5-1997

Development of Super-Dwarf Wheat Under Stress Conditions Simulation Those on the Space Station MIR

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DEVELOPMENT OF SUPER-DWARF WHEAT UNDER STRESS CONDITIONS SIMULATING THOSE ON THE SPACE STATION MIR

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science

UTAH STATE UNIVERSITY
Logan, Utah

1997

ABSTRACT

Development of Super-Dwarf Wheat Under Stress Conditions Simulating Those on the Space Station MIR

by

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Utah State University, 1997

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Super-Dwarf wheat plants were grown in simulation growth chambers under 12 treatments with three photoperiods (18 h, 21 h, 24 h) and four carbon-dioxide levels (360, 1200, 3000, and 7000 μmol/mol). Carbon-dioxide concentrations affected flower initiation rates of Super-Dwarf wheat. The optimum CO_2 level for flower initiation and development was 1200 $\mu mol \cdot mol^{-1}$. Super-optimum CO_2 levels delayed flower initiation, but did not decrease final flower bud number per head. Longer photoperiods not only accelerated flower initiation rates, but also decreased deleterious effects of super-optimum CO_2. Flower bud size and head length at the same developmental stage were larger under longer photoperiods. But final flower bud number was not affected by photoperiod. Stomatal densities on the abaxial surface were more sensitive to the variation of photoperiod and CO_2 level than those on the adaxial surface for Super-Dwarf wheat. Stomatal density did not

significantly change on the adaxial surface, but was significantly decreased on the abaxial surface under longer photoperiods and higher CO_2 levels at 27 day after planting (DAP). Cell-walls of both stem and leaf tissues did not significantly change with variation of photoperiod and carbon-dioxide levels at either seedling stage or mature stage. McDowell fixative was suitable for long-term storage of plant tissue for use in light microscopy. When stored up to 180 d, there was no significant change in leaf thickness, shape and size of mesophyll cells, and shape of chloroplasts for wheat leaves under the light microscope.

(75 pages)

To my wife, Xiaping Deng, my daughter, Daisy Jiang, and my parents in China, for their understanding and support during my academic research in Utah State University

ACKNOWLEDGMENTS

This research was supported by the National Aeronautics and Space Administration of the United States of America.

I would like to express the foremost appreciation to Dr. Frank B. Salisbury, my major professor, for his invaluable guidance and supervision throughout the course of this study. Great appreciation goes to Dr. William F. Campbell for his instructions in the laboratory. I would especially like to thank Dr. John G. Carman and Dr. Richard J. Mueller for their precious advice. I would also like to extend special thanks to Rubin Nan and Linda Gillespie for their help in my experiments.

Liming Jiang

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CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum L.*) is a basic food crop throughout the world. A species of grass, domesticated wheat is a major cereal crop; together with rice, these two constitute the basis of 40 % of all human energy (Norman and Stubbs, 1978). Wheat is well adapted to harsh climates. It is cultivated on windswept prairies too dry for rice or corn. Wheat can yield extremely well in controlled environments (Salisbury and Bugbee, 1988). Thus wheat is a strong potential crop to purify the air and provide food in future space exploration. Super-Dwarf wheat has been selected as a cultivar that might be grown through a life cycle (seed-to-seed) in a Russian/Bulgarian growth chamber called *Svet* (means light in Russian) in the Russian space station *Mir*. From August to November 1995, Super-Dwarf wheat plants were grown in Svet in the Krystal module of Mir for 88 d (project "Greenhouse 2"). One of the objects of the experiment was to grow Super-Dwarf wheat through a complete life cycle in space (Salisbury et al. 1995). Because of a series of equipment failures, the objective was not met, although the plants survived and remained alive during the entire 88 d until the final harvest. The plants did not complete the life cycle. They remained completely vegetative during the entire experiment. Because Super-Dwarf wheat plants have previously formed heads in space (later matured on the ground; Mashinsky et al, 1994), we do not ascribe this vegetative condition to microgravity. Rather, we feel that one or a combination of the following factors reversed the flowering process in our plants: low light level, short photoperiods, water logging of the substrate that occurred for intervals during the experiment (this could lead to ethylene production, which is known to inhibit flowering), and at least one

high-temperature event (35-37 °C , day 37). In 1996, another seed-to-seed experiment with environmental measurement and sampling was carried out in space station Mir. (NASA-3). Plants grew well and formed many heads, but the heads were all sterile. Much more work needs to be done to explain the results from the space experiments. The data for this thesis were obtained as one part of the base-line experiments carried out at Utah State University to support the space experiment. These experiments were designed to study development of Super-Dwarf wheat plants grown under conditions that partially simulate those in the space station. In addition, the study also investigated the effects of storing wheat-plant tissue for long periods in fixative, a technical problem arising from sampling and then storage before return from space.

The Svet, which has 0.1 m^2 of growing area, was built in Bulgaria and has been on the Russian Space Station *Mir* since 1990. In the original design, plants were exposed to cabin air, which was moved from below over the plants and through the fluorescent lamps to cool them (Salisbury et al. 1995; Bingham et al., 1996). Light levels were about 110 μmol $\cdot m^{-2} \cdot s^{-1}$, just enough to produce poor growth for most species. The root module is divided into two compartments, each containing an ion-exchange zeolite produced in Bulgaria called *Balkanine.* The zeolite was treated to provide the essential plant nutrients. Water is introduced into a foam material (the hydro-accumulator), and a wick then carries the water to the seeds (planted in a fold in the wick) and to a moisture sensor, which can automatically control addition of water to the hydro-accumulator.

Instrumentation was designed in the Space Dynamics Laboratory, Utah State

University, to be added to Svet (in space) so that several critical parameters of the environment could be monitored (Salisbury et al. 1995; Bingham et al. 1996). In the modified Svet with the Utah instruments, plants are enclosed in two plastic bags (cuvettes), one above each root-module compartment. Filtered air is circulated through the cuvettes, and sensitive infrared gas analyzers measure water vapor and CO_2 levels of air entering and leaving each cuvette. This Svet instrumentation system was used in space station Mir in 1995 and 1996.

The root module in Svet in the space station Mir is composed of two vegetation modules, each 319 mm long × 156.5 mm wide × 110 mm high, with two rows of seedlings per vegetation module. Each row contains up to 26 wheat seedlings. Super-Dwarf wheat plants are short enough for the Svet in which the plant growing height within the plant chamber is 30 cm.

An important goal of space experiments with plants is to examine the effects of microgravity and other space-related parameters on plant growth. The environmental stresses for the plants in the space station include the following:

1. Microgravity,

2. High carbon-dioxide concentration (up to 1.0–1.3%, compared with 0.034 % on earth),

3. Variable photoperiod (11–24 h in the 1995 space experiment; 18 or 23 in 1996),

4. Relatively low photosynthetic photon flux (PPF). It was about 110 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the space experiment in 1995 and 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the space experiment in 1996,

5. Possible water logging or dry substrate.

In this project, we use various carbon-dioxide levels and photoperiod levels in the simulating growth chambers to obtain experimental results of plant responses in:

1. Flower initiation and development — a critical response related to crop production,

2. Stomatal density — a factor to control CO_2 uptake and transpiration,

3. Cell-wall thickness of supporting tissue.

The results of cell-wall thickness will be compared with future observations on plant tissues from space, since there is little necessity for plant tissue to support itself under microgravity conditions.

The experiments in this project are designed to answer the following questions:

1. Is flower initiation in Super-Dwarf wheat delayed or accelerated under super-optimum CO_2 levels?

2. Is flower initiation in Super-Dwarf wheat accelerated under longer photoperiods?

3. Is there any significant difference in stomatal density for Super-Dwarf wheat plants under different CO_2 levels and photoperiods?

4. Is there any significant difference in cell-wall thickness for supporting tissue for the plants grown with various CO_2 levels and photoperiods?

