IN VIVO AND IN VITRO POLLEN MATURATION IN *LILIUM*: INFLUENCE OF CARBOHYDRATES

CHRISTOPHE CLÉMENT¹*, DANIEL AL-AWAD², JEAN-CLAUDE AUDRAN¹

¹Université de Reims Champagne-Ardenne, UFR Sciences,
Laboratoire de Biologie et Physiologie Végétales,
BP 1039, 51687 Reims Cédex 2, France

²Université de Tichrine,
Faculté des Sciences, Section de Sciences Naturelles, Lattaquie, Syria

ABSTRACT

The purpose of this study was to develop a protocol for *in vitro* pollen maturation, as a model to study the involvement of carbohydrates on pollen maturation in *Lilium*. *In vivo* and *in vitro* pollen maturation were followed and compared by transmission electron microscopy, and several *in vitro* parameters were tested in terms of carbohydrate physiology.

In vivo, pollen maturation was initiated at the vacuolated microspore stage, and consisted of two successive phases. The first phase was characterized by reactivation of microspore organelles, followed by microspore mitosis, starch synthesis and vacuole breakdown. During the second phase, starch was progressively degraded whereas lipid and phytine reserves accumulated. *In vivo*, pollen maturation occurred within 14 days and pollen germination rate was $73.6 \pm 2.2\%$.

We then attempted to realise *in vitro* pollen maturation starting from the vacuolated microspore stage. The best results were obtained with flower buds cultivated at 26°C, in 100 $\mu\text{mol/m}^2/\text{s}$ light, with a 16h/8h photoperiod on a modified Heller's medium supplemented with NAA (10^{-2} mg/l) and sucrose (M/6). In these conditions, pollen maturation occurred within 7 days only. *In vitro* matured pollen is cytologically comparable to *in vivo* developed pollen grains and the germination rate was $72.4 \pm 3.7\%$. When flower buds were cultivated in the dark, the germination rate decreased, but this could be compensated by providing high sucrose concentrations (1M) in the medium. Further, photosynthesis inhibitors had the same effect on pollen maturation than the darkness, strongly suggesting that photosynthesis occurs in the flower bud and is important for pollen maturation in *Lilium*.

KEY WORDS: pollen maturation, cytology, *in vitro*, *Lilium*, pollen mitosis.

INTRODUCTION

Pollen development consists of a complex succession of cytological events governed by two main steps: 1) meiosis leading to haploid microspores from microspore mother cells, 2) sporadic mitosis resulting in a bicellular pollen grain containing a vegetative cell and a reproductive cell. Sporadic mitosis is accompanied by genetic and physiological modifications affecting the behaviour of the gametophytic cells as well as the surrounding anther wall layers (Mascarenhas 1992, Wang *et al.* 1992). *In vivo*, these changes lead the microspore into the gametophytic way till pollen is prepared to germinate. This period is named the pollen maturation phase. *In vitro*, pollen grain can be reoriented from the initial gametophytic program to the androgenetic program, resulting in a haploid plant after regeneration (Brar and Khush 1994). Pollen natural maturation is poorly documented in comparison with androgenesis (Pareddy and Petolino 1992), although a fundamental approach would help solving number of androgenetic obstacles.

In *Lilium*, *in vivo* pollen maturation can be decomposed in several stages when studied at the optical microscopy level (Clément *et al.* 1994). Soon after mitosis, the generative cell is located against the young bicellular pollen grain wall. The vegetative cell (VC) further accumulates starch reserves while the generative cell (GC) moves towards the center of the amyloseous pollen grain. At the end of maturation, starch breaks down in the VC, and the GC flattens. We have previously demonstrated that pollen carbohydrate supply is accurately controlled by the anther wall layers during maturation (Clément and Audran 1995). Nevertheless, further experiments remain necessary to clarify the involvement of sugars on pollen maturation in the whole flower bud. Recovering *in vitro* matured pollen grains could be interesting in understanding the mechanisms governing pollen maturation and germination (Benito-Moreno *et al.* 1988, McCormick 1993). Rastogi and Sawhney (1989) have reviewed investigations undertaken to realise pollen grain maturation *in vitro*. They concluded that each species must be studied as a peculiar case in terms of medium optimization, especially concerning growth regulators and carbohydrates.

