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Simple Inexpensive Laboratory Equipment for Cultivation of Plants under Various CO_2 Concentrations

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Abstract. A simple equipment was developed to cultivate young cereal plants under enhanced CO_2 concentration. Cultivation system permits growing of about 40 barley seedlings for about 2 to 3 weeks. The system consists of two identical growth chambers (volume about 30 dm^3), gas conditioning circuit and measuring circuit with an infra-red CO_2 analyser. Capabilities of the whole equipment were tested by growing barley plants under 330 and 1000 cm^3 $(CO_2)m^{-3}$ and in combination with high or low nitrate level.

Artificial enrichment of the atmosphere with an extra CO_2 and benefit on the yield of many glasshouse crops is now common knowledge and commercial practice (MORTENSEN 1987). Although CO_2 enrichment in greenhouses has been used for nearly 100 years, relatively little is known about physiological consequences of CO_2 enrichment. The only generalization can be made that elevated CO_2 concentration influences both photosynthetic and development processes in plants, however, there is a very large variability in plant responses to elevated CO_2 (WITTWER 1985, MORTENSEN 1987). As to the development it seems probable that CO_2 (and O_2, too) affect plant growth *per se* independently of the effects *via* photosynthesis and photorespiration (QUEBEDAUX and HARDY 1975, GERBAUD *et al.* 1988). As to photosynthesis, acclimation of plants to high CO_2 concentration (PORTER and GRODZINSKI 1984), interaction of high CO_2 concentration with mineral nutrition (SIONIT 1983), CO_2-induced leaf injury (EHRET and JOLLIFFE 1985), feed-back inhibition (DELUCIA *et al.* 1985) as well as changes in leaf anatomy (APEL 1989) need further research to understand how plants respond to enhanced CO_2 concentration. In addition, rising atmospheric CO_2 concentration caused by mankind brings new research imperatives to physiology and ecology. From the point of view of the

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breeders, different response of different genotypes to elevated CO_2 concentration deserves special attention (APEL 1985, 1989, MUSGRAVE and STRAIN 1988).

There have been many studies conducted in greenhouses, field chambers or phytotrons (*e.g.* SASEK *et al.* 1985, DUCLOUX *et al.* 1987, GERBAUD *et al.* 1988, MUSGRAVE and STRAIN 1988), which need a rather sophisticated and expensive equipment. Also special chambers or systems constructed by individual investigators have been described (KHAVARI-NEJAD 1980, MAHON and DENNIS 1985, KNIGHT *et al.* 1988). The aim of our report is to describe simple and low-cost equipment for small-scale growth experiments with various CO_2 concentration.

DESIGN OF THE EQUIPMENT

Growth Chambers and Recirculation Circuit

A scheme of the whole equipment is illustrated in Fig. 1. Two identical growth chambers (0.22 × 0.3 × 0.4 m) are made of transparent acrylic glass. The chambers are detachable from their bottom parts, connection being made by 10 screws and sealed with rubber tape. Each chamber is stirred with fan (12 V DC long life motor, LI-COR, Nebraska, U.S.A., outside the chamber), two copper-constantan thermocouples and hair hygrometer. About 40 plants were grown hydroponically in two