5. Does McDowell fixative work well on plant material for long-term storage (90 d or longer)?

CHAPTER II

REVIEW OF THE LITERATURE

Stomatal density

Changes in stomatal density (number of stomata per unit leaf area) may contribute to documented increase in water use efficiency, photosynthetic rate, and biomass accumulation at high carbon-dioxide levels. The number, distribution, and morphology of stomata on the leaf surface may be important traits in the adaptation of plants to increasing atmospheric CO_2 levels because stomata largely control CO_2 uptake and transpiration (Muchow and Sinclair, 1987).

The literature is inconsistent on whether stomatal density changes in response to high CO_2. Stomatal density has been reported to decrease (Woodward, 1987; Woodward and Bazzaz, 1988; Penuelas and Matamala, 1990) in the following species: *Acer pseudoplatanus, Carpinus betulus, Fagus sylvatica, Populus nugra, Quercus petraea, Q. robur, Rhamnus catharticus, Tilia cordata, Nardus stricta, Vaccinium myrtillus, Amaranthus retroflexus, Ambrosia artemisifolia, Setaria faberii, Pinus uncinata, Pinus pinea, Alnus glutinosa, Betula pendula, Juniperus communis, Ceratonia siliqua, Buxus sempervirens, Pistacia lentiscus, Helleborus foetidus, Rhododendron ferrugineum, Amaranthus caudatus, Papaver alpinum, Cynodon dactylon, and Gentiana alpina*), or increase (Rowland-Bamford et al., 1990, species: rice) with increased CO_2. In several other species, no change in stomatal density in response to increased CO_2 could be detected (Koerner, 1988; Radoglou and Jarvis, 1992; Ryle and Stanley, 1992, species: poplar, ryegrass). Malone et al. (1993) also concluded that

individual plants of oats and wheat lack the plasticity to significantly alter stomatal density and aperture length in response to increasing atmospheric CO_2 in a single generation (annuals) or growing season (perennials). Nevertheless, none of the experiments above used as high as 3000 μmol/mol (0.3 %) CO_2. In this project, we grew wheat plants under CO_2 levels of 360, 1200, 3000, and 7000 μmol/mol in different cuvettes. Observations were made to see if stomatal densities changed under the super-optimal CO_2 concentrations.

Floral initiation

Photoperiods influence flower development in many species. Regarding flowering responses to photoperiod, plants are generally divided into three categories: (1) short-day plants, (2) long-day plants, and (3) day-neutral plants. Whether the responses of plants to photoperiods are positive or negative depends on the type of plant. For long-day plants, such as barley and wheat, short-day treatment (i.e., shorter than some minimum length; e. g., 12 to 16 h) delays flower development (Ellis et al., 1989). For short-day plants, the rate of flowering is higher under short photoperiods than under longer photoperiods; examples are *Aster* hybrids (Farina et al., 1994), Heliconias (Geersten, 1989), and perennial *Glycine* species (Kenworthy et al., 1989). Wheat is considered to be a quantitative long-day plant. Long photoperiods hasten flower initiation and spike differentiation. As far as we know, the first mention of photoperiodism in wheat seems to be by Wanser in 1922 (Pinthus, 1985). Later publications stated that spring wheat is a quantitative long-day plant (see for reviews: Salisbury, 1963; Hart, 1988). Most wheat cultivars are facultative long-day plants, flowering at virtually any daylength but more rapidly under long days. But not all cultivars of wheat

respond positively to longer photoperiods. Oosterhuis and Cartwright (1983) reported that a greater spikelet number per spike for semidwarf wheat was obtained under short day (8 h) than under long day (16 h). We did not know if Super-Dwarf wheat had a positive response to longer photoperiods. We also did not know whether Super-Dwarf wheat was a quantitative (facultative) or qualitative (absolute) long-day plant. If Super-Dwarf wheat is an absolute long-day plant, it would have a critical daylength, remaining vegetative at shorter daylengths. It is even possible that flower initiation is not influenced by day length but only occurs after a minimum development is reached. So what is the response of Super-Dwarf wheat to different daylengths? Is flower initiation accelerated or delayed under longer photoperiods? In this project, Super-Dwarf wheat was grown under different photoperiods (18 h, 21 h, 24 h) to determine if long photoperiods would promote floral development although the three daylengths were too long to determine if Super-Dwarf wheat might be an absolute long-day plant (with a critical daylength) or a facultative long-day plant.

High carbon-dioxide concentration has an obvious influence on wheat production. Many reports are available on this response (e.g., Kimball and Idso, 1983; Imai et al., 1985; Olson et al. 1988; Lawlor and Mitchell, 1991). Bugbee et al. (1994) reported that elevating CO_2 level from 340 to 1200 $\mu mol \cdot mol^{-1}$ can increase the seed yield of wheat by 30 % to 40 %; unfortunately, further CO_2 elevation to 2500 μmol/mol (0.25 %) has consistently reduced yield by 25 % compared with plants grown at 1200 μmol/mol. They also found that the yield increases in both rice and wheat were primarily the result of an increased number of heads per square meter, with minor effects on seed number per head and seed size. This finding is

consistent with CO_2 enrichment studies on other crop plants that have shown an increase in the number of reproductive structures rather than increased dry mass of individual seeds. This has been shown for wheat (Gifford, 1977), soybeans (Havelka et al., 1984), and rice (Baker et al., 1990). But is the decrease of wheat productivity under super-optimal CO_2 caused by the adverse effects on floral initiation or on seed set? This study was designed to investigate the effects of super-optimum CO_2 on flower initiation rate at early stages and on seed maturity at the seed-filling stage.

Cell-wall thickness

One of the crucial functions of the plant secondary cell wall is to give rigidity to the plant. But under microgravity there is not much necessity for plant tissue to support itself. So what would happen to cell-wall thickness of the supporting tissues of wheat plants in a space environment? Does the cell-wall thickness of a plant in space become smaller in a single life cycle compared with that on the earth? As a baseline study, cell-wall thicknesses of supporting tissues of wheat plants grown in simulating chambers were measured under the microscope so that the results could be compared with future observations on plant tissues from space. Because the major constituent of the secondary wall of supporting tissue for vascular plants is lignin, specific histological staining procedures for lignin were used to measure the cell-wall thickness.

Effects on plant tissue of long-term storage in fixative

The cell consists of a complex of substances in a highly organized state. Any disruption

of this organization causes changes to occur that ultimately affect the morphology of the cell. The purpose of the fixative is to prevent these changes from occurring. The cell is killed and fixed in a solution designed to inhibit the vital processes of the cell, to prevent autolysis, and to change the physical properties of the chemical substances in the cell such that they will not be removed in subsequent handling (Jensen, 1962). Ideally, the fixative would do this without changing the morphology of the cell from the living state. At the same time, it is desirable to maintain the size and shape, and to preserve the chemical reactivity of cellular constituents. Such an ideal fixative has, needless to say, never been found, especially for long-term storage. The commonly used fixing solutions represent a compromise between the good and the bad features of several possible reagents.

The reagents most commonly used in botanical fixing solutions are alcohol, formalin, acetic acid, chromic acid, potassium permanganate, and osmium tetroxide. Singly, each of the chemicals listed has properties that make it important as a fixative, yet no one is ideal. In our experiments, the tissue sample may remain in the primary fixative for 90 d or even longer. The primary fixative used by McDowell and Trump (1976), which consists of 4 % formaldehyde (37 % biological-grade) and 1 % glutaraldehyde (25 % biological-grade) in a ratio of 4F:1G buffered with monobasic sodium phosphate plus sodium hydroxide, pH 7.2 -7.4, may offer possibilities for long-term storage of plant tissues. The formaldehyde component penetrates the tissue rapidly, and this is followed by the more slowly diffusing glutaraldehyde, which stabilizes the tissue more thoroughly and more permanently. This combination may account for the good preservation that is observed. An added advantage

noted was that the 4F:1G-fixed tissue could be paraffin embedded, sectioned easily, and subjected to common histological and cytochemical staining techniques. McDowell and Trump successfully stored rat-kidney tissue in this fixative for up to 5 years. After 1 year, no serious structural changes could be detected with the electron microscope. The 4- and 5-year-stored samples gave only slight evidence of extraction of nucleoplasmic materials. Does the McDowell fixative work as well on plant material? In this project, we tested the effects of long-term storage in McDowell fixative on plant tissue.