*Correspondence and reprints

Fig. 1. – *In vivo* pollen maturation. A – Vacuolated microspore: most of the volume was occupied by the vacuole (V), arrowhead – dictyosome, E – exine, I – intine. Uranyl acetate/lead citrate, X 25,000. B – Vacuolated microspore: plastids (P) were undifferentiated with a single saccule (arrowhead). Uranyl acetate/lead citrate, X 19,000. C – Premitosis: Starch (S) appeared in the plastid, whereas mitochondria were dividing (arrowheads). Intine is bilayered, N – nucleus. Uranyl acetate/lead citrate, X 19,000. D – Premitosis: dictyosomes (D) were multiplied and numerous golgi vesicles could be detected (arrowheads). Uranyl acetate/lead citrate, X 19,000. E – Young bicellular pollen grain: a double membrane separated the vegetative cell (VC) from the generative cell (GC). Starch was still accumulating in the plastid (star). PATAg, X 9,000. F – Young bicellular pollen grain: pollen coat (star) was deposited on the exine. Uranyl acetate/lead citrate, X 19,000. G – Young bicellular pollen grain: the cytoplasm of the GC was dense but poorly provided with organelles, L – lipids, M – mitochondria. Uranyl acetate/lead citrate, X 16,000. H – Young bicellular pollen grain: vacuole was fragmented in the VC, as well as in the GC. Uranyl acetate/lead citrate, X 6,000.

Fig. 2. – *In vivo* pollen maturation. A – Young bicellular pollen grain: rough endoplasmic reticulum (arrowheads) surrounded plastid (P), VC – vegetative cell, GC – generative cell. Uranyl acetate/lead citrate, X 19,000. B – Amylaceous pollen grain: starch reached its maximum amount in the plastid (star), whereas lipids (L) began to accumulate in the cytoplasm of the VC. Uranyl acetate/lead citrate, X 16,000. C – Premature pollen grain: lipid synthesis increased in the VC, concurrently with starch degradation in the plastid. PATAg, X 19,000. D – Premature pollen grain: in the VC, reserve vacuoles (V) accumulated phytine reserves (arrowheads). Uranyl acetate/lead citrate, X 19,000. E – Premature pollen grain: vesicles could be observed between the VC plasmalemma and the intine (arrowhead). Uranyl acetate/lead citrate, X 19,000. F – Premature pollen grain: plastids divided (arrowheads), and were in close association with RER (double arrowheads). Uranyl acetate/lead citrate, X 23,000.

Fig. 3. – *In vivo* pollen maturation. A – Nearly mature pollen grain: the cytoplasm of the vegetative cell (VC) was mostly occupied by lipids (L) and phytine vacuoles (arrowheads), N – nucleus. Uranyl acetate/lead citrate, X 6,000. B – Nearly mature pollen grain: detail showing mitochondria (M) of minimal size and single starch grain plastid (P), arrowhead – phytine reserves. Uranyl acetate/lead citrate, X 23,000. C – Mature pollen grain: a PATAg positive membrane was detected between the VC and the generative cell (GC) (arrowheads). The organelles of the VC were concentrated around the GC, D – dictyosome, R – phytine reserves. PATAg, X 19,000. D – Mature pollen grain: plastids were undifferentiated and did not contain any starch left. Uranyl acetate/lead citrate, X 23,000.

In this paper, in order to further understand sugar physiology of pollen maturation in *Lilium*, we have first investigated the cytological events occurring during *in vivo* pollen maturation in greater details (transmission electron microscopy) from the premitosis stage until anthesis. Secondly, we have developed a protocol destined to obtain *in vitro* conform mature pollen grains from dissected flower buds. Then, the influence of several culture parameters was studied on pollen maturation.

MATERIAL AND METHODS

Plant material

Flower buds or isolated stamens of *Lilium* cv. 'enchantment' were collected at the vacuolated microspore stage. They were then fixed for cytology or sterilized for *in vitro* culture.