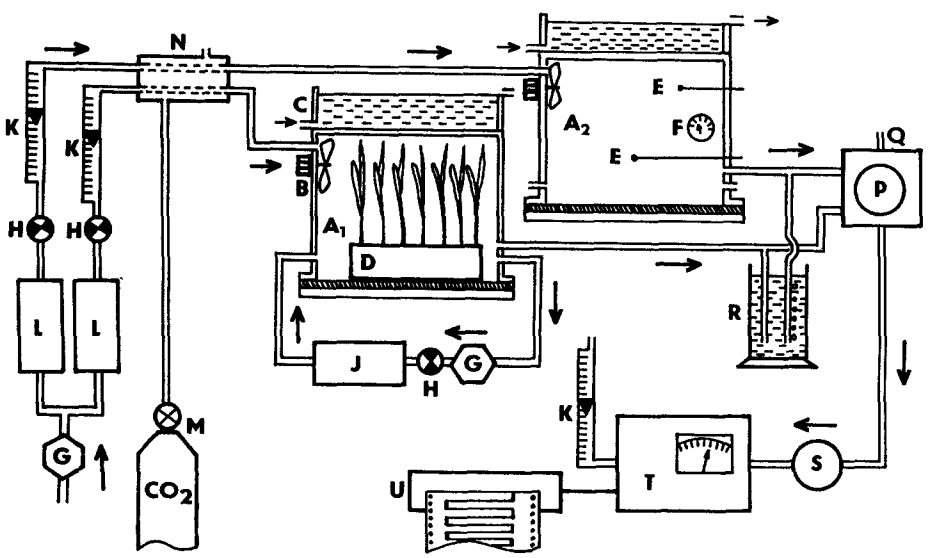

Fig. 1. Schematic diagram of the cultivation equipment. Growth chambers and recirculation loop: A_1, A_2 – two identical growth chambers, B – fan, C – water filter, D – cultivation vessel, E – thermocouple, F – hair hygrometer, G – membrane pump, H – regulatory valve, J – air drying column. Gas mixing circuit: K – float flow meter, L – CO_2 absorber, M – CO_2 bottle with electromagnetic valve, N – CO_2 permeation tubes. Gas measuring circuit: P – three-way sequential valve, Q – calibration port, R – overflow regulator, S – water vapour condenser, T – infrared gas analyser, U – line recorder.

vessels (0.8 dm^3). The chambers were placed in a room with partially controlled conditions, the light was provided by sodium high pressure lamp (Tesla SHC 400 W, Tesla Czechoslovakia); the average quantum irradiance (400–700 nm) of 200 to 300 $\mu mol\ m^{-2}s^{-1}$ can be achieved in the middle of the plant height through a water filter on the top of the chamber. The day/night temperatures inside and outside the chambers were similar (23/18 ± 2 °C). The air humidity was kept constant (70± 10%), excessive water vapour from transpiration was removed by $CaCl_2$ or silicagel in a closed loop with a very low air-flow rate. The leakage of the chambers was negligible, in spite of slightly higher pressure inside the system.

Gas Mixing Circuit

The air from the laboratory driven by a membrane pump passed through a CO_2 absorbing column with Natrocalcid and then was enriched with CO_2 by passing through the silicon-rubber permeation tubes installed in the glass vessel blown with pure CO_2 (APEL 1966). The air-flow rate was 80 $dm^3\ h^{-1}$, the length of permeation tubes was set to provide 330 and 1000 $cm^3\ (CO_2)m^{-3}$ mixtures in the first and the second channel, respectively. The variable CO_2 decrease in the chambers due to photosynthesis during cultivation period can be balanced by slight manipulation of flow rate. Float flowmeters (Pf-Pg, VEB MLW Prüfgeräte, Medingen, G.D.R.) and regulatory teflon-glass valves were used to measure and maintain flow rate through permeation tubes, CO_2 concentration was held constant (± 15 $cm^3(CO_2)\ m^{-3}$) throughout the light period. During the dark period atmopsheric air was blown through both chambers. The mixing of CO_2 into air and delivery into growth chambers started 80 min before the light was switched on, since this time period is necessary to remove the gas content of chambers and to attain the CO_2 concentration required inside the chambers. Light switching and gas mixing was controlled with a timer-switch and electromagnetic valve (ESPA 9200, Zita, Ruse, Bulgaria).

Measuring Circuit

Outgoing gas streams were sampled with a sequential valve (VEB Junkalor, Dessau, G.D.R.) and CO_2 concentration monitored alternately in both channels at a period of 4 min with an infra-red gas analyser with Peltier dew-point control (Infralyt IV, VEB Junkalor, Dessau, G.D.R.).

PERFORMANCE OF THE EQUIPMENT

Experiment I

The capability of the cultivation system was tested by growing spring barley plants (*Hordeum vulgare* L., cv. Spartan). Seeds were germinated for 3 d on wet filter

paper and then transferred into hydroponic vessels in chambers (day 0). In both chambers plants were grown in Hoagland No. 3 solution (full nutrition) for 14 d, solution being renewed on the 8th day. The chambers were blown with air with varying CO_2 concentration, equal in both chambers. The growth chambers were installed in a room with partially controlled conditions with 12 h photoperiod. Dry matter production of shoots and roots were compared with 10 plant samples after 14 d cultivation. No differences in growth occurred between the chambers (results not shown).

Experiment II

Two CO_2 concentrations, 330 and 1000 cm^3m^{-3}, in combination with two nutrient treatments using Hoagland solution No. 3 (15 mol(NO_3^-)m^{-3}, H3) and No. 1 (1.5 mol(NO_3^-)m^{-3}, H1). Solutions were renewed on the 7th and 12th day. Four plants from each treatment were harvested on the 8th and 16th day and dry matter production was estimated. Results were statistically evaluated by Student *t*-test (Fig. 2).

