

MAŁGORZATA KLIMKO¹, MAGDALENA KLUZA¹, ANNA KREFT²

MORPHOLOGY OF POLLEN GRAINS IN THREE VARIETIES OF *HELIANTHUS ANNUUS* L.

From ¹Department of Botany
August Cieszkowski Agricultural University in Poznań
and ²Department of Botany
Pedagogical Higher School in Słupsk

ABSTRACT. The paper reports results of a morphological study of pollen grain of three varieties of *Helianthus annuus*: Wielkopolski population variety and two interline hybrids Frankasol and Coril. The viability of the pollen grain was also determined. No significant differences were found between the population variety and the two interline hybrids.

Key words: *Helianthus annuus*, grains pollen, population variation, interline hybrid, sexine, nexine

Introduction

Sunflower (*Helianthus annuus* L.) originates from the area of south-western part of the USA. In Europe it is cultivated mainly in the belt spreading from Spain through France, to the Ukraine and Kubań. In Russia and in the Ukraine, Romania, Bulgaria, Hungary and Slovakia it has become the main oil plant (**Podsolnechnik** 1975).

The oil-bearing forms of sunflower occur as older population varieties and interline hybrids which have been obtained in the last 30 years by way of cross-pollination of selected inbred lines. New hybrids give much higher yield than the population varieties thanks to the effect of heterosis already in the first generation (F₁). For this reason such varieties displace the population forms even in the countries of less favourable soil and climatic conditions, also in Poland (**Kłoczowski** 1981). The interlinear hybrids differ from the population varieties by a number of morphological and utility features as well as by the varieties of the features in the community of cultivated plants.

Older population varieties were subjected to different kinds of study, e.g. of the varieties of their morphological features, their interrelations and the effect on the yield (**Kłoczowski** 1967, 1975). The analogous properties of the hybrid varieties are poorly

recognized, in particular in cultivation in Polish soil and climatic conditions. Among others the biology of flowering and fruit-bearing of the new hybrids and disturbances in these processes in the first generation of the interline hybrids have not been under close scrutiny. The first attempts at comparing different varieties in the aspect of morphological features of the stems, leaves and fruit, as well as in the course of the process of flowering, were made by **Kluza** (1997) and **Kluza** and **Muśnicki** (1998, 1999).

The results prompted this study on the differences in the morphology of pollen grain and its viability of two interline hybrid and a population variety.

Material and methods

The study was performed for three varieties registered by the Centralny Ośrodek Badania Odmian Roślin Uprawnych (State Centre for Investigation of Cultivated Plants), the Polish population variety of Wielkopolski obtained by the Institute of Plant Cultivation and Acclimatisation, and two interline hybrids: Frankasol from France obtained by Cargill and Coril from the USA concern Pioneer.

Measurements of pollen grain in the equatorial position without taking into regard the length of the spines were performed under a microscope coupled to a computer, with 30 repetitions for each varieties. The software used was Lucia 3.5 program with computer analysis of a microscopic image, devised by Laboratory Imaging Ltd. from Czech Republic. Mean values of the pollen grain length (in the polar plane) and width (in the equatorial plane) were calculated. The results allowed classification of the size of microspores. The shape of the pollen grains was classified according to the Erdtman scale (**Erdtman**1952) on the basis of the ratio of the polar length (P) to the equatorial length (E). The area of the grains was calculated as πr^2 , where r is the half of the equatorial length.

Pollen grains were closed in the Canadian balm and observed under a light microscope at a magnification of 1000x, under immersion. The observations allowed description of the microspores in the polar and equatorial positions. Detailed analysis was made of the external wall of the grains-exine, on the basis of determinations of its thickness and stratification.

The sculpture and aperture of the pollen grain were determined on the basis of a series of photographs taken under SEM microscope (at a magnification of 388x; 3240x; 2200x) in the Electron Microscope Laboratory at the A. Mickiewicz University, Poznań. Analysis was also made of the spines, furrows and pori. The total length of the spines including the pyramid-like base was measured. The length of the outgrowth and their number per a unit area of 100 μm^2 were determined.