Preparation for microscopy

Anthers were fixed in 1% glutaraldehyde (v/v) in a 0.1 M phosphate buffer at pH 7.2 in the presence of 4% sucrose (w/v) for 24 h. After 3 rinses (5 min each) with the buffer plus 4% sucrose (w/v), objects were post-fixed with 1% OsO₄ (w/v) in the same buffer with 4% sucrose (w/v) for 4 h. They were then rinsed 3 times (5 min each) with the buffer, dehydrated in the alcohol series, transferred to acetone, and embedded in araldite.

Ultra-thin sections, collected on copper or gold grids, were stained with uranyl acetate/ lead citrate for general staining or with PATAg test for polysaccharides according to Roland (1978), and examined on a JEOL JEM 100C microscope at 100 kV.

Culture

Flower buds were sterilized in calcium hypochlorite (7% w/v) during 10 min, rinsed in sterile distilled water and dissected. They were placed on Murashige and Skoog's (MS) or Heller's (H) medium, modified according to Al Awad (1993). Explant cultures were performed at 26°C, in 100 µmol/m²/s light, under 70% of relative humidity, with a 16h/8h photoperiod. The influence of several parameters was studied: 1) mineral medium: MS or H medium, 2) growth regulators: naphthalen acetic acid (NAA), kinetin (KIN), and gibberellic acid (GA₃), 3) carbohydrates with various concentrations: sucrose, glucose, fructose, maltose, mannitol, 4) darkness associated with various concentrations of sucrose. Moreover, in order to test a potential involvement of photosynthesis in the flower bud during pollen maturation, flower buds were placed on a modified Heller's medium without carbon source, but supplemented with photosynthesis inhibitors: dichlorodimethylurea (DCMU), sodium fluorure (NaF), sodium sulfite (Na₂SO₃).

After 3 and 7 days of culture, anthers were collected and fixed as described before. After 7 days of culture, pollen germinability was estimated according to Southworth (1983) with a sample of 1,000 pollen grains per flower bud, and 3 flower buds per experimental condition.

RESULTS

Pollen maturation in vivo

In order to obtain a complete view of pollen maturation, we started our study just before pollen mitosis. At the vacuolated microspore (VM) stage, the cytoplasm was narrow along the

pollen wall, most of the microspore being occupied by the vacuole (Fig. 1C). Plastids were small, undifferentiated and devoid of starch grain (Fig. 1B). Pollen wall exhibited complete exine and a bilayered intine (Fig. 1C). Just prior to mitosis, plastids accumulated small starch grains (1C) and were surrounded by rough endoplasmic reticulum (RER). Simultaneously, mitochondria and dictyosomes also multiplied intensely (Fig. 1C, 1D), and numerous golgi-vesicles could be observed (Fig. 1D). After mitosis had occurred, plastids continued accumulating starch (Fig. 1F). Simultaneously, mitochondria reached their maximal size (Fig. 4). Soon after mitosis, pollen coat was deposited in the interbacula spaces of the exine (Fig. 1F). The VC and the GC were separated by a double membrane (Fig. 1E), but no wall was distinguished. In the GC, the cytoplasm was dense and poorly provided with organelles (Fig. 1G). Nevertheless, dictyosomes, RER, and mitochondria were observed, but no plastids could be detected. At the bicellular pollen grain stage, the vacuole was fragmented in both VC and GC (Fig. 1H). Starch was still accumulating in the plastids, which were surrounded by RER (Fig. 2A).

At the amylaceous pollen grain stage, the vacuole had completely disappeared. Plastids reached their maximal size (Fig. 5), and contained up to 50 starch grains. In the cytoplasm of the VC, lipids began to accumulate (Fig. 2B). From this stage, dramatic modifications were observed in the behaviour of the VC organelles. In the plastids, starch progressively broke down, whereas lipid globules accumulated (Fig. 2C). In parallel, mitochondria decreased in size (Fig. 4), and reserve vacuoles including electron dense particules of phytine appeared in the cytoplasm of the VC (Fig. 2D). The plasmalemma of the VC had notched and a third layer was added on the internal face of the intine (Fig. 2E). During the end of maturation, amylaceous reserves were degraded in the plastids of the VC. This phenomenon was accompanied by plastid divisions, leading to single starch grain plastids (Fig. 2F). RER was still in close association with plastids (Fig. 2F). Lipid and phytine reserve accumulation and starch degradation were going on.

