Submicroscopic Morphology Comparisons between Hybrid F₁ by Wheat and Octoploid Triticale and the Parental Generation

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Abstract

F₁ hybrid generation comes from the hybridization of common wheat Jingdong 8 as the female parent and octoploid triticale Jinsong 5 as the male parent. It can be clearly seen from the submicroscopic morphology comparisons of pollen grains, leaf epidermis and stomas between hybrid F₁ and the parents that most of the shapes of the parents’ pollen grains are nearly circular, while those of the F₁ hybrid pollen grains are irregular and severely wizened. In addition, the pollen grains of the F₁ hybrid and parents are significantly different in the submicroscopic features of exine sculptures and germ pores. The common features of the three tested materials are stomatal apparatus, long cell and hair cell, and the cells are arranged in parallel with the vein. Leaf epidermal cells of the female parent which is the common wheat are clear outline, and they are also arranged tidily and smoothly, Pores are arranged in a straight line. Guard cells are dumbbell-shaped with bristles of different lengths. Pores of the male parent which is octoploid triticale have ridge-like heaves with horizontal and vertical alignment around them, and they are rectangular and large, also the general arrangement type is linear. Guard cells is very obvious, but with very few and short bristles. Both of the submicroscopic morphologies of leaf epidermis and stomas of the hybrid F₁ are more similar with those of the male parent, while the leaf epidermis have bristles and the size of pores ranges between the two parents. The F₁ hybrids and parents are significantly different in submicroscopic morphologies on pollens, leaf epidermis and stomas, so that the distinctions of submicroscopic morphologies can be used as a basis in the indicators and identification of the distant hybrids and their parents.

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1. Introduction

Wheat is an important food crop. In recent years, with the long-term directional selection and intensive production, the genetic basis of wheat varieties becomes single, which greatly limits the increase of wheat yield and quality. Wild relative species of wheat contain the valuable genetic resources which are lack in the gene pool of cultivated wheat species, so they are a tremendous gene pool for the improvement of wheat genes. It has become an important way for wheat breeders to obtain new germ plasms by using the wild relative species and common wheat to hybrid [1]. Rye is a good species in the family of wheat, with many good traits which the ordinary wheat doesn’t have, such as disease and insect resistance (rust, powdery mildew, aphids, etc.), resistance (cold, drought, salinity, resistance to dry-hot wind, etc.), lodging, strong tillering and high content of lysine. Rye is the earliest and most successful species used for improving wheat gene in the family of wheat, and it plays an important role in the improvement of wheat gene. But rye is difficult to directly cross-breeding with wheat[2]. Octoploid triticale is a synthetic new species by wheat and rye. It has all the genome of rye, retained the fine characteristics of rye as frost resistance, salinity, high protein content, and it is easy to hybridize with wheat. So it can be used as a bridge germ plasm to transfer the good genes of rye genome more effectively. Thus, in the production, octoploid triticale is usually used to hybridize with wheat breed new species with resilience and high yield[3].

This research gets the F1 hybrid generation from the hybridization of common wheat Jingdong 8 as the female parent and octoploid triticale Jinsong 5 as the male parent. Then it makes the submicroscopic morphology comparisons of pollen grains, leaf epidermis and stomas between hybrid F1 and the parents. There are already reports on the use of electron microscopy techniques in the field of plant taxonomy at present, but we not yet seen any reports about the use of electron microscopy on the identification of distant hybrids. This research makes the submicroscopic morphology comparisons of pollen grains, leaf epidermis and stomas between hybrid F1 and the parents, so that provides some basis for the use of electron microscopy techniques to identify distant hybrids as well as access to new germplasm of wheat.

2. Materials and methods

2.1. Materials

Tested octoploid triticale (Jinsong 5) is cited from Tai sub-centers in the National Wheat Improvement Center, and is planted in Agriculture Experiment Station in Hebei Normal University. The normal wheat Jingdong 8 has already been widely promoted in the eastern area, and now it is preserved in Crop Genetics and Breeding Research Group in Hebei Normal University. The F1 hybrid generation comes from the hybridization of Jingdong 8(♀) and Jinsong 5(♂).

