Beta 属植物の花粉の直径と微細構造

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Pollen diameter and ultrastructure in genus *Beta*

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Speciation of genus *Beta* has been investigated by Ulbrich[13] and Coons[2,3]. Hybridization of cultivated beet (*Beta vulgaris*) with the wild species is one of the important breeding techniques for sugar beet, since some of the latter species serve as new genes source. Some F_1 seedlings arising from crosses of sugar beets with each of the species exception with those of section *Vulgares* do not survive beyond the seedling stage, though F_1 seeding resulted. So it is very difficult to study their affinities between section *Vulgares* and other sections from their chromosomal behavior of the crossed F_1 seedling. Studies on pollen in genus *Beta* has been mainly carried out in *B.vulgaris* concerning pollen germination[6,7,11], male sterility[8] and ploidy[7]. In this investigation, in order to get additional information of the interrelationships between pollen morphology and speciation[10], diameter and surface ultrastructure of matured pollen grains of genus *Beta* and artificial 4n plant in *B.vulgaris* were observed by means of light microscope and scanning electron microscope (SEM).

Materials and methods

Species used for this investigation were shown in table 1. It consists of 3 sections 14 species. Before planting seeds of species except section *Vulgares* were scarified with conc. H_2SO_4 according to the procedure described by Stewart[12]. The plants were grown in the field or greenhouse of Agricultural Farm of Hokkaido University. Pollens at anthesis of each plant were collected on slide glass, stained with cotton blue, and the diameters were measured. Measurements were done 10 pollens of 12 anthers in each material. For the SEM, pollens were dried with silica gel in desicater. Number of apertures and aperture diameter were examined by SEM photographs.

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Results and Discussion

The results of this investigation are shown in table 1. Classification of genus *Beta*, summarized by Coons,[2,3] was described 4 sections and 13 species. *B.orientalis, B.perenis* and *B.campanulata* used in this study were not described by Coons.[2,3] According to Ulbrich,[13] *B.perenis* is included in subspecies of *B.vulgaris* and *B.orientalis* is a variety of *B.vulgaris*.

Because seeds of *B.nana*, which is in another section (section *Nanae*), did not germinate, this species was not investigated. In the case of *lomatogona* many of which pollens were sterile, normal stainable pollens were measured. Statistical analysis has revealed that there are highly significant differences in pollen diameter except among 4n sections, within 4n species of *vulgaris* and within 4n *Patellares* (table 2). Pollen diameters of *vulgaris, maritima, atriplicifolia, orientalis* and

Table 1. Some pollen characters in various species of genus *Beta*.

	Code#	Ploidy	Pollen diameter (μm)	Aperture diameter (μm)	No. of aperture	Surface area/ No. of aperture (μm^2)
Section *Vulgares*						
atriplicifolia	1	2	19.83	2.20	38-40	32
maritima	2	2	20.23	1.94	32-42	35
orientalis	3	2	19.88	2.40	32-42	34
patula	4	2	21.68	2.41	48	31
perenis	5	2	19.77	2.30	36-38	34
vulgaris TK76	6	2	20.09	2.77	32-34	37
HSY*		2	21.78			
MGM		2	22.28			
SP*		2	19.23			
macrocarpa	7	4	26.91	2.56	40	57
vulgaris	8	4	26.67	2.54	54-56	40
Section *Corollinae*						
corolliflora	9	4	25.46	3.11	30-36	60
lomatogona	10	4	28.25	3.16	40-50	57
trigyna	11	4	26.69	3.35	24-30	86
Section *Patellares*						
procumbens	12	2	23.69	2.75	30-36	53
webbiana	13	2	22.00	3.30	22-24	63
campanulata	14	4	26.58	2.47	40-44	53
patellaris	15	4	27.00	2.81	32-44	60

