Pollen and seed morphology of diploids and natural tetraploids of Trifolium pratense L. (Leguminosae)

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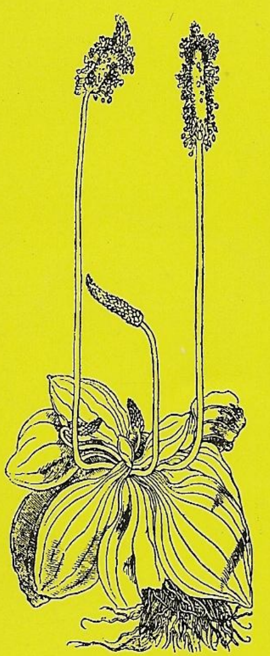

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Pollen and Seed Morphology of Diploids and Natural Tetraploids of *Trifolium pratense* L. (Leguminosae)

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Pollen and seed morphology were examined in diploids (2n = 14) and natural tetraploids (2n = 28) of *Trifolium pratense* L. The pollen morphology was generally correlated with ploidy level. Great variety in pollen size and apertures was noted in the tetraploid form. Exine structure was studied by TEM. Seed size, color and coat surface ornamentation can be distinguished between the diploid and tetraploid forms.

Key words: *Trifolium pratense* L., Leguminosae, pollen, seed.

INTRODUCTION

Red clover (*T. pratense* L.) is one of the most important forage legumes grown in many areas of the world. Tetraploid *T. pratense* L., which is grown naturally, is a feed plant of great economic value due to its production of seeds in large numbers and also its high protein content.

The natural tetraploid *T. pratense* L. is generally superior to the diploid forms in yield, disease resistance and persistence. However, it yields a very small amount of seed. It is very suitable for growing in large fields and can be used as animal feed. Its seed coat development (Algan and Bakar, 2000) has been investigated in detail.

The pollen morphology of *T. pratense* has received little attention since the light microscopy study by Najceuska and Speckmann (1968).

The present report describes the pollen and seed morphology of diploids and natural tetraploids of *T. pratense* L. using light (LM), transmission (TEM) and scanning electron (SEM) microscopy.

MATERIALS AND METHODS

Plants of natural tetraploids of *T. pratense* L. type E2 (2n = 28) were collected in 1982 by Elçi from the Tortum region of Erzurum (Turkey). Diploids of *T. pratense* (2n = 14) were collected in Ankara (Turkey).

Pollen slides were prepared using the technique of Wodehouse (1935). LM studies were made using a Leitz-Wetzlar microscope. Measurements were based on 20 or more pollen grains per population. Ten seeds from each plant (total 20 seeds) were measured for length and width under a binocular lens to the nearest 0.1 mm. In addition, ten seeds from each plant were pooled and weighed. For SEM studies, pollen grains and seeds were coated with gold for 4 min in a sputter-coater. For TEM studies, non-acetolyzed pollen grains were fixed in OsO_4, stained with uranyl acetate and embedded in araldite (Blackmore and Barnes, 1984). Ultrathin sections were poststained with lead citrate and uranyl acetate. A Jeol 100 CX11 electron microscope was employed for both TEM and SEM studies.

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TABLE 1. Pollen morphology of diploid and natural tetraploid *Trifolium pratense*. Values in μm

	Polar axes (P)			Equatorial axes (E)				Exine				Colpus		Por	
Cytotype	min	max	mean	min	max	mean	P/E ratio and shape	Thickness	Surface	No. of lumina/cm^2	Intine	Length	Width	Length	Width
Diploid (2n=2x=14)	33.2	36.4	35.4	26.0	32.2	30.2	1.17 perprolate	1.13	Suprareticulate, lumina angular interspersed with elongated lumina	6	0.43	26	4.2	9	5.2
Tetraploid (2n=4x=28)	25.0	39.5	35.2	23.9	37.4	30.1	1.17 perprolate	1.19	Suprareticulate, lumina angular interspersed with elongated lumina	10	0.30	27	4.2	12.9	9.1

