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POLLEN MORPHOLOGY AND TWO-DIMENSIONAL PATTERNS OF POLLEN COAT AND PROTOPLAST PROTEINS IN *AEGILOPS KOTSCHYI* × *SECALE CEREALE* AMPHIPLOIDS

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Amphiploid pollen of *Aegilops kotschyi* × *Secale cereale* was compared to the parental form by SEM, and comparatively measured using light microscopy. Pollen grains of amphiploids were larger and more variable in total and pore diameter than the parents. Amphiploid pollen was prolate, subprolate and prolate-spheroidal in shape. The exine of *Ae. kotschyi* AK-2 and AK-3 had a delicate verrucate surface, whereas *Secale cereale* S14 exine had a verrucate surface. Amphiploid pollen grain surfaces were more or less similar to those of the parents: delicate verrucate, verrucate and well verrucate. The sculpture of parental and amphiploid pollen grains showed conspicuous granulation. All amphiploids produced pollen with one pore with an operculum, surrounded by a well-defined annulus. Sporophytically produced peptides from the pollen coat and gametophytically produced peptides from the protoplast were analyzed separately by two-dimensional gel electrophoresis. Two accessions of *Ae. kotschyi* (AK-2, AK-3) showed differences in the 2-D patterns of peptides from both the pollen coat and the protoplast. The majority of pollen coat and protoplast peptides of the parents were detected in the amphiploids, but a number of parental peptides were absent. All the amphiploids possessed peptides in their pollen coat and protoplast not detected in the corresponding pollen fractions of the parents. No relation between colchicine and in vitro amphiploid production and its 2-D patterns was observed. The results of pollen morphology and pollen protein analysis are convergent.

Key words: Amphiploids, *Aegilops kotschyi*, *Secale cereale*, pollen morphology, protein, pollen coat, pollen protoplast.

INTRODUCTION

Intergeneric hybrids are interesting subjects for evolutionary, taxonomic, cytological and genetic studies on the joint action of different genomes. Their practical importance lies in the introduction of beneficial new genes into cultivated taxa.

The *Ae. kotschyi* genotype has been identified as a UUSS genome. The U genome has not been substantially modified during evolution, in contrast to the S genome which has undergone changes (Kimber et al., 1988; Kimber and Yen, 1989; Yen and Kimber, 1990). The genome of *S. cereale* (RR) is highly heterozygous because this plant is self-incompatible (SI). Now, after breeding programs, numerous lines of rye are self-compatible (SC), thus variability in SC is lower than in SI lines of rye.

The most distinctive features of pollen are the size of mature pollen grains, exine sculpture and the numbers of pores (Ertman, 1952; Fagri and Iversen, 1992; Klimko et al., 2004). The exine pattern is controlled by the sporophyte, whereas pollen size is determined by both the sporophytic and gametophytic genotypes (Ottaviano and Mulcahy, 1989; Bedinger, 1992; McCormick, 1993; Nepi et al., 1995). How the specific pollen wall patterns are determined is unknown, but exine sculpture is exclusively under sporophytic control and no segregation of the exine pattern is known (Heslop-Harrison, 1971a, 1972).

Pollen grain morphology and two-dimensional amphiploid protein patterns of *Ae. kotschyi* (TKK01) and *Ae. variabilis* (TKE12) with SI *S. cereale* and their parents were studied earlier. Pollen grains of *Ae. kot-*

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schyi × SI *S. cereale* amphiploids had two pores, while one pore was observed in the parents (Kalinowski et al., 2001).

Pollen walls and in particular their exine contain many low-molecular compounds (such as carotenes and flavonoids), lipids and proteins (Heslop-Harrison, 1971b; Knox, 1984). Pollen coat proteins and enzymes enabling contact between pollen and the stigma provide nutrition for pollen tube growth or protect against pathogens (Knox and Heslop-Harrison, 1970; Knox, 1971; Singh and Knox, 1984; Pacini et al., 1988; Pressey, 1991; Doughty et al., 1993, 2000; Hiscock et al., 1994; Bufe et al., 1995; Lavithis and Bhalla, 1995). Pollen coat proteins are synthesized in the tapetum and accumulated in exine cavities and on their surface in the last period of pollen maturation (Weber, 1992; Murphy and Ross, 1998; Suzuki et al., 1999). However, it has been suggested that gametophytically produced proteins are transported to the fluidal matrix within the anther loculus and deposited as components of the pollen coat (Doughty et al., 1998, 2000; Dickinson et al., 2000). Pollen coat compounds play essential roles in adhesion, hydration and pollen recognition on the stigma (Dickinson et al., 2000; Heizman et al., 2000). The composition of the pollen coat is not universal, making it a valuable taxonomic character, and it plays an important role in pollen-pistil interactions (Dickinson et al., 2000, and references therein).

The pollen coat, being easily diffusible, can be eluted with, for instance, an isotonic solution of mannitol (Howlett et al., 1975). For analysis of the protein in eluates, the use of cyclohexane proved effective in removing the pollen coat of *Brasscica napus* (Murphy and Ross, 1998), and diethyl ether for *Zea mays* (Suen et al., 2003). An isotonic solution containing sucrose or sucrose solution alone successfully eluted the pollen coat of two species of *Poaceae* (Kalinowski et al., 2002). This paper discusses some advances in the use of water sucrose solution instead of organic solutions for elution of the pollen coat.

Two-dimensional analyses of pollen proteins of *Aegilops* sp. × *Secale cereale* amphiploids were previously performed after extraction of proteins from both the pollen coat and protoplast (Kalinowski et al., 2001; Kalinowski and Wojciechowska, 2003). The pollen of amphiploids contained a majority of the parental peptides; however, some of the parental peptides were not expressed, and proteins specific to the amphiploids appeared. Similarly, a lack of parental isozymes and the presence of new ones were observed in leaves of *Ae. kotschyi* × *S. cereale* amphiploids (Kalinowski et al., 2003; Kalinowski and Wojciechowska, 2004).

Two accessions of *Ae. kotschyi* and self-compatible *S. cereale* were used to produce new F_1 hybrids and their amphiploids of *Ae. kotschyi* x *S. cereale* (Wojciechowska and Pudelska, 2002). One- and two-dimensional gel electrophoresis of enzymes of two accessions of *Ae. kotschyi* and their F_1 hybrids and amphiploids showed some differences (Kalinowski et al., 2003, 2004). On the basis of those previous results, amphiploids involving these two *Ae. kotschyi* accessions and the self-compatible *S. cereale* seem interesting subjects for morphological and molecular analysis of pollen, with the aim of making a more complete characterization of the amphiploids.