The viability of the pollen grains was studied in Belling fluid, 2% solution of carmine acetate mixed at the 1:1 ratio with glycerine, for the pollen collected in the time of peak flowering (July 17th, 1999). Some amount was frozen and studied 9 months later (April 17th, 2000) for the sake of comparison. The pollen grains were immersed in the Belling fluid and wet microscopic preparations were made. Determination of viability was performed on the basis of counting the grains which would turn pink (viable ones) in proportion to those which would not turn pink (dead), in 30 fields of the preparation, each of which containing minimum 10 objects.

Results

The size of the pollen grains of the varieties studied was assessed as average. The longest axis was the length measured in the polar plane. Sometimes in the interline hybrid Coril the width was slightly greater. This variety was characterised by the largest grains of all the varieties studied. The mean length of its grains was 32.90 μm and varied from 29.21 to 37.44 μm , while the mean width was 32.93 and varied from 29.41 to 37.85 μm (Fig. 1). The smallest grains were found in the line hybrid Frankasol – the mean length 31.25 μm (29.00 to 33.53 μm) and the mean width 30.74 (29.62 to 37.02 μm).

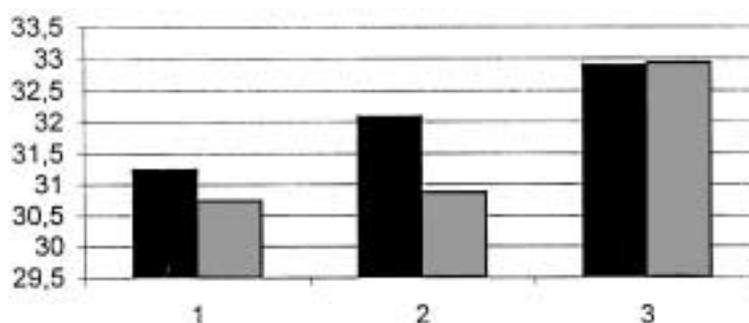


Fig. 1. Mean length in the polar plane (black) and width in the equatorial plane (grey) of the pollen grains of *Helianthus annuus* L. (1 – Frankasol, 2 – Wielkopolski, 3 – Coril)
Ryc. 1. Średnia długość w płaszczyźnie biegunowej (czarny) i szerokość w płaszczyźnie równikowej (szary) ziaren pyłku *Helianthus annuus* L. (1 – Frankasol, 2 – Wielkopolski, 3 – Coril)

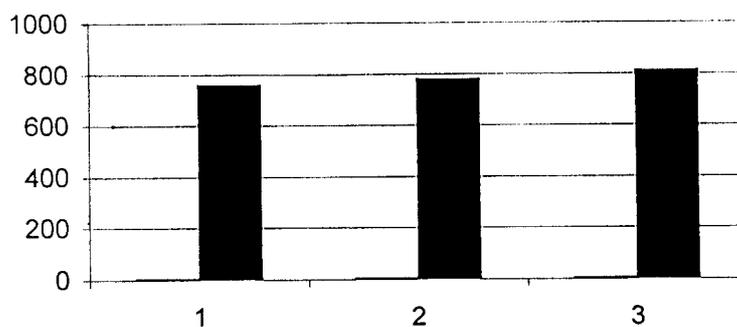


Fig. 2. Mean area of the pollen grains of *Helianthus annuus* L. (1 – Frankasol, 2 – Wielkopolski, 3 – Coril)
Ryc. 2. Średnia powierzchnia ziarn pyłku *Helianthus annuus* L. (1 – Frankasol, 2 – Wielkopolski, 3 – Coril)

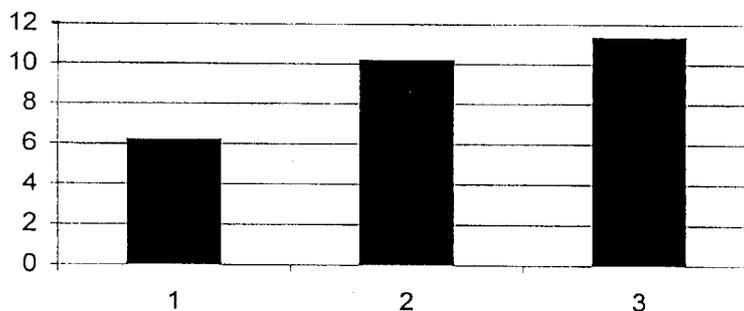


Fig. 3. Variability coefficient (V) of the area of the pollen grains of *Helianthus annuus* L. (1 – Frankasol, 2 – Wielkopolski, 3 – Coril)

