Cytological and Genetic Investigation of Abnormal Pollen Development in Sugarbeet

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ABSTRACT

Six unique sugarbeet (*Beta vulgaris* L.) mutations (*a1*, *a3*, *a4*, *a5*, *a6*, and *ap*) causing abnormal pollen development were investigated. Cytological examinations of *a1* and *a6* indicated that the male sterility caused by these mutations was due to degradation of microspores after initiation of pollen grain wall formation. Genetic investigations of *a1* and *a6* demonstrated that these mutations were non-allelic nuclear genes. Mutations *a3* and *a4* also resulted in male sterility and had monogenic inheritance. The male sterility controlled by *a5* is inherited as a digenic character. Cytological observations of *a3*, *a4*, and *a5* indicated that even though microspore degeneration begins during pollen wall formation for all three, the three mutations were not identical. The *ap* mutation (accreting pollen) was inherited as a single recessive nuclear gene causing fertile pollen grains to aggregate in the tetrads. All six mutations may be useful in molecular studies of pollen cell wall development. The *ap* mutation allows tetrad analysis in sugarbeet and the male sterile mutants may have utility in situations where hand emasculation is now required.

Additional Key Words: *Beta vulgaris* L., inheritance, male sterility, meiosis, microsporogenesis, mutation.

Cytological and genetic characterization of abnormal pollen development allows for the separation of microsporogenesis into stages and the investigation of genetic control mechanisms. Genetic investigation of male sterile sugarbeet provides a simple way to search for mutations controlling microsporogenesis. The first investigation of this type was a cytological characterization of the *a1* gene (Owen, 1952). Meiosis proceeded normally but microspores degenerated after liberation from quartets and formation of the thin exine (Artschwager, 1947). This degeneration is slightly

delayed in plants homozygous for the *a2* gene (Kaul, M.L.H., 1988). Later, a new male sterility gene was found in wild beet (*B. vulgaris* ssp. *maritima*). The gene from wild beet was not allelic to *a1* (Kinoshita, 1971). In male sterile plants from India, microsporogenesis and anther phenotype were identical to the *a1* gene, but allelic relationships were not determined (Kaul, M.L.H., 1988). Male sterile forms with nuclear control of the character were found in a Russian sugarbeet population (Peretyatko and Peretyatko, 1969) and in a primitive leaf beet population (Bosemark, 1998). Cytological observations of abnormal pollen development and tests for allelism with *a1* were not performed.

Mutations resulting in pollen sterility are not single class mutations acting in microsporogenesis. In sugarbeet, two mutations affecting microsporogenesis, *ap* (accretion pollen) and *ps* (parallel spindle), did not result in male sterility. In diploid sugarbeet, *ps* causes diploid pollen grain formation (Malyuta, 1980). Mutation *ap* leads to non-release of microspores from tetrads (Seilova et al., 1988); pollen consists of normal single grain pollen and pollen grains aggregated in tetrads.

This study highlighted the genetic and cytological behavior of six mutations affecting microsporogenesis in sugarbeet.

MATERIALS AND METHODS

A description of the mutations is presented in Table 1. The line with male sterility controlled by *a1* was provided by Dr. Michel Desprez (Florimond Desprez, Cappelle-en-Pevele, France). Sample 2285 was obtained from the Vavilov Institute of Plant Industry (VIR) beet collection (St. Petersburg, Russia). The male sterility of 2285 is controlled by a recently identified nuclear gene, *a6*. This male sterile form was found by Dr. Peretyatko in a Russian sugarbeet population (Peretyatko and Peretyatko, 1969). A mutation which results in complete male sterility, *a3*, was found by the author (Mglinets et al., 1998) in an inbred population created in the Laboratory of Populational Plant Genetics (Institute of Cytology and Genetics, Novosibirsk, Russia). Mutation *a4* was found in a self-pollinated progeny of fertile inbred line SLC-129 by the author. Mutation *a5* was found in inbred line SOAN-112 by Dr. Veprev (Laboratory of Population Plant Genetics, Institute of Cytology and Genetics, Novosibirsk, Russia). Mutation *ap* was obtained from Dr. Seilova (Institute of Botany, Almata, Kazakhstan).

Genetic analysis. To study inheritance, the flowers of male sterile plants were isolated before flowering, and pollinated 2 to 7 days after flowering with pollen from inbred lines in which there were no male sterile plants. In crosses between two male fertile plants, flowers of one were

Table 1. Characterization of six mutations causing abnormal pollen development in sugarbeet.

Mutation	Origin	Mode of inheritance	Anther phenotype	Description reference
Ap	Institute of Botany, (Almata, Kazakhstan)	- - -	normal	Seilova et al, 1988
a1	France	monogenic	white, empty	Owen, 1952
a3	IC&G,(Novosibirsk, Russia)	monogenic	light-brown	Mglinets et al, 1998
a4	IC&G,(Novosibirsk, Russia)	- - -	white, empty	- - -
a5	IC&G,(Novosibirsk, Russia)	- - -	white, empty	- - -
a6	VIR (sample 2285, St-Petersburg, Russia)	monogenic	white, empty	Peretyatko, 1975

hand emasculated before flowering, isolated by means of pergamine bags, and pollinated 2 to 4 days later. The F_2 generation was obtained from self-pollinated F_1 plants or F_1 sib-crosses.