This paper presents morphological observations of mature pollen grains, and separately analyzes sporophytically and gametophytically produced pollen proteins of *Aegilops kotschyi* × *Secale cereale* amphiploids and their parents. Pollen coat and protoplast proteins were analyzed by two-dimensional gel electrophoresis.

MATERIALS AND METHODS

PLANT MATERIAL

The plant material consisted of parental forms of *Ae. kotschyi* (accessions 14805 and 14408, here called AK-2 and AK-3, respectively) and *S. cereale* S14, a self-compatible line, here called S14, as well as seven *Ae. kotschyi* × *S. cereale* amphiploid plants. Amphiploids 103A and 103B (*Ae. kotschyi* AK-2 × *S. cereale* S14), as well as 84C and 84D (*Ae. kotschyi* AK-3 × *S. cereale* S14) were produced by colchicine treatment of F_1 hybrids. Amphiploids 103/4 (*Ae. kotschyi* AK-2 × *S. cereale*), 84E and 84F (*Ae. kotschyi* AK-3 × *S. cereale* S14) were obtained by F_1 hybrid chromosome doubling via in vitro culture propagation (Wojciechowska and Pudelska, 2002). For protein analysis, pollen was collected in Eppendorf tubes from glasshouse-grown plants and kept at -70˚C. Fresh pollen was taken for microscopy.

POLLEN MORPHOLOGY

Measurements of fresh pollen grains on the polar (P) and equatorial (E) planes were made under a light microscope (1600×). For each parent and amphiploid, 30 pollen grains were randomly selected, a total of 300 pollen grains. Observations of sculpture were made with a scanning electron microscope (Philips FEN 515) before and after the pollen grains were coated with gold. The descriptive terminology used in this report follows Erdtman (1952) and Fægri and Iversen (1992).

TWO-DIMENSIONAL GEL ELECTROPHORESIS

Samples of 10 mg frozen pollen were shaken gently 3 times with 400 µl isotonic extraction buffer (0.7 M sucrose, 0.5 M TRIS, 30 mM HCl, 50 mM EDTA, 10 mM KCl, 2 mM PMSF, 13 mM DTT). After each stage of elution the pollen suspension was centrifuged at 300 rpm. The pollen samples were eluted for 30 min the first time and then twice for 10 min to remove vestigial parts of the pollen coat fraction. Each of the 3 fractions

TABLE 1. Measures of location and dispersion for pollen grains and their pores of *Aegilops kotschyi* AK-2 and AK-3, *Secale cereale* S14, 103-amphiploids (AK-2 × S14) and 84-amphiploids (AK-3 × S14)

Character	Calculated characteristic	AK-2	S14	103A	103B	103/4	AK-3	84C	84D	84E	84F
P[µm]	minimum	39.53	39.53	46.51	58.14	51.16	44.18	53.48	53.48	51.16	51.16
(long axis)	maximum	58.13	55.81	62.79	74.42	62.79	51.16	67.44	72.09	65.12	62.79
	mean	49.48	47.44	55.23	63.95	55.81	47.29	56.77	60.00	56.97	55.12
E[µm]	minimum	34.88	23.25	37.21	44.18	34.88	34.88	39.54	39.54	39.54	41.86
(short axis)	maximum	47.28	34.88	60.46	60.46	53.86	41.86	51.16	53.48	51.16	46.51
	mean	44.10	31.39	45.35	54.18	41.56	39.38	43.25	48.87	46.51	44.18
P/E	minimum	1.00	1.13	0.96	0.96	1.15	1.05	1.14	1.00	1.09	1.14
	maximum	1.29	2.20	1.58	1.40	1.66	1.37	1.61	1.55	1.40	1.38
	mean	1.22	1.54	1.22	1.18	1.36	1.20	1.43	1.24	1.23	1.25
Annulus width	minimum	4.05	2.70	3.87	4.34	3.61	3.48	3.16	2.53	3.97	3.15
[µm]	maximum	5.40	3.78	6.31	5.42	4.69	4.65	4.42	4.33	4.69	4.74
	mean	4.65	3.24	4.61	4.51	3.79	4.64	3.79	3.46	4.45	4.02
Pore diameter	minimum	5.60	3.48	3.49	3.61	6.00	4.08	5.68	4.69	4.76	3.47
[µm]	maximum	6.22	4.93	6.66	6.50	6.14	4.86	6.94	6.25	5.42	6.94
	mean	5.91	4.33	4.97	4.69	6.07	4.62	6.32	5.34	5.06	5.07

was shaken with 400 µl phenol solution in water, to ensure the concentration of proteins from all eluates of a given pollen sample in a small volume of phenol (Hurkman and Tanaka, 1986). Rinsed pollen was homogenized with 500 µl extraction buffer and the next steps were performed as above. The proteins from the phenol phase were precipitated in a solution of 0.1 M ammonium acetate in methanol, and after drying they were dissolved in 10 µl and 20 µl resolving buffer (9 M urea, 4% NONIDED NP-40, 2% DTT, 2% Servalyte, pH 2–4) for the pollen coat (*pc*) and protoplast (*pt*), respectively. Pollen coat protein elution, two-dimensional gel electrophoresis, protein staining and gel analysis were described in detail in Kalinowski et al. (2001). Each extraction procedure and two-dimensional gel electrophoresis (2-DE) was performed twice, except for 103A, done only once because this amphiploid produced little pollen. All the 2-D patterns were highly repeatable.

The peptides on the gels were compared in terms of their isoelectric points (pI) and molecular weights (MW) in kilodaltons. A Pharmacia 2-D calibration kit (17–0582–01) and Sigma Silver Stain SDS Molecular Weight Mixture (M–5630) were used to compare with the peptides after two-dimensional gel electrophoresis.

RESULTS

MORPHOLOGY OF POLLEN GRAINS

Pollen grains of *Ae. kotschyi* AK-2 were larger than those of AK-3; in the P plane their diameters were more similar than in the E plane. The range of diameters in both the P and E planes was greater in AK-2 than in AK-3. Pollen grain shapes (P/E) were variable for both maternal plants. In AK-2, 50% of the pollen were subprolate, 30% were prolate-spheroidal, and 20% were prolate. In AK-3, 70% were subprolate, 20% were prolate-spheroidal, and only 10% were prolate. The pollen of *S. cereale* S14 was smaller than that of *Ae. kotschyi*, especially in the E plane. The range of diameters was greater in the P than in the E plane. About 50% were prolate, 25% were subprolate, and 25% were prolate-spheroidal.

The 103-amphiploids produced larger pollen, and their range of diameters in both the P and the E planes was greater than that of the parents. The shapes (P/E) of colchiamphiploids 103A and 103B were more similar to the shape of AK-2. The shape of the slightly smaller pollen of 103/4 was intermediate between the parents. The range of diameters of the 103-amphiploids in the P plane was equal to or slightly less than that of the parents, whereas in the E plane it was greater than that of the parents.