Histological stains

The interesting history of biological staining techniques is almost as long as the history of the microscope. Between the older natural dyes and the more recently developed synthetic dyes, hundreds of dyes were available and in use. Of these, few have achieved wide use; fewer still have been adapted to use in routine botanical microtechnique, including safranin, fast green, hematoxylin, orange G, and aniline blue. Dyes are used either singly or in combinations of two or more. The stain depends on the structures to be observed. Safranin-fast green is a good combination for general plant tissue, especially meristematic tissue (Jensen, 1962; Berlyn and Miksche, 1976; Clark, 1981). In the safranin-fast green combination, the tissue is first overstained with safranin. All the dye-binding sites are filled, and unbound dye is adsorbed at the tissue surfaces. The tissue is then washed to remove the unbound dye. Next, the tissue is destained in acid alcohol, which removes the dye not tightly bound, namely, the dye bound by the cytoplasm, cellulose, and nuclear material other than the nucleolus and the chromosomes. The lignin in the cell wall, the nucleoli, and the

chromosomes hold the dye tightly. The acid alcohol is not particularly selective and will effectively remove all of the stain if applied long enough. So plant tissue should remain in the acidified alcohol only for a short time. When most of the color has left the cytoplasm, however, the tissue is dehydrated by rapid transfer through a series of alcohols of increasing concentration (in which safranin is soluble) to fast green. The fast green stains the cytoplasm and cellulose walls, which now act as positive dye-binding sites. Absorbed, excess fast green is removed with a differentiating solution consisting of clove oil, absolute alcohol, and xylene, in which the dye is slightly soluble. Finally, the tissue is placed in xylene after which the section is mounted under a cover slip with a permanent mounting medium. With the safranin-fast green staining method, lignin and chromatin show red; chloroplasts pink to red; cellulose walls and cytoplasm green. To show lignin specifically, we used Phloroglucinol (saturated aqueous) : 20 % Hydrochloric Acid = 1 : 1. Either fresh tissue or fixed tissue will appear red-violet. To test the starch at the seed-filling stage, we used KI-I staining: 0.2 g iodine + 2 g potassium iodine (add first) + 100 mL water. The mature seeds will appear blue to black in a few minutes. Newly formed seeds will appear red to purple.

CHAPTER III

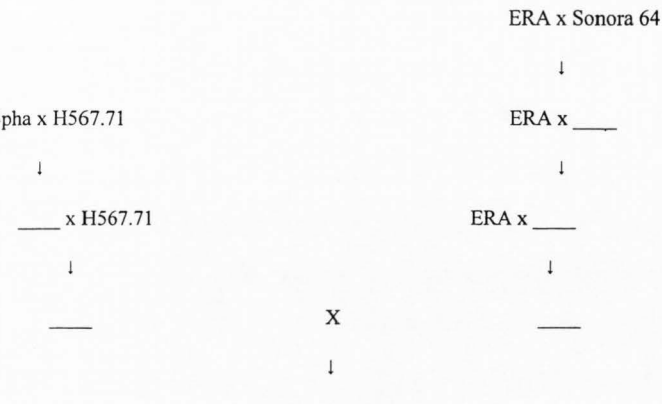
MATERIAL AND METHODS

Seeds and growth chambers

The name and selection of Super-Dwarf wheat are:

Triticum aestivum CMH79.481-1Y-8B-2Y-2B-0Y.

The pedigree chart of Super-Dwarf wheat is shown below:



Super-Dwarf wheat was originally obtained from CIMMYT in 1984, by Dr. Bruce Bugbee. It is normal wheat (42 chromosomes), except for extra dwarf genes. This cultivar is about 15 cm tall in the field, and about 25 cm tall in cool, high-nitrogen condition in the greenhouse. It has small round seeds rather than long seeds typical of wheat.

The simulating facility consisted of 12 growth chambers constructed of foamboard,

each with the same dimensions as Svet. The growth chambers were set up in the Gravity Laboratory in the research greenhouse of Utah State University, 1400 N. 800 E., Logan, Utah. Each Svet-simulating growth chamber included two vegetation modules (cuvettes), each 319 mm long, 175 mm wide, and 90 mm high (Fig. 1). The plants growing in each module were enclosed in separate transparent bags into which air was introduced at controlled temperatures and CO_2 levels. Carbon-dioxide cylinders were used to provide CO_2 to the growth chamber through hoses and flow meters. Infrared sensors were set up in the transparent bags. The sensors were connected through wires to a CO_2 analyzer to measure CO_2 levels of air entering and exiting each cuvette. The CO_2 analyzer was designed and developed by Dr. Gail E. Bingham and his student Timothy Paul Abel. A description of the CO_2 analyzer is available in Abel's thesis (Abel, 1997). A computer was used to monitor CO_2 level and accumulate other environmental data (Fig. 2). The computer model was AMDEK Color 710, and the program for monitoring data was GREENHOUSE 2 provided by Dr. G. E. Bingham. The facilities were set up by Dr. G. E. Bingham's students in the Department of Electrical and Computer Engineering at Utah State University. When CO_2 fluctuated during the experiment, we manually adjusted the flow meter for individual cuvette to maintain a specific CO_2 level. On the top of the mockups were fluorescent lamps to provide light. In each module, 52 wheat seeds were sown in two rows, each row containing 26 seeds.

A 3 × 4 factorial experiment with three photoperiods (18 h, 21 h, 24 h) and four carbon dioxide levels (360, 1200, 3000, and 7000 $\mu mol \cdot mol^{-1}$) was replicated with one module in one room and another in a different room. All environmental conditions for plant

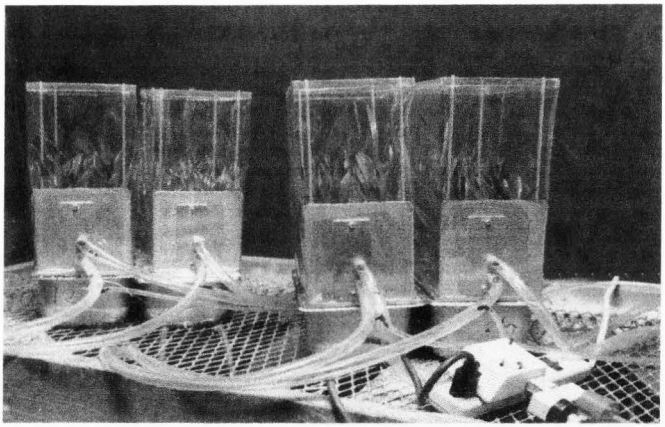


Fig. 1: Two mock-ups with outer boxes removed, also showing a time clock.

