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The Application Of Methods Of Cytoembryological Analysis Considering The Causes Of Alfalfa Infertility (For Example Medicago Romanica Prod.)

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ABSTRACT

In order to increase the seed productivity of Medicago romanica Prod. it is important to know all the reasons affecting on the seed formation. The study of the anatomy and the morphology of reproductive organs by the method of cytoembryological analysis will help to solve the problem of low seed productivity associated with the early shedding of flowers and the fall of the ovaries.

Keywords: alfalfa, ovary, material, ovule, fertility.

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INTRODUCTION

High fodder values of alfalfa provide a wide spread of this culture. Besides that, alfalfa plays a leading role in the accumulation of biological nitrogen, prevention of erosion and secondary salinization of soils. The task to create and the implement new varieties for specific environmental conditions (considering biological characteristics) take an important direction in the feed production. Varieties that give high yields of green mass with protein content increase are very necessary for the South of Russia. They should be characterized by a high level of seed productivity, good regrowth and drought resistance. The creation of new varieties and hybrids with the necessary properties largely depends on the source material. The local wild species having both haying and pasture use can be used as starting material for the creation of new varieties and hybrids.

Medicago romanica Prod. is a steppe form of yellow alfalfa. Wild perennial plant has drought resistance and salt tolerance due to the powerfully developed root system and dense pubescence. It can be found in floodplains, on the slopes of mountains, it is part of the fescue-feather grass formations in steppes. This valuable forage plant for steppe areas is used as hay and pasture plant. It is important to know all the reasons that affect the seed formation, in order to increase the seed productivity of alfalfa. A sufficient number of works are devoted to the biology, agricultural engineering and seed production of alfalfa.
The question regarding the anatomy and morphology of alfalfa and her reproductive organs is often overlooked, because of paying attention to features of development of above-ground mass and seed productivity. The study of this particular issue will help to solve the problem of low seed productivity associated with the early shedding of flowers and the abscission of the ovaries [2].

The application of methods of cytoembryological analysis allows to analyze the causes of infertility associated with the development peculiarities of the reproductive organs with the greatest objectivity. There is no information in the literature about anatomy of the studied alfalfa sample Medicago romanica Prod. therefore, the present studies aim to fill this gap.

MATERIALS AND METHODS

The biology of flowering and fertilization of wild-growing alfalfa sample Medicago romanica Prod. were evaluated In the unstable moistening zone of Stavropol region.

Pre-soaked and bathed in paraffin microscopic sections of the investigated objects (due to the small size of the alfalfa ovaries (up to 8 mm)) were used in the study of histological material of ovaries and ovules. Preparation of the material for microscopic examination included a number of stages:

1. Fixation, this meansthe mortification of the tissue with preservation of its structure and cell contents as unchanged as possible, corresponding to the living state. Fixation of the ovaries was performed using formalin which retains its shape, color and structure of the drug, it is easy to use. Fixation duration is 24 hours. Then the objects were washed with water to remove formalin.

2. Sealing and storage of material. This washed in water material was placed in alcohol, starting with a very diluted, because the alcohol causes damage of objects when it is a quick transaction. Objects were kept for 30-40 minutes in alcohol from 10^0 to 60^0, and were kept for an hour in each starting from 60^0.

3. Preparation of microscopic sections. It was used the fill of materials in paraffin wax. Completely dehydrated preparations were passed through three portions of benzene. Soaked in benzene objects were transferred to benzene which was saturated with paraffin wax. The preparations were transferred with a heated spatula in pure melted paraffin after sufficient impregnation (30-40 min), as the first portion of the paraffin is contaminated by residues of benzene, so the objects are transferred from it in the second cup after some time. After the drug is completely impregnated with wax, it is poured. Smooth metal frames were used as molds. Heated to 60^0 paraffin was poured to the edges of the form, trying not to make air bubbles. The object to be filled was transferred into it with a heated metal spatula and heated dissecting needle, it was oriented to a position in which it can be cut. The form filled with paraffin was transferred to a small bath. Water in it was been adding so carefully, that it did not fill in paraffin.