Amphiploids 84C-F produced larger pollen than AK-3 and S14, but colchiamphiploids 84A and 84D produced slightly larger pollen than 84E and 84F obtained via in vitro culture. Among the 84-amphiploids, the range of P and E diameters indicated high variability in 84E pollen, lower variability in 84C and 84D pollen, and lowest variability in 84F pollen. The shapes (P/E) of 84C were slightly more similar to those of S14 than to those of AK-3, whereas those of 84D, 84E, and 84F were similar to those of AK-2.

The pores of *Ae. kotschyi* AK-2 were slightly larger than those of AK-3, surrounded by annuli identical in diameter. The variation of pore diameter was higher in

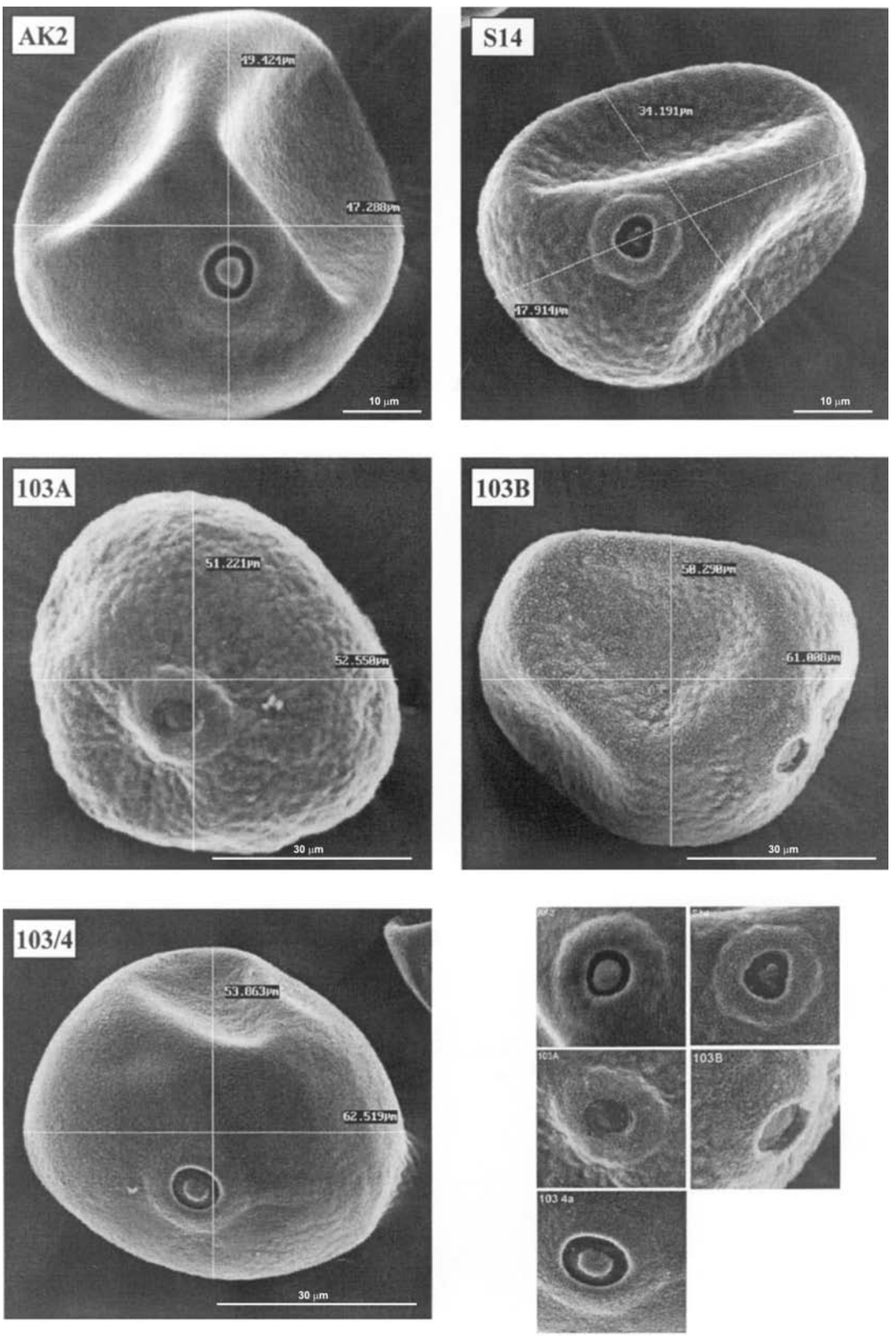

Fig. 1. Pollen grains and their pores from *Aegilops kotschyi* AK-2, *Secale cereale* S14, their colchiamphiploids 103A and 103B, and amphiploid 103/4 obtained via in vitro culture.

AK-3 than in AK-2 pollen, unlike annulus width, which varied more in AK-2 than in AK-3. Both pollen diameter and annulus width were distinctly less in *S. cereale* than in *Ae. kotschyi* accessions, but the range of variation of annulus diameter was lower in AK-2 and AK-3 than in S14. In contrast, the range of pore diameters in S14 was twice that of the maternal forms. Pore diameter in colchiamphiploids 103A and 103B was intermediate between that of the parents, whereas amphiploid 103/4 had larger pores surrounded by thinner annuli than in the parents. The pollen of amphiploid 103/4 varied less in pore and annulus diameter than that of 103A and 103B. Colchiamphiploid 84C had larger pores and colchiamphiploid 84D had only slightly larger pollen than the amphiploids obtained via in vitro culture (84E and 84F). The pores of the colchiamphiploids were surrounded by annuli thinner than in those obtained by doubling chromosomes via in vitro culture. Among the four 84-amphiploids, the highest variation of pore and annulus diameters was observed in 84F, and the lowest in 84E. Table 1 presents pollen diameters in the P and E planes, pollen shapes (P/E ratio), and pore and annulus diameters.

The exine surfaces of *Ae. kotschyi* AK-2 and AK-3, *S. cereale* S14 and the amphiploids differed in ornamentation (Fig. 1). The exine sculpture of AK-2 and AK-3 were delicately verrucate on both the operculum and the annulus, whereas in AK-3 the verrucate sculpture was additionally granulate. The exine ornamentation, the small operculum and the annulus of *S. cereale* were irregularly verrucate, with conspicuous granulation.

AK-2 × S14 amphiploids produced pollen with different exine sculpture and different pore traits. Pollen of colchiamphiploids 103A and 103B was more similar in sculpture to the paternal plant (S14), while that of 103/4 was more similar to the maternal pollen (AK-2). The 103A amphiploid pollen sculpture was coarsely verrucate, as was that of the annulus, which was prominent and had an irregular outer margin. The sculpture of 103B pollen was delicately verrucate and granulate, and its inconspicuous annulus had a regular margin. The sculpture of 103/4 pollen was slightly verrucate, and their pores were surrounded by slightly prominent, delicately verrucate, rather thin annuli.