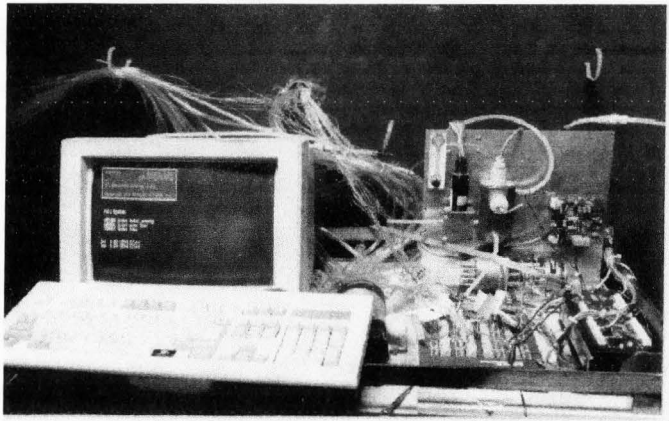


Fig. 2: CO_2 analyzer and the computer that accumulates environmental data.

growth, including temperature and soil type, were kept the same in the two rooms. The other environmental conditions in the modules were temperature 20 °C; irradiance approximately 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ or 8.64 $mol \cdot m^{-2} \cdot d^{-1}$ (photosynthetic photon flux; PPF) provided by 8 fluorescent lamps (8 W Sylvania cool white) in each chamber; relative humidity 65 % ± 10; rate of air flow 7 $L \cdot min^{-1}$; root substrate peat:pearlite = 1:4 by volume. The plants were watered every 2 to 4 d depending on soil conditions; at each watering, the same amount of nutrient solution was supplied to each module (500—750 mL for each cuvette). The nutrient solution was made with 20-10-20 Peat-Lite Special, Scotts-Sierra Horticultural.

Sampling stages

Flower initiation was studied at 15 d, 18 d, 21 d, and 24 d. Four sample plants were taken per treatment at each sampling date, two from room 1 and two from room 2. One sample plant was taken from each row. Sampling was undertaken from one end of a row to the other end so that thinning effects were the same for all treatments. Longitudinal sections of the apical meristem tissues were made with a standard paraffin technique (Jensen, 1962; Clark, 1981, see Appendix II). The number and size of flower buds were determined under the light microscope. Cell-wall thickness samples (four plants) were taken at 8 d (seedling stage) and 55 d (mature stage). At 55 days after planting (DAP), the head length of four main shoots per treatment was determined . The shoots were selected at random, and two shoots were taken from each room. The head length was measured from the base to the top of the head, not including awns. To study the seed-filling stage, two sample plants were taken for each treatment at 74 DAP. Two seeds from the base of the head in the main stem in each

sample plant were tested for starch accumulation with KI-I method (Berlyn and Miksche, 1976) to observe the degree of maturity of the seeds.

Measurements and statistical analysis

To study flower initiation, longitudinal sections close to the median were made of the apical meristem. On each section, flower bud number was counted, and flower buds (spikelets) were measured with an eye-piece micrometer. The data for four plants (four sample values) per treatment were analyzed as a three-way factorial experiment using analysis of variance. The three factors tested were replicate (room), photoperiod, and CO_2 level. Significant differences were established at the alpha = 0.05 level. The statistical analysis was done with SigmaStat 2.0 software in a microcomputer.

To measure stomatal density, four sample plants, two from each room, were refrigerated (15 °C) for 2 h and warmed to room temperature (25 °C) for 10 min, and the largest mature leaf was cut from each sample plant. A 1.5-cm piece was then cut from the middle, placed between two slides, and stomatal density was observed directly under the light microscope. Six fields of view were chosen by moving the slides without looking through the eye-piece before observation on each side (abaxial side and adaxial side) of a leaf. The area of the field of view in the microscope was determined, and the number of stomata per square millimeter was calculated. Sample values from four plants per treatment were subjected to analysis of variance as above.

The major constituent of the secondary wall of vascular plants is lignin. The mixture of phloroglucinol (saturated aqueous) and 20 % hydrochloric acid (1:1) was used to visualize

lignin. This is a specific reaction. The cell wall appears red-violet when stained with the mixture. Standard paraffin technique was used to make slides. The sections were stained with phloroglucinol + 20 % HCl (1:1) for 5 min before microscopic measurement. The thickness of two adjacent vessel-element walls was measured under the microscope with an eye-piece micrometer. Half of the measurement is the cell-wall thickness of one vessel element.

Four sample plants were taken at 8 d and 55 d for each treatment for cell-wall thickness measurement. Three cross-sections were randomly chosen for each plant. The stem tissue sample was in mature regions and the leaf sample was taken in the middle part of a leaf. Cell-wall thickness was measured for a vessel element in the outer and inner ring of each stem sample and the main vein and a branch vein of each leaf sample. Six measurements were obtained for each stem and leaf sample. The data of the four sample plants were subjected to analysis of variance as above.

In another experiment, 13-d-old leaves of Super-Dwarf wheat cultivated under the same conditions in a large growth chamber were used for histological sectioning experiments conducted at 24 h, 90 d, and 180 d after fixation in McDowell fixative. The fully expanded leaves (two leaves) were cut from two sample plants for each treatment. The middle part of the leaves was cut into 1-cm pieces and fixed in McDowell fixative. The McDowell fixative was made by 4 % formaldehyde and 1 % glutaraldehyde with monobasic sodium phosphate buffer at pH 7.2-7.4 (see Appendix I). The samples were dehydrated and embedded in paraffin, and cross sections (10 μ) were then made. The shape of chloroplasts in mesophyll cells, the size of mesophyll cells and bulliform cells, and the leaf thickness were then

determined for 50 samples per treatment. A two-sample *t* statistic was computed and significant differences were established at the alpha = 0.05 level.

CHAPTER IV

RESULTS

Flower initiation

Effects of photoperiod and CO_2 level were tested by statistical analysis with the four sample plants for each treatment. Because two sample plants were taken from each room, the effects of room difference were also tested by three-way analysis of variance.

No flower buds were observed at 15 DAP. Effects of room, photoperiod, CO_2 level at 18, 21, and 24 DAP and their interactions are shown in Tables 1, 2, 3, 4, and 5. Both photoperiod and CO_2 level affected flower bud number at 18 and 21 DAP. The differences between 18-h and 21-h treatments were not significant. But flower bud numbers under 24-h treatments were significantly different from 18-h and 21-h treatments at 18 and 21 DAP (Tables 2 and 4). The mean of flower bud number in Fig. 3 showed that flower bud numbers under 24-h treatments were significantly larger than 18-h and 21-h treatments at 18 and 21 DAP. There were significant differences in flower bud number between 1200 μmol/mol CO_2 treatments and other CO_2 treatments at 18 and 21 DAP (Tables 2 and 4). The average flower bud number in Fig. 3 showed that super-optimum CO_2-level treatments (3000 and 7000 μmol/mol) resulted in fewer flower bud numbers than 1200 μmol/mol CO_2 treatments. At 24 DAP, however, both photoperiod and CO_2 level did not affect flower bud number (Table 5). There was no significant difference in average flower bud number at 24 DAP (Fig. 3). The results also indicated that there were no room effects on flower bud number at 18, 21, and 24 DAP.

Table 1: Three-way ANOVA for flower bud number at 18 DAP.

Source of Variation	DF	SS	MS	F	P
Photoperiod	2	80.375	40.188	62.226	<0.001
CO_2	3	115.563	38.521	59.645	<0.001
Room	1	0.0208	0.0208	0.0323	0.859
Photoperiod x CO_2	6	7.125	1.188	1.839	0.134
Photoperiod x Room	2	0.041	0.0208	0.0323	0.968
CO_2 x Room	3	0.229	0.0764	0.118	0.948
Photoperiod x CO_2 x Room	6	0.458	0.0764	0.118	0.993
Residual	24	15.500	0.646		
Total	47	219.313	4.666		

Table 2: Comparisons for effects of photoperiod and CO_2 level on flower bud number at 18 DAP.

Comparison	Diff of Means	p	q	P<0.05
Photoperiod				
24 h vs. 18 h	2.938	3	14.621	Yes
24 h vs. 21 h	2.500	3	12.443	Yes
21 h vs. 18 h	0.438	3	2.178	No
CO_2 level				
1200 µmol vs. 7000 µmol	4.000	4	17.243	Yes
1200 µmol vs. 3000 µmol	3.500	4	15.087	Yes
1200 µmol vs. 360 µmol	2.917	4	12.572	Yes
360 µmol vs. 7000 µmo	1.083	4	4.670	Yes
360 µmol vs. 3000 µmol	0.583	4	2.514	No
3000 µmol vs. 7000 µmol	0.500	4	2.155	No

Table 3: Three-way ANOVA for flower bud number at 21 DAP.

