In vitro associations of tomato plants and *Rhizobium;* induction of nitrogenase activity

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Summary. In vitro associations of the non-legume *Lycopersicum esculentum* and *Rhizobium* sp. cowpea 32H1 were established. Tomato plants induced rhizobial nitrogenase activity. Induction of nitrogenase activity was possible through a membrane which was impermeable for bacteria.

Non-leguminous tissues and plants (*Petunia*[1,2], *Triticum*[3]) are able to induce nitrogenase activity in rhizobia[4,5]. In associations of *Portulaca* callus tissues and *Rhizobium* 32H1, induction of nitrogenase activity was possible through a membrane which was impermeable for bacteria[6]. Conversely, as revealed by ^{15}N-analysis and accumulation studies, part of the N fixed by the bacteria was excreted in the form of ammonia, passed the membrane, and was channeled into the normal plant pathway of ammonia utilization[7,8].

Tomatoes are close relatives of *Petunia hybrida,* a species successfully used in induction experiments[1,2,9]. Therefore, it seemed to be possible that they would induce nitrogenase activity as well. For possible practical use, we tested the inducing capabilities of plants, not of tissue cultures. Furthermore, we studied the possibility of nitrogenase induction in transfilter associations.

Rhizobium sp. cowpea 32H1 was cultivated on yeast-mannitol agar[10]. Tests for the contamination of the *Rhizobium* strain were routinely carried out[9]. Additionally, tests on contaminations were performed at the end of each association experiment. These tests confirmed the absence of microbial infections. As plant partner *Lycopersicum esculentum* var. Benarys Gartenfreude was used. Seeds were sterilized with Domestos (Unilever) / H_2O (vol: vol, 2:1), containing 0.25% Captan (Orthocid), for 20 min. Thereafter the seeds were washed extensively with sterile water for 1 h, and placed in plastic petri dishes on WH medium[11]. The seeds were allowed to germinate and grow at 27 °C in light (5000 lx) for 15 h per day. Associations were established in gas-tight glass vessels (2550 ml), containing 500 ml WH medium. 30 tomato plantlets were transferred at an age of 5 days from the petri dishes into each association vessel. They were allowed to grow for several further days under the conditions mentioned above. At the times indicated, 10 ml *Rhizobium* suspension (10^8 cells/ml) were added. 8 days after initiation of the association 4% of the atmosphere was replaced by acetylene. Nitrogenase activity was determined by the acetylene reduction test[12]. Transfilter associations were established on WH medium in the apparatus used with *Portulaca* callus[6]. 3 tomato plants were transferred at an age of 5 days from the petri dishes into one of its chambers, and at the same time rhizobia (0.5 ml; 10^8 cells/ml) were added into the other chamber.

Plants and bacteria were separated by a membrane with a pore size of 150-200 nm. 6 transfilter apparatus were placed on moistened sand in the 2550-ml vessels, and the acetylene reduction test was performed as described above. In each experimental series, the following assays were run: rhizobia, tomatoes, and acetylene; rhizobia; tomatoes; rhizobia and acetylene; tomatoes and acetylene; rhizobia and tomatoes.

Under the conditions used neither rhizobia, nor tomatoes, nor associations of rhizobias and tomatoes showed a detectable endogenous ethylene production. Tomatoes alone showed no nitrogenase activity, rhizobia alone showed a very low or no nitrogenase activity. In associations of tomatoes and rhizobia, however, nitrogenase activity was induced or greatly enhanced (fig. 1). As with *Petunia*[2,5], the inducing capability increased with the age of the tomato plants. Nitrogenase activities, obtained with tomato plants which were inoculated at an age of 9, 16 and 22 days,

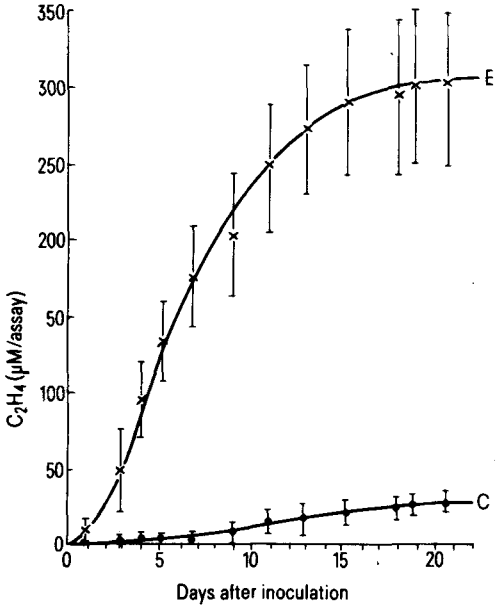

Figure 1. Time-course of nitrogenase activity in associations of *Lycopersicum esculentum* and *Rhizobium* 32H1. E (experiment): ethylene accumulation in associations of tomato plants and *Rhizobium,* C (control): ethylene accumulation in control rhizobia not associated with tomatoes. The tomato plantlets were inoculated at an age of 22 days. Standard deviations are indicated.