The 84-amphiploids (AK-3 × S14) produced pollen varying in sculpture (Fig. 2), like the 103-amphiploids. In the 84C colchiamphiploid, the ornamentation of the exine surface, the annulus and the operculum was verrucate with marked granulation. In the second colchiamphiploid (84D) the pollen exine was delicately verrucate. The pores of 84C were surrounded by well developed and prominent annuli. In 84D the annulus was less prominent. Two amphiploids produced via in vitro culture (84E and 84F) produced pollen differing in exine sculpture. The exine sculpture of 84E was slightly verrucate and delicately granulate. In 84E, the annulus margin was slightly prominent and poorly defined, while in 84F it was undulate and clearly prominent. The exine sculpture of 84F was classified as coarsely verrucate and granulate. The structure of the surface of the annulus and operculum of the 84E and 84F amphiploids was the same as their exine ornamentation.

2-D PATTERNS OF POLLEN COAT AND PROTOPLAST PROTEINS OF PARENTS

The 2-D patterns of pollen coat and protoplast proteins showed quantitative and qualitative differentiation of the two accessions of *Ae. kotschyi* and the self-compatible *S. cereale*. The pollen coat and protoplast of AK-2 contained more peptides than the corresponding fractions of AK-3, and the peptides also differed. The pollen coat of AK-2 had 34 high molecular weight peptides with MW ranging from 33.0 to 48.5 kDa (at pH 6.2–8.0), six with MW 44.0–60.0 kDa (pH 4.9–5.5), and ten over 76.0 kDa (pH 5.4–6.8). The pollen coat of AK-3 had, for example, three peptides with MW 20.0–21.0 kDa (at pH 5.0–5.1), nine with MW 13.5–17.0 kDa (at pH 8.1–8.9) and five with MW 23.0–25.5 kDa (at pH 8.5–8.9). The protoplast of AK-2 had a group of eight peptides with MW lower than 9.0 kDa (pI at pH 6.1–7.6). Other groups of AK-2 protoplast peptides differing from those of the AK-3 protoplast were as follows: four with MW 14.2–17.0 kDa (at pH 4.9–5.0), four with MW 20.0–22.5 kDa (at pH 4.8–5.4), eight with MW 19.0–23.0 kDa (at pH 8.0–8.7), four with MW 15–17.5 kDa (at pH 6.4–6.8), and five with MW 33.0–49.0 kDa (at pH 8.4–8.8). The pollen protoplast of AK–3 had ten peptides with MW ranging from 41.0 to 47.0 kDa (at pH 5.3–6.2) and six with MW 60.0–68.0 kDa (at pH 5.5–6.1).

2-D PATTERNS OF POLLEN COAT PROTEINS OF AMPHIPLOIDS

The 2-D *pc* patterns of *Ae. kotschyi* AK-2 and *S. cereale* S14 showed 382 and 426 peptides, respectively. The synthetic pattern for the parents was composed of 477 spots. A total of 22 *pc* peptides (5.8%) of AK-2 and 46 (9.6%) of S14 were not detected in three 103-amphiploids. In the pollen coat of amphiploids 103A, 103B and 103/4, 438, 271 and 259 peptides were present, respectively, and 39, 24 and 37 peptides were new, that is, not on the synthetic 2-D diagram for the parents. Also, a number of parental pc peptides were absent in the amphiploids (Fig. 3). As can be seen in Figure 3, the 2-D pattern of 103A was more similar to the parental forms than were the other two 103-amphiploids. There was no correlation between the 2-D patterns and the method of amphiploid production. Of all the characteristic pc peptides of colchiamphiploids, four in 103A and two in 103B were low-molecular.

Two-dimensional analysis of the *pc* protein pattern of *Ae. kotschyi* AK-3 showed 278 peptides, includ-

