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MEETINGS

MEETING OF THE NETHERLANDS SOCIETY FOR PLANT CELL AND TISSUE CULTURE ON 10 MARCH 1983

F. QUAK (*Instituut voor Planteziektenkundig Onderzoek (IPO), Postbus 42, 6700 AA Wageningen*)
Tissue culture and phythopathology

F. A. HAKKAART (*Rijksproefstation voor de Bloemisterij in Nederland, Linnaeuslaan 2a, 1431 JV Aalsmeer*)
Disease control of floricultural crops using meristem culture techniques

J. VAN AARTRIJK and G. J. BLOM-BARNHOORN (*Laboratorium voor Bloembollenonderzoek, Postbus 85, 2160 AB Lisse*)
Use of meristem culture to produce virus-free bulb crops

D. BAKKER (*Nederlandse Algemene Keuringsdienst (NAK) van de Noordoostpolders, Randweg 14, 8304 AS Emmeloord*)
Meristem culture and in vitro propagation of potato

G. STARITSKY, I. H. W. HOVERS and E. A. ZANDVOORT (*Vakgroep Tropische Plantenteelt, Landbouwhogeschool, Postbus 341, 6700 AH Wageningen*)
Rifampicin, an effective antibiotic against bacteria in cultures in vitro

The isolation of bacteria-free explants, mostly buds or nodal segments as starting material for vegetative propagation in vitro is often problematic at the department. The bacteria in question are in general non pathogenic *Pseudomonas, Flavobacterium, Alcaligenes* and *Escherichia* spp. which do not seriously inhibit the growth of vigorous cultures. However, under less favourable conditions, e.g. during storage of germplasm, the bacteria may overgrow the cultures. Moreover, contaminated cultures are a menace to clean ones in the collection.

Stimulated by the success of PHILLIPS et al., (1981) a number of antibiotics were tested on their effect on bacterial growth in contaminated cultures of *Cinchona pubescens* and a *Cryptocoryne* sp. Filter sterilised solutions of oxytetracycline, streptomycin, chloromycetin (= chloroamphenicol), penicillin-G and rifampicin were added to autoclaved medium cooled down to c. 60 °C, at concentrations of 10 and 50 mg/l. Rifampicin and the more stable gentamycin were, in concentrations of 10, 50 and 100 mg/l, autoclaved together with the medium for 20 min. at 115 °C. To facilitate the observations on bacterial growth the crystal-clear gelling agent 'Gelrite' was used to solidify the medium.

Oxytetracycline, chloromycetin and penicillin-G inhibited the growth of the explants; only in the oxytetracycline 50 mg/l treatment bacterial growth also was inhibited. Streptomycin and gentamycin affected neither the growth of the explants nor that of the bacteria. Only rifampicin inhibited the growth of the bacteria without affecting the development of the explants in all treatments.

Rifampicin does not usually kill the bacteria and after transfer of the cultures to medium without antibiotic, re-establishment of the bacterial population is possible. Nevertheless, after repeated treatment bacteria-free cultures can be isolated.

PHILLIPS, R., S. M. ARNOTT and S. E. KAPLAN (1981): Antibiotics in plant tissue cultures: Rifampicin effectively controls bacterial contaminants without affecting the growth of short-term explant cultures of Helianthus tuberosus. *Plant Sci. Lett.* 21: 235–240.

A. F. CROES (*Botanisch Laboratorium, Toernooiveld, 6525 ED Nijmegen*)

Role of hormones in the initiation and development of tobacco flower buds in tissue culture

Explants consisting of epidermis and several layers of subepidermal cortex are cut from flower stalks of tobacco and plated on Murashige-Skoog medium. Primordia appear after approximately one week on the surface of the tissue and can be recognized as flower bud primordia three days later.

Development is dependent on the presence of a metabolizable sugar (glucose 30 g l^{-1}), auxin (naphthalene acetic acid) and cytokinin (benzyladenine).

The cytokinin concentration is the critical factor in determining the number of buds per explant formed. A low auxin concentration (10^{-7} mol l^{-1}) promotes flower bud initiation but further development is supported by a higher auxin level (10^{-6} mol l^{-1}). In some experiments explants were shifted after different periods of incubation to non-inductive medium (concentrations of both hormones: 10^{-7} mol l^{-1}). The results show that physiological processes leading to flower bud initiation take place in the first days after explanting. No specific morphological changes are observed in the tissue by that time.