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Objects which were filled with paraffin were cut with a knife in the form of a cube. A wooden block was smeared by a hot metal scalpel with a small amount of paraffin and a cube was quickly pressed into it. The sides were also melted with a hot spatula and immersed in cold water. Cutting the paraffin block was carried out on a sled microtome, the cut was removed with a soaked in water brush and transferred to warm water for straightening.

The slide glasses were kept in 96^0 alcohol and wiped with a non-fibrous cloth. A drop of a solution of chicken protein with glycerin was applied to the prepared glass and rubbed with a thin layer. The glass was heated until whitish vapors appeared and after cooling a little alcohol was applied to the prepared layer with a brush and the preparation was applied on it after that. Researched objects were dried in a drying cabinet at a temperature of 42^0-45^0C for several days.

4. Coloring cuts. The preparations were stained with hematoxilin with an alcohol solution of eosin, which gives a lilac shade.

RESULTS AND DISCUSSION

Studies were carried out when plants entered the budding phase - the beginning of flowering, which coincides with the stage VIII - IX of organogenesis. The inflorescences of alfalfa were characterized by different quality in the development of buds and flowers, and the upper part lags behind the lower one. It was observed withering away and falling flowers already in this phase, in our opinion, this may be due to the fact that a large number of tying flowers cannot be fully provided with nutrients. Conducted morphological studies have shown that the alfalfa ovary is upper, single-celled, curved, slightly flattened thin and weak. This is quite consistent with the already known in the literature data on the morphology of the structure of the alfalfa flower. The longitudinal histological sections of the ovaries show that the outer and inner walls of the ovaries are covered with epidermis (Figure 1). This primary covering multifunctional fabric gives strength and protects internal tissues from damage, regulates gas exchange, protects against excessive evaporation. The sinuous outlines of the side walls increase strength.

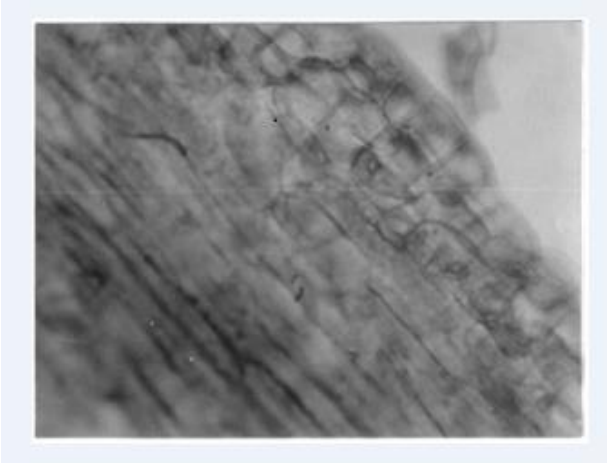

Figure 1: Epidermis wall of the ovary. Increase 140 times

The walls of the ovaries make up the cells of the parenchyma (Figure 2), which are a loose tissue with poorly specialized, different in shape cells, preserving its embryonic character.

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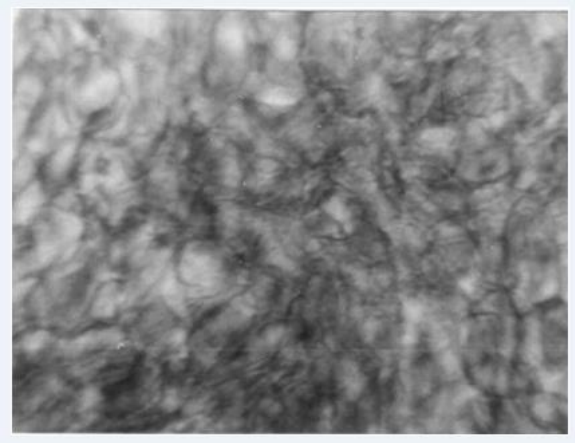

Figure 2: Parenchymal cells of the ovary. Increase 140 times

Opinions about the role of parenchymal cells of the ovary are divided. Carrying and storing functions were credited with it, however, chloroplasts weren't discovered to confirm the assimilative functions.

We have identified large thin-walled cells with a granular structure among the parenchymal cells of the sample. We tend to assume that they perform the storage function and accumulate nutrients for the further development of the ovule.

