37.0	30.0	1.11

From the maternal plants of the previous generation with sterile pollen, 279 rye offspring were reproduced. The spikes of every plant were covered with paper cages before the beginning of flowering. One hundred eighty-eight plants produced normal pollen under the paper cages. Most likely, these plants were produced as a result of cross pollination of male-sterile flowers by rye pollen in the previous generation. Under self pollination, an average of 182 plants with fertile pollen produced one caryopsis/spike. The autosterility genes of these plants worked normally. Another six self-pollinated plants had from 32 to 65 caryopses/spike. These were self-fertile plants. Pollen fertility is a dominant feature. The distinctive feature of the 58 plants with sterile pollen was the ability to set seed without pollination under the paper cages. The other 33 plants with sterile pollen did not set seed under the paper cages. Small, nonviable germination of plants in the shape of a lamina rosette were produced instead of pistils within one such offspring. Apomictic reproduction and sterile pollen were inherited by the rye offspring as linked features. The distribution of rye plants with sterile pollen in the various series by seed-set rate under paper cages is given in Table 2. On average, 12.2 caryopses were produced per spike within the best group of plants. The ratio of produced seeds to flowers/spike was 18.4%.

Table 2. Distribution of rye plants in various series by the number of caryopses produced/spike.

Grain number class	No of families studied	Grains/spike	Flowers/spike	Seed set
0	33	0.0	60	0.0
< 1–3	36	0.7	58	1.2
> 3	22	11.2	61	18.4

Productivity rates of the eight best apomictic plants are presented in Table 3. High variation was discovered in the manifestation of all features excluding 1,000-grain weight.

Table 3. Rate of seed sete and development of spike productivity features of apomictic rye plants under paper cages without pollination.

Plant number	Seed set (%)	Flowers/spike	Grains/spike	1,000-kernel weight (g)	Spike productivity (g)
1	82	73	60.3	22.9	1.38
2	37	70	27.3	26.5	0.72
3	34	67	23.0	17.2	0.40
4	29	64	18.4	32.0	0.59
5	25	68	16.7	33.3	0.56
6	19	65	12.4	27.8	0.34
7	22	60	13.4	25.0	0.33
8	18	56	10.1	14.6	0.15

Pistils of the apomictic rye produced develop with normal germinating ability. Hybrid F_1 plants, produced after pollination of paternally sterile flowers by pollen from other plants, produce fertile pollen, indicating that the fetal sac cells in the rye are reduced. Diploid plants produced without a paternal parent inherit the sterile pollen feature, which is recessive. Apomictic plants with sterile pollen can be reproduced without pollination on isolated plots. They can be used for production of heterosis plants and genotypes, which are resistible to some pathogens. The production and identification of such offspring in allogamous species are complicated. Tetraploid apomicts can be produced by duplication of the chromosome set. High seed set is possible in the produced tetraploids. At the same time, they will be unable to cross with the source diploid rye. The ability of rye for apomictic reproduction may be transferred to other cultivated cereals species using genetic engineering techniques. Haploids are not seen among plants with both sterile and fertile pollen. The phenomenon of polyembryony was noticed in the apomictic offspring. Up to five viable germs are formed in a caryopsis without pollination.

The study of apomictic offspring was continued under glasshouse conditions. From an unknown cause, anthers with pollen formed in approximately a half of the studied offspring under insufficient light conditions, a surprising fact. However, almost all the pollen in these anthers was nonviable. Up to 12 caryopses were set in unpollinated flowers of each spike. Analysis of the genetic organization of the described rye apomixis is hampered by the fact that autosterility genes are manifested in the phenotype. The mechanism of embryogenesis also remains unknown. Perhaps, the endosperm of apomictic rye is diploid. Starch grains in apomictic rye endosperm are smaller to those in the endosperm of amphimixis plants. The aleurone layer is thinner than in amphimixis plants and is missing in some. The caryopsis coat is contiguous with the starchy endosperm in these irregular places. Apomict endosperm and embryo are not produced in every flower. Seed set under paper cages varies from 0 to 82% of the number of flowers in the spike. Sometimes endosperm or embryo remains abortive. When there is no budlet in the embryo, a scutellum will normally develop. If there is no embryo, but there is an endosperm in a caryopsis, the micropyle will be occluded thoroughly. As a result of the proliferation of external seed coat cells, a tightly closed protuberance in the shape of denticle or papilla, called a caruncle, is formed around the micropyle. The micropyle of apomict plants, as a rule is, occluded (Shishkinskaya 2005). In nearly all ripe, apomictic caryopses, the micropyle cavity and space between the embryo bottom and caryopsis coat is filled with a pink, vitreous substance. This pigment is not usually present in amphimict rye. Amphimict rye, unlike normal rye, has very thin floral glumes, especially the inner glumes.

Diploidy of apomictic rye offspring produced without a paternal parent indicates that they can develop from unreduced cells of the fetal sac or nucellus, such as in *Poa bulbosa* (Kordum 1970). There is no digenesis in these cases. Offspring completely identical to the maternal parent are produced. The nature of origin of adventive fetal sacs from the nucellus cells is similar to the origin of the budlet in the sporophyte phase. In apomictic plants, haploid cells of the fetal sac can give birth to a diploid embryo as a result of chromosome endoduplication (Maheshwari 1954). Apomictic rye plants have a prolonged period from the beginning of stem formation until anthesis. Perhaps that is why the pyles of the stigma and flower lodicules are overgrown. Kyzlasov (2006) discovered earlier that in rye with polygynous flowers, lodicules are converted into pistils. With no pollination, the life of the pistil in apomictic rye plants was 10–15 days longer; with no pollination, the stigmas die earlier than the ovaries.

In apomictic caryopses, the seed coat and endosperm are wrinkled. The seed coat hangs down from the endosperm. A translucent bubble of fetal integument forms at the bottom of embryo. In cross section, the caryopsis covering is porous and vesicular. Apomictic rye has a tendency to break between the cell proliferation layer of the seed coat and other parts of the caryopsis. The endosperm grows together poorly with a caryopsis cover and only partly occupies the pericarp. Sometimes a gap in the lower part of the suspensor of a ripe caryopsis consists of dark, mortified tissue that does not fasten at the micropyle and does not adjoin the caryopsis covering. A cavity often forms in the middle of the caryopsis. Caryopses are flat and compressed from two opposite sides and sometimes only a caryopsis cover without endosperm is formed in them. A large part of the caryopsis rises to the surface if submerged. The main root is often abortive.

The produced apomictic rye has the ability for sexual reproduction but differs from normal rye by pollen sterility. Seeds form after pollination with pollen from other plants. Normal rye plants are produced under such conditions. However, with no pollination, overriping and perishing pistils transform into caryopses without the participation of a paternal parent. The offspring are diploid plants. The study of winter rye apomict described in this article will be continued.

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