Morphological anomalies in pea (*Pisum sativum* L. cv. Hamil.) pollen grains under high doses of zinc

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Abstract

The morphology of pea (*Pisum sativum* L. cv. Hamil.) pollen grains was studied after adding zinc to the substrate in doses of: 0, 1, 500 and 1000 mg/pot. The observations were carried out using both light and scanning electron microscopy. The largest pollen grains were found at a dose of 500 mg/pot. Approximately 10% of the grains with such abnormalities in sporoderm structure as various types of swellings, mostly ring-shaped, were found under high concentrations of Zn (500 and 1000 mg/pot). The greatest number of deformed grains (over 40%), deeply plicated or strongly flattened, were observed at the dose of 1000 mg Zn. All of the described deformations related to excess Zn are documented photographically.

Key words: pollen grain morphology, anomalies, *Pisum sativum* L. cv. Hamil., zinc

INTRODUCTION

Many studies have described di- and polymorphism of pollen grains in numerous plant species. Differences in the morphology of pollen grains coming from the same anther were analyzed mainly in terms of the size, shape, number of apertures, sporoderm thickness and exine ornamentation of the grains. A distinct and frequently encountered sign of polymorphism is variation in the size of pollen grains, reported among others by: Belkina (1972) in several species from the family of *Lamiaceae*, Ater (1985) in *Phragmites* and Sawickij et al. (1987) in several *Rosa* species. Along with differences in size, difference in the number of apertures occurred in *Thymus* (Belkina 1972), in representatives of the genus *Sideritis* (Carrion and Alcaraz 1988) and in *Fraxinus* and *Rumex* (Till et al. 1989). Differentiation of grain size and exine ornamentation

was noticed in several species from the genus *Erythrina* (Hemsley and Ferguson 1985) and in *Iris chamaeris* (Till et al. 1989).

It is still not satisfactorily explained if the morphological variability of pollen has an exclusively genetic basis or if it may be dependent on other factors.

Studies of the morphology of pea pollen grains were aimed at checking if high doses of zinc in the substrate could result in disturbances in the formation of pollen grains. Attention was paid to the size and shape of these grains, the position of apertures, exine sculpture and sporoderm thickness.

MATERIAL AND METHODS

The study was conducted on pea (*Pisum sativum* L.) cv. Hamil pollen. The plants were cultured in Mitscherlich vases in quartz sand, 7,6 kg per vase. After the plants germinated, zinc was introduced into the substrate in the form of a solution of $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$ in the following amounts: 0, 1, 500 and 1000 mg per vase. A detailed description of the substrate and fertilization used can be found in the paper by Łyszcz and Ruszkowska (in press).

Pea flowers were fixed in 70% ethanol, after which slides of pollen in glycerin-gelatin were made. They were used for observations and measurements under a light microscope. The viability of pollen from fresh anthers was studied using Belling's solution.

Preparations for scanning electron microscopy (SEM) were made by first dehydrating the material in an alcohol series. Dry preparations from stamens were coated with gold using a CS 100 Sputter Coater. Observations on pollen morphology were made using a BS-300 Tesla Scanning Electron Microscope.

The mean size of pollen grain was calculated on the basis of the measurements of 300 grains from 4 different plants from each dose of zinc. Terminology according to Erdtman (after Dyakowska 1959) was used to describe the morphology of the pollen grains.

Statistical analysis was done using variance analysis in a system of completely randomized singular classification.

The mean values were compared with the aid of multiple Tukey confidence intervals at a 1% risk of error.

RESULTS

POLLEN GRAIN SIZE

Pollen grains significantly differing among each other in size were found in the control material and in that treated with various doses of zinc. The largest grains were found after applying doses of 500 mg/vase. The differences in the mean size of these grains and the means of the control and with the remaining

doses of zinc were found to be statistically significant (Table 1). The largest grains from the 500 mg dose were characterized by especially large dimensions: the lengths of the polar and equatorial axes were 30% greater than that of the largest pollen grains in the control (Figs. 1, 2).

Table 1

The size of pea pollen grain (*Pisum sativum* L. cv. Hamil) at various doses of zinc in the substrate

Zinc dose in mg per vase	Average size of grains μm		Minimal size of grains μm		Maximal size of grains μm	
	length of polar axis	length of equatorial axis	length of polar axis	length of equatorial axis	length of polar axis	length of equatorial axis
0 control	46.7 a	26.2 a	34.6	19.0	57.1	34.6
1	45.5 a	26.3 a	36.7	18.3	55.8	33.6
500	51.9 b	31.0 b	37.5	25.0	75.0	45.0
1000	46.8 a	25.4 a	36.7	18.4	58.1	36.7

Results not differing significantly are denoted by the same letter.