Ryc. 3. Współczynnik zmienności (V) ziarn pyłku *Helianthus annuus* L. (1 – Frankasol, 2 – Wielkopolski, 3 – Coril)

The area of the pollen grain was the largest for Coril (mean value $811.87 \mu\text{m}^2$), and the smallest for Frankasol (the mean value $757.44 \mu\text{m}^2$) (Fig. 2, 3).

The ratio of the polar to equatorial axis (P/E) was on the average 1.02, which according to the Erdtman scale (l.c.) corresponds to elongated shape of the grains. Ball-shaped forms prevailed for Coril (P/E = 1.00), and elongated forms for Wielkopolska variety (P/E = 1.04).

In the polar aspect the shape of the pollen grains was ball-triangular, while in the equatorial aspect it was slightly elliptical. The photographs taken for the polar position revealed the presence of clearly marked polar fields free from furrows (Fig. 4).

The thickness of exine depended on the variety and site of measurement on the grain, it was much thinner in the vicinity of the furrows. The thickest was the exine in the pollen grain of the Wielkopolski variety for which it was $4.34 \mu\text{m}$, its thickness in the pollen grain of the interline hybrids was similar and equalled $3.95 \mu\text{m}$ and $3.98 \mu\text{m}$ in Frankasol and Coril, respectively. The exine was composed of two distinct layers (films) of which the external one called sexine was much thicker than the inner one – nexine. These two layers (films) were also the thickest in Wielkopolski variety: $2.38 \mu\text{m}$ and $1.96 \mu\text{m}$, respectively. In Frankasol grains the difference in thickness between these two layers was the smallest: $2.04 \mu\text{m}$ and $1.91 \mu\text{m}$, while in Coril grains the corresponding values were: $2.11 \mu\text{m}$ and $1.87 \mu\text{m}$. The type of pollen for which the columellae in sexine were invisible (Fig. 4) under a microscope was called *Aster* (Moore et al. 1991).

Particular attention was paid to the spines, furrows and pori. The grains were covered with fully developed tectum excreting abundant amounts of yellow pollen balm. The sculpture of the tectum surface was smooth, apart from protruding spines with a broad pyramid base and slim outgrowth. According to Faegri and Iversen (1978), grains of this type are classified as spiny so *Echinatae*. These type spines were characteristic of many representatives of the subfamily *Tubiflorae*, and referred to as wide conical. The longest outgrowths were found in the interline hybrid Frankasol. The mean total length of the spine, together with the pyramid-like base was $6.95 \mu\text{m}$ and varied from 5.49 to $8.51 \mu\text{m}$. The shortest spines were observed for the population variety, the mean length was $5.47 \mu\text{m}$, and it varied from 4.20 to $7.09 \mu\text{m}$. Similarly the longest outgrowths were observed for

Frankasol, the mean value was $4.52 \mu\text{m}$ ($3.31\text{-}5.57 \mu\text{m}$), and the shortest for the Wielkopolski variety – the mean value $3.46 \mu\text{m}$ ($2.54\text{-}4.49 \mu\text{m}$). Some of the spines were slightly bent at the top. The outgrowths in the interfurrow area were slightly longer than those near the furrows. The number of spines per a unit area ($100 \mu\text{m}^2$) was the highest in the population variety – 5.1, while the lowest in Frankasol – 4.64. The pollen grain aperture was complex, with pori hid in the furrows. There were three furrows running across the equatorial zone and their length relative to the polaraxis was $3/4$. The furrows were depressions in exine, of sharp ends and narrow elongated shape. In each of them there was one porus of elliptical shape arranged with its wider axis across the furrow, along the equatorial axis (Fig. 5).