2.2. Methods

Take leaves of three tested materials (cut into small pieces) and fix them with FAA. Then clean them, and dehydrate them step by step with ethanol ion (30%, 50%, 70%, 85%, 95%, 100%), each step 10 to 15 minutes. Gel them on the double faced adhesive tape after natural drying.

After the spraying treatment by the ion sputtering going with KYKY-2800 scanning electron microscope, they should be observed under the electron microscope (working voltage is 15 KV), and the observed images will go directly into SEM image analysis system home was observed[5]. Determinate 5 pollens for each material and take the average value, taking photographs of the representative pollens.
3. Results and Analysis

3.1. Submicroscopic Morphology Characteristics of the Pollens of Tested Materials

It can be clearly seen under the electron microscopy that most of the shapes of the parents’ pollen grains are nearly circular, while those of the F1 hybrid pollen grains are irregular and severely wizened, shaped in deflated triangular or irregular in shape. This may be greatly related to abnormal stamen (Figure 1-3). At the same time, a small number of parent pollens exists the phenomenon of shrinkage, so it can be inferred that this may be related to the production methods used[6]. The characters of pollen exine sculptures and germ pores are one of the main basis for identifying pollen grains[7-8]. The germ pores of the tested pollens of the parents and the F1 hybrids in this experiment are nearly circular, bulge around the openings, and with apertures cover on the pore. But the openings of germ pores, sizes and shapes of germ pores (such as cracks, punch) are different between the parents and the F1 hybrid, and the openings of germ pores of the F1 hybrid are smaller than those of the parents. In the observed materials, the surfaces of all the pollen are granule glyph. The parents’ are composite granule glyph, and the arrangement is relatively loose while the size is smaller. The F1 hybrid’s is single granule glyph, with uniform and single size (Figure 4-6, Table 1).
Table 1. Submicroscopic Morphology Characteristics of the Pollens between parents and Hybrid F1

<table>
<thead>
<tr>
<th>Tested Materials</th>
<th>Cover Shapes of Germ Pores</th>
<th>Surface Features</th>
<th>Size(μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jingdong 8</td>
<td>apertures is convex, pore cover has cracks</td>
<td>rough, mostly composite granules, granules are larger, tightly packed</td>
<td>72.85 × 44.29</td>
</tr>
<tr>
<td>Jinsong 5</td>
<td>apertures is convex, pore cover has or doesn’t have cracks</td>
<td>composite granules, granules are larger, relatively loosely packed</td>
<td>72.78 × 52.85</td>
</tr>
<tr>
<td>Hybrid F1</td>
<td>apertures is convex, pore cover has lots of cracks</td>
<td>single particles, granules are smaller, loosely packed</td>
<td>65.17 × 53.49</td>
</tr>
</tbody>
</table>

3.2. Submicroscopic Morphology Characteristics of Leaf Epidermis and Stomas of Tested Materials

The parts of all materials observed on microstructure in this experiment are the lower epidermis of the leaves. In general, the common features of the three tested materials are stomatal apparatus, long cell and hair cell, and the cells are arranged in parallel with the vein. Specifically speaking, Leaf epidermal cells of Jingdong 8 are clear outline, and they are also arranged tidily and smoothly. Pores are arranged in a straight line. Guard cells are dumbbell-shaped, but are not so obvious as the other two materials. They have bristles with different lengths (Figure7). Pores of the Jinsong5 have ridge-like heaves with horizontal and vertical alignment around them, and they are rectangular and large, also the general arrangement type is linear. Guard cells is very obvious, but with very few and short bristles. Octoploid may be a great cause to the large single hole[2] (Figure 8). Both of the submicroscopic morphologies of leaf epidermis and stomas of the hybrid F1 are more similar with those of the male parent, and the differences are that the leaf epidermis have bristles and the size of pores ranges is smaller than the male parent (Figure 9-12, Table 2).

