Application Note

Understanding the diversity of pollen morphology and detecting of responsible genomic signatures in Eternal Rye

Seeing beyond

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The Plant Reproductive Genetics group has come together around Dr. Steven Dreissig to focus on the impact of genetic and environmental factors on the sexual reproduction of plants in general. In this paper, the so-called eternal rye plays a special role in understanding, which genomic signatures are responsible for the size and morphology of the rye's pollen and therefore its reproduction behavior. Ultimately, we will consider how answers to these questions can be transferred into the overall development of improved breeding methods. For this task Dr. Dreissig and his team are using stereo and fluorescence microscopes, among other techniques, on a regular basis.

A Brief History of Eternal Rye
When people settled down around 10,000 years ago, they began to cultivate grain starting with wheat. Cultivation of rye only came along some 5,000 years later, owing to its "unstructured" biological behavior in reproduction.

Today, in times of climate change and with a burgeoning world population, it's precisely this behavior that makes rye of special interest to plant researchers who wish to transfer its unstructured reproductive behavior into other "inbreeding" grains and thus improve hybrid breeding.

The long-term field trial at Halle was already underway in 1878 when the agronomist Julius Kühn laid out a trial field of winter rye (*Secale cereale L.*). The purpose of the "Eternal Rye Cultivation" project, as he put it, was to prove Justus von Liebig's nutrient theory, and the original size of the trial field was six plots of 1,000 m².

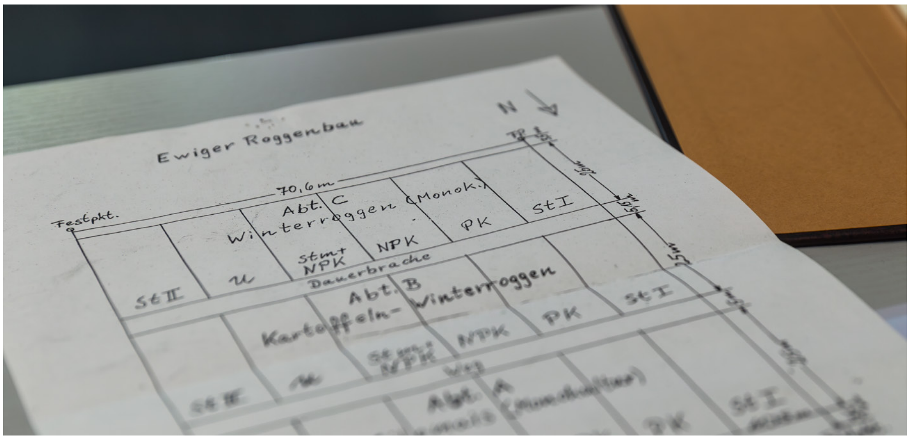

Figure 1 *Plan of the trial fields for eternal rye cultivation.*

Stable manure fertilization	mineral PK fertilization	mineral NPK fertilization	mineral NPK fertilization + manure	without fertilization	without fertilization (1893 – 1953 manure)
silage maize monoculture (Dept. A)					
Black fallow					
Crop rotation winter rye / potatoes (Dept. B)					
Black fallow					
Winter rye monoculture (Dept. C)					

Table 1 *The trial design since 1990*

Figure 2 *Each individual plant is labeled so the results can be assigned later. Copyright of Martin-Luther-University Halle-Wittenberg, Germany*

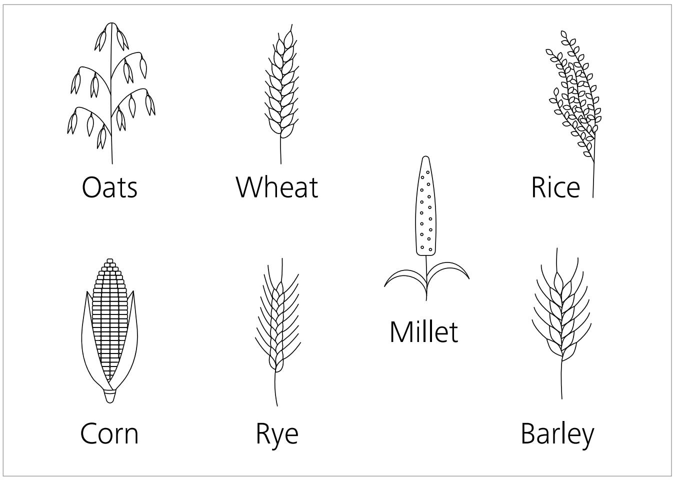

Figure 3 *Types of grain*

In 1961, faced with a strong propagation of field horsetail (*Equisetum arvense*), the area was divided into three large parts with black fallow areas in between. Since then, the field size has been 6 plots of 290 m².