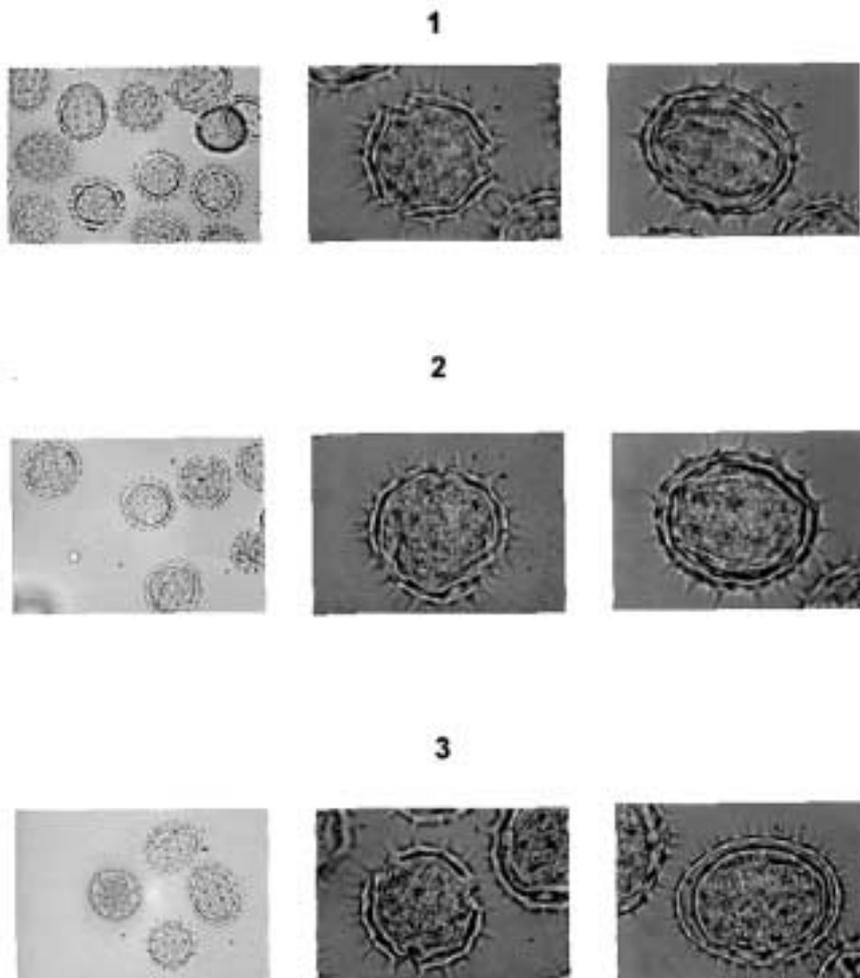


Fig. 4. The pollen grains of *Helianthus annuus* L. under a light microscope (1 – Frankasol, 2 – Wielkopolski, 3 – Coril)

Ryc. 4. Ziarna pyłku *Helianthus annuus* L. spod mikroskopu świetlnego

(1 – Frankasol, 2 – Wielkopolski, 3 – Coril)

