Acta Bot. Neerl. 35(4), November 1986, p. 521–528.

MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR PLANT MORPHOLOGY, -ANATOMY AND -CYTOLOGY ON 25 OCTOBER 1985

F. BOUMAN* and W. MEIJER** (**Hugo de Vries laboratorium, Universiteit van Amsterdam, **University of Kentucky, Lexington, USA*)

Comparative seed morphology in Rafflesiaceae

The parastic family Rafflesiaceae comprises 8 genera and about 50 species. The ovule and seed characters support the subdivision of the family by H. HARMS (1935, *Die natürllichen Pflanzenfamilien*, vol. **16** b).

The ovules show two main evolutionary trends, viz. a change from anatropous towards orthotropous ovules (Rafflesieae and Cytineae) and one from bitegmic, as in Apodantheae, towards unitegmic ovules. The tribes Rafflesieae and Cytineae have rudimentary outer integuments, while the Mitrastemoneae are fully unitegmic. The nucellus is small and tenuinucellate throughout and degenerates early.

In all genera the outer layer of the inner of the single integument becomes sclerotic, so that the seeds are in fact exotegmic. Only in the Apodantheae is the exotegmen covered by uni-layered testa. The exotegmic cells are thickened all around or have U-shaped thickened walls (in Rafflesieae and Mitrastemoneae). In *Rafflesia* the testal and tegmic parts of the seed coat are clearly distinguishable. the seeds are relatively small, have an oily endosperm and an undifferentiated embryo consisting of a few cells only. The mature seeds are often embedded in a slimy or pulpy endocarp and mainly dispersed by endozoochory, in some genera possibly also epizoochorously.

MARGARIDA VENTURELLI *(Instituto de Biociências, Universidade de São Paulo, Brasil)*

Embryology and seed structure in Mayacaceae

The Mayacaceae were embryologically unknown and *Mayaca fluviatilis* Aubl. was studied for its embryology and seed development. The flowers are at least partly cleistogamous. The anther is tetra-sporangiatae and opens by a pore. Anther wall formation follows the basic type. An endothecium does not differentiate, but the epidermal cells show U-thickened walls and form an exothecium. The tapetum is glandular with uninucleate cells. The microspore mother cells undergo successive reduction divisions to form isobilateral and decussate tetrads. The ovules are orthotropous, bitegmic and tenuinucellate. The megaspore mother cell forms a T-shape tetrad of which the chalazal megaspore develops into a *Polygonum* type of embryo sac. The endosperm is initially nuclear but later becomes cellular. The endosperm cells accumulate starch, the outermost layer, which is proteinaceous, excepted. The embryogeny follows the Onagrad type. The mature endosperm is small and undifferentiated. A hypostase is present in the mature seed. Both the inner and the outer integument are of dermal derivation and remain mainly 2-layered. After fertilization the thin-walled cells of the inner layer of the outer integument and the outer layer of the inner integument show a considerable radial extension, thus closing the micropyle. The mechanical layer of the seed coat originates from the outer cell layer of the outer integument that acquired U-thickened walls. The seed has no operculum.

The family Mayacaceae shows a singular combination of characters. Although it shares most embryological characters with the other families of the Commeliniflorae, none of them has a comparable set of characters. Derived mayaceous characters are: the poricidal anthers and the lack of

an endothecium, the orthotropous and tenuinucellate ovules, and the absence of an endotestal or exotegmic layer. The family deviates from the other Commelinales by the lack of an endothecium, its Onagrad type of embryogeny and the structure of the exotesta.

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Three-dimensional reconstruction of the generative cell and its wall connection in mature bicellular pollen of Rhododendron

Pollen grains of *Rhododendron* spec. were germinated *in vitro*, and stained with DAPI (4,6 dimidino-2 phenyl indole). In the pollen grain there is a bright stained generative cell and a less deeply stained vegetative nucleus. The germination shows that the vegetative nucleus mostly preceeds the generative cell in the tube. After about 50 hrs the generative cell divides into two sperm cells, presumably connected. Three dimensional reconstruction (McConchie et al. 1985) of the generative cell in the mature grain shows a complex elongated spindle shaped cell, with two long tips curling around the cell. One of these tips is connected to a PAS positive wall ingrowth, connecting the outer tangential wall of the intine to the generative cell. Cytochemistry (PA-TCH-SP staining) showed it is a much convoluted polysaccharide wall ingrowth. No linkage to the vegetative nucleus was found in the mature stage.