* HSY ; Half Sugar Yellow
SP ; SP561001-0

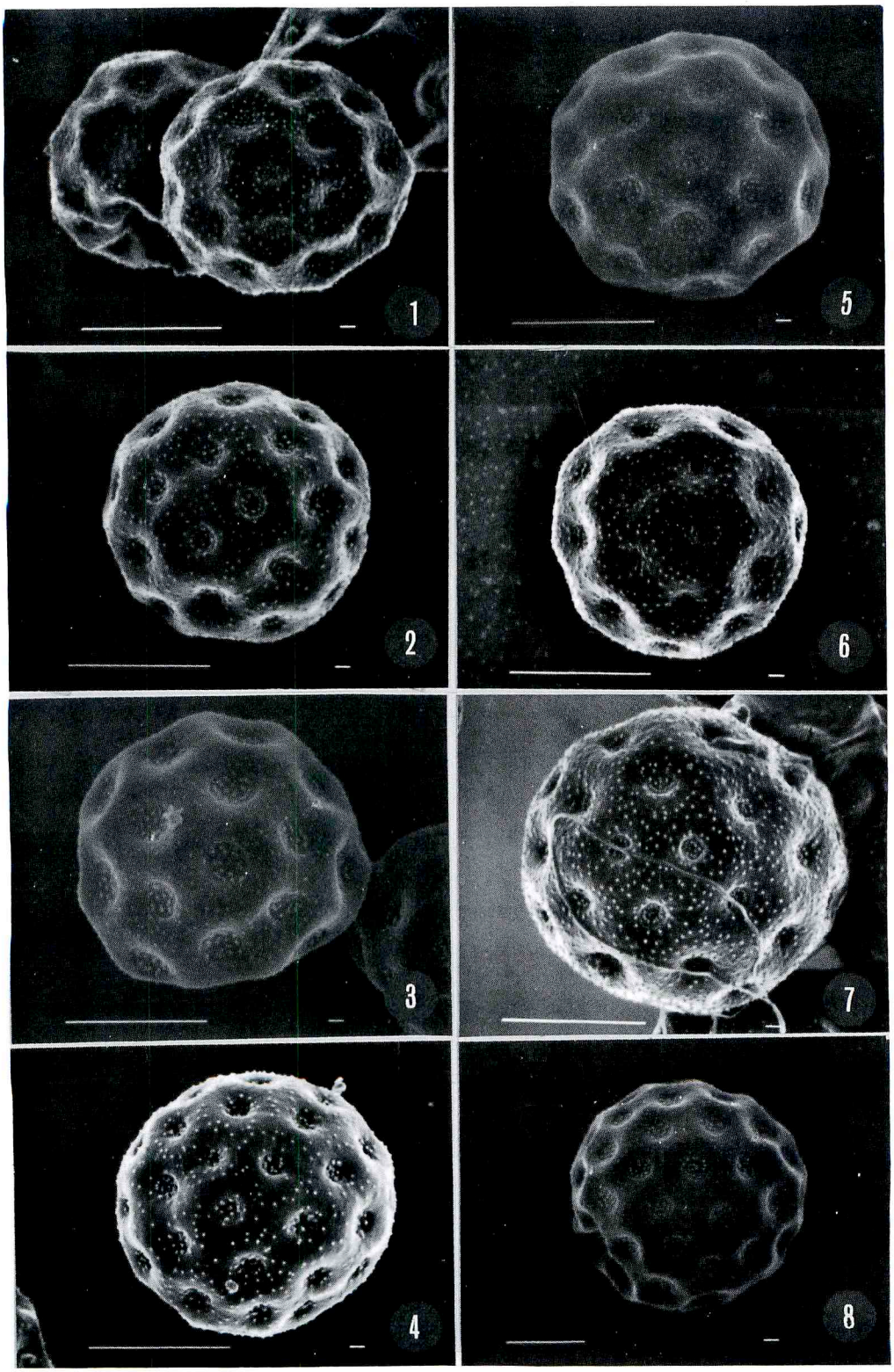
\# Code number shows figure number.

Table 2. Analysis of variance for pollen diameter of genus *Beta*.

Source of variation	df	Mean square
Species and/or Lines	17	119.7695***
Diploid (D) vs. Tetraploid (T)	1	1752.0586***
Within (D)	10	23.5617***
Vulgares (V) vs. *Patellares* (P)	1	105.3072***
Within (V)	8	14.1529***
vulgaris vs. others	1	8.6116***
Within *vulgaris*	3	24.5234***
Within others	4	7.7594***
Within (P)	1	17.0860***
Within (T)	6	8.0676***
Among section	2	0.0030
Within (V)	1	0.3504
Within *Corollinae*	2	23.4780***
Within (P)	1	1.0966
Error	198	0.5228