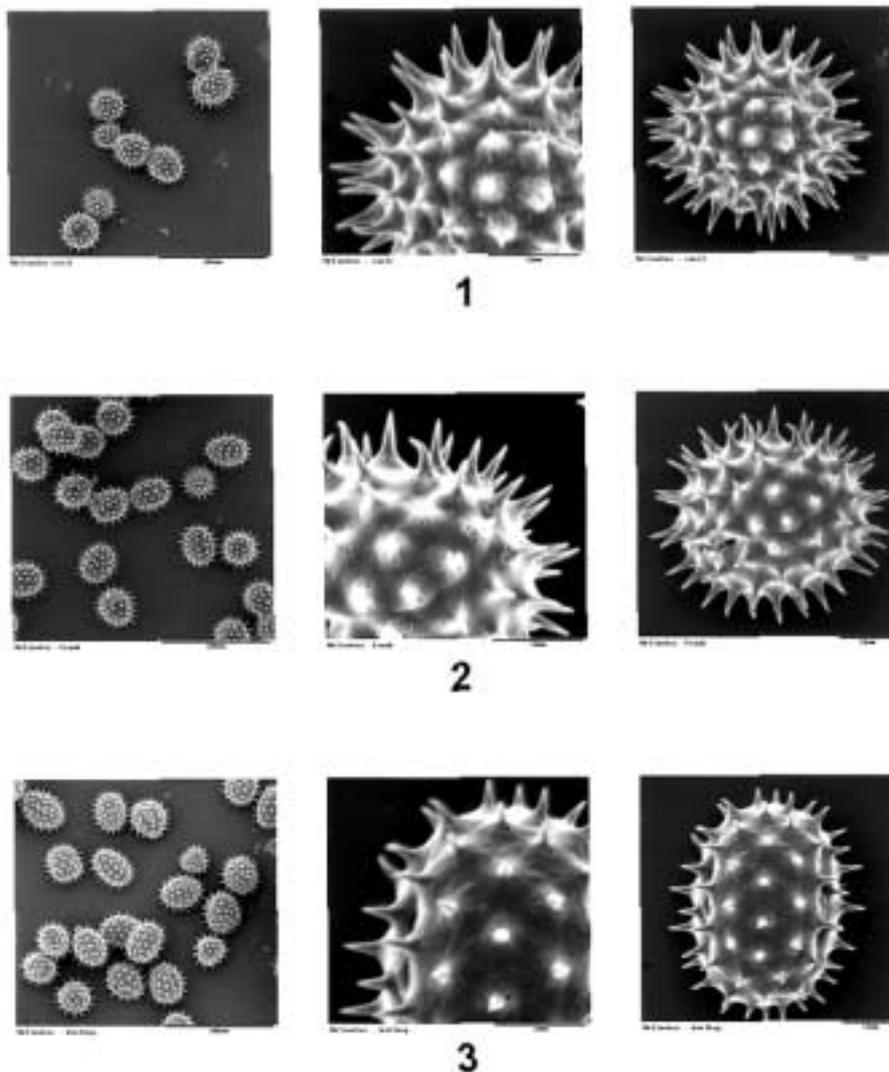


Fig. 5. The pollen grains of *Helianthus annuus* L. under SEM microscope
(1 – Frankasol, 2 – Wielkopolski, 3 – Coril)

Ryc. 5. Ziarna pyłku *Helianthus annuus* L. spod mikroskopu świetlnego
(1 – Frankasol, 2 – Wielkopolski, 3 – Coril)

The viability of pollen grains (Fig. 6) at the peak flowering was high, and the highest of 97.1% in Wielkopolska population variety. In Frankasol it was 96.7%, while in Coril 96.6%. After 9 months of storage in refrigerator the viability dropped but was still the greatest for the Wielkopolski variety – 92.4% and the lowest in Frankasol – 86.1%.

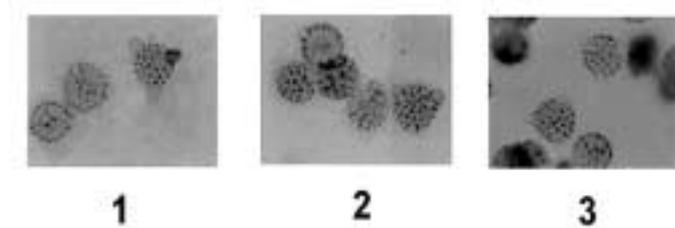


Fig. 6. The vitality of the pollen grains of *Helianthus annuus* L.
(1 – Wielkopolski, 2 – Coril, 3 – Frankasol)
Ryc. 6. Żywotność ziarn pyłku *Helianthus annuus* L.
(1 – Frankasol, 2 – Wielkopolski, 3 – Coril)

Concluding remarks

The study on the morphology of pollen grains of three varieties of *Helianthus annuus* L. proved that they were of average size and elongated-ball shape. They were classified as spiny – *Echinata*, and the shape of the spines was characteristic of the subfamily *Tubiflorae*.

The largest grains were found in the interline hybrid Coril, while the smallest in Frankasole, the most ball-like shaped were those of Coril, and the most elongated those of Wielkopolski population variety.

Analysis of the internal structure of the pollen grains revealed changes in the thickness of exine, which varied along the whole circumference. The highest number of spines per a unit area was established for the population variety, while the smallest for Frankasole. As far as the spine length was concerned, the situation was the reverse the longest spines had Frankasole and the shortest Wielkopolski population variety.