Three dimensional reconstruction of the generative cell and vegetative nucleus in the pollen tube shows a highly complex generative cell, with a lot of finger-like tails around the vegetative nucleus. The part of the generative cell containing the nucleus is behind the vegetative nucleus, but a large amount of the cytoplasm preceeds the vegetative nucleus. In this stage the connection is broken.

McConchie, C. A., S. Jobson & R. B. Knox (1985): Computer-assisted reconstruction of the male germ unit in pollen of Brassica campestris. *Protoplasma* **127**: 57–63

H. J. WILMS and H. B. LEFERINK-TEN KLOOSTER *(Vakgroep Plantencytologie en -morfologie, Arboretumlaan 4, 6703 BD Wageningen)*

Computer-assisted reconstruction of the male germ unit in pollen of Spinacia oleracea

To be published in *Acta Bot. Neerl.*

C. J. VENVERLOO *(Vakgroep Moleculaire Plantkunde, Nonnensteeg 3, 3211 VJ Leiden)*

The role of cytoplasm and nucleus in the regulation of the plane of cell division in epidermis cells of Nautilocalyx leaf explants

A high percentage of the first cell divisions in the highly vacuolated epidermis cells is periclinal and symmetrical. The extract "division site" is already determined two or more hours before prometaphase as deduced from the presence of a phragmosome and a band of microtubules at that time. The formation of the phragmosome is a process taking many hours. It is always preceeded by the migration of the nucleus to the centre of the cell.

When explants are grown in the presence of drugs that interfere with the cytoskeleton, the migration of the nucleus as well as the formation of a phragmosome are strongly inhibited. When such a premitotic inhibition was followed by release from this inhibition shortly before mitosis, a high percentage of the cells formed new cell walls in an abnormal orientation or position: after treatment with colchicine many oblique cell walls were formed, some of these clearly resulted from cell plates growing successively in an anticlinal and a periclinal plane. The disturbances produced by cytochalasin B were more severe. Most divisions were asymmetrical.

These results suggest 1) that F-actin is important for the localization of the new cell wall, and 2) that the cell plate is able to follow the guidance of a pre-existing "division site" even after a long premitotic colchicine treatment.

A. A. BOMINAAR, L. GOOSEN-DE ROO and C. L. DÌAZ *(Vakgroep Moleculaire Plantkunde, Botanisch Laboratorium, Nonnensteeg 3, 2311 VJ Leiden)*

Immunogold and immunofluorescence localization of pea-lectin in developing root hairs

By use of immunofluorescence staining, it was shown that lectin is present at the surface of tips of only a number of intact immature root hairs (DÌAZ et al. 1986).

This finding raised the question whether the non-stained hairs do not excrete lectin or whether they do not synthesize it.

To resolve this problem immunogold labelling was performed on ultrathin Lowicryl K4M sections of the root zone with developing root hairs.

Initial trials showed a heterogenous gold distribution over the root together with a remarkable background staining. This result however, was inconclusive with respect to the distribution of lectin and possible relocation of lectin during the processing of the material could not be excluded. The main problem was that an appropriately specific lectin-positive tissue control was not available.

Subsequent experiments in which lower concentrations of the primary antibody were used together with different concentrations of the blocking agent BSA, suggested that most of the gold labelling was due to non-specific staining rather than to relocation of lectin.

Results from the improved method indicated that lectin was predominantly present at the outer side of the plasmamembrane of lectin-positive root hairs and that lectin-negative hairs did not synthesize lectin at all.

DÌAZ, C. L., P. C. VAN SPRONSEN, R. BAKHUIZEN, G. J. J. LOGMAN, E. J. J. LUGTENBERG & J. W. KIJNE (1986): Surface lectin of pea (Pisum sativum) roots localized by immunofluorescence microscopy. *Planta* (in press).