TABLE 2. Seed morphology of diploid and natural tetraploid *Trifolium pratense*. Seed length and width (mm) are based on mean values; seed weight (gram per seed) is based on weight of 10 seeds from each form

	Length			Width			Length/Width	Weight				
Cytotype	min	max	mean	min	max	mean	mean	min	max	mean	Outline	Color
Diploid (2n=2x=14)	2.00	2.50	2.2	1.25	1.5	1.4	1.57	0.0001	0.0015	0.0010	narrowly elliptic	yellow
Tetraploid (2n=4x=28)	1.25	1.75	1.4	–	–	1	1.4	0.0001	0.0010	0.0003	narrowly elliptic	dark brown

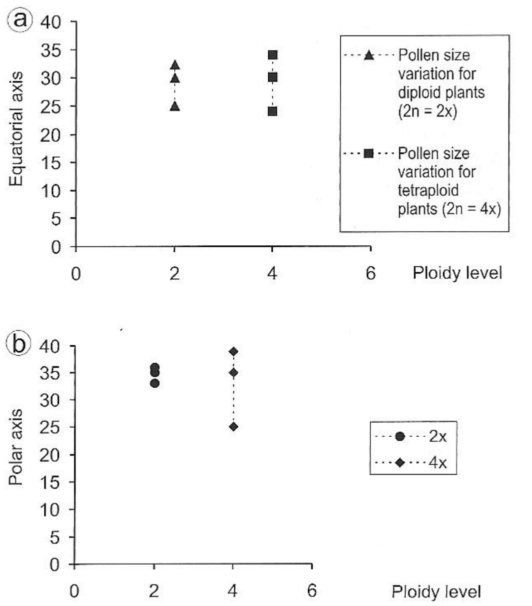

Fig. 1. Minima, maxima and means of polar and equatorial axes of pollen of diploid and tetraploid *Trifolium pratense*.

Terminology follows that of Faegri and Iversen (1975), Ghazali and Krzywinski (1989) and Punt et al. (1994).

RESULTS

POLLEN MORPHOLOGY

Pollen size

The size of pollen grains (polar axis × equatorial axis) of *T. pratense* in Turkey ranges from 35.4 μm (33.2–36.4 μm) × 30.2 μm (26–32.2 μm) in the diploid plant to 35.2 μm (25–39.5 μm) × 30.1 μm (23.9–37.4 μm) in the tetraploid plant (Fig. 1a–b). The diploid plant and the tetraploid plant have pollen grains similar in mean size (Tab. 1).

Symmetry and shape

The pollen grains of the diploid form are radially symmetrical and isopolar. The tetraploid form sometimes shows asymmetry. The ratio of P (length of the polar axis) to E (equatorial axis) is 1.17 (subprolate in shape). The outline is elliptic in equatorial optical section and circular in meridional optical section (Fig. 2).