No significant differences were found between the properties of the population variety and the interline hybrids, expected to be more profitable in cultivation. The Wielkopolski variety was characterised by the highest viability of pollen from among the varieties studied, of 97.1% during flowering and 92.4% after 9 months of storage. The size and area of its pollen grains was average. Their shape in the equatorial position was the most elliptical of those considered.

References

- Erdtman G.** (1952): Pollen morphology and plant taxonomy. Angiosperms. An introduction to palynology 1. Almqvist and Wiksell, Stockholm.
- Faegri K., Iversen J.** (1978): Podręcznik analizy pyłkowej. Wyd. Geol., Warszawa.
- Kluza M.** (1997): Kwitnienie, zapylanie i zapłodnienie u populacyjnych i mieszańcowych odmian oleistych

słonecznika zwyczajnego (*Helianthus annuus* L.) In: Mater. Konf. Nauk. „Biologia kwitnienia, nektarowania i zapylania roślin”. Lublin: 72-76.

Kluza M., Muśnicki Cz. (1998): Zmienność i odziedziczalność niektórych cech morfologicznych u dwóch mieszańców liniowych słonecznika zwyczajnego (*Helianthus annuus* L.) na tle odmiany populacyjnej. *Rośliny Oleiste – Oilseed Crops* 19, 2: 423-428.

Kluza M., Muśnicki Cz. (1999): Jakość niełupek różnych typów odmian słonecznika zwyczajnego (*Helianthus annuus* L.) w zależności od miejsca ich położenia w koszyczku. *Rośliny Oleiste – Oilseed Crops* 20, 1: 275-280.

Kłoczowski Z. (1967): Charakterystyka ważniejszych cech i właściwości niektórych zagranicznych odmian słonecznika oleistego w warunkach ekologicznych Polski. *Biul. IHAR* 6: 81-88.

Kłoczowski Z. (1975): Studia nad niektórymi cechami słonecznika oleistego i ich znaczenie w hodowli tej rośliny w Polsce. *Hod. Rośl. Aklim. Nasien.* 19, 2: 89-131.

Kłoczowski Z. (1981): Ważniejsze problemy heterozyjnej hodowli słonecznika oleistego. *Biul. IHAR* 144: 109-115.

Moore P.D., Webb J.A., Collison M.E. (1991): *Pollen analysis*. Blackwell Scientific Publications, London.

Podsolnechnik. (1975). Ed. W.S. Pustovojt. Kołos, Moskwa.

MORFOLOGIA ZIARN PYŁKU TRZECH ODMIAN *HELIANTHUS ANNUUS* L.

S t r e s z c z e n i e

W badaniach nad morfologią ziarn pyłku słonecznika wykorzystano trzy odmiany: polską odmianę populacyjną Wielkopolski, wyhodowaną przez Instytut Hodowli i Aklimatyzacji Roślin oraz dwa mieszańce międzyliniowe: Frankasol pochodzący z Francji, wytworzony przez firmę Cargill i amerykański Coril otrzymany przez Koncern Pioneer.

Analizę biometryczną oparto na pomiarach pyłków w płaszczyźnie biegunowej i równikowej oraz na pomiarach ich powierzchni. Szczegółowej obserwacji poddano eksynę, kolce, bruzdy i porusy. W wyniku badań stwierdzono, że pyłki *H. annuus* są średniej wielkości, z typu kolczastego *Echinata*. Największe ziarna występowały u mieszańca międzyliniowego Coril, a najmniejsze u Frankasol. Zróżnicowanie zaznaczyło się również w kształcie ziaren pyłku; najbardziej kuliste występowały u odmiany Coril, natomiast wydłużone u formy Wielkopolski.

W budowie wewnętrznej ziarna pyłku różniły się grubością eksyny, liczbą kolców i ich długością. Na podstawie obserwacji żywotności ziarn pyłku stwierdzono, że odmiana populacyjna odznacza się największą żywotnością w czasie kwitnienia (97,1%). Nie stwierdzono istotnego zróżnicowania pomiędzy odmianą populacyjną a przedstawianymi w bardziej korzystnym świetle mieszańcami międzyliniowymi.