CATHARINA J. VENVERLOO (*Vakgroep Moleculaire Plantkunde, Nonnensteeg 3, 2311 VJ Leiden*)

The influence of colchicine and other inhibitors on the plane of cell division in Nautilocalyx explants

Mitosis in the highly vacuolated epidermis cells of leaf explants of *Nautilocalyx* was preceded by the formation of a phragmosome (PS), a process taking some hours, and probably starting after the migration of the nucleus to the centre of the cell. A nearly continuous PS, dividing the cell into two parts was already present 1–2 hours before the appearance of the mitotic apparatus. During mitosis and cytokinesis the PS remained in position; it guided the growing cell plate to the cell wall. A band of microtubules (BMT = PPB) was also found in these cells; it was present before mitosis. Both the PS and the BMT give an early indication of the plane of cell division and of the exact place where the new cell wall will meet the old walls.

Inhibitors of cytokinesis were tested in low concentrations for their ability to interfere with the formation of PS or BMT. 2,6-Dichlorobenzonitrile, an inhibitor of cellulose synthesis, did not change PS-formation or the plane of cell division; it prevented the finishing of the cell plate and produced binucleate cells without a cell wall or with a partial wall. Colchicine (15–50 μM) inhibited the migration of the nucleus to the centre of the cell and the formation of a premitotic PS; it changed the predominant direction of cell division from periclinal to anticlinal. The new cell walls were often curved and incomplete. The function of BMT and PS will be discussed.

ANNEKE M. HEMRIKA-WAGNER and L. H. W. VAN DER PLAS (*Vakgroep Plantenfysiologie, Biologisch Laboratorium, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam*)

Callus induction of potato tuber tissue at different temperatures and the effect on the energy metabolism

When potato tuber discs were placed on a callus inducing medium at a high temperature (28 °C), callus induction started within a few days. The fresh weight increased exponentially with a doubling time of about 20 days. During incubation at a low temperature (8 °C) the fresh weight of the discs increased hardly during the first three weeks, after this lag phase growth started with a doubling time of about 35 days.

On the first days of incubation at 28 °C respiration was highly insensitive to malonate, while during incubation at 8 °C the period of malonate insensitivity extended over the first weeks of incubation. These results indicate that during the lag phase of growth glucose is mainly oxidized via the pentose phosphate pathway.

A high capacity of alternative oxidase pathway respiration developed during callus induction

at 28 °C (75% of respiration proved to be azide-resistant), while during callus induction at 8 °C only 40% of respiration was resistant to azide. In the lag phase of growth the activity of the alternative pathway was low, during the exponential phase of growth the activity reached its maximal rate. At both culture temperatures activities of alternative pathway respiration were of the same order of magnitude.

MEETING OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR WILD FLORA PROTECTION ON 30 OCTOBER, 1980

M. T. JANSEN (*Kerkewijk 188, 3904 JK Veenendaal*)

The flora of the bridge embankments and dikes in the Fluviatile District in The Netherlands

The wild flora of the Fluviatile District in The Netherlands decreased enormously during recent decades. Many wild flowers of the slopes of the dikes disappeared as a consequence of fertilizer treatment, whereas those of the flood meadows lost their habitat due to both sand- and clay-pits and agricultural activities. Fertilization and egalisation of the area of the flood meadows especially influenced the flora.

At present many interesting plant species can be found only on the embankments which are not so intensively influenced by man. These embankments are higher and as a consequence drier than the surrounding landscape. Therefore many plant species of a more southern and eastern distribution area can be found on these sites.