The research focuses on the long-term effects of different types of fertilization (industrial and farmyard manure) on the cultivated plants and soil. A strip of rye on this test field also offers exciting insights for organic farming, as it has not been fertilized since 1878. The grain is harvested annually and then replanted. The results have been archived since 1949 in regular soil samples. Among other things, this involves further research into how the grain copes with stress and how that affects yields. This longest continuous field test in Germany is now under official protection as a "cultural monument".

Grain types and characteristics

Grains are sweet grasses and the awn itself sits at the end of long stalks. The grain family consists of seven different types – wheat, rye, barley, oats, corn, rice and millet – and each with different subspecies.

Figure 4 *Typical appearance of rye. Copyright of Martin-Luther-University Halle-Wittenberg, Germany*

Figure 5 *Wheat grain, acquired with ZEISS Stemi 305 stereo microscope*

The mature rye plant (*Secale cereale*) has a slender, tough, fibrous stem (straw) and elongated leaves. Typically it reaches sizes from 30 centimeters up to 200 cm. The inflorescence (spike) is long and slender with stiff long awns (beards). Rye grain (caryopsis) is arranged in pairs alternately on a zig-zag shaped rachis. The grain is covered with a lemma, a palea, and a glume (chaff), which is normally awned. Rye grain (kernels) ranges in length from 4.5 to 10 mm and in width from 1.5 to 3.5 mm. The grains are normally grayish-yellow, but can vary widely depending on the rye cultivar, region of cultivation or harvesting conditions. A crease extends the full length of the ventral side of the grain. The surface of the grain is usually shriveled and has a rough texture. A single grain weighs ~20 mg.

Despite the enormous differences between the grain types, all grains share a similar structure. Rye grains – like wheat grains – comprise three distinct morphological parts: starchy endosperm, bran (pericarp and testa), and germ (embryo and scutellum). Rye comes in both summer and winter forms, although only the winter form is of great economic importance.
(Rye – an overview | ScienceDirect Topics)

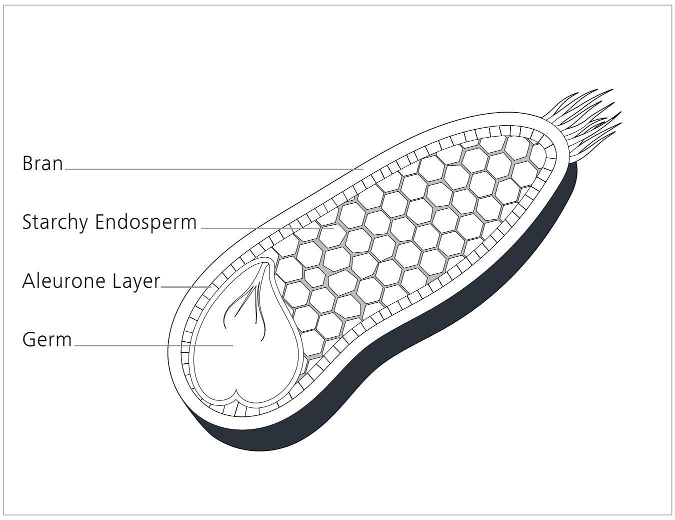

Figure 6 *Structure of grain*

Figure 7 *Ear of rye*

Sample preparation and investigation

Rye flowers from April to early September and sets its pollen freely. Being self-incompatible and unable to fertilize itself, it must depend on neighboring plants for pollen. Therefore, the plant sheds a lot of pollen itself to increase its chance of fertilization. According to Vries (1971), an ear of rye produces 4.2 million pollen, more than nine times as many as a self-pollinating ear of wheat. The flight behavior and mobility of the pollen grains is determined by their size and surface, and is therefore decisive for the successful propagation of the plant.