Cytological analysis. To observe the effects of *a1*, *a3*, *a4*, *a5*, and *a6* on microsporogenesis, branches with flowers at different stages of pollen formation were collected from male sterile plants, fixed in Karnoy's fixative (acetic acid and ethanol, 1 : 3), and stored in 80% ethanol. Microspores for observation were obtained by squashing anthers in a drop of 4% acetocarmine, adding a drop of Fora-Berlize liquid (50 distilled water : 200 chloralhydrate : 20 glycerol : 30 hummiarabic), covering with a coverslip, and staining for 1 to 2 months. For observation of the *ap* mutation, a standard squash method and staining with aceto-carmine was used. Photographs were made using a Carl Zeiss Jena microscope and Micrat-300 film. Magnifications of 600x or 990x were used for all cytological observations

RESULTS AND DISCUSSION

Inheritance of the *a1* mutation obtained from France and the male sterility in sample 2285 were described by Owen and Peretyatko, respectively (Owen, 1952; Peretyatko, 1975). Cytological studies revealed that microspores degenerated at the uninucleate stage after release from the tetrads and thin exine formation, in both sources (Fig. 1). At the end of degeneration, only empty pollen walls stuck inside the anther are visible. A male sterile line from France (*a1, a1*) was pollinated by heterozygotes from 2285. All of the resulting progeny were male fertile. Hence, the male sterility in sample 2285 is not controlled by the *a1* gene. Based upon these results, this gene was designated *a6*.

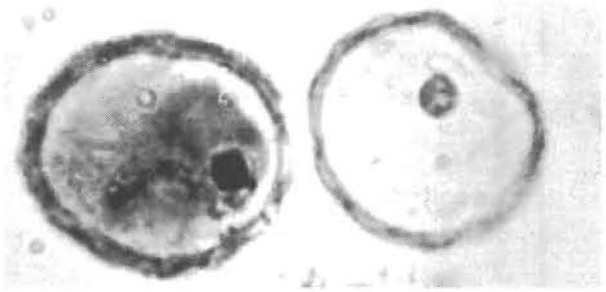


Figure 1. Microspore degeneration caused by *a1* and *A6* genes (magnification 990x).

Genetic investigations indicated that *a3* has monogenic inheritance (Mglinets et al., 1998). The cytological analysis showed that microsporogenesis proceeds normally prior to the development of pollen walls with distinct pores (Fig. 2A). Then the pollen wall thickens and subdivides into several layers. The pollen cell becomes shapeless but its cytoplasm stained well with acetocarmine (Fig. 2C). Mature anthers contain conglomerates of shapeless pollen grains, each with a single nucleus.

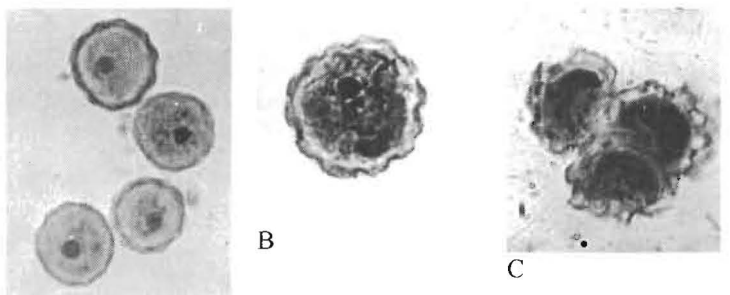


Figure 2. Microspore degeneration caused by *a3* gene (magnification 990x).

To study the inheritance of male sterility caused by *a4*, male sterile plants were pollinated with pollen from SOAN-98. All F_1 plants had a normal fertile phenotype. In the F_2 generation, segregation into two phenotypes with a ratio of 12 male sterile to 33 normal was observed. This ratio is consistent with monogenic segregation (χ^2=0.07) and implies control by a single nuclear gene, *a4*. Cytological observation has shown that degeneration of microspores begins after the initiation of pollen grain wall formation. Exine is formed as convexities (Fig. 3). Distinctly stained pollen grains and nuclei were visible. However, continued development of the pollen wall was not observed and the degree of staining of the pollen grain nuclei gradually decreased. Finally, only empty sticky walls of pollen grains, without the pollen grain nucleus, are observed inside of the anther.

All F_1 plants obtained from pollinated *a5* male sterile parents had normal (male fertile) phenotype. The F_2 segregation ratio was 13 male sterile to 146 fertile (Dr. Veprev personal communication). This segregation ratio corresponded to digenic inheritance with complementary interaction. Cytological observation of mutation *a5* showed that the first deviation occurs after tetrad disintegration and the start of exine formation. In the process of exine formation, convexities were formed on the surface of pollen grains (Fig. 4A). The exine lost its distinct outline; becoming visible as a veil around the pollen cytoplasm (Fig. 4B).