Microscopic study of ovary sections in the early stages of development of generative organs (VII-VIII stages of organogenesis) showed that the ovaries are laid along the abdominal suture of the ovary and the embryo of alfalfa is represented by a typical meristem tissue limited by the epidermis. The investigated meristem or educational tissue consists of undifferentiated thin-walled cells that can divide multiple times. The cavity of each cell is filled with dense cytoplasm and relatively large nucleus, which occupies a central position. The thinness of the cells of this tissue is characterized by the ability to stretch and increase in volume. The division of meristem cells is accompanied by the division of epidermal cells and leads to a significant growth of the rudiment.

The flowering phase coincides with the IX stage of organogenesis. The samples that entered this phase were characterized by a straight pestle, a rounded stigma, characteristic color of the corolla and formed ovules. The average number of ovules in the ovary is 8.8 pieces. The studied ovaries differed in the number of sterile and fertile ovules. The visual sterility of the ovules was determined by very small size (about 0.5 mm). Histological studies have established that the number of primary sterility investigated ovules expressed protruding from the nucellus intigumenta (Figure 3). That is a serious obstacle for the penetration of pollen tube into the embryo bag [3].

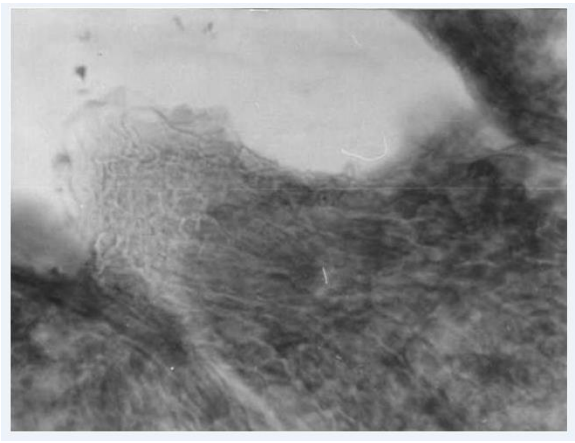

Figure 3: Sterile ovule integument with protruding from the nucellus. Increase 70 times.

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Ovules with fetal embryonic bags are characterized by the presence of internal and external integuments, they form the microscopically zigzag channel in the upper part. The fertilized ovule endosperm contains a large amount of coarse starch (Figure 4), the main part of which is accumulated in the halase part of the ovule surrounded by regenerating epidermis [4]. Fertility ovules per ovary was on average 84.4%.

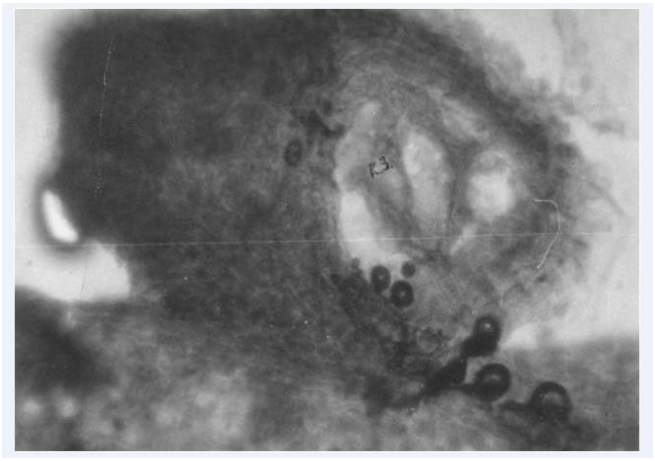

Figure 4: Fertile ovule with starch grains. Increase 70 times

CONCLUSION

The histological analysis of the fallen ovaries revealed the following anomalies in the development:

1. The absence of a clear differentiation of conductive beams, which adversely affects for the supply of nutrients by the ovules;
2. Vague outline of the epidermal cells leads to a violation not only protective function, but also the gas exchange;
3. Weak expression of the parenchymal tissue suggests the presence of abnormalities in the assimilation and storage processes, which are occurring in the walls of the ovary.

These anomalies limit yield of Medicago romanica Prod. seed. However, the high fertility of ovary ovaries affects favorably on seed sample productivity.

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