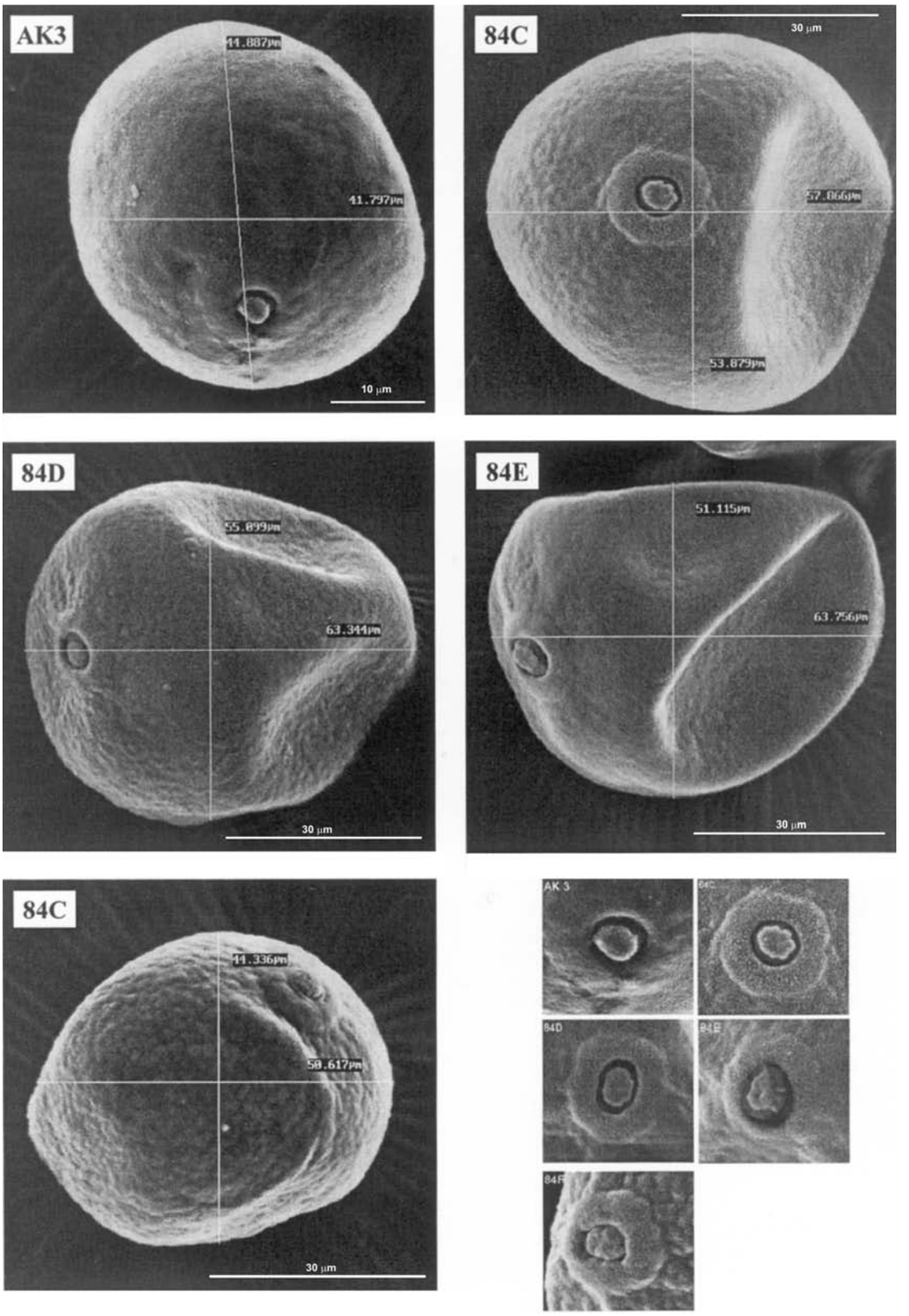

Fig. 2. Pollen grains and their pores from *Aegilops kotschyi* AK-3, and their amphiploids (AK-3 × S14): colchiamphiploids 84C and 84D, and amphiploids 84E and 84F obtained via in vitro culture.

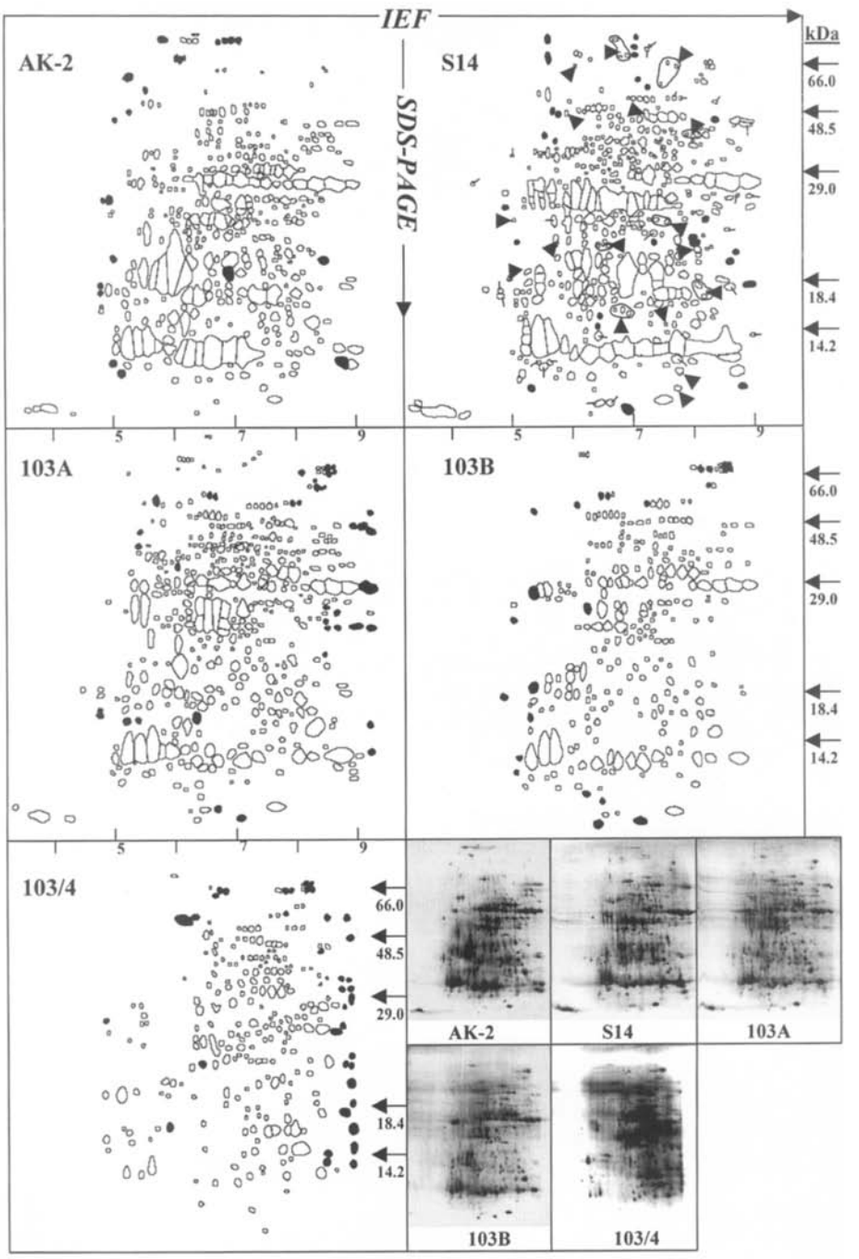

Fig. 3. 2-D protein patterns of pollen coat from *Aegilops kotschyi* AK-2, *Secale cereale* S14, colchiamphiploids 103A and 103B, and 103/4 obtained via in vitro culture. Filled circles in AK-2 mark peptides absent in 103-amphiploids; filled circles in S14 mark peptides undetected in all 103- and 84-amphiploids; filled circles mark new amphiploid peptides lacking in the parents; arrows mark S14 peptides undetected in 103-amphiploids; strokes mark peptides of S14 undetected in 84-amphiploids.

ing 12 (4.3%) not detected in 84C-F. As many as 44 (10.1%) of *S. cereale* S14b peptides were absent in these amphiploids. The synthetic 2-D pattern for *pc* of the parents (AK-2 and S14) was composed of 428 spots. Numerous parental peptides were not detected in 84C-F amphiploids; however, characteristic ones, which were undetected in the parental *pc*, were detected here (Fig. 4). In the *pc* of 84C and 84D colchiamphiploids, 355 and 414 peptides were detected, respectively. The pollen coat of 84C had 14 (3.9%) and that of 84 D had 27