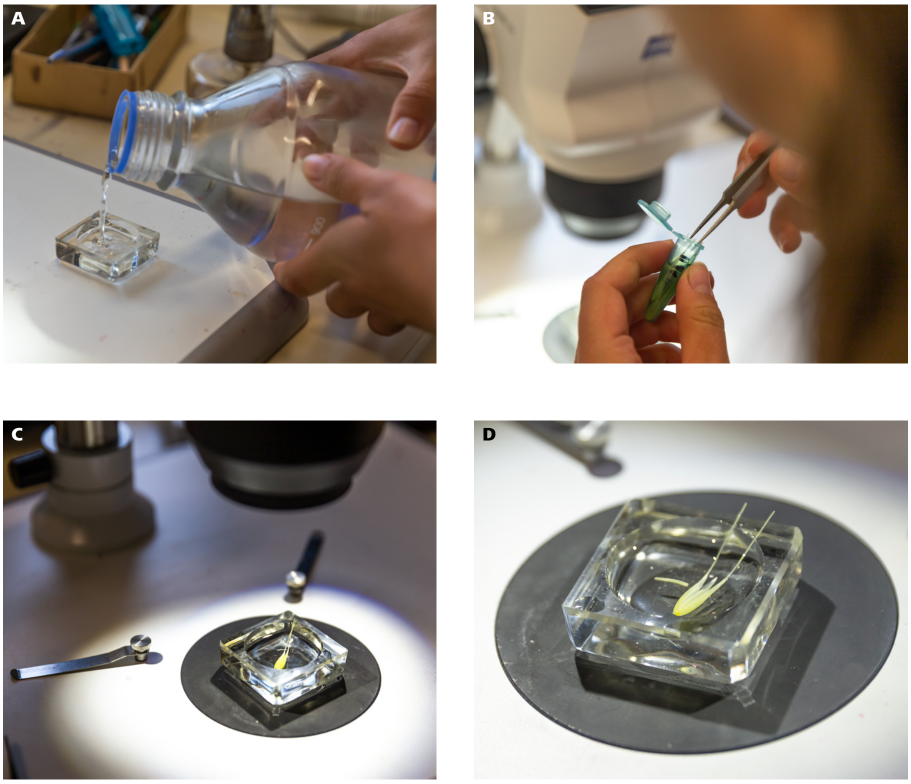

Figure 9 A – D *To investigate the pollen, the anthers are manipulated out of the flowering plant under a stereo microscope. They are kept in a fixative consisting of Ethanol and acetic acid.*