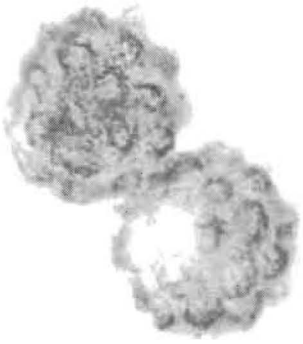
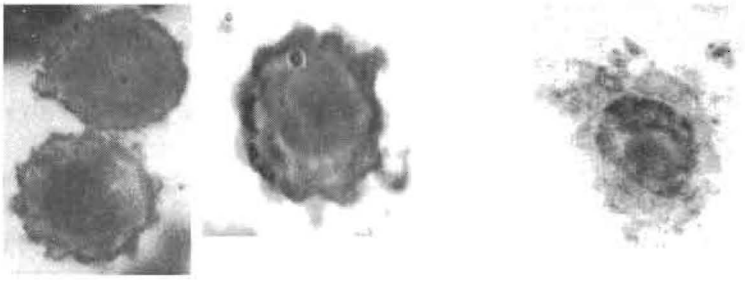


Figure 3. Pollen degeneration caused by *a4* gene (magnification 990x).



A B

Figure 4. Expression of the *a5* gene (magnification 990x).

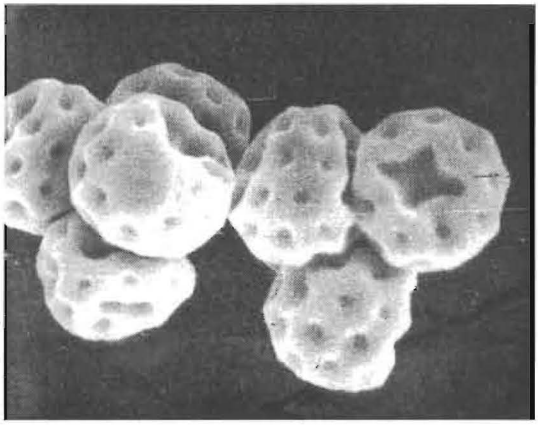


Figure 5. Pollen grains homozygous for *ap* gene (magnification 600x).

In order to investigate the inheritance of the *ap* mutation, the F_1 generation was created by pollinating inbred line SOAN-19 with mutated pollen grains. The F_1 plants produced normal pollen grains. The F_2 generation segregation ratio was 33 *ap* to 89 normal. This ratio corresponds to the monogenic model of inheritance (χ^2=0.27). The *ap* mutation apparently is inherited as a single recessive nuclear gene. Microscopic observations revealed that the development of pollen grains proceeds without deviation up to the tetrad stage. However, after the disappearance of the callose cover, tetrads did not split and pollen grains stuck together. Further development of pollen grains assembled in tetrads appeared normal.

The known sugarbeet mutations acting in microsporogenesis may be divided into three groups (Fig. 6). The first group consists only of a single mutation (*ps*). This mutation results in the formation of unreduced pollen and self-pollinated progenies consist of diploid and triploid plants. Similar mutations have been described in ray (Lelley et al, 1987), red clover (Parrott and Smith, 1986), *Solanum* species (Yerk and Peloquin, 1989), vitis (Zhang et al., 1998), and a few other species. Some researchers propose that these mutations be used to create tetraploid plants. The second group consists of mutation *ap*. This type of mutation previously was described only in *Arabidopsis* (Preuss et al, 1994). This mutation makes tetrad analysis possible. The last group is composed of mutations acting in microspore development at the uninucleate pollen stage (*a1*, *a3*, *a4*, *a5*, and *a6*). In sugarbeet, all these mutations result in male sterility and most of them impair pollen wall development. Similar mutations also cause male sterility in other species, such as *Arabidopsis thaliana* and *Zea mays* (Kaul, M.L.H., 1988).

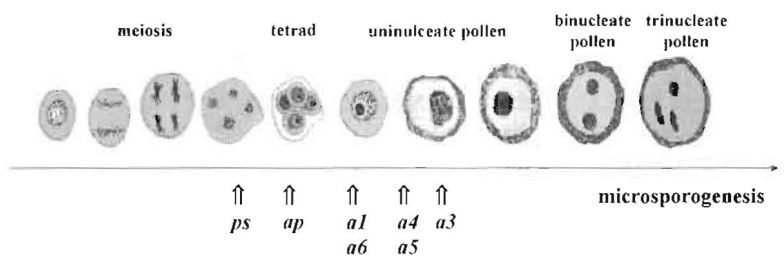


Figure 6. Microsprogenesis and action of seven genes.

ACKNOWLEDGMENTS

I appreciate the assistance of Dr. S.G. Veprev in performance of the given research and in providing the results on the inheritance of *a5*. Thanks to Z.A. Osipova for her technical help in providing cytological

analysis. This investigation was supported by a Young Scientist Investigations Support Grant from the Siberian Branch of the Russian Academy of Sciences.

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