W. A. VAN HEEL *(Rijksherbarium, Postbus 9514, 2300 RA Leiden)*

On the development of gynoecia with septal nectaries

In Asparagales and Liliales comparison shows how – superior – gynoecia having septal nectaries differ from those not having septal nectaries, as regards the early development. The lateral parts of the carpels grow inwards more freely ("hemisynplicate" sensu Leinfellner), leaving space for the septal nectaries. The wall of the gynoecium develops by meristematic fusion, leading to syncarpy. In the centre of the gynoecium the free lateral parts fuse by epidermal fusion, leading to closure of the young gynoecium and style formation. Between these different zones of fusion the slits for the nectaries result from non-fusion. The openings of the nectaries are on different levels of the ovary, depending on the degree of meristematic fusion.

In Alismatiflorae septal nectaries are more open and diffuse. The two kinds of fusion are slight and late, for instance in *Butomus*, or absent in others, in correspondence with the less syncarpous gynoecia. Evolutionary lines from open to localized septal nectaries are discovered by comparing developmental processes.

This work is in line with a review of septal nectaries by RUDOLF SCHMID (see *Acta Botanica Neerlandica* 34(1), 1985, p. 125–128).

M. T. M. WILLEMSE and M. A. W. FRANSSEN-VERHEIJEN *(Dept. of Plant Cytology and Morfology, Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands)*

Structural changes in the style during pollination of Gasteria verrucosa

During circa five days the stylar development in the open flower of *Gasteria verrucosa* was examined by ultrastructural analysis.

During about four days after the opening of the flower the style stretches, mainly in its central part. The style is covered by a thick undulated cuticle. During this period vacuole formation occurs as described for *Linum* by AMELUNXEN and coworkers (1984, 1985). In the style, the cells show dictyosomes that produce vesicles. The relative quantity of starch and lipid decreases. The stylar canal is nearly closed by a solid thick wall at the top and a more viscose wall at the basal part of the style. During the development in the open flower, lipid droplets appear along the stylar canal

cells, especially in the region beneath the top. Also the stigmatic papillar cell and subpapillar stylar canal cells show a difference in number of mitochondrial cristae and, later on, also in the production of dictyosome vesicles, this in comparison with the stylar canal cells in the basal part of the style.

After about four days stylar development stops and the stigma will accept compatible pollen. In the stylar cells, a great number of smooth endoplasmic reticulum appears while the number of cytoplasmic vesicles decreases, which suggests an activity in the preceding excretion. After germination of the pollen tubes, the stylar cells near the tip of the pollen tube excrete. After passing the pollen tube the number of lipid droplets in the stylar canal is diminished. In the upper part of the stylar canal with its solid wall, the pollen tube can collapse (WILLEMSE & FRANSSEN-VERHEIJEN 1984).

Near the ovules the epidermis of the placental tissue shows the development of a papillar transfer cell in the same period of five days. This cell probably functions in guiding the pollen tubes to the ovules. The composition of the cell is very close to these of the stigmatic papillar cell, except their walls.

AMELUNXEN, F. & U. HEINZE (1984): Zur Entwicklung der Vacuole in Testa-Zellen des Leinsames. *Eur. J. Cell Biol.* **35**: 343–354.

HILLING, B. & F. AMELUNXEN (1985): On the development of the vacuole II. Further evidence for endoplasmic reticulum origin. *Eur. J. Cell Biol.* **38**: 195–200.

WILLEMSE, M. T. M. & M. A. W. FRANSSEN-VERHEIJEN (1984): Pollen tube penetration in the style of Gasteria. In: M. T. M. WILLEMSE & J. L. VAN WENT (comp.): *Sexual reproduction in seed plants, ferns and mosses.* Proceedings 8th Int. Congress, Wageningen, Pudoc, Wageningen, p. 118.

F. H. A. WILMS *(Botanisch Laboratorium, Katholieke Universiteit, Nijmegen)*

Origin and development of floral buds in tobacco explants

Floral bud formation in *Nicotiana tabacum* was studied *in vitro*. Strips consisting of the epidermis and several layers of cortical cells were cut from floral stalks and cultured on solid Murashige & Skoog medium supplied with 150 mM glucose, 10^{-7} M Naphthaleneacetic acid and 10^{-6} M Benzylaminopurine.