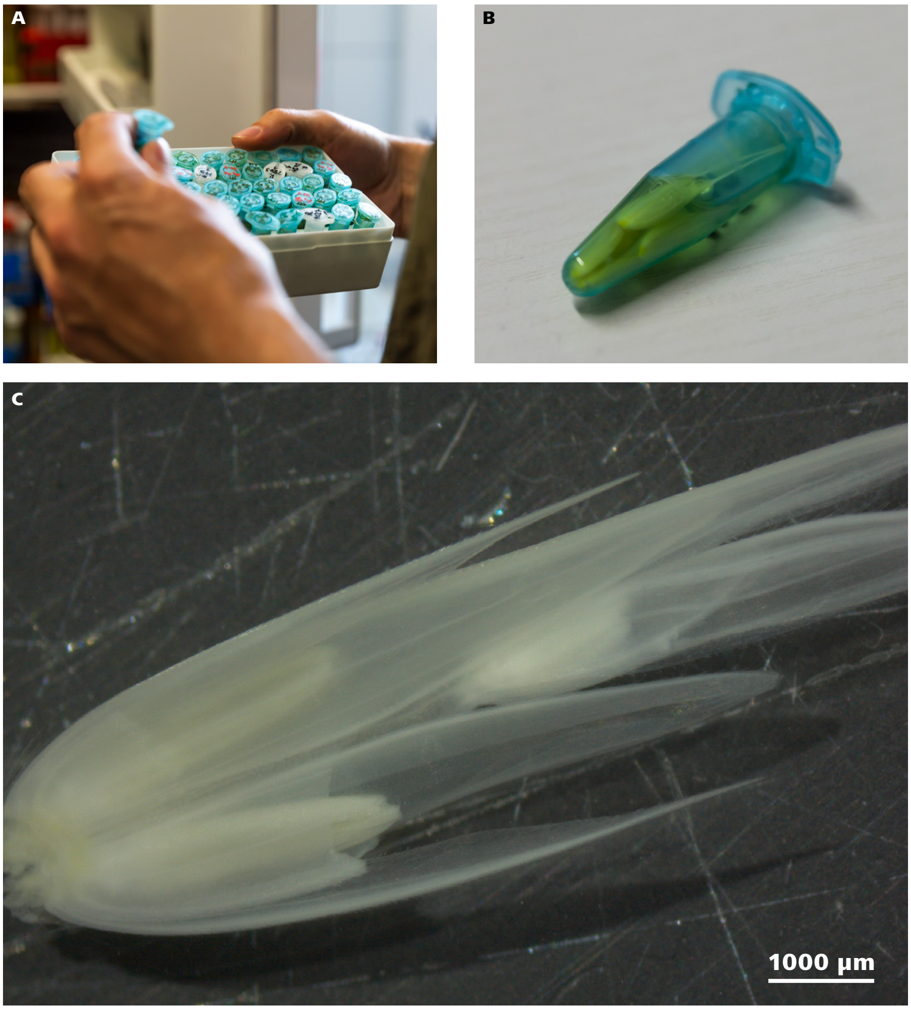

Figure 8 A – C *Anthers containing the pollen are clearly visible. Copyright of Martin-Luther-University Halle-Wittenberg, Germany (Figure 8C)*

The optimum size of pollen is a compromise between flight distance and the ability to "hit" surfaces. The smaller and lighter the pollen, the more it integrates into the airflow and tends to flow around objects. The larger the pollen, the greater its ability to settle on surfaces and the more energy can be given to the pollen to grow through the pistil. If you know, which genes are responsible for the pollen size and formation, you can intervene in the development of correspondingly fertile, high-yielding varieties.

The pollen grain is the male germ cell of the flowering plant. They are formed in the pollen sac of the anther (dust bag). Each flowering plant consists of six anthers, each containing several, usually four, pollen sac chambers called microaporangia.

Figure 10 *The six anthers are manipulated out of the flowering plant. Copyright of Martin-Luther-University Halle-Wittenberg, Germany*

Observing pollen with a fluorescence microscope will give you the first hints of vitality and size. The diameter of rye pollen varies by 41 to 48 µm on the short axis and 59 to 68 µm on the long axis. It consists of of three nuclei, normally two longer shaped and one round. Dead pollen can be detected by their modified morphology. The pollen then are counted, whether with the fluorescence microscope or with flow cytometry. This evaluation is based on hundreds of plants to reach conclusions on:

- fertility in connection with environmental conditions
- average pollen size in connection with the genome sequence.