Source of Variation	DF	SS	MS	F	P
Photoperiod	2	35.37	17.688	18.867	<0.001
CO_2	3	29.729	9.910	10.570	<0.001
Room	1	0.188	0.188	0.200	0.659
Photoperiod x CO_2	6	1.958	0.326	0.348	0.904
Photoperiod x Room	2	0.125	0.0625	0.0667	0.936
CO_2 x Room	3	0.22	0.0764	0.0815	0.969
Photoperiod x CO_2 x Room	6	0.208	0.0347	0.0370	1.000
Residual	24	22.50	0.938		
Total	47	90.313	1.922		

Table 4: Comparison of effects of photoperiod and CO_2 on flower bud number at 21 DAP.

Comparison	Diff of Means	p	q	P<0.05
Photoperiod				
24 h vs. 18 h	2.000	3	8.262	Yes
24 h vs. 21 h	1.563	3	6.455	Yes
21 h vs. 18 h	0.438	3	1.807	No
CO_2 level				
1200 µmol vs. 7000 µmol	2.000	4	7.155	Yes
1200 µmol vs. 3000 µmol	1.833	4	6.559	Yes
1200 µmol vs. 360 µmol	1.417	4	5.068	Yes
360 µmol vs. 7000 µmol	0.583	4	2.087	No
360 µmol vs. 3000 µmol	0.417	4	1.491	No
3000 µmol vs. 7000 µmol	0.167	4	0.596	No

Table 5: Three-way ANOVA for flower bud number at 24 DAP.

Source of Variation	DF	SS	MS	F	P
Photoperiod	2	0.292	0.146	0.11	0.889
CO_2	3	9.229	3.07	2.503	0.083
Room	1	0.0208	0.020	0.0169	0.898
Photoperiod x CO_2	6	3.208	0.535	0.435	0.848
Photoperiod x Room	2	0.542	0.271	0.220	0.804
CO_2 x Room	3	0.396	0.132	0.107	0.955
Photoperiod x CO_2 x Room	6	0.292	0.0486	0.0395	1.000
Residual	24	29.500	1.229		
Total	47	43.479	0.925		

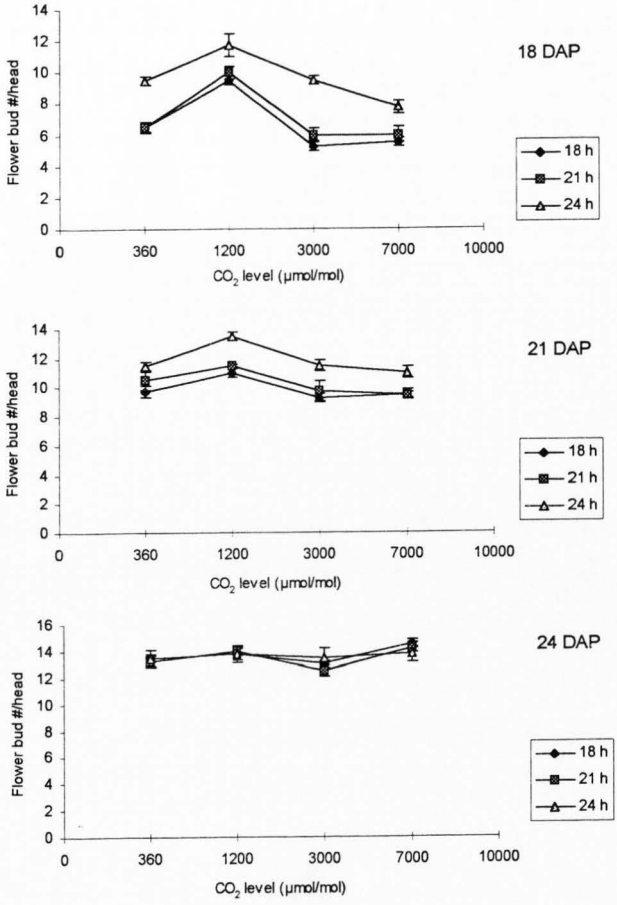


Fig. 3: Mean of flower bud number per head with S. E. under different photoperiods and CO_2 levels at three stages.

Differences of flower bud size among treatments were much more distinctive than flower bud number. Analysis of variance for effects of room, photoperiod, and CO_2 are shown in Tables 6-11. Photoperiod and CO_2 affected flower bud size at 18, 21, and 24 DAP (Table 6, 8, and 10). The 24-h treatments were always significantly different from other photoperiod treatments (Tables 7, 9, and 11). The average flower bud size in Fig. 4 showed that longer photoperiods resulted larger flower bud size at the three stages. Carbon dioxide treatments of 1200 μmol/mol level were always significantly different from other CO_2 treatments (Tables 7, 9, and 11). Average flower bud size in Fig. 4 showed that 1200 μmol/mol CO_2 treatments resulted in larger flower bud size than other CO_2 treatments under the same photoperiods. The statistical data also indicated that there were no room effects on flower bud size at the three stages.

Head growth

At 35 DAP, one typical sample plant was taken for each treatment and heads were dissected and photographed (Fig. 5). Differences of head length and size at 35 DAP stage were distinctive with variation of photoperiod. These results suggested that there might be a grand growth between 24 DAP and 35 DAP for the plants with 24-h treatments, which resulted in much larger and longer heads than 18-h and 21-h treatments.

The results of head length at 55 DAP are showed in Tables 12 and 13. The 24-h treatments were significantly different from 18-h and 21-h treatments. But there were no significant differences between 18-h and 21-h treatments (Table 13). Average head length in Fig. 6 showed that 24-h treatments resulted in longer heads than 18-h and 21-h treatments

under the same CO_2 levels. For carbon dioxide effects, 1200 µmol/mol treatments were significantly different from 360, 3000, and 7000 µmol/mol treatments. The average head length in Fig. 6 showed 1200 µmol/mol treatments resulted in longer head length than other CO_2 treatments under the same photoperiods. But ANOVA results showed no significant difference among treatments with 360, 3000, and 7000 µmol/mol CO_2 treatments (Table 13).

Table 6: Three-way ANOVA for flower bud size at 18 DAP.

Source of Variation	DF	SS	MS	F	P
Photoperiod	2	4739.542	2369.771	34.543	<0.001
CO_2	3	4799.063	1599.688	23.318	<0.001
Room	1	7.521	7.521	0.110	0.743
Photoperiod x CO_2	6	125.625	20.938	0.305	0.928
Photoperiod x Room	2	0.542	0.271	0.00395	0.996
CO_2 x Room	3	126.229	42.076	0.61	0.613
Photoperiod x CO_2 x Room	6	65.958	10.993	0.160	0.985
Residual	24	1646.500	68.604		
Total	47	11510.979	244.914		

Table 7: Comparisons of effects of photoperiod and CO_2 level on flower bud size at 18 DAP.

Comparison	Diff of Means	p	q	P<0.05
Photoperiod				
24 h vs. 18 h	24.250	3	11.711	Yes
24 h vs. 21 h	10.313	3	4.980	Yes
21 h vs. 18 h	13.938	3	6.731	Yes
CO_2				
1200 µmol vs. 3000 µmol	25.583	4	10.700	Yes
1200 µmol vs. 7000 µmol	22.833	4	9.550	Yes
1200 µmol vs. 360 µmol	18.833	4	7.877	Yes
360 µmol vs. 3000 µmol	6.750	4	2.823	No
360 µmol vs. 7000 µmol	4.000	4	1.673	No
7000 µmol vs. 3000 µmol	2.750	4	1.150	No

Table 8: Three-way ANOVA for flower bud size at 21 DAP.