At the mature pollen grain stage, most of the VC cytoplasm was occupied by lipidic and phytic structures (Fig. 3A, 3B). Mitochondria presented their minimal size (Fig. 4), and dictyosomes were scarce (Fig. 3C). Plastids exhibited undifferentiated features, were devoid of starch (Fig. 3D), and reached their minimal size (Fig. 5). The intercellular space between the VC and the GC was not modified from the young bicellular pollen grain stage until the mature pollen grain stage (Fig. 3C). The organelles of the VC were preferentially concentrated around the GC (Fig. 3C).

In vivo, pollen maturation occurred within 12 to 14 days and the germination rate was $73.6 \pm 2.2\%$. This value was used as a control in further experiments, in order to test the quality of maturation.

Pollen maturation in vitro

The influence of mineral medium was first tested on pollen maturation. In absence of mineral salts, with or without sucrose, pollen grains were almost totally unable to germinate after 7 days of culture (Fig. 6). The best results were obtained with Heller's medium supplemented with sucrose (M/6), since the germination rate was $51.3 \pm 4.3\%$. The MS medium was found to be less favourable to pollen maturation *in vitro*, since pollen germination did not exceed 30% after 7 days of culture.

The influence of growth regulators at different concentrations was then studied on Heller's medium supplemented with sucrose (M/6), and only the most interesting results are presented here. In presence of gibberellic acid GA₃, pollen matu-

Mitochondria (μm)

Fig. 4. – Mitochondria diameter variations during pollen maturation. VM – vacuolated microspore, Mi – mitosis, BPG – young bicellular pollen grain, APG – amyloplastic pollen grain, MPG – mature pollen grain.

Plastid (μm)

Fig. 5. – Plastid diameter variations during pollen maturation. VM – vacuolated microspore, Mi – mitosis, BPG – young bicellular pollen grain, APG – amyloplastic pollen grain, MPG – mature pollen grain.

Fig. 6. – Effects of mineral medium on the germination rate of *in vitro* matured pollen after 7 days of culture in flower bud. H – Heller, MS – Murashige and Skoog, S – sucrose.

Fig. 7. – Effects of growth regulators on the germination rate of *in vitro* matured pollen after 7 days of culture in flower bud. 0 – without growth regulator, 1 – GA3 = 1 mg/l, 2 – ANA = 10^{-2} mg/l + KIN = 10^{-2} mg/l, 3 – ANA = 10^{-2} mg/l.

ration was 3 times less effective than without growth regulators (Fig. 7). The use of a combination of auxin (NAA 10^{-2} mg/l) and cytokinin (kinetin 10^{-2} mg/l) induced higher pollen germination rate, but the best results were obtained with NAA alone (10^{-2} mg/l). The pollen germination rate then reached $72.4 \pm 3.7\%$ after 7 days of culture. This condition was used for further experiments.

Several carbohydrates at different concentrations were next studied (Fig. 8). Glucose and fructose were the less suitable

sugars tested for pollen maturation, since the germination rate did not exceed 15%. Sucrose (M/6) appeared to be the best carbon source for *in vitro* pollen maturation of *Lilium*, with a germination rate of $72.4 \pm 3.7\%$.

The effect of darkness on pollen maturation was estimated in combination with increasing concentrations of sucrose (Fig. 9). In the medium containing sucrose (M/6), the germination rate was only $21.2 \pm 3.4\%$ in the dark, against $72.4 \pm 3.7\%$ in the light. This percentage increased in parallel with increasing

Germination rate

Fig. 8. – Effects of carbohydrates on the germination rate *in vitro* matured pollen after 7 days of culture in flower bud. S – sucrose, G – glucose, F – fructose, Ma – maltose, Mo – mannitol.

Germination rate

Fig. 9. – Effects of darkness and sucrose concentration on the germination rate of *in vitro* matured pollen after 7 days of culture in flower bud. S – sucrose.

Germination rate

Fig. 10. – Effects of photosynthesis inhibitors on the germination rate *in vitro* matured pollen after 7 days of culture in flower bud.

sucrose concentrations. Pollen germination was $71.1 \pm 5.4\%$ when flower bud was cultured on sucrose (1M) medium.