SPORODERM THICKNESS

The pollen of the control plants was characterized by a uniformly thick sporoderm, both in the polar regions as well as in the equatorial regions of the grains (Fig. 1). However, differences were noticed in the thickness of the pollen wall among the particular grains, which was 1.5–2.0 μm .

Plants which received a 500 mg dose of zinc also had pollen grains characterized by differences in sporoderm thickness, which usually ranged from 1–1.5 μm and was thinner than in the case of control plants (Figs. 1, 2). In most of the grains from plants treated with 500 mg Zn/vase distinct differences in wall thickness were observed: the sporoderm was thicker on the poles of the grains (apocolpia) while between the fissures (mesocolpia) — it was thinner (Figs. 6–11).

MORPHOLOGICAL TRAITS OF PROPERLY SHAPED PEA POLLEN GRAINS

Pea pollen grains, classified as belonging to type *Vicia*, are elliptic or an elongated oval in shape. They are characterized by a complex aperture since round pores (pori) are situated in the middle of meridional fissures (colpi) (Figs. 12, 13, 15). Due to the presence of three such fissures and the same number of pores, these grains are classified as tricolporate (granum pollinarium tricolporatum). The sculpture of the exine between the fissures (mesocolpia) is reticulate with thick walls (reticulum latimuratum) having irregular polygonal

areolas (lumina) (Figs. 15–16). The surfaces around the poles (apocolpia) also have a reticulate sculpture of the exine, and are characterized by very thick walls but with smaller areolas that run into each other. The fissures are narrow, relatively shallow, regularly spaced. The edge of the fissure (margo) is characterized by a more compact sculpture than the mesocolpia and apocolpia, in it reticulate bulges passing into papillary (gemmae) or granular structures are arranged close to each other. The interior of the fissure is also covered by exine with papillary structures, more sparsely distributed and larger than on the margo (Fig. 15). The pores are oval in shape, slightly equatorially elongate (apertura alongata) with a diameter larger than the width of the fissure. They are covered with a thin layer of exine which has a sculpture similar to that of the margo. In swollen grains the elastic layer of the sporoderm covering the pores usually begins to bulge outwards (Figs. 5-10, 12, 13).

ANOMALIES IN SPORODERM FORMATION AT HIGH ZINC DOSES

Deviations in the form of small thickenings in the sculpture of exine were observed in about 1.5% of the pollen grains of control plants. At zinc doses of 500 g/vase, 10%, while at 1000 mg/vase, about 11% of the pollen grains were found to have various types of thickenings on the surface of the exine (Figs. 5-11) near one of the poles.

In many of the grains the thickenings were ring-shaped, which gave the impression that they were a reinforcement around an additional pore. Observations made by SEM indicate that the surface within the ring is probably covered by a thinner layer of exine (Fig. 19). Grains were also found in which there was a gap in the exine in certain places as well as in its thickening nearby, on the opposite side of the pole (Fig. 11). It seems that this could be the result of local cracking of the exine.

Round depressions found in various positions on pea pollen grains may also be classified as anomalies (Fig. 9), along with thickenings and protrusions of various sizes and shapes (Figs. 17-19).

Polen grains showing the anomalies described above stained intensely with Belling's solution, which indicates that they remained viable.

POLLEN GRAIN DEFORMATIONS AT EXCESSIVE ZINC DOSES

Toxic doses of zinc (500 and 1000 mg/vase) also influenced the formation of greater numbers of strongly deformed pollen grains, which was manifested in their irregular, intense creasing (Figs. 3, 4, 20, 23). In the control material, only 12% of the grains were deformed, while at a dose of 500 mg zinc per vase, 30% deformed grains were found, at 1000 mg/vase, over 40%. These grains did not stain with Belling's solution, which indicates that they were sterile.

PLATE I

Pea pollen grains at various doses of Zn, light microscope: Fig. 1 — control, Fig. 2 — 500 mg Zn per vase, treated with Belling's solution, differences in sporoderm thickness (arrows) and grain size visible; Figs. 3-4 — 1000 mg Zn per vase, deformed pollen grains in polarized light, x 900

PLATE II

Pea pollen grains at 500 mg Zn per vase (light microscope) demonstrating varied size, different wall thickness and anomalies in exine structure (arrows): Figs. 5-8 — pollen grains in polarized light, Figs. 9-11 — pollen grains in transmitted light, x 1200

PLATE III

Pollen grains from 500 mg Zn per vase (SEM): Fig. 12 — no visible morphological deviations, x 1090; Fig. 13 — normally developed grain with visible fissure and pore along with a mesocolpium with a reticulate sculpture of the exine, x 1722; Fig. 14 — deformed grain, x 2000