Some of these higher parts in the landscape along the rivers IJssel, Rhine, Lek, Waal and Maas can be mentioned together with a number of interesting plant species:

a. River IJssel – Railway dike near Westervoort. This site is extremely rich in *Coronilla varia*. Besides, a number of species are found which are rare in this part of the country, e.g. *Astragalus glycophyllos, Koeleria cristata, Brachypodium pinnatum, Anthyllis vulneraria,* and *Sedum telephium* ssp. *maximum*. In 1981 *Chrysanthemum corymbosum*, a plant new for the Dutch flora, was found on this site.

b. River Rhine – Railway dike near Arnhem. The part of this dike on the Southern bank is the richest in species: *Malva alcea, Bunias orientalis, Euphorbia seguieriana, Viola hirta,* and *Brachypodium pinnatum* may be mentioned.

c. River Rhine – Road bridge Rhenen – Kesteren. Southern bank: *Campanula rapunculus, Bunias orientalis,* and *Inula brittannica.*

d. River Lek – Railway bridge near Culemborg. Northern bank: *Conium maculatum, Peucedanum carvifolia,* and *Berteroa incana*. The embankment on the northern bank is extremely rich in rare species. Very abundant are *Silene vulgaris* and *Origanum vulgare*. Furthermore *Geranium pyrenaicum, Lamium maculatum,* and the grasses *Brachypodium pinnatum* and *Bromus erectus.*

e. River Waal – Zaltbommel – Waardenburg. Two bridges, a railway and a road bridge are situated close together. As a consequence a rather large area of non-agricultural grassland is found here. Especially the Southern embankments are rich in species, e.g. *Stenactis strigosa, Sanguisorba officinalis, Erigeron acer, Tragopogon pratensis* ssp. *orientalis* and *Verbascum nigrum*. Due to the use of large quantities of salt under winter conditions on the main road the salt marsh species *Puccinellia distans* occurs abundantly on the slopes of the road dike.

f. River Maas – Road bridge (former railway bridge) near Gennep. Eastern bank: *Berteroa incana, Vicia dasycarpa, Sisymbrium altissimum, Echium vulgare, Oenothera biennis,* and *Reseda lutea*. This assortment of species is perhaps influenced by the sub-continental characteristics of the climate in this part of the country.

g. River Maas – Two bridges, a railway and a road bridge, near 's-Hertogenbosch. The slope of the Southern embankment is dominated by *Cynodon dactylon*. Here also *Saponaria officinalis* occurs. On the Northern bank: *Armoracia rusticana* and *Sanguisorba officinalis.*

It may be concluded that the slopes of the embankments in the Fluviatile District can be considered as important refugia for a number of rare and endangered plant species in The Netherlands. Authorities of both Rijkswaterstaat (Ministry of State for Waters) and Dutch Railways are interested in

the maintenance of the species diversity of these sites and as a consequence try to preserve these floristically important sites.

J. T. R. KALKHOVEN (*Rijksinstituut voor Natuurbeheer, Postbus 46, 3956 ZR Leersum*)

The flora of the riverdelta in The Netherlands

The delta of the great rivers (Maas, Waal, Rijn (Rhine), IJssel, and some minor ones) in The Netherlands belongs phytogeographically to the Fluviatile District. Among the c. 1000 species occurring in this district c. 300 of them are characteristic for the river valley; part of these can also be found in the coastal dunes and in the southernmost part of the province of Limburg (Chalk District). From these 300 fluviatile species 26% belong to the ecological group of species of cultivated fields on more or less chalky soils and species of dry forelands; another 26% belong to the species of grasslands on dry neutral and basic soils: these form the vegetation on point bars, river dunes and dikes. About 15% of these species find their specific environment in hedgerows and at forest borders.

Comparing the distribution of the species over frequency classes (number of square km plots, cf. VAN DER MAAREL 1971) before 1950 and in the seventies one can see that nearly half of the typically fluviatile species is lowered one class and 17% two or more classes. Only 5% have become more widespread (mainly introduced species).

A new source from which data about the flora in the Fluviatile District may be taken is the Atlas of the extinct and very rare species of the Netherlands flora (MENNEMA et al., 1980). Of the 332 taxa mentioned in this volume 189 taxa occurred in the Fluviatile District before 1950. Up to now 79 taxa have disappeared from the area. This decline expressed in number of species is heaviest in the ecological groups of fields, dry forelands and relatively dry grasslands.

The disappearance of weeds of cultivation is a common feature in the whole country, mainly caused by purification of seed and the use of herbicides. The predominant influences on the fluviatile flora of the grasslands are: – intensification of agricultural management (fertilizers, early mowing, high density grazing);
- land development projects (removing of hedgerows & c.);
- dike body enlargement (changing of slope and soil material);
- excavation (mining of sand from point bars).