Photosynthesis inhibitors affected *in vitro* pollen maturation (Fig. 10). In the light without photosynthesis inhibitors and without sugar in the medium, the germination rate was $34.3 \pm 4.2\%$. In the presence of DCMU 50 μ M and DCMU 100 μ M, the germination rate decreased respectively to $9.3 \pm 2.1\%$ and $17.2 \pm 1.6\%$. Using NaF or Na₂SO₃, less than 1% of pollen grains germinated after 7 days of culture.

Pollen behaviour during *in vitro* maturation was also examined at the microscopical level. In the best culture conditions (Heller's medium, sucrose M/6, NAA 10^{-2} mg/l and light), pollen mitosis occurred within 2 days. Pollen grains reached the

amylaceous stage after 3 days of culture and exhibited similar features to *in vivo* developed pollen. Vacuole resorbed and starch was visible within plastids (Fig. 11A). After 7 days of culture, *in vitro* and *in vivo* developed pollen grains exhibited similar features (11B). When the culture conditions were not favourable, mitosis usually occurred, but amylogenesis in the VC was less important (Fig. 11C). Furthermore, abnormal microvacuoles were detected, which grew progressively, thus producing highly vacuolated pollen grains (Fig. 11D), with a weak germination rate.

Attempts of pollen development *in vitro* from earlier stages of ontogenesis with the same culture conditions were not successful. When flower buds were collected at the young vacuo-

Fig. 11. – *In vitro* pollen maturation. A – Flower bud cultivated in the light on Heller's medium supplemented with NAA (10^{-2} mg/l) and sucrose (M/6) after 3 days of culture: mitosis had occurred. In the cytoplasm of the vegetative cell (VC), vacuole (V) was fragmented, lipids accumulated (arrowheads), whereas starch was present in the plastids (P), GC – generative cell, N – nucleus. PATAg, X 6,000. B – Flower bud cultivated in the light on Heller's medium supplemented with NAA (10^{-2} mg/l) and sucrose (M/6) after 7 days of culture: starch had disappeared from the plastids, but lipids (L) and phytine corpuscles (arrowheads) were abundant. PATAg, X 9,000. C – Flower bud cultivated in the light on water supplemented with sucrose (M/6) after 3 days of culture: abnormal vacuoles were abundantly distributed in cytoplasm of the VC. PATAg, X 9,000. D – Flower bud cultivated in the light on water supplemented with sucrose (M/6) after 7 days of culture: vacuoles were the most well developed organelle in the VC. PATAg, X 15,000.

lated microspore stage, most of microspores did not enter sporadic mitosis and later degenerated. After 7 days of culture in the best culture conditions (Heller's medium, sucrose M/6, NAA 10^{-2} mg/l and light), the germination rate was $4.8 \pm 0.6\%$.

DISCUSSION

In vivo pollen maturation

Before pollen mitosis, in the cytoplasm of the VC, the division of organelles such as mitochondria, plastids and dictyosomes is quickly followed by sporadic mitosis. These results are in accordance with previous reports dealing with appearance of new proteins (Wang *et al.* 1992) and new mRNA (Mascarenhas 1992) in the gametophytes at the vacuolated microspore stage. They confirm that a new program is initiated from the microspore stage, and governs pollen maturation.

In *Lilium*, *in vivo* pollen maturation can be decomposed in two physiological periods based on reserve metabolism, especially on the cycle of amylogenesis/amyolysis. The first one leads from mitosis to the amylaceous pollen grain. During this first period, plastids accumulate high amounts of starch, whereas the vacuole is resorbed simultaneously. Owing to the fact that there is no polysaccharides left in the locular fluid at this time of pollen development (Clément *et al.* 1994), our observations could suggest that the microspore vacuole contains some carbohydrates, which are next accumulated in starch within the plastids.