*** ; Significant at 0.5% level

perenis are about 20μm, those of *patula* and *webbiana* are about 22μm. *B.procumbens* shows about 24μm in diameter, a little larger than the above diploid species. On the other hand, those of tetraploid species show about 27μm. The artificial tetraploid *B.vulgaris* shows about the same value as spontaneous tetraploid species. Nagao and Takahashi[7] has reported that pollen diameter of tetraploid plant is larger than that of diploid one in *B.vulgaris*. Buttler[1] has reported that population of wild beet are not uniform with regard to pollen size. He has shown about 20-25μm in diameter. Ford-Lloyd[5] has reported about 8-18μm, and shown a schatter diagram of pollen grain diameter and pore diameter. Photographs of these pollen by the SEM were shown in plate. Pollen grains of genus *Beta*, belonging to forate type[9] have many apertures ranged from 22 to 56 in number, and many thorns about 0.1μm in high on the surface. The diameter and numbers of pollen aperture examined also shown in table 1. *B. maritima* has the smallest aperture about 1.94μm in diameter, and *trigyna* has the largest about 3.35μm. The number of aperture ranged from 22 of *webbiana* to 48 of *patula* in diploid species and from 32 of patellaris to 56 of artificially induced *vulgaris* in tetraploid ones. It showed the tendency that tetrapolid has more aperture than diploid species. In diploid species of section *Vulgaris*, *patula* tends to have more aperture than others. Dale and Ford-Lloyd[4] have shown that this species is self-compatible. In *vulgaris* pollen diameter of tetraploid plants show much larger than that of diploid one. On the other hand, the diameter of aperture does not show the difference between both ploidy levels. To discuss more pre-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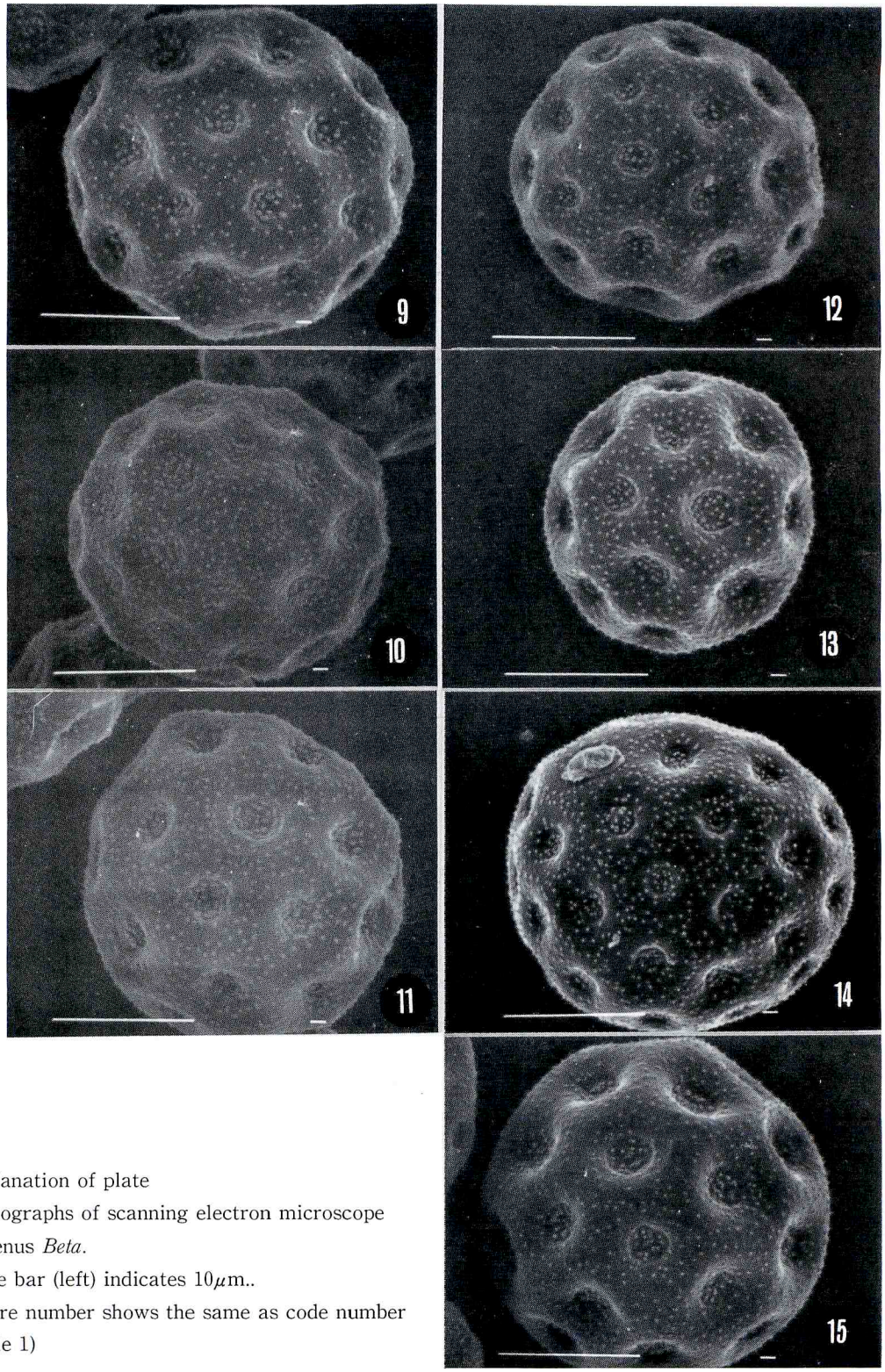