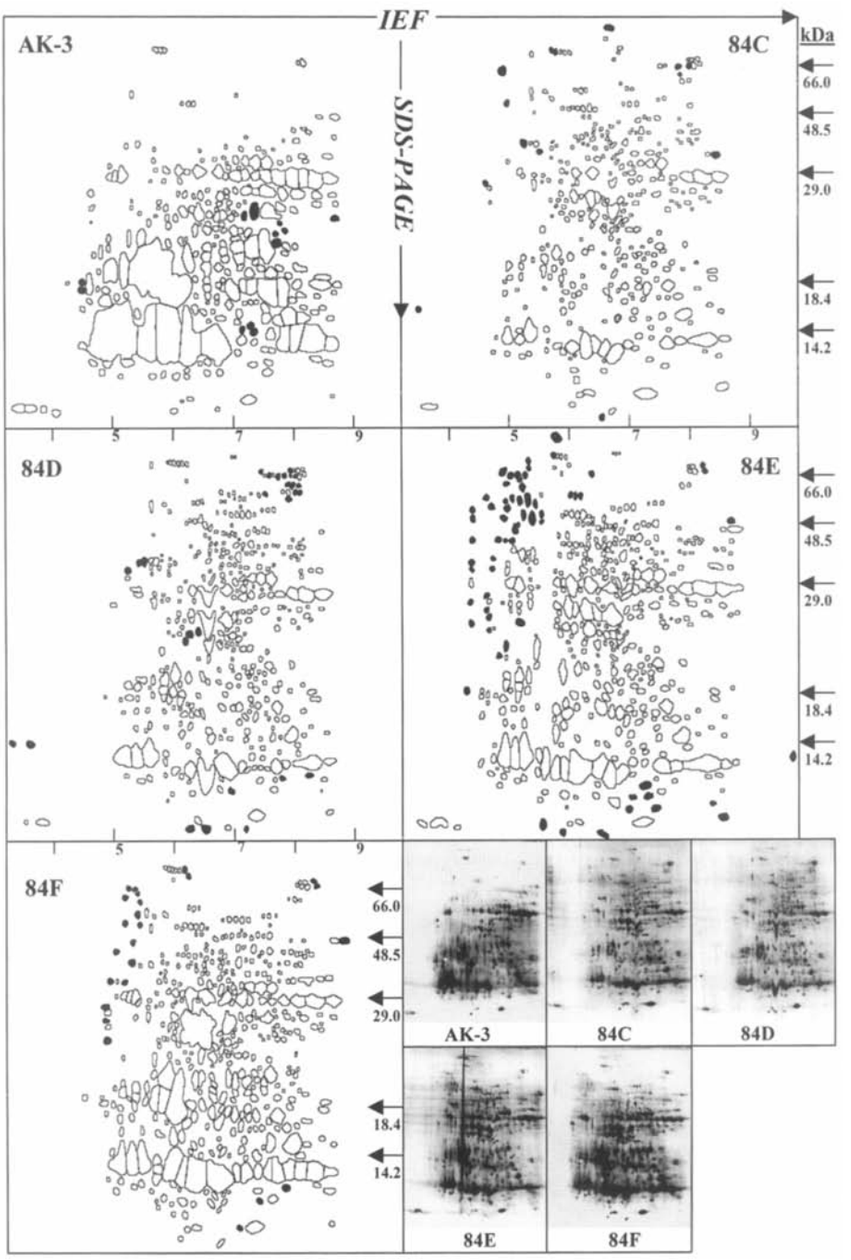

Fig. 4. 2-D protein patterns of pollen coat from *Aegilops kotschyi* AK-3, colchiamphiploids 84C and 84D, and 84E nd 84F obtained via in vitro culture. Filled circles in AK-2 mark peptides undetected in 84-amphiploids; filled circles in amphiploids mark peptides undetected in the parents.

(6.5%) new peptides, when compared to the parents. The pollen coat of the 84E amphiploid had 476 peptides and 63 (13.2%) of them were not detected in the *pc* of the parents. The second amphiploid 84F had 437 peptides and 24 (5.4%) of them were new compared to the *pc* of the parents. The colchicine and callus-derived amphiploids had a number of characteristic peptides separated at the pH 4.4–6.3 ranges. The other characteristic *pc* peptides of 84-amphiploids were not dependent on the method of amphiploid production. The 2-D patterns of 84-amphiploids are shown in Figure 4, where characteristic peptides are marked.

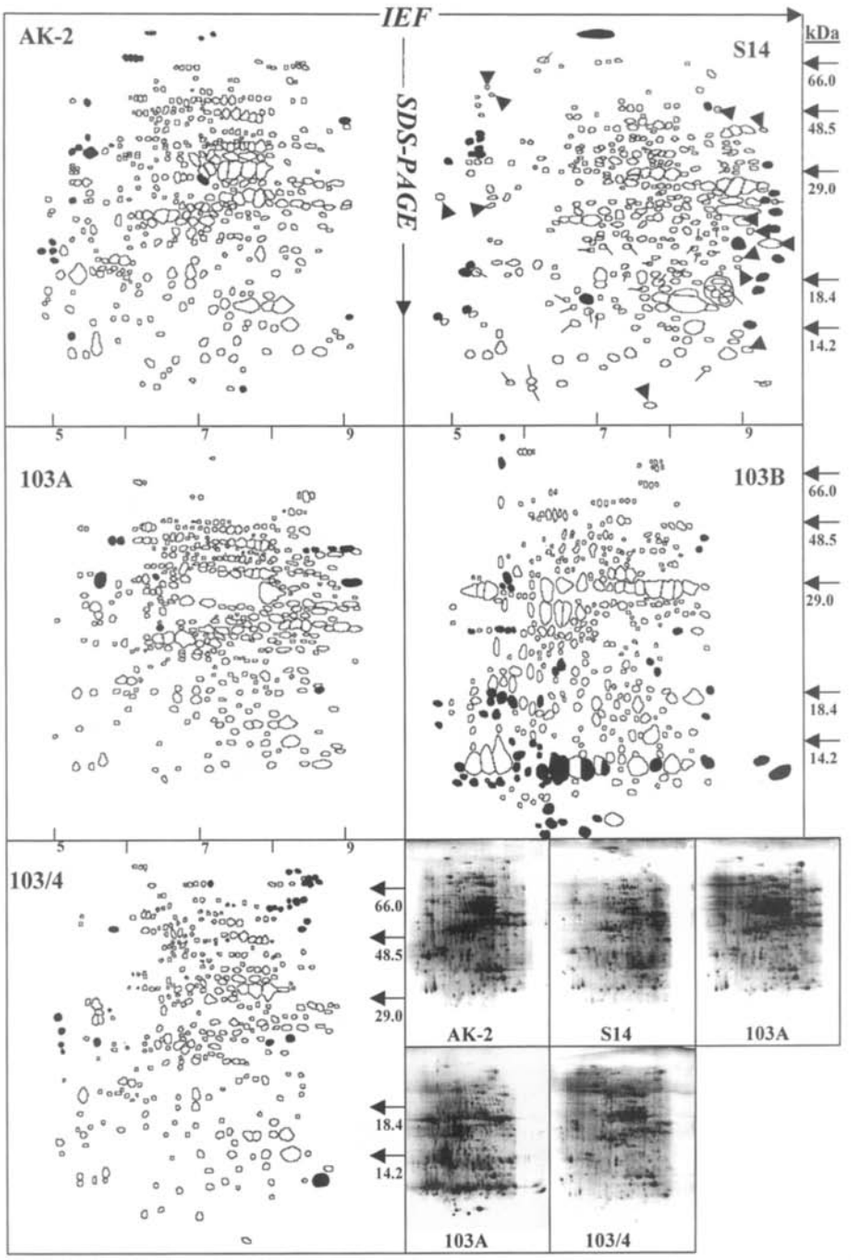

Fig. 5. 2-D protein patterns of pollen protoplast from *Aegilops kotschyi* AK-2, *Secale cereale* S14, and colchiamphiploids 103A and 103B, and 103/4 obtained via in vitro culture. Filled circles in AK-2 mark peptides absent in 103-amphiploids; filled circles in S14 mark peptides undetected in all 103- and 84-amphiploids; filled circles mark new amphiploid peptides lacking in the parents; arrows mark S14 peptides undetected in 103-amphiploids; strokes mark S14 peptides undetected in 84-amphiploids.