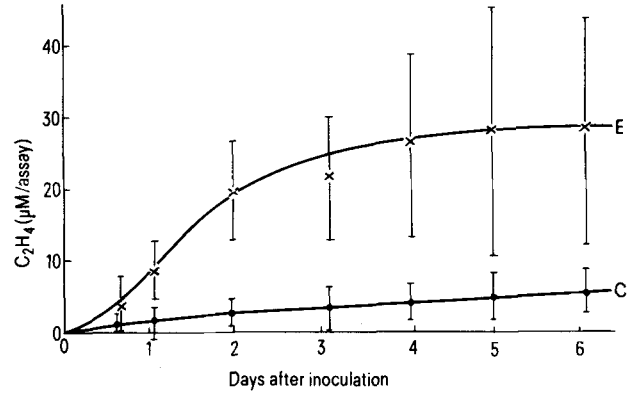

Figure 2. Time course of nitrogenase activity in transfilter associations of *Lycopersicum esculentum* and *Rhizobium* 32H1. Plantlets and rhizobia were separated by a dialysis membrane which was impermeable for bacteria. The transfilter association was initiated with 5-day-old tomato plantlets. 8 days later acetylene was added, and the first determination of ethylene was carried out. E and C as in fig. 1. Standard deviations are indicated.

respectively, were 0.11, 0.46 and 0.56 μM C_2H_4/plant × time (time = days needed till the final level of ethylene accumulation was attained). Older tomato plants, which were inoculated at an age of 31 days, showed no further increase of enzyme activity. This failure, however, could be due to the deteriorated status of the plants which were growing on increasingly more exhausted media.

In transfilter associations the tomato plants were inoculated at an age of 5 days. The bacteria concentrated along the separating membrane just as in transfilter associations of *Portulaca* callus and *Rhizobium*[6], and nitrogenase activity was induced (fig. 2). 0.26 μM C_2H_4/plant × time were produced. A correct comparison of associations and transfilter associations was not feasible, because both systems differed in several details. Nevertheless, the nitrogenase activity induced in transfilter associations was in the range obtained in associations without separation of plants and bacteria. These data indicate that tomato plants are able to induce easily nitrogenase activity even through a membrane which was impermeable for bacteria.

Thus, the tomato plant, which yields our most important canned fruit[13], is able to induce nitrogenase activity in rhizobia. However, before discussing, the possible practical use of tomato/*Rhizobium* associations, one should clarify the mechanism of this induction. One possibility would be the production of inducing factors. Another, less probable, possibility would involve the removal of inhibiting substances from the medium by the growing plants and subsequent derepression of the rhizobial nif-operon. Transfilter inductions could be explained best by inducing substances. They were essential for further experiments in which we are trying to get direct evidence for the existence or non-existence of inducing factors.

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Influence of oestradiol on tissue respiration of the Indian garden lizard, *Calotes versicolor*

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Summary. Ovariectomy reduced the respiratory rates of liver and skeletal muscle homogenates of the Indian garden lizard, *Calotes versicolor*. Administration of oestradiol dipropionate elevated the rate of tissue respiration of ovariectomized animals. This finding lends support to the view that oestradiol, like testosterone, is able to stimulate the oxidative metabolism of this animal.

It has recently been shown that orchidectomy reduces, and testosterone administration increases, the rate of respiration of tissues of *Calotes versicolor*[2,3]. Since oestrogens in general are known to have physiological actions opposite to those of testosterone[4], the effects of ovariectomy and oestrogen therapy on tissue respiration were studied in this species.

Adult female lizards were collected from nature and acclimated to laboratory conditions[3]. After a fortnight 25 animals were bilaterally ovariectomized and 10 sham-operated under open ether anesthesia. 1 month later the ovariectomized animals were divided into 3 groups of 6 animals each. Groups I and II received 20 μg and 40 μg of oestradiol dipropionate (Ovocyclin®, Ciba India Ltd.) in olive oil respectively and group III received olive oil alone. One group of sham-operated animals served as control. 5 intramuscular injections were given on alternate days in 0.1 ml of vehicle. 24 h after the last injection all animals were killed by decapitation and respiratory rates of liver and skeletal muscle measured manometrically[3]. During the course of experimentation all animals were provided with maggots and water ad libitum. Statistical analysis of the data was done using Student's t-test[5].

Results. Ovariectomy significantly reduced the respiration of liver ($p < 0.01$) and of skeletal muscle ($p < 0.05$). 20 μg of oestradiol increased the respiratory rate of liver and skeletal muscle ($p < 0.02$ and $p < 0.05$ respectively) of ovariectomized animals and 40 μg of the steroid when injected into ovariectomized animals increased the respiratory rate of liver only ($p < 0.01$, see table 1).

Discussion. Results indicate that ovariectomy significantly reduced the respiratory rates of tissues of *Calotes versicolor* and that oestradiol was able to stimulate tissue respiration of ovariectomized animals. This effect is similar to that of orchidectomy and testosterone administration on the respiratory rates of liver and skeletal muscle of *Calotes versicolor*[3], *Hemidactylus flaviviridis*[6] and *Natrix piscator*[7]. The present study shows that oestradiol is as effective as testos-

Effect of ovariectomy and oestradiol on respiratory rate of liver and skeletal muscle of *Calotes versicolor*

Status*	Oxygen consumption (μl O_2/mg wet tissue/h)	
	Liver	Skeletal muscle
Sham-operated + oil	0.725 ± 0.068	0.877 ± 0.381
Ovariectomized + oil	0.315 ± 0.045[a]	0.411 ± 0.071[b]
Ovariectomized + 20 μg oestradiol	1.144 ± 0.232[c]	1.174 ± 0.204[d]
Ovariectomized + 40 μg oestradiol	1.133 ± 0.172[e]	

* 6 animals per group. Compared to sham-operated + oil: [a] $p < 0.01$; [b] $p < 0.05$. Compared to ovariectomized + oil: [c] $p < 0.02$; [d] $p < 0.05$; [e] $p < 0.01$.