Source of Variation	DF	SS	MS	F	P
Photoperiod	2	6743.375	3371.688	72.607	<0.001
CO_2	3	3244.563	1081.521	23.290	<0.001
Room	1	7.521	7.521	0.162	0.691
Photoperiod x CO_2	6	209.625	34.938	0.752	0.614
Photoperiod x Room	2	1.542	0.771	0.0166	0.984
CO_2 x Room	3	7.729	2.576	0.0555	0.982
Photoperiod x CO_2 x Room	6	47.458	7.910	0.170	0.982
Residual	24	1114.500	46.438		
Total	47	11376.313	242.049		

Table 9: Comparison for effects of photoperiod and CO_2 on flower bud size at 21 DAP.

Comparison	Diff of Means	p	q	P<0.05
Photoperiod				
24 h vs. 18 h	27.500	3	16.142	Yes
24 h vs. 21 h	21.813	3	12.804	Yes
21 h vs. 18 h	5.688	3	3.338	No
CO_2				
1200 µmol vs. 7000 µmol	21.167	4	10.760	Yes
1200 µmol vs. 3000 µmol	18.917	4	9.616	Yes
1200 µmol vs. 360 µmol	13.667	4	6.947	Yes
360 µmol vs. 7000 µmol	7.500	4	3.813	No
360 µmol vs. 3000 µmol	5.250	4	2.669	No
3000 µmol vs. 7000 µmol	2.250	4	1.144	No

Table 10: Three-way ANOVA on flower bud size at 24 DAP.

Source of Variation	DF	SS	MS	F	P
Photoperiod	2	74519.792	37259.896	438.352	<0.001
CO_2	3	4793.833	1597.944	18.799	<0.001
Room	1	24.083	24.083	0.283	0.599
Photoperiod x CO_2	6	2902.542	483.757	5.691	<0.001
Photoperiod x Room	2	17.542	8.771	0.103	0.902
CO_2 x Room	3	133.417	44.472	0.523	0.670
Photoperiod x CO_2 x Room	6	38.458	6.410	0.0754	0.998
Residual	24	2040.000	85.000		
Total	47	84469.667	1797.227		

Table 11: Comparison for effects of photoperiod and CO_2 level on flower bud size at 24 DAP.

Comparison	Diff of Means	p	q	P<0.05
Photoperiod				
24 h vs. 18 h	90.625	3	39.319	Yes
24 h vs. 21 h	74.063	3	32.133	Yes
21 h vs. 18 h	16.563	3	7.186	Yes
CO_2				
1200 µmol vs. 7000 µmol	24.250	4	9.112	Yes
1200 µmol vs. 360 µmol	23.583	4	8.861	Yes
1200 µmol vs. 3000 µmol	20.833	4	7.828	Yes
3000 µmol vs. 7000 µmol	3.417	4	1.284	No
3000 µmol vs. 360 µmol	2.750	4	1.033	No
360 µmol vs. 7000 µmol	0.667	4	0.250	No

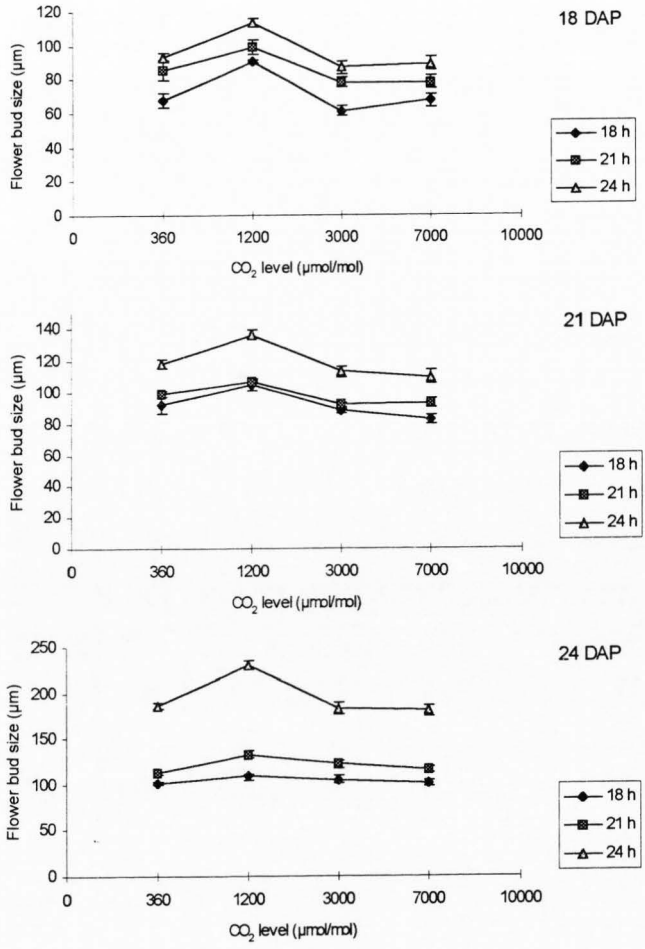


Fig. 4: Mean of flower bud size with S. E. under different photoperiods and CO_2 levels at three stages.

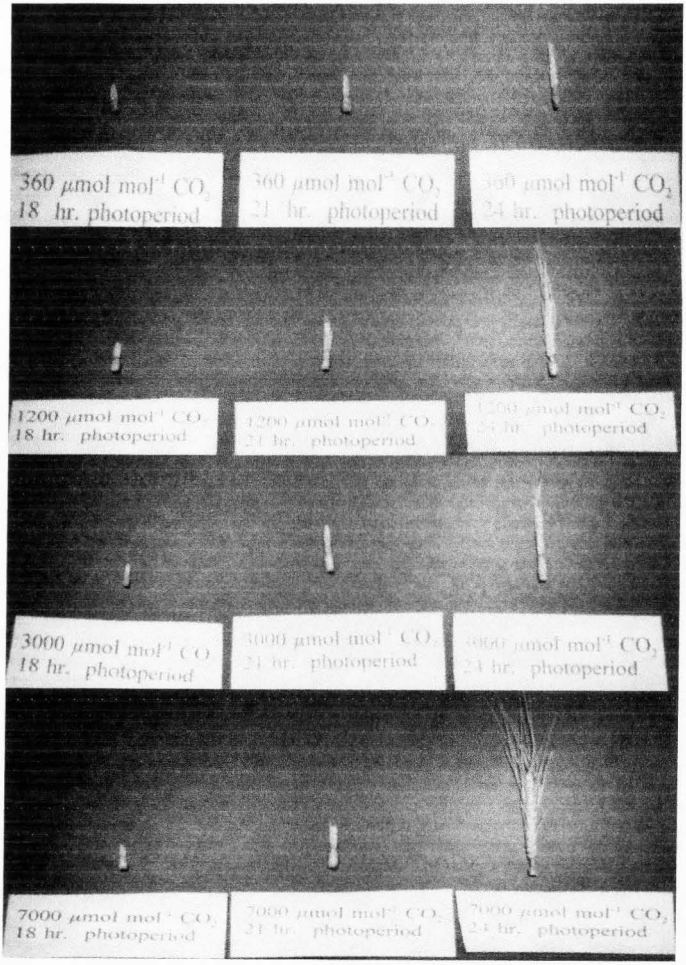


Fig. 5: Head length of Super-Dwarf wheat at 35 DAP.

Table 12: Three-way ANOVA for head length at 55 DAP.