2-D PATTERNS OF POLLEN PROTOPLAST PROTEINS OF AMPHIPLOIDS

The 2-D patterns of the protoplasts of *Ae. kotschyi* AK–2 and *S. cereale* S14 showed 402 and 348 peptides, respectively. The synthetic diagram for the parents was composed of 494 spots. A total 22 (5.5%) of the *pt* peptides of AK-2 were lacking in the 103-amphiploids. Some of the protoplast peptides (36 peptides, 10.3%) of S14 were not detected in these amphiploids and, as can

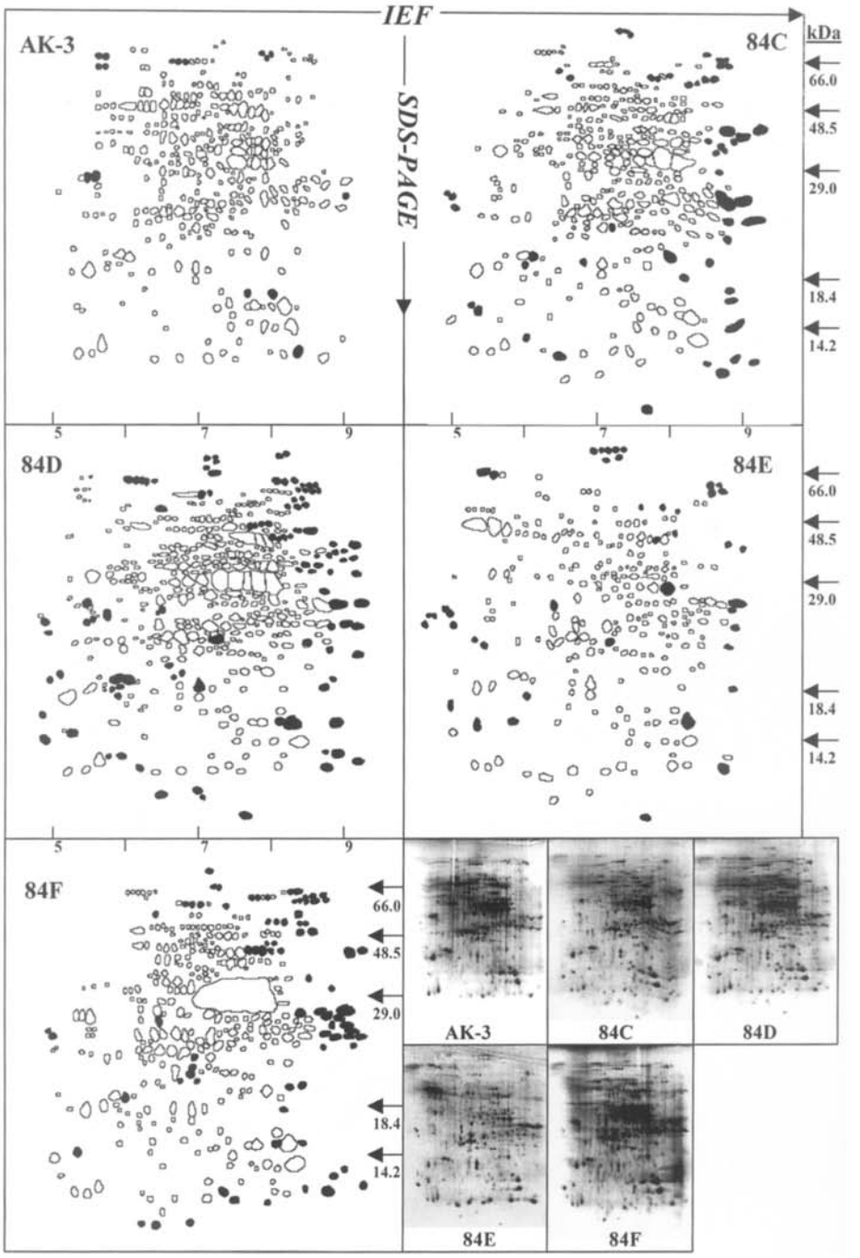

Fig. 6. 2-D protein patterns of pollen protoplast from *Aegilops kotschyi* AK-3, colchiamphiploids 84C and 84D, and 84E and 84F obtained via in vitro culture. Filled circles in AK-3 mark peptides undetected in 84-amphiploids; filled circles in amphiploids mark peptides undetected in the parents.

be seen in Figure 5, these peptides separated at low and high pH. The numbers of *pt* peptides for 103-amphiploids were approximately similar: 387 for 103A, 398 for 103B, and 350 for 103/4. However, the 2-D patterns of these amphiploids were qualitatively different, as shown in Figure 5. A number of parental peptides were lacking in 103-amphiploids, and some new ones were detected. The 103A protoplast had 11 (2.8%), 103B had 59 (14.8%) and 103/4 had 25 (7.1%) peptides not detected in the parents. Many new peptides in the *pt* of 103B had MW lower than 14.2 kDa, and their pIs were in a wide pH range.

Among the 340 *pt* peptides of AK-3, 16 (4.7%) and 44 (12.6%) were not detected in the protoplasts of 84-amphiploids. The peptides of 84-amphiploids differed both quantitatively and qualitatively from those seen on the 2-D synthetic diagram of the parents, composed of 466 spots (Fig. 6). These differences were not correlated with the method of amphiploid production. The 84-amphiploids had more new peptides than the 103-amphiploids. Of the 358 *pt* peptides of 84C, 55 (15.4%) were present in only in 84C. The highest number of peptides in 84-amphiploids were visualized in 84D; of its total 448, 92 (20.5%) were new. The lowest number of peptides (275) were detected in 84E, and 39 (14.2%) of them were lacking in the parents. Amphiploid 84F had the same number of peptides as in the *pt* of 84C (358), but their 2-D patterns differed in quality. In the 84F *pt*, 78 (21.8%) were lacking in the *pt* of the parents. Many new peptides of amphiploids were separated as groups of peptides, meaning that they were separated at a narrow pH range and their MW were in narrow ranges.