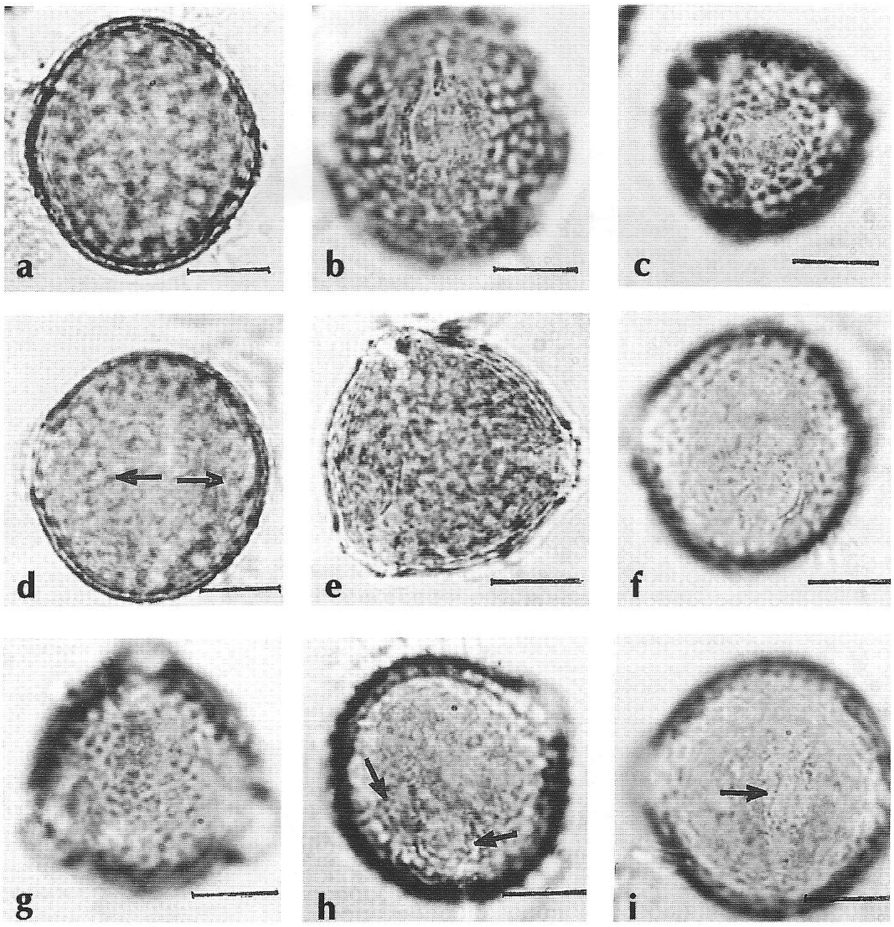

Fig. 2. Pollen morphology of *Trifolium pratense* (LM). (**a–c**) Diploid form. (**a**) Equatorial view, (**b**) Aperture in equatorial view, (**c**) Ornamentation of polar view. (**d–i**) Tetraploid form. (**d**) Pantotricolporate (arrows) in equatorial view, (**e**) Polar view, (**f**) Zonotricolporate equatorial view, (**g**) Ornamentation of polar view, (**h**) Diporate (arrows) in equatorial view, (**i**) Colpate (arrows) in equatorial view. Bar = 10 μm.

Pollen aperture

Pollen grains in the diploid form are zonotricolporate. In the tetraploid form they are generally zonotricolporate, and rarely pantotricolporate and tetracolporate (4%) with lalongate ora. The ora are elliptic (Fig. 2e,h). The longest and the widest ora were observed in the tetraploid form. In addition, the tetraploid form has shown heterocolporate characteristics. Pollen grains have three types of apertures: (1) colpate pollen (Fig. 2i), (2) colporate pollen (Fig. 2e), and (3) colpodiporate pollen (Fig. 2h). The apocolpium is 16.6–20 mm (Fig. 2c,f,g).

Exine

Exine sculpturing is suprareticulate under SEM and psilate near the margins of the aperture (Figs. 3a, 4a,b). The number of lumina per cm^2 ranges from 6 to 10. Other prominent features include these: exine thickness of 1.13–1.19 μm; tectum 0.54–0.55 μm thick, pertectate; columellae 0.3–0.4 μm high; foot layer thin, 0.1–0.2 μm; endexine thin, 0.075–0.15 μm, continuous (Figs. 3b–c, 4c). Intine thickness is 0.3–0.43 μm; intine thickens greatly under the aperture (Fig. 3b).

