Ethylene Effects on Tissue Cultures of *Nicotiana tabacum*

Ethylene was known for long time to have effects on plants and was considered a plant regulator[1]. It plays a role in many different processes, such as the onset of ripening in fruits[2], the production of transverse expantion of cells[2], the abscission of leaves and fruits[3] and the inhibition of auxin polar transport in etiolated pea stem sections[4]. Only recently, ethylene has been shown to be produced by plants[5]. Such a production of ethylene gas is related to the applied auxin. In each tissue an optimum of auxin concentration is found: low concentrations are generally promotive and high concentrations inhibitory of growth. The reason for such inhibition was unknown until[6] it was discovered that certain high critical concentrations of auxin induce endogenous ethylene. These studies were generally performed on etiolated stem sections of peas and sunflowers. It appears interesting to know if a similar behaviour of auxin and ethylene, established for a developed plant, is reproducible in cultured cells.

With this in mind, cells of *Nicotiana tabacum* var. Barley were isolated from seeds and subcultured for 1 year period on Steward and coll[7] medium. For growth studies Murashige and Skoog (MS)[8] medium was used, supplemented with auxins: 0.1 and 1 mg/l of 2–4 dichlorophenoxyacetic acid (2–4 D) or 0.1, 1 and 5 mg/l of Indol-3-acetic acid (IIA). To determine the effect of ethylene on cell growth, petri dishes containing callus fragments of 150 mg of fresh weight were placed in dessicators into which ethylene gas (2.35 v.p.m. of air) was injected through side arms. Control cultures, in the same experimental conditions, were placed in similar dessicators but insuffled with air. Callus fragments grown in different experimental conditions were transferred every 15 th day and fresh weight measured.

Figure 1 shows the fresh weight increase in presence of different concentrations of 2–4 D and constantly applied ethylene. Figure 2 shows the fresh weight increase in presence of different doses of IAA and constantly applied ethylene.

It is known[9] that bud inhibition growth of plant caused by IAA is reversed by kinetin. If this inhibitory action accounted for IAA is mediated by endogenous production of ethylene, it should be possible to demostrate, in vitro, a kinetin reversal effect of ethylene-auxin inhibition. For this purpose callus fragments were grown on MS supplemented with kinetin alone (5-furfurilaminopurine (K) 0.1 and 1 mg/l) and kinetin with different doses of 2–4 D, always in the presence of constantly applied ethylene (Figure 1).

From the data presented we found indications that ethylene gas has on cultured cells of *Nicotiana tabacum* the same effect described for in vivo plants. It is very difficult to judge if constantly applied ethylene alone has any inhibitory effect, because callus fragments cannot survive for a long period without any hormonal addition[10]. Instead it was possible to show that both auxins used, 2–4 D

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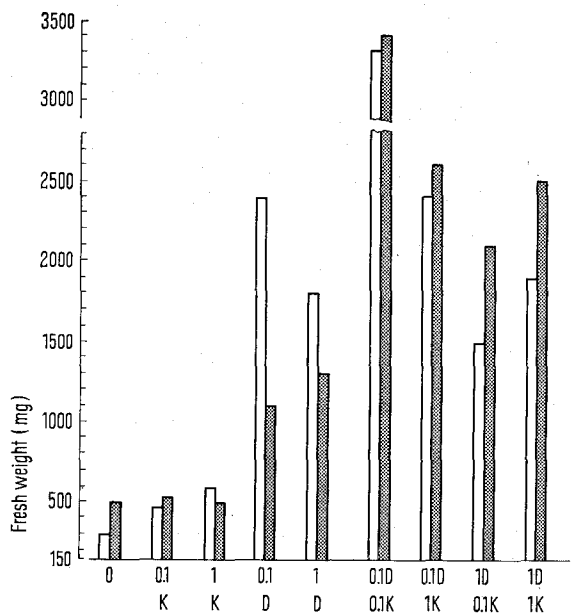

Fig. 1. Fresh weight increase of *Nicotiana tabacum* cells grown in presence of constantly applied ethylene (dotted column) and air (white column) and different doses of kinetin alone (0, 0.1 mg/l and 1 mg/l K) and 2–4 dichlorophenoxyacetic acid alone (0, 0.1 mg/l and 1 mg/l D) and various doses of kinetin and 2–4 dichlorophenoxyacetic acid after 15 days of culture.

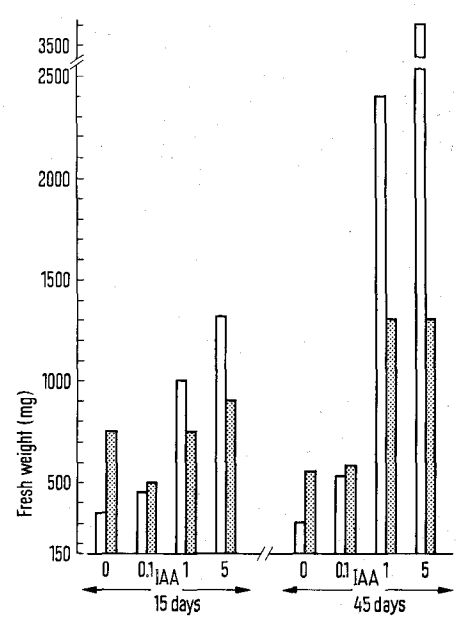

Fig. 2. Fresh weight increase of *Nicotiana tabacum* cells in presence of constantly applied ethylene (dotted column), air (white column) and different doses of indol-3 acetic acid (0, 0.1 mg/l, 1 mg/l and 5 mg/l IAA) after 15 and 45 days of culture.