Table 2. Submicroscopic Morphology Characteristics of Leaf Epidermis and Stomas between parents and Hybrid F1

<table>
<thead>
<tr>
<th>Materials</th>
<th>Leaf Epidermis Characters</th>
<th>Stoma Size(μm)</th>
<th>Stoma Characters</th>
<th>Seta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jingdong8</td>
<td>cells arranged in neat rows, Relatively smooth</td>
<td>44.29×10.12</td>
<td>slender, oval, arranged in straight line, interval is larger, guard cells is clear</td>
<td>less, different lengths</td>
</tr>
<tr>
<td>Jinsong5</td>
<td>cells arranged in neat rows, not smooth</td>
<td>97.29×30.12</td>
<td>rectangular, surrounded by ridge-like bulges, large intervals between the pores</td>
<td>little, short</td>
</tr>
<tr>
<td>HybridF1</td>
<td>cells arranged in irregular, has bristles</td>
<td>86.35×26.31</td>
<td>Rectangular, subsidence, similar with the shape of the male parent, ridge-like bulges was not as clear as the male parent</td>
<td>much, long</td>
</tr>
</tbody>
</table>
4. Discussions

The genetic background of octoploid triticale is relatively simple, which is just attached the rye genome R to the basis of wheat genome ABD. This provides a good genetic basis to import the good traits of rye into common wheat. The hybridization of octoploid triticale and wheat is more likely to be successful, and their hybrid offspring happens with the translocation between homologous chromosomes has a high rate. So it is very suitable for breeding of wheat - rye translocations to breed new varieties with disease-resistant, drought tolerance and higher yield than parents[2].

Electron microscopy is a recently developed new technology. It consists of electro-optical technology, vacuum technology, fine mechanical structure and modern computer control technology. Scanning electron microscopy (SEM) has a magnification far beyond the limits of 1,000 times by optical microscopy, which can be up to 30 times or more. It also has many other advantages such as high resolution, clear imaging, three-dimensional images, and simple sample preparation. The application of electron microscopy makes the research of plant morphology into the submicroscopic level. It can be clearly observed with scanning electron microscope the surface and cross-section morphology and characteristics of plant which can identify unknown plants[4]. Researches with electron microscopy technology in recent years on the surface of the cuticle in plant taxonomy corolla, stoma of citrus plants, spores morphologies of four Selaginellaceae plants produced in Tibet all show that the stoma type of leaves, the size and morphology of stomas, surface ornamentation and cuticle features of petals, external ornamentation characteristics of spores, micromorphological features of pericarp and seed coat exist differences between genus, species and also species and varieties. They all have significance in taxonomic as the basis of the classification between families, genera and species of the genus level[5-8]. Identification of plant species with the plant surface micro-morphological characterizations is a method used widely in the identification of medicinal plants[9].

This research gets the F1 hybrid generation from the hybridization of common wheat Jingdong 8 as the female parent and octoploid triticale Jinsong 5 as the male parent. Then it makes the submicroscopic morphology comparisons of pollen grains, leaf epidermis and stomas between hybrid F1 and the parents. The results showed that parents and the F1 hybrid have significant differences in the microscopic characteristics of exine sculptures and germ pores.

Though there are common features in micromorphology of leaf epidermis. But leaf epidermal cells of the female parent which is the common wheat are clear outline, and they are also arranged tidily and smoothly, Pores are arranged in a straight line. Guard cells are dumbbell-shaped with bristles of different lengths. Pores of the male parent which is octoploid triticale have ridge-like heaves with horizontal and vertical alignment around them, and they are rectangular and large, also the general arrangement type is linear. Guard cells is very obvious, but with very few and short bristles. Both of the submicroscopic morphologies of leaf epidermis and stomas of the hybrid F1 are more similar with those of the male parent, while the leaf epidermis have bristles and the size of pores ranges between the two parents. The results of the research showed that parents and the F1 hybrid have significant differences in the microscopic characteristics of pollen, leaf epidermis and germ pores. Submicroscopic morphology can be used as a basis on the identification of the distant hybrids from octoploid triticale and wheat. In recent years, the observed research of plant submicroscopic morphology with scanning electron microscopy (SEM) has become one important means to the exploration of the surface structure of plants. Its application leads human into a new phase of plant knowledge and understanding[10]. But micro-morphological characteristics are still only the complement of macro-morphological features. In the actual identification of the plant, integrated applications with a variety of properties should be used, making comparative analysis in order to get the most objective and accurate results.
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