Nitrogen nutrition treatments were chosen in demonstration experiment because of the high importance of nitrogen as a limiting factor for photosynthesis and growth, hence availability of N affects plant response to CO_2 enrichment (WOO and WONG 1983, MAREK and FRANK 1984, HÁK and NÁTR 1987). As expected, higher CO_2 concentration considerably increased shoot dry matter on the 8th day as well as the 16th day of cultivation. Surprisingly, shoot dry matter on the 8th day of 1000 H1

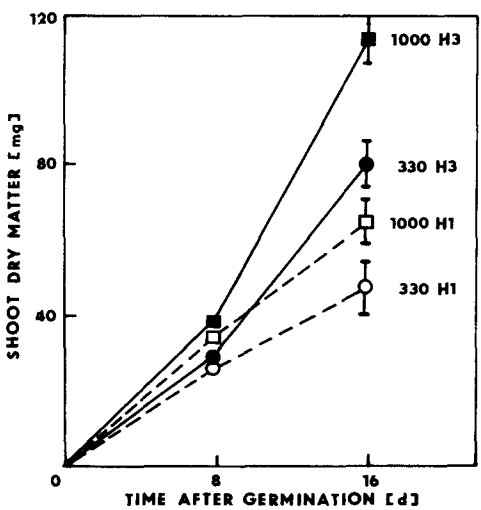

Fig. 2. Shoot dry matter production of barley plants after 8 d and 16 d of cultivation in the atmosphere with 330 or 1000 cm^3 (CO_2)m^{-3} in combination with two nutrient levels, either low (H1) or high (H3) nitrate concentrations. One point represents a mean of 4 plants, vertical bars show 95% confidence interval.

variant was 118% of the control variant 300 H3 (Fig. 2). However, this difference between small samples was not significant. On day 16, higher CO_2 concentration caused 42% and 36% increase in shoot dry matter of H3 and H1 variants, respectively. Higher CO_2 concentration stimulated plants growing under low N treatment (1000 H1) to reach 80% of 300 H3 dry matter. Stimulating effect of higher CO_2 concentration in spite of low nitrogen supply would seem rather paradoxical. Nevertheless, there are reports that the limiting effect of nutrition can be partly compensated by an increase in CO_2 concentration (SIONIT 1983, WITTWER 1985, APEL – personal communication). Besides, the beneficial effect of higher CO_2 concentration on growth is maintained under shortage of water (GOUDRIAAN and BIJLSMA 1987). However, much more experimental data are needed to understand better the combined effect of high CO_2 concentration and various environmental factors on plant growth.

CONCLUSION

The above experiments proved that the chambers and accessory equipment are sufficient to cultivate enough plants under homogeneous controlled conditions for elementary growth analysis and additional leaf gas-exchange and biochemical analyses, which will be done in our future programme. Cultivation equipment described and tested here, although much simpler than others has sufficient potential for small-scale growth experiments under modified atmospheric conditions, which can be useful in physiological as well as agricultural research programmes.

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BOOK REVIEW

FRALEY, R. T., FREY, N. M., SCHELL, J. (ed.): GENETIC IMPROVEMENTS OF AGRICULTURALLY IMPORTANT CROPS. Progress and Issues. Current Communications in Molecular Biology. – Cold Spring Harbor Laboratory 1988. 116 pp. $ 25.05.

The last few years have seen the rapid development of new methodologies in genetic improvements of agriculturally important crops. This publication is based on lectures presented at Cold Spring Harbor Laboratory, Banbury Center, 1988. The aim was to bring together various academic, agricultural researchers, and officials from governmental agencies to discuss all aspects of modern plant breeding, as well as questions how safe our future agricultural production will be for consumption. The introductory papers review plant transformation using *Agrobacterium* and direct DNA uptake methods. Further articles focus upon topics such as the strategies for practical gene transfer into agriculturally important crops, plant defense gene regulation, transformation to produce virus-resistant plants, engineering insect and herbicide resistant crops, altering protein and oil-quality traits into seeds, field testing of genetically engineered plants etc. Of interest are articles on regulatory processes controlling the release of new varieties and on the importance of governmental agencies in formulating specific regulations dealing with the commercialization of genetically engineered plants.

All those concerned with the risks associated with the introduction of genetically engineered plants will find a stimulating amount of material in this publication.

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