and IAA, inhibit explant growth in cultured cells. Such growth inhibition is evident also at 2–4 D low concentrations (Figure 1) which cannot be increased for the toxicity of synthetic product. IAA inhibition of weight is nearly linear with the time as long as concentration of applied ethylene is maintained constant: the growth inhibition by low concentrations is smaller than high ones as reported in Figure 2. The reversal effect of ethylene-auxin inhibition is related to kinetin/auxin ratio[11]. As shown in the Table, the Student t-test on fresh weight of callus fragments grown in the presence of constantly applied ethylene and different hormonal conditions of kinetin and auxin is not significantly different from controls grown in the same experimental conditions but in presence of air. On the contrary, a significant difference exists when callus fragments are grown in presence of constantly applied ethylene and auxins.

Student t-test applied on fresh weight of callus fragments of *Nicotiana tabacum* cells grown in different hormonal conditions of 2–4 D (0.1 and 1 mg/l) and kinetin (0.1 and 1 mg/l) after 15 days of culture.

Media	Air	Ethylene
–	0.01	0.01
0.1 2–4 D	0.1	0.05
1 2–4 D	0.1	0.05
0.1 K	0.2	0.1
1 K	0.1	0.05
0.1 2–4 D 0.1 K	0.3	0.2
0.1 2–4 D 1 K	0.7	0.5
1 2–4 D 0.1 K	0.8	0.7
1 2–4 D 1 K	0.5	0.3

Riassunto. Si è studiato l'effetto dell'etilene su culture di cellule di *Nicotiana tabacum*, in vitro. I risultati ottunuti mostrano che le auxine in presenza di etilene inibiscono la crescita di frammenti di callus, e che tale inibizione viene rimossa dalla kinetina.

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Isolation and Classification of Water Leptospira Strains: Identification of Three New Serotypes

The authors have isolated from stagnant waters of the Friuli zone (Italy) 5 leptospira strains which they have first purified and then serologically compared with the leptospira serotypes so far described in the literature.

Materials and methods. Isolation. The isolation of the strains has been obtained by seminating the collected water samples in Zuelzer's medium and filtering the positive cultures through Millipore membranes at a porosity of 0.22 $\mu\mu$[1]. The filtrate was either seeded in KORTHOF's medium, or on COX's solid medium, obtaining in this way leptospira colonies. The cultures in fluid medium have been trcated according to BABUDIERI[2] to avoid the possibility of mixed cultures. The isolation and the first passage of the strains have been effected at environmental temperature.

Classification. The classification of the strains has been done by preparing the immune sera in rabbits and testing them with the reference strains of all serotypes belonging to the complex 'biflexa', and then setting the specific immune sera for the last ones, against the isolated strains. A first screening has been effected with cross-agglutination tests. In the cases in which an affinity was recognized, we have had recourse to the agglutinines cross absorption test, according to the methods suggested by the OMS Expert Committee on leptospirae (1967)[3].

Results. The 5 isolated strains have been given the following names: Friuli 8, Friuli 35, Friuli 37, Friuli 44, Friuli 48. One of these, Friuli 48, was unable to grow at a temperature of 30 °C or more, optimal for leptospirae. But from this strain, Friuli 48, after some passages, we have obtained a mutant able to grow at 30 °C. In Table I only the reference strains of the serotypes of the 'biflexa complex' which gave some positive cross-agglutination with the strains under study are reported. In some instances (Friuli 8 and 35 and Doberdò 1 and RPE) the well-known phenomenon of the unilateral agglutination was observed. The strains which, by the agglutination test, showed an antigenical affinity, were checked through the cross agglutinin absorbtion test. The results are reported in Table II.

According to the results of the cross absorption tests, 2 of our strains (Friuli 8 and 35) belong to the serotype 'San Giusto'; the 3 other strains, on the contrary, are antigenically independent and can be considered reference strains of 3 new serotypes. One of these, however (Friuli 37), shows some affinity with the Bulgarian serotype 'Maritza' and can be considered as belonging to the same serogroup Maritza. In Table III systematic of our 5 strains is reported.

Conclusions. Of the 5 new strains, 2 have shown their appurtenance to already known serotypes, while for the others we have observed only feeble agglutinations scattered with some strains isolated from the zone of Trieste and from Bulgaria. The existence of strains antigenically related such as Friuli 37 and Bulgaria 16, isolated in geographically distant zones, confirms once again the antigenic cosmopolitanism of the leptospirae. The finding of water leptospirae that do not multiply at 30 °C but at lower temperatures is a fact to be borne in mind, both from a practical and a theoretical point of view; it is ad-

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