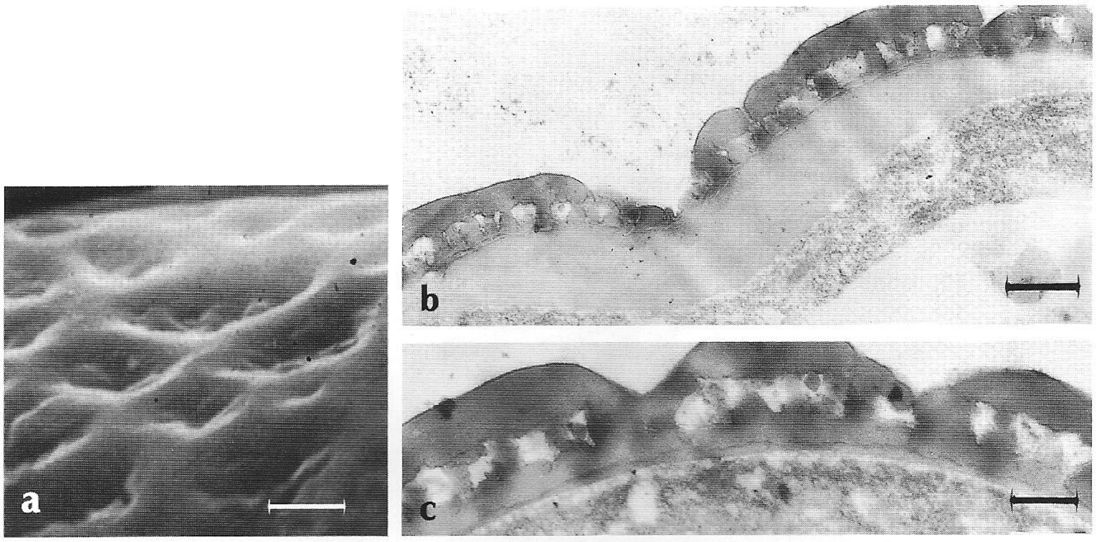

Fig. 3. SEM and TEM of pollen grain of diploid *Trifolium pratense*. (**a**) SEM showing suprareticulate exine ornamentation. Bar = 1 μm, (**b**) TEM showing exine structure in apertural zone. Bar = 1 μm, (**c**) TEM showing exine structure. Bar = 0.6 μm.

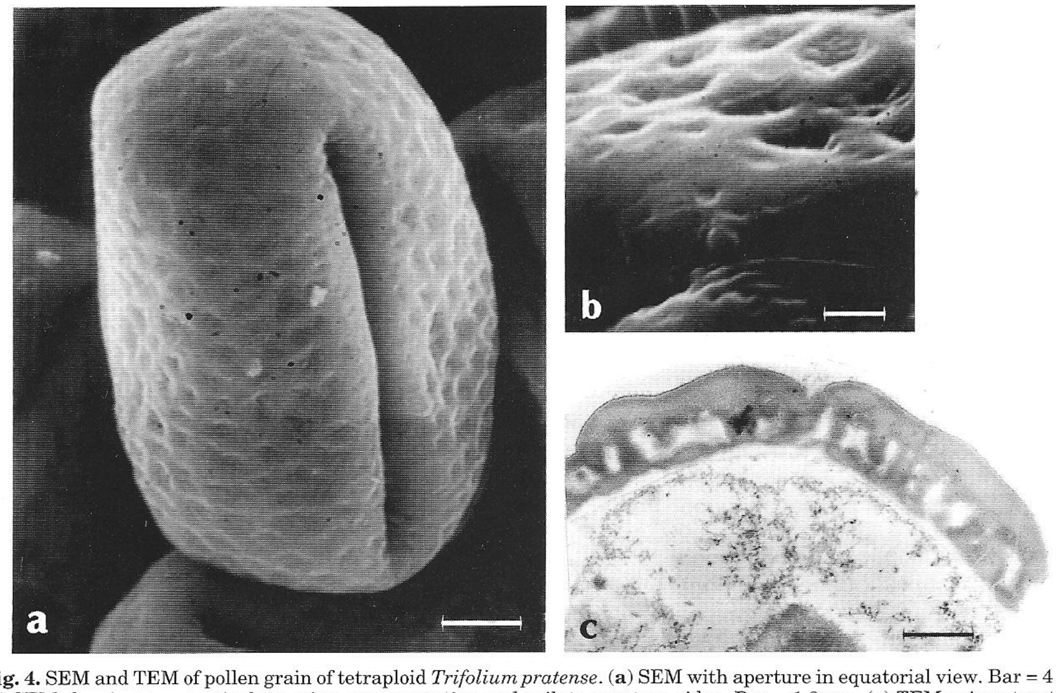

Fig. 4. SEM and TEM of pollen grain of tetraploid *Trifolium pratense*. (**a**) SEM with aperture in equatorial view. Bar = 4 μm, (**b**) SEM showing suprareticulate exine ornamentation and psilate aperture ridge. Bar = 1.2 μm, (**c**) TEM exine structure. Bar = 1 μm.