The morphogenesis of floral buds can be divided in 3 stages:

- The first stage (0 to 4 days) is marked by the onset of the wound reaction leading to cell divisions at the wounded surfaces. These cell divisions continue throughout the three stages and eventually lead to the formation of wound periderm. The end of the first stage is marked by the formation of division centres in the epidermis and sub-epidermal cortical cells visible at the surface of the explants as primary outgrowths (up to 5 per explant).
- In the second stage (5 to 7 days) the primary outgrowths further expand and from their surface secondary outgrowths or protuberances begin to emerge. They are the result of new division centres formed by epidermal and sub-epidermal cells of the primary outgrowths. The central cells of the division centres in the cortex differentiate into tracheary elements.
- The third stage (8 to 15 days) is marked by the differentiation of the protuberances into organized flower buds. The differentiation occurs centripetally. First bracts are initiated and consecutively sepals, petals, anthers and pistils are formed. Inside the explant tracheary elements develop from the original tracheary centres into the different parts of the flower.

H. M. P. KENGEN, J. A. TRAAS and J. DERKSEN *(Botanisch Laboratorium, Katholieke Universiteit, Nijmegen)*

The cytoskeleton in protoplasts of Nicotiana plumbaginifolia

Using western blotting and immunofluorescence, protoplasts of *Nicotiana plumbaginifolia* were probed for cross-reactions with commercially available antibodies against desmin and vimentin, (intermediate filaments), α-tubulin (microtubules), actinin and actin (microfilaments). Also rhodamin-phalloidin (kindly provided by prof. Th. Wieland, Heidelberg) was used as a probe for microfilaments. The antibodies against vimentin and desmin, and actinin did not show cross-reactions in both western blots and immunofluorescence preparations. In western blots both anti-tubulin and

anti-actin showed a strong reaction with a single band (55.000 D and 43.000 D respectively, indicating a specific reaction with plant tubulin and plant actin. In immunofluorescence preparations dense arrays of randomly oriented microtubules were observed. In dry-cleave preparations for electron microscopy these microtubules are seen lying close to the membrane. Microfilament bundles showed identical distributions in preparations stained with anti-actin and rhodamin-phalloidin. Actin filaments seem to form a meshwork around the nucleus from where they run in bundles to other parts of the cell. These bundles are not strictly oriented, occasionally hoop-like arrangements were observed. As shown for microtubules, gold-labelled anti-bodies can also be used for the detection of cytoskeletal elements in cleaved preparations for electron microscopy.

A. M. C. EMONS *(Botanisch Laboratorium, Universiteit Nijmegen, Toernooiveld, 6525 ED Nijmegen)*

Differences in cell wall texture of root hairs supporting the division of the genus Equisetum into two subgenera

The only existing family of the Equisetinae, the Equisetaceae, is represented by the single genus *Equisetum*. Ten different species of the genus are mentioned in the Flora Europaea. These species were studied with respect to the cell wall texture of the root hairs using polarizing- and electron microscopy.

The present study concludes the presence of two categories of cell wall texture types in *Equisetum*. This distinction parallels the division of the genus *Equisetum* into the subgenera *Equisetum* (horsetails) and Hippochaete (scouring rushes). All species of the subgenus *Equisetum, E. arvense, E. fluviatile, E. palustre, E. pratense, E. sylvaticum* and *E. telmateia*, have a helicoidal cell wall texture in their root hairs, in young as well as in full-grown developmental stages. The helicoidal cell wall texture consists of a stack of parallel-fibred lamellae, each lamella one microfibril thick. Each subsequent lamella is progressively rotated with respect to the previous lamella. All species of the subgenus Hippochaete, *E. hyemale, E. ramosissimum, E. scirpoides* and *E. variegatum*, deposit an additional inner cell wall layer against the helicoidal layer when growth has stopped. The microfibrils in this additional layer do not produce a helicoidal texture, but are arranged in a Z-helix. A helical layer is positive birefringent in the polarizing microscope, while a helicoidal layer is isotropic.

