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MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON APRIL 6 1976

M. G. C. SCHOUTEN and M. J. NOOREN *(Botanisch Laboratorium, Katholieke Universiteit, Nijmegen)*

Coastal vegetation types and soil features in South-East Ireland

The study reported here was carried out at the SE coast of Ireland (Co. Wexford) during the summer of 1974.

The SE coast of Ireland has a relatively rich maritime flora: 60% of the total number of Irish maritime species have been recorded here, and again 60% of the total number of Irish maritime Atlantic and Atlantic-Mediterranean species. These phenomena can be explained from the great variety of habitats along the SE coast (rocky headlands, dunes, sandy and gravelly beaches, salt-marshes) and from climatic conditions (the SE of Ireland is the driest and the warmest part of the country).

Three of the most important areas which have been studied are: the dunes of Ballyteige Burrow, the salt-marshes along Ballyteige Lough, and the reclaimed polder area of Kilmore Slob.

A. The dunes of Ballyteige Burrow

Only a relatively small part of the dune area is occupied by communities of the Ammophiletea: the association Euphorbio-Agropyretum juncei is represented on the lower part of the first dune range, and towards the top of the dunes of this first range, communities with *Ammophila arenaria* appear, belonging to the association Euphorbio-Ammophiletum. Relatively very high amounts of Ca and P have been recorded in this coastal dune ridge, whilst the figures for especially C and N are rather low. The differences between the mineral contents of the soils of the Euphorbio-Agropyretum juncei stands and those of the *Ammophila arenaria* communities are not very obvious, since many of the combinations (especially those of Na and K) are washed out from the coarse sand very easily. Broadly speaking, however, the contents of minerals recorded (Ca, P, K, Mg, Na) amount to somewhat higher figures in the Euphorbio-Agropyretum juncei zone, which is frequently reached by the sea water. Although the figures for N and C remain rather low, they are in a number of cases nearly twice as high in the latter zone and this may explain why *Matricaria maritima* ssp. *inodora* var. *salina* occurs abundantly in the Euphorbio-Agropyretum juncei stands and is almost completely absent from the Euphorbio-Ammophiletum zone.

By far the largest part of the dune area, however, is stabilized and is covered by communities of the Galio-Koelerion. There is a clear difference between the vegetation types of the S-exposed and those of the N-exposed ones. On the S-exposed slopes, the association Tortulo-Phleetum arenarii is represented; two subassociations could be distinguished (-typicum, differentiating species a.o. *Phleum arenarium* and *Vulpia membranacea*, on somewhat mobile sand; -cladonietosum, differentiating species a.o. several *Cladonia* ssp. and *Peltigera canina*, on more stabilized sand). A variant with *Frullania tamarisci* of the subassociation -cladonietosum occurs on the oldest landinward dunes.

The N-exposed slopes show a community of *Rosa pimpinellifolia* and *Festuca rubra* (a.o. *Rosa pimpinellifolia*, *Thymus drucei*, *Galium verum*, *Scilla non-scripta*, *Anacamptis pyramidalis* and *Viola riviniana*), also belonging to the alliance Galio-Koelerion. A variant with *Asparagus officinalis* var. *prostratus* is represented on the lee slope of the first dune range.

The stabilized part of the dune system shows, in comparison to the coastal dune ridge, an

increase of the humus content and a decrease of the lime content; these phenomena are the most obvious in the soils of the N-exposed slopes.

B. The salt-marshes along Ballyteige Lough

The silty shore of Ballyteige lough shows a zonation of several vegetation types according to the elevation of the shore and the connected frequency of inundation by the sea water.

The Spartinetum × townsendii or the Thero-Salicornion are represented in the lowest zone extending from below up to just above the mean high water line; relatively very high contents of Ca, P, Mg, Na, and K have been recorded here.

The next zone extending from the mean high water line up to the high water line of spring tide is occupied by Puccinellietum maritimae stands; a decrease of the mineral contents has been measured in this zone.