DISCUSSION

Microscope observations showed that pollen grain morphology varied in the studied group of amphiploids, and did not depend on the method of amphiploid production. The variability could be the result of the action or interaction of genes in the UUSS and RR genomes of *Ae. kotschyi* and *S. cereale*, respectively.

The shapes of *Ae. kotschyi* × *S.cereale* amphiploid pollen grains were intermediate between the shapes of the parent pollen. In an earlier study, the pollen grain surface was moderately and irregularly folded, and all pollen grains had two pores (Kalinowski et al., 2001). In that study, different accessions of *Ae. kotschyi* and self-incompatible *S. cereale* were used for amphiploid production. In this paper the maternal plants were two other accessions of *Ae. kotschyi*, and the paternal plant was self-compatible *S. cereale*. All amphiploids in the present study produced pollen grains with one pore only, probably because the higher homozygosity of self-compatible than self-incompatible rye may result in the formation of single-pore pollen grains in amphiploids. Different ornamentation of the amphiploid exine was observed, intermediate or more or less similar to that of their parents. The tapetal cells secrete sporopolenin precursors for exine formation, and the specific pollen wall patterns, determined by the sporophyte, do not segregate in the F_1 of the cross between *Lycopersicon esculentum* and *Solanum pennelli* (Quiros, 1975); thus our results are convergent. The different ornamentation of the exine in amphiploids obtained from the same parent probably is a result of their genetic instability. Each genetically unstable amphiploid may be able to produce different patterns of exine, mainly intermediate between the parental patterns. There was no relationship between the method of amphiploid production and the ornamentation of the exine.

Pollen size is determined by both the sporophytic and gametophytic genotypes. Irrespective of the *Ae. kotschyi* accession and the method of amphiploid production, all amphiploids possessed larger pollen than their parents. After chromosome doubling of the F_1 hybrid (USR), the amphiploid possesses a UUSSRR genome and each of the sets is homozygous. The additive activity of sporophytic and gametophytic genes in *Ae. kotschyi* and *S. cereale* makes the pollen grains of amphiploids larger than those of their parents.

Pollen protein of *Aegilops* sp. × *S. cereale* amphiploids was analyzed earlier (Kalinowski et al., 2001; Kalinowski and Wojciechowska, 2003); sporophytically and gametophytically produced peptides were analyzed together, and interactions between pollen coat and protoplast proteins may have occurred during extraction and been detected after electrophoresis. Also, post-translational modification of proteins (Zannis and Breslow, 1981), for example glycosylation or phosphorylation, may change the isoelectric points of proteins, finally changing the 2-D patterns. When proteins from the pollen coat and the protoplast were analyzed together (Kalinowski et al., 2001), lower numbers of peptides were separated using the same procedure of extraction and the same 2-DE method as in this study. This suggests that the number of pollen peptides separated together by 2-DE electrophoresis is not an arithmetic sum of the pollen coat and protoplast peptides. Thus, it seems reasonable to analyze pollen coat and protoplast proteins separately.

In 2-DE analysis of five enzymes from leaves of the same material as in this study, some parental isozymes were not detected in amphiploids, and some isozymes present in amphiploids were lacking in the parents (Kalinowski and Wojciechowska, 2004). Similar results were obtained in this analysis. Compared to the 2-D patterns of the parents (synthetic 2-D diagrams), both the pollen coat and protoplast of amphiploids had fewer peptides. Because both parental genomes are heterozygous, amphiploids should be regarded as sums of the doubled haploid genomes; thus, fewer peptides are synthesized. Somaclonal variation initiated in vitro (Tonelli, 1990) and colchicine acting as a mutagen (Pickett-Heaps, 1967) cannot be ruled out here.

This study also showed that numerous peptides detected in the parents were lacking in the pollen coat and protoplast of the amphiploids. This may be explained as a result of gene suppression (Galili and Feldman, 1984). It is probable that homologous genes of *Ae. kotschyi* control suppressors inhibiting some structural genes of the haploid genome of *S. cereale*, and vice versa. Thus, the lack of many parental peptides in amphiploids suggests a high level of suppres-

sion in amphiploids. In newly formed allopolyploids obtained from crossing *Aegilops* and *Triticum*, Ozkan et al. (2001) observed rapid elimination of chromosomes and genome-specific sequences in allopolyploids, and initiation of these processes in F_1 plants. Also, the formation of wheat amphiploids may be associated with the elimination of some loci (Shaked et al., 2001).

New peptides found in amphiploids may be a result of silent gene activation or of neutralization of suppression. The appearance of new peptides in amphiploids may also be an effect of inducing changes in the cytoplasm of doubling F_1 hybrid chromosomes (Bahrman et al., 1988). Detection of new peptides in amphiploids may also be a result of recombination between different alleles.

Variation in the number of amphiploid peptides has been attributed to the effects of regulators controlling the expression of structural genes (Colas des Francs and Thiellement 1985; Thiellement et al., 1986; Zivy et al., 1992). A relationship between those genes and the intensity of spots was observed in *Triticum durum* × *T. tauschii* amphiploids (Aragoncillo et al., 1978). Our results were obtained for amphiploids formed after crossing species from two genera, so each gene in the pollen protoplast was represented by a single allele and in the pollen coat by the same two alleles. Thus, some parental peptides as heteromers may not be produced, or may be produced in amounts insufficient for detection. In this study, different 2-D spectra of peptides were documented for the same fraction, that is, the pollen coat of amphiploids obtained after crossing the same parents by the same modality of doubling F_1 hybrid chromosomes. The results showed no relationship between the protein spectra of the parents and the method of amphiploid production. It seems that the regulators of gene action are random, and involve somaclonal variability, the mutagenic activity of colchicine, and the activity of genes of different genomes. It may also be a result of genetic instability of the amphiploids. Chromosomal aberrations, in particular translocation, may cause changes in the 2-D patterns of amphiploids. Translocations are common in rye, wheat and triticale (Lukaszewski and Gustafson , 1983; Figureias et al., 1984; Marais and Marais, 1994).

The amphiploid peptide patterns of both the pollen coat and protoplast fractions were more similar to the maternal than to the paternal species. This reflects the important influence of the maternal cytoplasm on amphiploid pollen proteins.

Differences in the size and morphology of amphiploid pollen were not dependent on the method of their production. Nor was there any correlation between the method of amphiploid production and the 2-D patterns of 84-amphiploids. Thus, the pollen morphology findings converged with the results of 2-DE analysis.

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