In order to study the possibility of environmental influences i.c. the consistency of the substratum, root hairs of plants grown in water were studied as well as root hairs of plants grown in soil. In addition, for *Equisetum hyemale*, root hairs of plants grown in several agar concentrations were studied. However, it was shown that the wall texture is not influenced by the consistency of the substratum

The two aquatic species, *E. fluviatile* and *E. palustre* have only helicoidal wall texture, but the presence of a helical layer in full-grown hairs is not a prerequisite for life in soil.

MEETING OF THE NETHERLANDS SOCIETY FOR PLANT CELL AND TISSUE CULTURE, ON 15 NOVEMBER 1985

R. J. BOGERS *(Stichting Laboratorium voor Bloembollenonderzoek, Postbus 85, 2160 AB Lisse)*

Tissue-culture research at the Bulb Research Centre

Two important objectives of flower-bulb research are:
- improvement of quality
- introduction of new crops and cultivars.

Substantial attention is given to:
- the possibility to obtain virus-free plants by meristem culture and the development of specific, sensitive tests that can be applied on a large scale
- factors that influence the formation and growth of new bulblets; this work is aimed at obtaining large-scale methods for rapid multiplication (twin-scaling, chipping, tissue culture, etc.) of flower-bulb crops.

Research to develop methods for rapid multiplication in vitro (to be applied in commercial production) will be done in the Central Research Laboratory for Tissue Culture of Horticultural Crops. In this laboratory at Lisse (to be opened in the spring of 1986) the Bulb Research Centre and the Research Stations for Floriculture (Aalsmeer) and Arboriculture (Boskoop) will join their efforts.

Fundamental research on the rapid multiplication in vitro of tulips is being done in the Department of Plant Molecular Biology at the University of Leiden (Prof. Dr. K. R. Libbenga), in cooperation with the Bulb Research Centre.

P. G. ALDERSON *(Nottingham University School of Agriculture, Sutton, Bonington, Loughborough, Leiscester U.K.)*

In vitro propagation of bulbous plants

There is increasing interest in the use of tissue culture techniques for the commercial propagation of bulbous plants in the families Amaryllidaceae, Iradaceae and Liliaceae. These techniques may permit the rapid multiplication of healthy stocks of bulbs and new varieties produced in breeding programmes. For some species, e.g. *Tulipa, in vitro* propagation will eventually offer an alternative to the only method of vegetative propagation currently available, viz. natural daughter bulb formation. For other species *in vitro* propagation may permit rapid mass propagation without the seasonal constraints of traditional methods of propagation.

In culture many bulbous plants regenerate adventitious plantlets from explants of bulb scales, leaves, floral stems and axillary buds. The success of a culture is influenced by the genotype, the origin of the explant, the physiological state of the donor plant, and the chemical and physical environment of the cultured explant. Plantlets may arise on explants from the outer layer of cells or from internal tissues. In order to multiply plantlets in culture it may be necessary to manipulate them physically prior to subculture on media containing elevated levels of cytokinin. In some genera multiplication may be achieved by enhanced axillary shoot formation. Transfer of cultured progeny to the soil environment requires the initiation and development of bulbs at the base of plantlets which may be enhanced by specific temperature, light, plant growth regulator and carbohydrate regimes. After the progeny have been established in soil their genetic uniformity can be assessed.

The *in vitro* culture of bulbous plants will be reviewed to evaluate the requirements of different genera at the different stages in the propagation process. Special consideration will be given to current research on *Tulipa*.

P. C. G. VAN DER LINDE *(Stichting Laboratorium voor Bloembollenonderzoek, Postbus 85, 2160 AB Lisse)*

In vitro propagation of lily, iris and hyacinth

In vitro propagation of bulbous crops is used as a method to rapidly build up commercial stocks of plants, which are selected for quality, e.g., virus-free specimens or new cultivars. *In vitro* propagation of lily, iris and hyacinth starts by excision of a piece of scale from a surface-sterilized bulb. For iris this piece of scale is still attached to a part of the basal plate. These explants are subsequently cultured under conditions, that favour adventitious plantlet formation. The number of plantlets per explant is depending on many factors, such as pretreatment of the parent tissue, intensity of the surface sterilization, cultivar, size of the explant, polarity within the explant, and culture conditions. For lily and hyacinth the plantlets directly exhibit bulblet growth. For iris bulblet growth of the plantlets has to be induced by a cold treatment.