Explanation of plate
Photographs of scanning electron microscope in genus *Beta*.
Scale bar (left) indicates 10μm..
Figure number shows the same as code number (table 1)

cisely, the surface area per one aperture in each were calculated by the following formular.

Surface Area of Pollen (μm^2)/No. of Aperture

They were shown in the last column of table 1. From this, the species of section *Vulgares,* except *macrocarpa,* shows that one aperture locates in 30-40μm^2 each. On the other hand, those of section *Corollinae* and *Patellares*, except *trigyna*, show 50-60μm^2 irrespective of ploidy level. It is very interesting that this value of *macrocarpa* shows the difference from others in section *Vulgares.* Dale and Ford-Lloyd[4] have also shown that this species is self-compatible. The diameter of self-compatible species tends to have larger pollen than those of self-incompatible ones. Abe has studied the speciation of genus *Beta* by the isozyme banding pattern. They also show the differences in *macrocarpa* in comparison with other species of section *Vulgaris* (personal communication). Pollen aperture of *webbiana* is a very peculiar appearance showing fewer and larger than the others. The differences of pollen characters were obvious among sections. It is presumed that the pollen characters are different from the ploidy levels and the breeding behavaviors, such as compatibility.

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Summary

The diameters and the surface ultrastructure of matured pollen grains of various species in the genus *Beta* are observed by means of light microscope and scanning electron microscope. Clear differences in pollen diameter are observed between two ploidy levels (2n and 4n), with variations among species.

The diameters of aperture varies from 1.94μm in martima to 3.35μm in *trigyna.* The number of aperture in tetraploids are more than that of diploids in *vulgaris,* but the surface area per one aperture is about the same. In section *Vulgares, patula* (self-compatible species) tends to have more apertures than the others. A pollen aperture is located in every 30-40μm^2 of pollen surface in species of section *Vulgares,* except *macrocarpa* (57).

In species of section *Corollina* and *Patellares* except *trigyna* (86) which has fewer apertures and larger area per aperture, and *webbiana* which has fewer and larger apertures. A aperture is located in every 50-60μm^2 irrespective of ploidy level. This result presumes that the pollen characters differ from the ploidy levels and the breeding behaviors.

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要　約

糖用作物のテンサイの属する *Beta* 属植物の3節14種の成熟花粉の直径と表面構造が光学顕微鏡と走査型電顕を用いて観察された。花粉直径は種間で変異し、とくに倍数性レベルを異にした場合、4倍体種の花粉が明らかに大きかった。発芽孔の直径は *maritima* の 1.94 μm から *trigyna* の 3.35 μm まで変異していた。*vulgaris* では発芽孔数は4倍体の方が2倍体種の花粉に較べて多かったが、1発芽孔当たりの表面積は両者ほぼ等しかった。*Vulgares* 節では自殖性とされている *patula* が発芽孔数が多く、また *macrocarpa* では発芽孔当たりの表面積が大きかった。しかし他の種ではよく似た発芽孔数と発芽孔当たりの表面積の値を示した。*Corolljnae* 節と *Patellares* 節の種では、特に *trigyna* で発芽孔数が少なく、発芽孔当たりの表面積が大きく、また *webbiana* では発芽孔の数は少なく、直径は大きかった。花粉の形質は倍数性レベルと繁殖行動により異なっていた。