Source of Variation	DF	SS	MS	F	P
Photoperiod	2	274.625	137.313	7.431	0.003
CO_2	3	459.063	153.021	8.281	<0.001
Room	1	0.521	0.521	0.0282	0.868
Photoperiod x CO_2	6	50.375	8.396	0.454	0.835
Photoperiod x Room	2	15.542	7.771	0.421	0.661
CO_2 x Room	3	6.396	2.132	0.115	0.950
Photoperiod x CO_2 x Room	6	26.792	4.465	0.242	0.958
Residual	24	443.500	18.479		
Total	47	1276.813	27.166		

Table 13: Comparison for effects of photoperiod and CO_2 level on head length at 55 DAP.

Comparison	Diff of Means	p	q	P<0.05
Photoperiod				
24 h vs. 18 h	5.688	3	5.292	Yes
24 h vs. 21 h	4.063	3	3.780	Yes
21 h vs. 18 h	1.625	3	1.512	No
CO_2				
1200 µmol vs. 7000 µmol	7.250	4	5.842	Yes
1200 µmol vs. 3000 µmol	7.167	4	5.775	Yes
1200 µmol vs. 360 µmol	7.000	4	5.641	Yes
360 µmol vs. 7000 µmol	0.250	4	0.201	No
360 µmol vs. 3000 µmol	0.167	4	0.134	No
3000 µmol vs. 7000 µmol	0.0833	4	0.0672	No

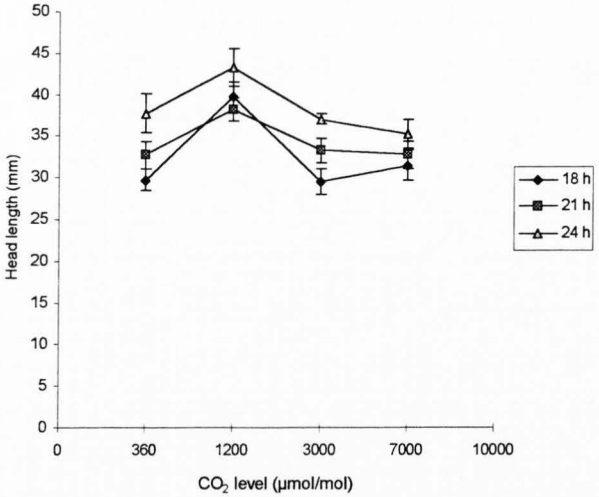


Fig. 6: Mean of head length with S. E. at 55 DAP.

Seed maturity

The KI-I test is a qualitative test. Seeds of plants exposed to a 24-h photoperiod were more mature at 74 DAP than seeds from other treatments (Table 14). The 21-h and 24-h photoperiods produced more mature seeds regardless of CO_2. But the differences were not striking. They were only between purple and dark purple, not between purple and blue (or even black). There were no distinct differences among the different CO_2 treatments.

Table 14: Results of I-KI test for Super-Dwarf wheat seeds at 74 DAP (blue or black = mature seed; red or purple = less mature seeds).

CO_2 level		Photoperiod		
(µmol/mol)	Sample	18 h	21 h	24 h
360	plant 1 seed 1	purple	dark purple	dark purple
	seed 2	purple	purple	dark purple
	plant 2 seed 1	dark purple	purple	dark purple
	seed 2	purple	purple	dark purple
1200	plant 1 seed 1	purple	purple	dark purple
	seed 2	dark purple	dark purple	dark purple
	plant 2 seed 1	blue	dark purple	dark purple
	seed 2	purple	dark purple	dark purple
3000	plant 1 seed 1	purple	dark purple	dark purple
	seed 2	purple	dark purple	blue
	plant 2 seed 1	dark purple	dark purple	dark purple
	seed 2	purple	dark purple	dark purple
7000	plant 1 seed 1	purple	dark purple	dark purple
	seed 2	dark purple	dark purple	dark purple
	plant 2 seed 1	purple	dark purple	dark purple
	seed 2	purple	dark purple	dark purple

Stomatal density

Stomatal densities were significantly different between the two sides of Super-Dwarf wheat leaves. Adaxial stomatal densities were always higher than abaxial densities. At 15 DAP, adaxial stomatal densities were 44 to 62% higher than abaxial stomatal densities. At 27 DAP, adaxial stomatal densities were 33 to 60% higher than abaxial stomatal densities.

At 15 DAP, photoperiod and CO_2 did not affect stomatal density on either side ($P>0.1$). At 27 DAP, however, photoperiod and CO_2 did affect stomatal density on abaxial side (Table 15), but not on adaxial side ($P>0.1$). The effects of interaction for photoperiod and CO_2 were also significant ($P<0.001$). The results in Table 16 showed that 18-h photoperiod treatments were significantly different from 21-h and 24-h treatments and 360 μmol/mol carbon dioxide treatments were significantly different form higher CO_2 level treatments. The average stomatal densities in Figs. 7 and 8 showed that abaxial stomatal density decreased with increasing photoperiod and CO_2 level at 27 DAP.

Cell-wall thickness

Cell-wall thickness of vessel elements of Super-Dwarf wheat leaves at 8 DAP and 55 DAP were measured for each sample plant. Results of three-way analysis of variance showed no significant effects of photoperiod and CO_2 level on cell-wall thickness of vessel elements for either stem or leaf at both 8 and 55 DAP ($P>0.05$). Mean values of cell-wall thickness of vessel elements with standard error are shown in Tables 17 and 18.

Table 15: Three-way ANOVA for abaxial stomatal density at 27 DAP.

Source of Variation	DF	SS	MS	F	P
Photoperiod	2	1600.543	800.271	8.760	0.001
CO_2	3	2447.891	815.964	8.932	<0.001
Room	1	0.603	0.603	0.00660	0.936
Photoperiod x CO_2	6	6849.718	1141.620	12.496	<0.001
Photoperiod x Room	2	16.121	8.061	0.0882	0.916
CO_2 x Room	3	35.272	11.757	0.129	0.942
Photoperiod x CO_2 x Room	6	11.395	1.899	0.0208	1.000
Residual	24	2192.582	91.358		
Total	47	13154.126	279.875		

Table 16: Comparisons for effects of photoperiod and CO_2 level on abaxial stomatal density at 27 DAP.

Comparison	Diff of Means	p	q	P<0.05
Photoperiod				
18 h vs. 24 h	12.879	3	5.390	Yes .
18 h vs. 21 h	11.504	3	4.814	Yes
21 h vs. 24 h	1.374	3	0.575	No
CO_2				
360 µmol vs. 1200 µmol	18.050	4	6.542	Yes
360 µmol vs. 3000 µmol	15.864	4	5.750	Yes
360 µmol vs. 7000 µmol	14.939	4	5.414	Yes
7000 µmol vs. 1200 µmol	3.111	4	1.127	No
7000 µmol vs. 3000 µmol	0.925	4	0.335	No
3000 µmol vs. 1200 µmol	2.186	4	0.792	No

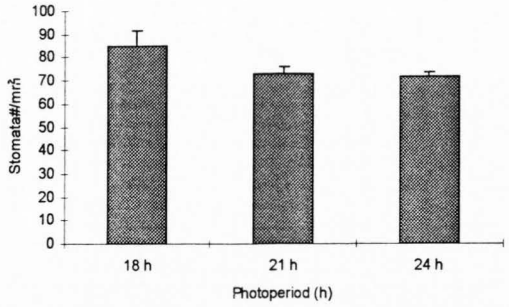


Fig. 7: Mean of abaxial stomatal density with S. E. under different photoperiods at 27 DAP.

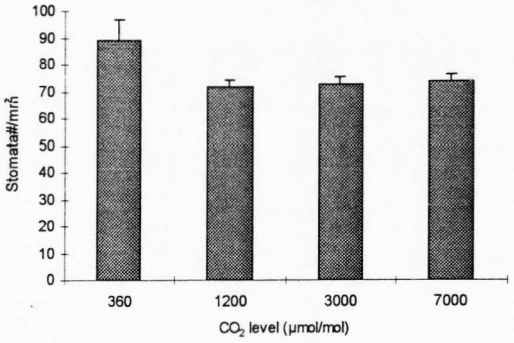


Fig. 8: Mean of abaxial stomatal density with S. E. under different CO_2 levels at 27 DAP.