Further propagation is accomplished by subculture of the initially formed plantlets. For lily a scale of the bulblet is used and for hyacinth a piece of scale-leaf. Subculture of iris is started with longitudinally divided shoots.

Before planting bulblets of lily and hyacinth are stored at 5° C and iris at 30° C for some weeks to guarantee a normal and homogenous development in soil.

Due to differences in the number of explants per bulb, in the number of plantlets per explant and in further propagation of the initially formed plantlets, the yield after two cycles of propagation is different for these three genera. For lily and hyacinth more then 10,000 bulblets per bulb are obtained, whereas for iris this figure is less then 1000. Further research on the needs of the tissue

in subsequent propagation cycles and on the growth of the bulblets is necessary to be able to optimize the propagation schemes for these crops.

G. M. S. HOOYKAAS-VAN SLOGTEREN *(Vakgroep Moleculaire Plantkunde MOLBAS, Biochemisch Laboratorium, Wassenaarseweg 64, 2333 AL Leiden)*

Can Agrobacterium tumefaciens be used for genetic manipulation of bulbous crops and other monocotyledons

The soil bacterium *Agrobacterium tumefaciens* is a member of the bacterial family of *Rhizobiaceae*. Virulent *Agrobacterium* strains are able to induce tumors on dicotyledonous plants. The mechanism leading to tumor induction has largely been elucidated in recent years. In addition to its chromosome *A. tumefaciens* carries a self-replicating circular DNA molecule, the Ti plasmid, which carries the genes involved in tumor induction. After infection a distinct part of the Ti plasmid – the T-DNA – is incorporated in the genome of the cells of the infected plants. The T-DNA harbors genes coding for enzymes involved in the synthesis of the plant hormones auxin and cytokinin and a number of tumor specific compounds called "opines". The production of large quantities of plant hormones leads to an unlimited growth of both transformed plant cells and neighbouring untransformed cells. The opines which are excreted by the tumor cells can be used by agrobacteria as a source of nutrition. Therefore, agrobacteria genetically manipulate plants in order to create a favourable niche for themselves. In the laboratory this property of agrobacteria can be used for the introduction of foreign genes into plants, namely by introducing these foreign genes first into the T-DNA of the Ti plasmid.

After infection with virulent agrobacteria tumors are formed on dicotyledonous plants but not on monocots. This was the reason that until two years ago it was generally believed that monocots could not be transformed by *Agrobacterium*. This turned out to be a wrong assumption. We hypothesized that even though no tumor formation occured, still transformation might have taken place. Therefore, we tested a number of monocotyledonous plants for the presence of the T-DNA encoded tumorspecific opines in cells around the wound sites that had been infected with *Agrobacterium*. In this way indeed, opines were found in infected *Narcissus, Hyacinthus* and *Chlorophytum* plants. No opines were found in uninfected plants, nor in plants that had been infected with *Agrobacterium* strains, carrying a mutation affecting virulence, even if an intact T-DNA was present.

From these findings it can be concluded that the genetic engineering of at least certain monocots via *Agrobacterium* is no longer utopic.

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Tissue culture in wheat and rye

J. B. M. CUSTERS *(Instituut voor de Veredeling van Tuinbouwgewassen, Postbus 16, 6700 AA Wageningen)*

Tissue culture in lily and tulip

At the IVT interspecific crosses in *Lilium* and *Tulipa* are made to breed new valuable cultivars. In these crosses embryo abortion often occurs. The embryo culture method of Asano was used to solve this problem.

With lily a number of crosses between unrelated species belonging to different sections succeeded. Thus *L. candidum* and *L. rubellum* germplasm was introduced in *L. longiflorum*, *L. longiflorum* germplasm into the Asiatic hybrids, and *L. henryi* germplasm into the Oriental hybrids. Embryo culture appeared to be a prerequisite for bridging the sectional boundaries. Thereafter, in the backcrosses and in the combinations between plants from backcrosses embryo culture was not necessary, but yielded a 10–1000 fold improvement of the crossing results.