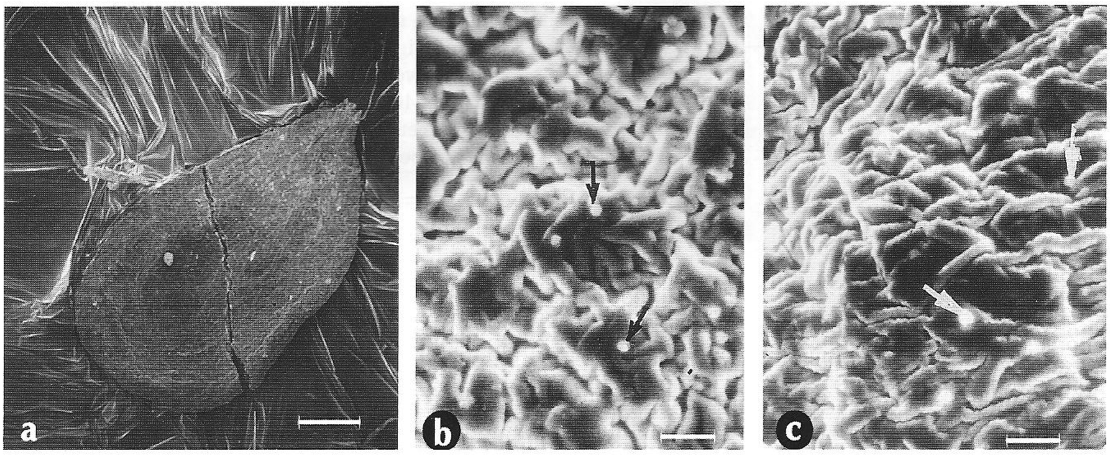

Fig. 5. SEM of seeds of diploid and tetraploid *Trifolium pratense* plants. (**a**) Seed morphology of tetraploid form. Bar = 0.2 μm, (**b**) Seed coat ornamentation of diploid form (arrows). Bar = 0.02 μm, (**c**) Seed coat ornamentation of tetraploid form (arrows). Bar = 0.02 μm.

SEED MORPHOLOGY

Seed macromorphology

Most of the variation in seed size and weight was found among diploid and tetraploid forms (Tab. 2). Seed weight was correlated with seed width and length (Tab. 2). The largest and heaviest seeds occur in the diploid form: average 2.3 mm in length, 1.4 mm in width, and weight 0.001 g (Fig. 6). The seeds are smaller in the tetraploid form: 1.4 mm in length, 1 mm in width, and weight 0.0003 g (Tab. 2; Fig. 6). In both of them the seed outlines are narrowly elliptic. The seeds of the diploid form are yellow, whereas they are dark brown in the tetraploid form. In the tetraploid form the seed coat is darker in color as it contains an ample amount of tannin.

Seed micromorphology (SEM)

The seed coat surface is rough and shows rugulate-granulate sculpture, with 5–6 granules per cm^2 in the diploid form. The seed coat surface of the tetraploid form is rugulate-striate-granulate, with 1–2 granules per cm^2 (Fig. 5).

DISCUSSION

Pollen and seed morphology are important characters in the diploid and tetraploid forms of *Trifolium pratense*. Brochmann (1992) reports that pollen grain size is strongly correlated with ploidy level, but the study presented here does not confirm such a correlation. The mean values for P and E are similar in the diploid and tetraploid forms: P = 35.5, E = 30.1 and P = 35.4, E = 30.2, respectively. However, greater variation in the maximum and minimum values occurs in the tetraploid form: P = 25–39.5 and E = 23.9–37.4 (Tab. 1; Fig. 1). Chaturvedi et al. (1990) reported significant variation of pollen grain size in triploids, tetraploids and hexaploids of *Arachis* L. (Leguminosae). In all three cases the pollen grains are perprolate in shape.

Brochmann (1992) stated that seed size generally correlates well with chromosome number. We observed that the tetraploid form has smaller seeds than does the diploid form. Seed color distinguishes diploid from tetraploid forms (Tab. 2).