At this moment there are nearly 40 nature reserves, higly important for the fluvatile flora. They are, however, very small and isolated from each other; consequently the diversity in vegetation types within the grasslands and other formations is very difficult to maintain.

MAAREL, E. VAN DER (1971): Florastatistieken als bijdrage tot de evaluatie van natuurgebieden. *Gorteria* 5: 167–188.

MENNEMA, J., A. J. QUENÉ-BOTERENBROOD & C. L. PLATE (Eds.) (1980): Atlas of the Netherlands Flora 1. The Hague/Atlas van de Nederlandse Flora 1. Amsterdam.

H. M. VAN DE STEEG (*Botanisch Laboratorium, Toernooiveld, 6525 ED Nijmegen*)

The decline of the river valley grassland flora near Nijmegen since the middle of the 19th century

According to the 'Flora Novomagensis' by P. M. E. Gevers Deynoot and T. H. A. J. Abeleven (1848) the flora of Nijmegen was rich in grassland species of dry, lime-rich soils in the middle of the nineteenth century. The detailed descriptions of locations and habitats in the flora Novomagensis enable the reconstruction of the flora, and to a certain extent also the vegetation, of certain parts of it.

Of special interest for the flora of flood-sensitive river valley grassland were four habitats. In order of richness in characteristic species these habitats are: areas recently covered by river sand after bursting of the dike (in the flora the locations 'Oosterhoutse Bos' and 'Griend at Weurt'), the fortifications of the town of Nijmegen, natural levees in the river foreland, and the dikes.

Most of the species mentioned for the dikes belong to the alliance Arrhenatherion. A special group on dikes are species of the alliance Trifolion medii: *Origanum vulgare, Agrimonia eupatoria*

and *Trifolium medium*. Most of the flood-sensitive species mentioned for the river foreland, like *Medicago sativa* ssp. *falcata*, *Thalictrum minus*, *Veronica teucrium*, and *Salvia pratensis*, have their optimum in the alliance Mesobromion.

Largely different was the flora of the earth works and walls of the fortifications of Nijmegen. Besides species of rather dense grassland such as *Medicago sativa* ssp. *falcata*, *Knautia arvensis*, and *Astragalus glycyphyllos*, many species of low, open vegetation are mentioned, e.g. *Sedum album*, *S. acre*, *Alyssum alyssoides*, *Saxifraga tridactylites*, *Vicia lathyroides*, *Artemisia campestris*, *Scabiosa columbaria*, and even *Armeria maritima* var. *elongata*.

Characteristic for the two sand covered areas, the Griend at Weurt, probably an area rich in gravel, and the woodland Oosterhoutse Bos, damaged after the 1820 dike break, are species of open grassland on coarse dry sand like *Sedum sexangulare*, *S. reflexum*, *S. album*, *Alyssum alyssoides*, *Saxifraga tridactylites*, *Petrorhagia prolifera*, *Artemisia campestris*, *Euphorbia cyparissias*, and *E. seguieriana*. For the location Griend at Weurt almost only those species with an optimum in Festuco-Sedetalia communities are mentioned. In the location Oosterhoutse Bos, floristically the richest place in the Flora area, most of the river valley grassland species have been recorded.

The rich pioneer flora of the Oosterhoutse Bos location was partly lost soon after the middle of the nineteenth century in consequence of mining of the sand, and partly by recovery of the wood. The rich flora and vegetation of the fortifications of Nijmegen disappeared with the levelling of the earthworks and most of the walls soon after 1874 as a consequence of the expansion of the town.

The formerly well-developed flood-sensitive grassland vegetation on natural levees in the river forelands, especially in the Ooy and near Weurt and Beuningen, with abundant *Salvia pratensis*, is now almost totally gone. Possible causes are levelling of high natural levees to improve discharge of water and ice, intensification of agricultural management, and recently higher summer floods.

Up till now a particular part of the river valley grassland flora and vegetation could maintain itself on the less managed steep slopes of the dikes. But now the characteristic flora and vegetation in this habitat is also vanishing, locally in consequence of fertilising, and in the near future will almost totally vanish in consequence of the strengthening of the dikes followed by intensive agricultural use.