The second period begins at the amylaceous pollen grain stage and achieves at the mature pollen grain stage. During progressive starch degradation, many sugar consuming activities are detected in the cytoplasm of the VC, as for instance lipid accumulation. It was previously showed that monomers from starch breakdown are used at least partly for lipid biosynthesis (Miki-Hirosige and Nakamura 1983). Simultaneously, the accumulation of phytine reserves, earlier described by several authors (Dickinson and Lin 1986, Baldi *et al.* 1987), as well as the formation of the third layer of the intine, are also sugar consuming events and could possibly use starch degradation products. The association of RER with plastids during whole pollen maturation can be interpreted as 1) a way of sugar incorporation in the plastid during amylogenesis (Pacini *et al.* 1992) and 2) as a route for starch breakdown products towards the cytoplasm (Polowick and Sawhney 1993) or towards the GC (Luegmayr 1993) during amyolysis. The GC in *Lilium* pollen is rather poor in organelles as in other species (Schröder and Oldenburg 1990, Van Aelst and Van Went 1991), although it was shown that the GC presents ATP consuming activities (Knox 1984), especially mRNA translation into proteins (Chaboud and Perez 1992). The VC organelles concentrated around the GC could provide energy and metabolites through the thin double membrane interspace.

The two physiological periods of pollen maturation defined in this paper coincide with the cycle of amylogenesis/amyolysis occurring in the plastids. It seems that each cytological event detected during pollen maturation is dependant upon starch accumulation or degradation.

In vitro pollen maturation

Our experiments showed that pollen maturation in *Lilium* can occur *in vitro*. The best results were obtained with flower buds, on Heller's medium supplemented with NAA (10^{-2} mg/l) and sucrose (M/6). In these conditions, cytological features and germination rate of *in vitro* grown pollen are identi-

cal to naturally matured pollen, strongly suggesting that artificially developed pollen is conform to *in vivo* grown pollen. Further, *in vitro* maturation occurs within 7 days instead of 14 *in vivo*, indicating that the culture conditions described in this paper are optimal for *Lilium* pollen maturation. Nevertheless, the fertilization ability of artificial pollen needs to be verified, in order to obtain a better estimation on the quality of pollen maturation (Benito-Moreno *et al.* 1988).

From our results, the influence of growth regulators is decisive for pollen maturation in *Lilium*. The auxin NAA alone (10^{-2} mg/l) leads to conform mature pollen grain *in vitro*. Numerous structural and biochemical modifications occur in the pollen grain during maturation. Auxin may contribute to these transformations, especially to the acquisition of germination ability (Cresti *et al.* 1990).

Concerning carbohydrates, sucrose seems to be the most well adapted sugar for pollen maturation from flower buds of *Lilium*. These data are in agreement with results registered previously. Sucrose is known to be 1) a convenient carbon source for *in vitro* development of *Lycopersicon esculentum* flower buds (Rastogi and Sawhney 1987), and 2) an adapted osmoticum for microspore culture in *Lilium longiflorum* (Tanaka *et al.* 1980).

From our results, it seems that the darkness and photosynthesis inhibitors have a negative influence on pollen maturation. But this effect can be compensated by providing higher amounts of sucrose in the medium. These data strongly suggest that 1) photosynthesis occurs in the flower bud *in vivo*, and 2) assimilates produced by photosynthesis in the buds are necessary to pollen maturation. The involvement of flower organ photosynthesis in the development of reproductive structures was previously reported in *Malus* (Vemmos and Goldwin 1993), and in many other species (Bazzaz *et al.* 1979).

In order to have a better comprehension of whole pollen development, it would be interesting to obtain conform development of pollen *in vitro* from the earliest stages of pollen ontogenesis (microspore mother cell or sporogenous tissue). Stapleton and Bedinger (1992) realised complete pollen development from premeiosis to mature pollen grain in *Zea mays*. The conditions used in our study did not allow entire development of *Lilium* pollen. Especially, microspores would not pass through mitosis. Further, the other flower organs of *Lilium* also stop their development at the same stage (data not shown). An additional factor(s) seems to be necessary to induce all metabolic changes leading to pollen mitosis and, next, pollen maturation. This unknown condition may be of importance not only for pollen, but also for the whole flower bud development.

Further studies are still necessary to overcome pollen mitosis *in vitro*. Elucidating this problem would bring useful informations at a fundamental level, but also at a biotechnological level. The success of androgenesis depends upon the competence of the microspores to modify their gametophytic program to a new sporophytic program at the time of sporadic mitosis (Kieffer *et al.* 1993). Success in this task may help androgenesis failure in recalcitrant species.

ACKNOWLEDGEMENTS

We thank Dr M. Burrus for helpful advices in writing the English manuscript.