A still further decrease has been recorded in the successive zone extending from the mean high water line of spring tide up to the extreme high water line of spring tide. This zone shows a community of *Parapholis strigosa* and *Plantago maritima* (Armerion maritimae).

The highest part of the lagoon shore forms a transition to the dunes backing the salt-marsh area. This transitional zone is occupied by communities of the Saginion maritimae. The superficial silt layer shows still lower mineral contents; the coarse sand appearing at some depth, however, contains a very high amount of lime.

C. The reclaimed polder area of Kilmore slob

This polder has been reclaimed from the sea about 1840. It was mostly under water in winter between 1900 and 1960 until a new drainage system was installed. In parts of the polder there is a complex pattern of low, more or less silty areas, rather often flooded by brackish streams and higher, somewhat drier, fresh grasslands. On the more or less saline sites, a community occurs representing a transition of the Armerion maritimae and the Nanocyperion flavescentis; this vegetation type has been called a community of *Carex distans* and *Scirpus cernuus* (a.o. *Carex distans*, *Scirpus Glaux maritima*, *Juncus gerardii*, *Centaurium pulchellum*, and *Odontites verna*). The soils are still rather immature and they contain relatively high amounts of Ca, P, and Na whilst the figures for C and N are rather low. According to the salinity and the humidity of the soils three variants of the community of *Carex distans* and *Scirpus cernuus* could be distinguished.

On the higher sites which are hardly ever inundated by brackish water, the alliance Cynosurion is represented. The soil has a clear A-C profile and lower contents of Ca, P, and Na have been recorded. According to the humidity of the soils several variants of the Cynosurion cristati occur.

SYMPOSIUM: GENETIC MANIPULATION IN PLANTS, ON NOVEMBER 19, 1976

R. L. M. PIERIK *(Laboratorium voor Tuinbouwplantenteelt, Wageningen)*

In vitro culture of higher plants as a tool for genetical manipulations

In vitro culture of higher plants, the cultivation of protoplasts, cells, tissues, organs, embryo's and plants on artificial culture media under sterile conditions, has become an important tool for geneticists, plant breeders and molecular biologists. The technique of in vitro cul-

ture has been applied in the following fields: generative propagation (e.g. sowing of orchids), embryo culture (to prevent embryo abortion and to induce "seeds" to germinate), vegetative propagation (the building up of clones by shoot tip, meristem-, explant-, callus-, single cell- and protoplast culture), test-tube-fertilization (fertilization of flowers and ovula in vitro), the production of haploids by means of anther and/or pollen grain culture, protoplast culture for biochemical engineering or other purposes.

During the symposium special attention was paid to vegetative propagation in vitro. Quite a number of plant species can now be propagated vegetatively by means of several techniques. However, the following requirements should be fulfilled when plants are reproduced in vitro:

1. They should be genetically identical to original material (no mutations).
2. The regeneration ability, especially in callus cultures, should not disappear.
3. The technique should not be too complicated.

In vitro culture of higher plants can also be a very helpful tool in the following studies:

1. Regeneration of plants from cultured protoplasts.
2. Production of haploid plants by means of anther culture.
3. Realisation of test-tube-fertilization in those cases where no fertilization in vivo (due to incompatibility) occurs.
4. The induction and easy detection of mutations in tissues, single cells and protoplasts.
5. Embryo culture to prevent embryo abortion or to induce seed germination.
6. Germination of seeds (e.g. orchids).
7. Meristem culture to produce pathogen-free plants.
8. Realisation of somatic hybridization by regeneration from fused protoplasts of sexually incompatible plants.
9. Production of polyploid plants by means of colchicine treatment.
10. Solving the chimaera problem; culture of cell layers and regeneration of plants.
11. Test-tube culture as a gene bank.