According to Chaturvedi et al. (1990), the shapes of the lumina in the reticulum are of basic significance. Both tetraploid and diploid forms have suprareticulate ornamentation, angular lumina interspersed with elongated lumina. The number of lumina per cm^2 is 6 in the diploid form and 10 in the tetraploid form.

SEM observations indicated that seed coat sculpture may be used to discriminate between diploids and tetraploids of *T. pratense*. Diploid forms showed rugulate-striate-granulate sculpture (Fig. 5).

Chaturvedi et al. (1990) reported that the effect of hybridization is well pronounced in triploids and hexaploids as to types of apertures and their orientations. Najceuska and Speckmann (1968) also indicated that the pollen grains of tetraploid *Trifolium*

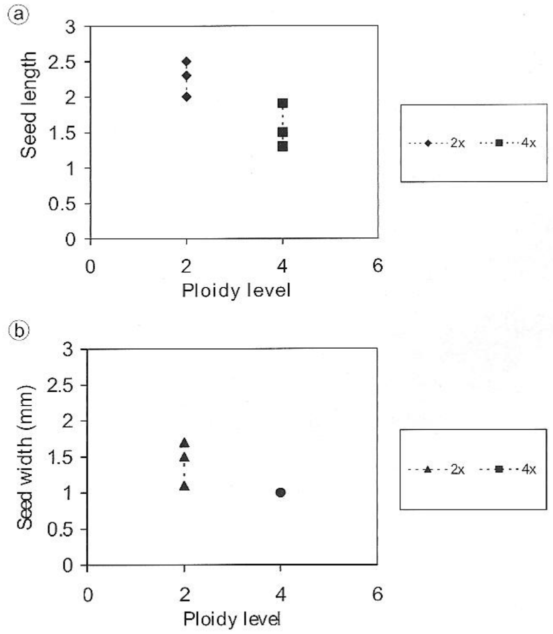

Fig. 6. Correlation of seed size with level of ploidy. Seed length and width (mm) are based on 10 seeds per plant.

sp. mostly have more than three germinal pores. We observed that pollen grains of the tetraploid form may be trizonocolporate, tetrazonocolporate, tripantocolporate and heterocolpate, whereas those of the diploid form are only trizonocolporate (Fig. 2). Our TEM investigations showed exine structure to be similar between the tetraploid and diploid forms (Figs. 3b–c, 4c).

Apparently there are specific morphological changes in pollen grains and seeds that result from ploidy level.

LITERATURE

ALGAN G, and BAKAR BÜYÜKKARTAL HN. 2000. Ultrastructure of seed coat development in the natural tetraploid *Trifolium pratense* L. *Journal of Agronomy and Crop Science* 184: 205-213.

BLACKMORE S, and BARNES SH. 1984. Transmission electron microscope preparations from unacetolysed herbarium pollen: A comparison of methods. *Pollen et Spores* 26: 137- 146

BROCHMANN C. 1992. Pollen and seed morphology of Nordic *Draba* (Brassicaceae) phylogenetic and ecological implications. *Nordic Journal of Botany* 12: 657-673.

CHATURVEDI M, YUNUS D, and NAIR PK. 1990. Cytopalynological studies of *Arachis* L. (Leguminosae). Cultivated and wild species and their hybrids. *Grana* 29: 109-117.

FAEGRI K, and IVERSEN J. 1975. *Textbook of pollen analysis*. Hafner Press, New York.

GHAZALI GEBE, and KRZYWINSKI K. 1989. An attempt to clarify the term heterocolpate. *Grana* 28:179-186.

NAJCEUSKA CM, and SPECMANN GJ. 1968. Number of chloroplasts and pollen grain pores in diploid and tetraploid varieties of some *Trifolium* species. *Euphytica* 17: 357-62.

PUNT W, BLACKMORE S, NILSSON S, and LE THOMAS A. 1994. *Glossary of pollen and spore terminology*. LPP Foundation, Utrecht.

WODEHOUSE RP. 1935. *Pollen grains*. Mc Grow-Hill, New York.