LITERATURE CITED

- AL AWAD D. 1993. Etude de la production pollinique en conditions artificielles et analyse *in vitro* de l'expression des potentialités organogènes des pièces florales chez *Lilium* cv. 'enchancement' à partir de boutons floraux d'âges variés, conservés ou non à 4°C. Ph.D. thesis of the University of Reims, (France) 169 p.
- BALDI B.G., FRANCESCHI F.A., LOEWUS F.A. 1987. Localization of phosphorus and cation reserves in *Lilium longiflorum* pollen. *Plant Physiol.* 83: 1018-1021
- BAZZAZ F.A., CARLSON R.W., HARPER J.L. 1979. Contribution to reproductive effort by photosynthesis of flower and fruits. *Nature* 279: 554-555
- BENITO-MORENO R.M., MACKE F., ALWEN A., HEBERLE-BORS E. 1988. *In situ* seed production after pollination with *in vitro* matured, isolated pollen. *Planta* 176:145-148
- BRAR D.S., KHUSH G.S. 1994. Cell tissue for plant improvement. In: Mechanisms of plant growth and improved productivity. A.S. Basra (ed). Marcel Dekker Press, New York, pp. 229-278
- CHABOUD A., PEREZ R. 1992. Generative cells and male gametes: isolation, physiology and biochemistry. *Int. Rev. Cytol.* 140: 205-232
- CLÉMENT C., AUDRAN J.C. 1995. Anther wall layers control pollen sugar nutrition in *Lilium*. *Protoplasma* 187: 172-181
- CLÉMENT C., CHAVANT L., BURRUS M., AUDRAN J.C. 1994. Anther starch variations in *Lilium* during pollen development. *Sex. Plant Reprod.* 7: 347-356
- CRESTI M., MILANESI C., SALVATICI P., VAN AELST A.C. 1990. Ultrastructural observations of *Papaver rhoeas* mature pollen grains. *Bot. Acta* 103: 349-354
- DICKINSON D.B., LIN J.J. 1986. Phytases of germinating lily pollen. In: Biotechnology and ecology of pollen. Mulcahy D.L., Ottaviano E., Mulcahy G.B. (eds). Springer-Verlag, New York, Berlin, Heidelberg, Tokyo, pp. 357-362
- KIEFFER M., FULLER M.P., CHAUVIN J.E., SCHLESSER A. 1993. Anther culture of kale (*Brassica oleracea* L. convar. *acephala* (DC.) Alef). *Plant Cell Tiss. Org. Cult.* 33: 303-313
- KNOX R.B. 1984. The pollen grain. In: Embryology of Angiosperms. Johri B.M. (ed). Springer-Verlag, New York, Berlin, Heidelberg, Tokyo, pp. 197-271
- LUEGMAYR E. 1993. The generative cell and its close association with endoplasmic reticulum of the vegetative cell in pollen of *Cyrtandra pendula* (Gesneriaceae). *Protoplasma* 177: 73-81
- MACCORMICK S. 1993. Male gametophyte development. *The Plant Cell* 5: 1265-1275
- MASCARENHAS J.P. 1992. Pollen gene expression: molecular evidence. *Int. Rev. Cytol.* 140: 3-18
- MIKI-HIROSIGE H., NAKAMURA S. 1983. Growth and differentiation of amyloplasts during male gamete development in *Lilium longiflorum*. In: Pollen: biology and implications for plant breeding. Mulcahy D.L., Ottaviano (eds). Elsevier Science Publisher, Biomedical, New York, pp. 141-147
- PACINI E., TAYLOR P.E., SINGH M.B., KNOX R.B. 1992. Development of plastids in pollen and tapetum of rye-grass, *Lolium perenne* L. *Ann. Bot.* 70: 179-188
- PAREDDY D.R., PETOLINO J.F. 1992. Maturation of maize *in vitro*. *Plant Cell Rep.* 11: 535-539
- POLOWICK P.L., SAWHNEY V.K. 1993. An ultrastructural study of pollen development in tomato (*Lycopersicon esculentum*). 1. Tetrad to early binucleate microspore stage. *Can. J. Bot.* 71: 1039-1047
- RASTOGI R., SAWHNEY V.K. 1987. The role of plant growth regulators, sucrose and pH in the development of flower buds of tomato (*Lycopersicon esculentum* Mill.) cultured *in vitro*. *J. Plant Physiol.* 128: 285-295
- RASTOGI R., SAWHNEY V.K. 1989. *In vitro* development of Angiosperms floral buds and organs. *Plant Cell Tiss. Org. Cult.* 16:145-174
- ROLAND J.C. 1978. General preparation and staining of thin sections. In: Electron microscopy and cytochemistry of plant cell. Hall J.L. (ed). Elsevier/North Holland Biomedical press, Amsterdam, Oxford, New York, pp. 1-62
- SCHRÖDER M.B., OLDENBURG H. 1990. Ultrastructural studies on plastids of generative and vegetative cell in *Liliaceae*. 7. Plastid distribution during generative cell development in *Tulbaghia violacea* Harv. *Flora* 184: 131-136
- SOUTHWORTH D. 1983. Developmental changes in UV absorbance by pollen exine layers in *Gerbera* (Asteraceae). *Grana* 22:115-118
- STAPLETON A.E., BEDINGER P.A. 1992. Immature maize spikelets develop and produce pollen in culture. *Plant Cell Rep.* 11: 248-252
- TANAKA I., TAGUCHI T., ITO M. 1980. Studies on microspore development in Liliaceous plants. II The behavior of explanted microspores of the Lily, *Lilium longiflorum*. *Plant Cell Physiol.* 21: 667-676
- VAN AELST A.C., VAN WENT J.L. 1991. The ultrastructure of mature *Papaver dubium* L. pollen grains. *Acta Bot. Neerl.* 40: 319-328
- VEEMOS S.N., GOLDWIN G.K. 1993. Stomatal and chlorophyll distribution of Cox's orange pippin apple flowers relative to other cluster parts. *Ann. Bot.* 71: 245-250
- WANG C.S., WALLING L.L., ECKARD K.J., LORD E.M. 1992. Patterns of protein accumulation in developing anthers of *Lilium longiflorum* correlate with histological events. *Am. J. Bot.* 79:118-127