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J. G. TH. HERMSEN *(Instituut voor Plantenveredeling, Landbouwhogeschool, Wageningen)*

Induction and potential of haploids in higher plants

Haploids are plants obtained from reduced gametes. They may originate in vitro from male gametes and in vivo either from unfertilized female gametes (parthenogenesis) or from a generative nucleus (androgenesis). Haploids in higher plants use to be rare in nature. They may occur in combination with poly-embryony.

Scientists have succeeded in increasing the frequency of in vivo haploid production by selection of superior pollinators which stimulate parthenogenesis. In addition efficiency of detection of haploids has greatly been improved by using genetic seed or seedling markers (maize, potato, alfalfa, cotton). In barley and wheat haploids are being obtained from crosses with *Hordeum bulbosum*, followed by natural selective elimination of all chromosomes of *Hordeum bulbosum* from hybrid embryos. In vivo androgenesis occurs at extremely low frequencies (0.00125% in maize) but a recessive mutant in maize gave a 2000 fold increase to 2.3%.

In vitro production of haploids through anther or pollen culture has been successful in nearly 60 species, especially in species of Solanaceae and Gramineae. Up to now it has become a routine method for *Datura* and *Nicotiana* only.

The potential of haploids for basic research and practical breeding is: 1. to create completely homozygous lines by colchicine treatment of haploids from diploid and allopolyploid species; 2. to simplify and speed up genetic research and breeding of autotetraploid species, because it can be carried out at the diploid level; 3. to speed up and refine existing breeding methods; 4. to apply breeding methods in crops in w hich these methods without haploids would not be feasible. Somatic hybridization in combination with haploids opens up completely new possibilities for breeding.

R. A. SCHILPEROORT *(Laboratorium voor Biochemie, Leiden)*

Somatic hybridisation in plants

Somatic hybridisation in plants by the fusion of plantcell protoplasts is a fascinating possibility to perform somatic cell genetics. Genetics at the cellular level will contribute in studies on the molecular basis of cell regulation and celldifferentiation. Moreover agricultural application is expected. If at will plants can be regenerated from cells of plants species which are of economic value somatic hybridisation opens perspectives for plant improvement.

Protoplasts, which are cells freed from their cell wall, can be isolated from different organs of plants of many species as well as from cells growing in suspension culture. Commercially available enzymes are used to this end. Cell wall regeneration and cell division leading to callus formation have been achieved for protoplasts of several species. In several cases plants could be regenerated from these calli (1).

Intraspecific, interspecific and intergeneric fusion of protoplasts is induced by agents. Very high fusion frequencies are obtained with the use of polyethyleneglycol (PEG) at pH 7.0 in the presence of Ca^{2+} ions (2). PEG causes the aggregation of protoplasts and consequently induces close membraneous contact needed for fusion. Protoplasts from different origin can nowadays be fused without much problem. The culture of protoplasts, however, is more difficult but recently well balanced media have been developed (3) which meet the special requirements for protoplast culture.

Hybrids only arise after nuclear fusion and this still seems to be a rather rare event. Because of this a generally applicable selection procedure for the isolation of hybrids is badly needed. Auxotrofic and drug resistant mutants would be useful as is demonstrated for somatic hybridisation with mammalian cells. Unfortunately non leaky mutants of this type are still missing in the case of plant cells. So far selection procedures are used based on naturally occurring differences in the sensitivity of cultured plant protoplasts to growth media and drugs (4). Also use has been made of visual markers like green chloroplasts of mesophyl cells and cytoplasmic strands of cultured cells in fusion products obtained from both types of cells. In this case, however, fusion products have to be isolated by micromanipulation in time, since the chloroplasts degenerate after a few cell divisions. Hybrids can than not be distinguished anymore from parental cells. In all cases the hybrid nature should be established firmly e.g. by chromosome analysis or by using differences in isoenzymes of the parental cells (5).