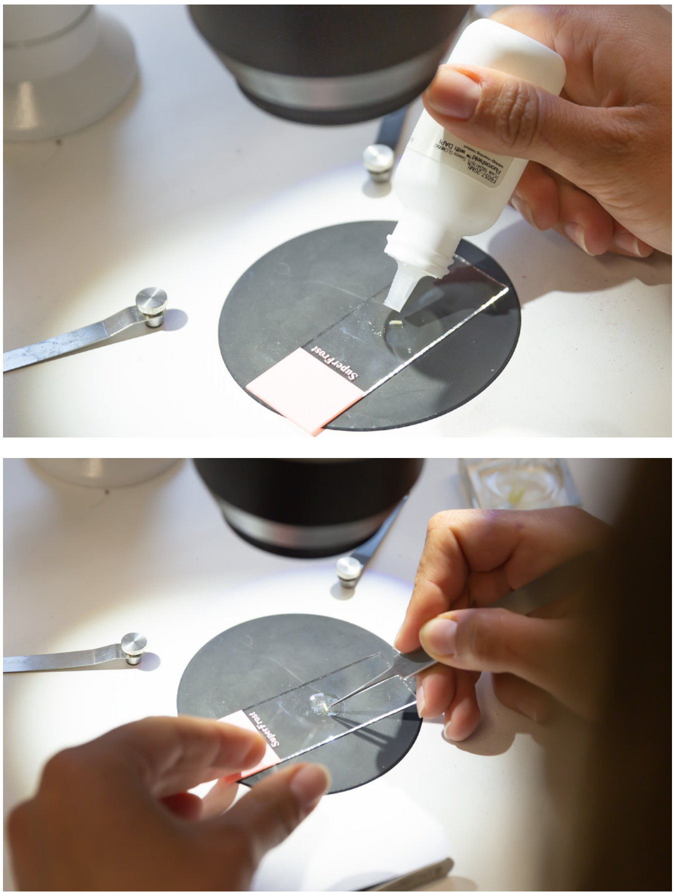

Figure 11 *Pollen is released out of the anthers by gently shaking and moving. The anther is removed. Then the pollen grains are stained with DAPI and fixed on a glass slide.*

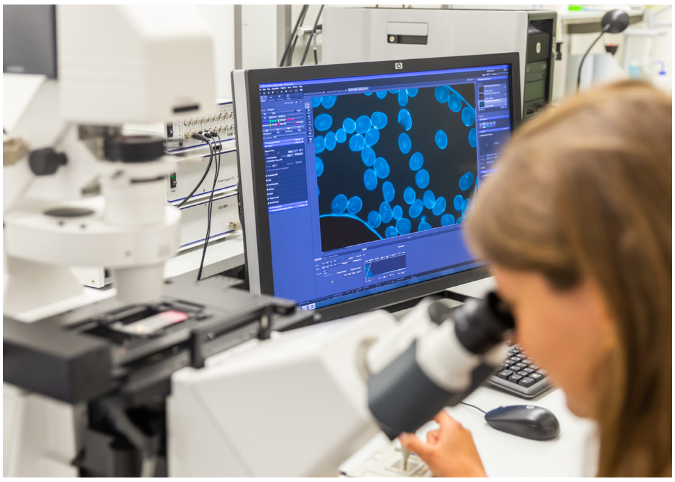

Figure 12 *The pollen and stained nuclei are clearly visible with the help of a fluorescence microscope*

This second point is of special importance. If you know the responsible genome and understand the mechanism of pollen size building, the next step is a targeted transfer to crops.

Conclusion

Breeding research is used to improve plant breeding methods and develop climate-resistant varieties to support future food security. Rye is a good candidate for this, as it is extremely hardy. It has good cold tolerance, is disease-resistant and makes no great demands. Rye is cultivated worldwide, right up to the Arctic Circle and at up to 4,000 m above sea level. It can grow in sandy soils with low fertility. Because of its lower value, rye crops need less fertilizer than other temperate cereals. All that, and rye is also more resistant to disease than wheat. In order to understand the mechanisms that influence the cross-breeding behavior of rye, you must investigate the plant's flowering and fertilization behavior as well as the factors influencing pollen size and distribution. Once the responsible genome is known and the mechanism of pollen size building is understood, the next step should be a targeted transfer to other crops.

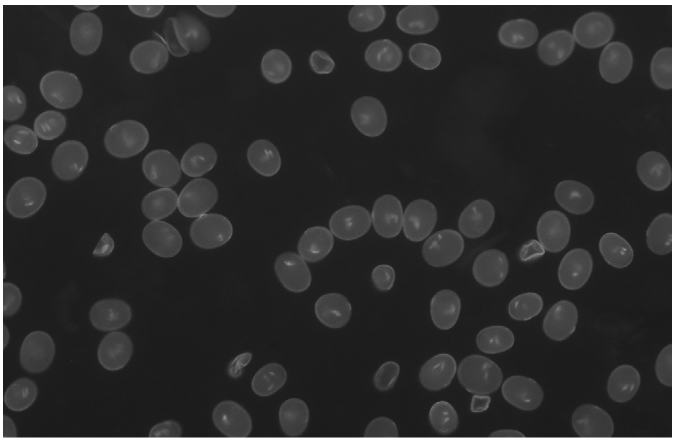

Figure 13 *Pollen grains normally contain three nuclei. Copyright of Martin-Luther-University Halle-Wittenberg, Germany*

References

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