Interspecific crosses in tulip exhibited the same types of divergent embryo development as in lily; normal sized embryos which locally start to degenerate, medium sized embryos in juicy endosperm or very small embryos without endosperm. All these embryos had a high rate of survival after incubation in vitro, but their development was very abnormal, for instance secondary embryoid formation occurred on a very large scale. On medium containing NAA 0.001 mg l^{-1} as growth

regulator and using temperature treatments derived from the normal thermoperiodicity in tulip, only sporadically seedlings or bulblets were obtained which could be transplanted into soil. Non-aborting embryos showed the same problems after culture in vitro.

G. J. BLOM-BARNHOORN, P. C. G. VAN DER LINDE and J. VAN AARTRIJK
(Stichting Laboratorium voor Bloembollenonderzoek, Postbus 85, 2160 AB Lisse)

Production of virus-free plants by meristem culture

Virus-free plants of several varieties of lily, hyacinth and iris were produced by culture of excised meristems, formed adventitiously on bulb-scale explants (ASJES et al. 1974; BLOM-BARNHOORN & VAN AARTRIJK 1985). Induction of the meristems was performed as described for *in-vitro* propagation of bulbous crops (VAN AARTRIJK & BLOM-BARNHOORN 1979, PIERIK & RUIBING 1973).

We have investigated the influence of storage temperature of the motherbulbs, of exogenous auxin and of culture temperature on growth and bulblet formation of excised meristems.

We also studied the effects of Virazole (= ribavirin: 1,β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, ICN Pharmaceuticals, Inc.) in the meristem-inducing medium on the growth of adventitiously formed meristems and on the percentage of virus-free plants.

Storage of bulbs of hyacinth "Pink Pearl" at 2°C during 8 weeks resulted in a better growth of meristems excised from bulb scales as compared to storage at 30°C. Meristems excised from explants of bulbs of iris "Prof. Blaauw" stored at 30°C, formed bigger bulblets than meristems from bulbs stored at 20°C or 9°C.

For hyacinth the auxin indole-3-butyric acid (IBA) had distinct effects on bulblet formation of excised meristems and on bulblet weight (BLOM-BARNHOORN et al. 1986).

Bulblets of lily and iris grown on media with 1-naphthylacetic acid (1-NAA) were heavier than those grown on media without 1-NAA. In all cultures the survival rate was increased by auxin.

Meristems of iris and hyacinth grew better at 15°C than at 20°C.

Addition of up to 40.0 μM Virazole to the meristem-inducing medium did not reduce induction and growth of the meristems.

Virazole reduced the percentage of meristem cultured, LSV-infected plants of *Lilium longiflorum* "Arai" from 38.2% to 10.8%. The percentages of virus-infected plants were not reduced in the other cultures: hyacinth "Rosalie" infected with hyacinth mosaic virus, a number of iris cultivars (infected with iris mosaic virus), and *Lilium* "Enchantment" infected with lily symptomless virus and tulip breaking virus.

Up to now, for all cultivars virus-free plants were obtained by meristem culture.

AARTRIJK, J. VAN & G. J. BLOM-BARNHOORN (1979): Some influences of 1-naphtylacetic acid on the differentiation of meristems of Lilium speciosum "Rubrum" nr. 10 in vitro. *Acta Hortic.* **91**: 269–279.

ASJES, C. J., M. H. BUNT & D. H. M. VAN SLOGTEREN (1974): Production of hyacinth mosaic virus-free hyacinths and lily symptomless virus-free lilies by meristem-tip culture. *Acta Hortic.* **36**: 223–228.

BLOM-BARNHOORN, G. J. & J. VAN AARTRIJK (1985): The regeneration of plants free of LSV and TBV from infected Lilium bulb-scale explants in the presence of Virazole. *Acta Hortic.* **164**: 163–168.

—, — & P. C. G. VAN DER LINDE (1986): Effects of Virazole on the production of hyacinth plants free from hyacinth mosaic virus (HMV) by meristem culture. *Acta Hortic.* **177**. In press.

PIERIK, R. L. M. & M. A. RUIBING (1973): Regeneration of bulblets on bulb-scale segments of hyacinth in vitro. *Neth. J. Agric. Sci.* **21**: 129–138.

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View of flower-bulb breeders on the application of in vitro techniques