The need of a selection procedure based on the use of mutants can be overcome by the use of micromanipulation in order to fuse protoplasts in any desired combination when the frequency of nuclear fusion is not too low.

In plant regeneration from hybrids obtained from both intraspecific and interspecific fusion a few promissing results have been published. The plants used in these studies were also sexual compatible. So, "parasexual-hybrids" could be compared with the "sexual-hybrids" to be sure of their hybrid nature. Plants were regenerated from hybrids of two *Nicotiana tabacum* mutants (6), *N. langsdorffii* + *N. glauca* (7) and *Petunia hybrida* + *P. parodii* (4).

Somatic cell genetics can only be achieved when chromosome elimination of one of the parents takes place in the hybrids (5). However, so far no clear indications have been ob-

tained for this. From the experience with mammalian cell hybridisation chromosome elimination might possibly only occur in intergeneric fusions. Intergeneric hybrids are of interest for plant improvement. For this it is not essential that the chromosome complement of both parents is present completely. One or a few chromosomes carrying the information for some required properties of one of the parents in the chromosomal background of the other might even be more valuable.

Somatic hybridisation might be a way to get hybrids which can not be obtained along the sexual way. It should be taken in mind however, that also with this new technology certain crosses will fail because of incompatibility between protoplasts of different species.

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A. J. KOOL *(Laboratorium voor moleculaire genetica, Vrije Universiteit, Amsterdam)*

Genetic modification of plants by molecular biological methods

In 1944 Avery proved that DNA purified from a certain strain of bacteria could be taken up and was stably expressed by a second, closely related strain. This phenomenon has been called transformation. If such a transfer of genetic information could be realized in higher plants then this technique would have the potential to improve yield, increase storage protein quality, introduce properties such as disease resistance, ability to fix nitrogen, etc. without altering desirable characteristics such as morphology and growth habit.

A number of authors (e.g. BENDICH & FILNER 1971, LEDOUX & HUART 1969, OHYAMA et al. 1972) have reported on the successful uptake of exogenously-supplied DNA by intact plant material, cultured plant cells and protoplasts. In some instances data are obtained that suggest that foreign DNA integrates into the plant chromosomes and that phenotypic expression of this foreign DNA occurs (LEDOUX & HUART 1974, HESS 1969).

However, one should be extremely careful in interpreting these data because a number of these experiments are not reproducible or important controls are missing: Other scientists are not able to reproduce the experiments of LEDOUX & HUART (1969) while KLEINHOFS et al. (1975) claim that the results of LEDOUX & HUART (1969) are due to bacterial contamination. HESS (1969) obtained red flowering wild type plants from a white flowering mutant of *Petunia hybrida* upon treatment with wild type DNA. However, when this modification is the

result of transformation, one must assume that two anthocyanin-inducing alleles are incorporated in at least 6 initials in the two tunica layers of the shoot apex (BIANCHI & WALET-FOEDERER 1974). Furthermore, these authors obtained evidence for a high rate of spontaneous reversion in this mutant *Petunia* line. Kool & Pelcher (J. Expt. Cell. Res., in preparation) showed that binding and uptake of radioactive DNA by cultured cells or protoplasts. as reported by several authors, is most probably just binding of the DNA to cellulose (cell wall or fragments thereof) and uptake of DNA degradation products. Therefore, despite the numerous reports on transformation of intact plant material or cultured plant cells, I still believe that these claims of transformation of higher plants are not justified by the data presented up to now. Instead of using just pieces of foreign DNA, a more feasible approach could be the use of vectors such as the plant viruses or the bacteria *Rhizobium* and *Agrobacterium*. These vectors, or at least (partially) their genetic material, are known to enter plant cells. By using restriction endonucleases and DNA ligase, foreign genetic information could be coupled on the DNA of these vectors and thus introduced into cells of higher plants.

It is obvious that as yet these types of experiments are only of interest for fundamental research while in the near future the practical application of these techniques for agricultural purposes is not very likely.

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