PLATE IV

Fragments of pollen grains at 500 mg Zn per vase (SEM): Fig. 15 — a grain with normal morphology with a reticulate mesocolpium and papillary (granular) sculpture of the exine within the fissure and pore, x 4920; Fig. 16 — a deformed grain with a depression along the fissures, x 4920

PLATE V

Figs. 17-19. Pea pollen grains at 500 mg Zn per vase showing anomalies in exine structure (arrows).
 Fig. 17 — x 2900, Fig. 18 — x 2580, Fig. 19 — x 2770 (SEM)

PLATE VI

Figs. 20-22. Deformed pea pollen grains at 1000 mg Zn per vase (SEM): Fig. 20 — x 804, Fig. 21 — x 1630; Fig. 22 — x 1020

PLATE VII

Figs. 23 and 24. Pea pollen grains at 1000 mg Zn per vase: Most of the grains show distinct deviations in their morphology. Fig. 23 — x 2360, Fig. 24 — x 2030 (SEM)

DISCUSSION

Most researchers accept that pollen grain polymorphism and deformations are genetic in nature. Belkina (1972) and Till et al. (1989) believe that polymorphism in respect to the number of apertures is genetically controlled and Belkina (1972) also accepts similar argumentation in the case of the size and shape of pollen grains. Carrion and Alcaraz (1988), however, explain the existence of grains with anomalies of apertures as the effect of biological variation which is marginal in the given taxonomic group. Till et al. describe seven types of exine ornamentation in *Iris chamaeiris* — from reticulate to papillary. These authors claim that it is possible to delineate within a population several populations of individuals which differ in terms of genetically determined pollen grain structure. According to the authors of the cited study, the existence of such differentiation may be related to evolutionary changes from one type of pollen to another.

The occurrence of deformed grain, usually sterile, is generally attributed to genetic factors related to the formation of hybrids (Belkina 1972, Carrion and Alcaraz 1988). The latter authors also claim that deformed grains may occur with increased frequency in polyploids and aneuploids and in individuals with an additional chromosome.

The results of other studies suggest that deviations in the formation of pollen may be caused by environmental factors. Blanca (1981) describes aberrations in the structure of *Centaurea* pollen grains in plants occurring on soils formed from slate.

Carrion and Alcaraz (1988) mention plant nutrition as a factor determining abnormal formation of pollen in *Sideritis glauca*. These abnormalities were expressed as a reduction in pollen size, in their asymmetry, formation of a thick exine and an indistinct or narrowed fissure.

One of the reasons for the formation of deformed and sterile pollen is the lack of copper in the medium. The effect of copper deficiency on deformations in wheat (Graham 1975, Alloway et al. 1986), oat (Weryszko et al. 1983) and barley (Alloway et al. 1986) has been described.

It therefore seems possible that excess zinc in the medium did influence the formation of the anomalies in exine sculpture of pea pollen grains described in this study and that it was responsible for increasing the number of deformed grains.

It is known that zinc plays an important role in blooming and seed formation (Riceman and Jones 1958). The studies of Polar (1970, 1975) have shown that the highest zinc content in *Vicia* and *Nicotiana* is found in stamens, more precisely, in pollen grains.

The data presented in the paper by Łyszcz and Ruszkowska (in press) indicate that at excessive doses of zinc (500 mg per vase), the dry mass of pea shoots decreased almost three-fold, and the mass of the produced seeds

decreased to the same degree as well. At the same time, the zinc content in the shoots of this plant increased significantly (about 40 times). If we assume that the distribution of Zn is similar in pea shoots as in species that have a high Zn content in pollen grains (Polar 1975), then increasing zinc in the substrate would cause primarily an increase in the amount of this element in pollen grains and could be the reason for the abnormalities in their morphology.

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*Anomalie morfologiczne ziarn pyłku grochu (*Pisum sativum* L.)
odm. Hamil przy wysokich dawkach cynku*

Streszczenie

Badano morfologię ziarn pyłku grochu (*Pisum sativum* L.) odm. Hamil po wprowadzeniu do podłoża różnych dawek cynku: 0, 1, 500 i 1000 mg na wazon. Obserwacje przeprowadzono w mikroskopie świetlnym oraz elektronowym skaningowym. Przy dawce cynku 500 mg na wazon znaleziono ziarna pyłku największych rozmiarów. Przy wysokich stężeniach cynku (500 i 1000 mg) stwierdzono występowanie ok. 10% ziarn z anomaliami w budowie sporodermy, w której zaobserwowano występowanie różnorodnych zgrubień, najczęściej w formie pierścieni. Przy dawce Zn 1000 mg na wazon notowano największą liczbę ziarn zdeformowanych (ponad 40%), mocno pofałdowanych lub silnie spłaszczonych. Opisane odchylenia w skulpturze sporodermy wywołane nadmiarem cynku zilustrowano licznymi fotografiami.