DOJRZEWANIE PYŁKU *LILIAM* *IN VIVO* I *IN VITRO*;
WPŁYW WĘGLOWODANÓW

STRESZCZENIE

Opracowaliśmy metodę dojrzewania pyłku *Lilium in vitro* w celu zbadania wpływu węglowodanów na dojrzewanie. Pyłek zaczyna dojrzewać w stadium zwakuolizowanej mikrospory. Okres dojrzewania składa się z dwu faz. W pierwszej fazie po reaktywacji organelli następuje mitoz, syntetyzuje się skrobia i wakuola ulega fragmentacji. Podczas drugiej fazy skrobia jest stopniowo degradowana a gromadzone są lipidy i fityna. Okres dojrzewania pyłku trwa do 14 dni, a $73,6 \pm 2,2\%$ ziaren pyłku jest zdolne do kiełkowania.

Dojrzały pyłek powstał *in vitro* ze zwakuolizowanych mikrospor. Najlepsze rezultaty otrzymano, kiedy paki kwiatowe hodowano w 26°C w $100 \mu\text{mol/m}^2/\text{s}$ przy fotoperiodzie 16h/8h na zmodyfikowanej pożywce Hellera z dodatkiem NAA (10^{-2} mg/l) i sacharozy (M/6). W tych warunkach pyłek dojrzewał już w 7 dni. Dojrzałe ziarna pyłku *in vitro* są cytologicznie do siebie podobne i mają podobną zdolność kiełkowania (*in vitro* – $72,4 \pm 3,7\%$). Pyłek dojrzewający w pakach kwiatowych hodowanych *in vitro* w ciemności ma zmniejszoną zdolność kiełkowania. Brak światła można skompensować zwiększając stężenie sacharozy (1M) w podłożu. Inhibitory fotosyntezy miały podobny wpływ na dojrzewający pyłek jak brak światła. Stąd można wnioskować, że fotosynteza odbywa się w paku lilii i jest ważnym czynnikiem potrzebnym do dojrzewania pyłku.

SŁOWA KLUCZOWE: dojrzewanie pyłku, cytologia, *in vitro*, *Lilium*, mitoz, pyłek.