Table 17: Mean of cell-wall thickness (μm) of vessel elements with S. E. at 8 DAP.

	CO_2 level (μmol/mol)	18 h	21 h	24 h
Stem	360	5.228±0.37	5.620±0.16	5.518±0.19
	1200	5.635±0.15	5.082±0.26	5.700±0.14
	3000	5.087±0.20	5.085±0.16	5.345±0.25
	7000	4.982±0.16	5.290±0.03	5.520±0.10
Leaf	360	4.837±0.28	4.775±0.25	4.930±0.15
	1200	4.785±0.17	4.873±0.20	5.150±0.16
	3000	4.765±0.13	4.970±0.23	5.190±0.12
	7000	5.050±0.30	5.357±0.30	5.425±0.19

Table 18: Mean of cell-wall thickness (μm) of vessel elements with S. E. at 55 DAP.

CO_2 level (μmol/mol)		18 h	21 h	24 h
Stem	360	5.5700±0.20	5.800±0.15	6.5625±0.45
	1200	5.8975±0.50	5.8900±0.16	6.2125±0.26
	3000	5.7800±0.19	5.8900±0.20	5.9250±0.37
	7000	5.7750±0.20	5.6650±0.25	6.0700±0.17
Leaf	360	5.1825±0.22	5.2825±0.90	5.0200±0.30
	1200	4.9875±0.19	5.0515±0.14	4.9350±0.07
	3000	4.9800±0.20	4.5200±0.20	4.8300±0.17
	7000	4.6975±0.28	5.2400±0.25	4.8000±0.18

Effects of long-term storage of plant tissue in McDowell fixative

For effects of long-term storage of plant tissue in McDowell fixative, no distinctive changes in shape of chloroplasts were observed for both 90-d and 180-d-stored leaf tissue compared to the 24-h fixation treatment. Data of leaf thickness, size of mesophyll cells, and bulliform cells are shown in Table 19. Statistical results of two-sample *t*-test for the effects of long-term storage of plant leaves in McDowell fixative indicated that there was no significant change in leaf thickness and size of mesophyll cells for the long-term storage up to 180 days ($P>0.2$). For the size of bulliform cells, no changes were detected for 90-d-stored leaf tissue. When stored up to 180 d, the bulliform cells were larger ($P<0.02$).

Table 19: Statistical data (means±S. E.) for effects of McDowell fixative on plant tissue in long-term storage.

Comparative items	24 h	90 d	180 d
Leaf thickness (μm)	237±3.38	233±3.32	234±3.34
Size of mesophyll cells (μm)	22.9±0.44	22.7±0.47	23.4±0.45
Size of bulliform cells (μm)	29.1±0.47	29.6±0.62	32.0±0.77

* Sample size=50

CHAPTER V

DISCUSSION

Flower initiation

Photoperiod is an important environmental condition for flower initiation in many species. Wheat (spring wheat) is a quantitative long-day plant. Although there is no critical day length for flower initiation for the wheat cultivars that have been studied, the flower initiation rate for wheat increases with increasing photoperiod length. Rawson and Richards (1993) reported that wheat plants reached ear emergence more quickly as photoperiod lengthened from 9 to 15 h. All photoperiods in my experiment were longer than those in Rawson's experiment. It was still clear that longer photoperiods favored flower initiation, especially flower bud size. Photoperiods affected flower bud size at all observed stages. Lengthening the photoperiod even decreased the deleterious effects of super-optimum CO_2 level. In Fig. 4, the 7000 μmol/mol CO_2 treatment with a 24-h photoperiod resulted in larger flower bud size than the treatment with 1200 μmol/mol CO_2 and 21-h photoperiod. The results suggested that longer photoperiods should be used in space experiments to reduce the deleterious effects of super-optimum CO_2 levels. Flower bud number, however, was affected by photoperiod only at the early stage (18 DAP). There was no significant difference ($P>0.05$) for flower bud number under different photoperiods at the 24 DAP stage (Fig. 3). At 35 DAP stage, head length appeared to be enhanced by longer photoperiods At 55 DAP, head length was increased by longer photoperiods. But the interaction of photoperiod and CO_2 did not show effects on head length, which indicated that super-optimum CO_2 levels

were unfavorable for head growth. Thus the results suggested that shorter photoperiods slowed the rate of apical development and increased the duration of the primordium initiation phases. These results are consistent with already reported data (Wanser, 1922; Craufurd and Cartwright, 1989; Rawson and Richards, 1993). The new information we can obtain from this experiment is that longer photoperiods not only accelerate flower initiation, but also overcome the unfavorable effects of super-optimum CO_2 levels. Note, however, that the final number of flower buds was not affected by photoperiod.

The quality of controlled-environment crops can be improved by controlling the aerial carbon-dioxide in the greenhouse (Enoch and Zieslin, 1988). Elevated carbon-dioxide has obvious effects on crop growth and yield. Many reports are available on this aspect (e.g., Kimball and Idso, 1983; Imai et al., 1985; Olson et al., 1988; Lawlor and Mitchell, 1991). In this project, Super-Dwarf wheat grew under a large range of CO_2 levels from 360 to 7000 $\mu mol \cdot mol^{-1}$. At 1200 $\mu mol \cdot mol^{-1}$ CO_2, the flower bud size was increased 9 % to 18 % compared with those at 360 μmol/mol CO_2 between the 18 DAP and 24 DAP stage. But further increased CO_2 levels (3000 and 7000 $\mu mol \cdot mol^{-1}$) resulted in 13 % to 36 % decrease in flower bud size compared with those under 1200 $\mu mol \cdot mol^{-1}$ CO_2 between the 18 DAP and 24 DAP stage. In this experiment, a super-optimum CO_2 concentration slowed the rate of flower initiation and apical development. Similar to photoperiod, however, the final number of flower buds in the shoot tip was not affected by CO_2 level (Fig. 3). At 24 DAP, there were no significant effects of CO_2 level on flower bud number. The results suggested that super-optimum carbon dioxide in the space station might decrease growth rates of the

reproduction organ, but not decrease the flower number per head. However, continuous light could be used in the space station to decrease these deleterious effects.

Starch formation is a measurement of seed maturity for wheat plants. The IK-I staining test is a specific test for wheat seed maturity. Newly formed seeds appear red to purple. Mature seeds appear blue to black. Although this is not a quantitative method, we can still determine the relative degree of maturity of wheat seeds at the 74-d stage. The results showed that seeds with 24-h photoperiod treatments were more mature than 21-h and 18-h treatments (Table 4). This suggested that longer photoperiods would shorten the total period of the life cycle (seed-to-seed) of Super-Dwarf wheat. Carbon dioxide level, however, did not show effects on seed maturity at the 74-d stage.

From the above results, we can see that both photoperiod and CO_2 concentration affect the rate of flower initiation. On space station Mir, there is a high CO_2 level with low PPF and variable photoperiods. Development of Super-Dwarf wheat plants might be delayed. Longer growth periods would be required to complete their life cycle (seed-to-seed) compared with wheat plants grown under normal conditions on the earth.

Stomatal density

The stomatal density on the wheat leaf surfaces may be an important trait in the adaptation of Super-Dwarf wheat to fluctuating CO_2 levels aboard the Mir station. So far there are no reports available regarding the correlation of photoperiod and stomatal density. The literature is inconsistent on whether stomatal density changes in response to elevated CO_2 concentration. Stomatal density has been reported to decrease with increasing carbon-

dioxide level (Penuelas and Matamala, 1990; Rowland-Bamford et al., 1990; Beerling and Chaloner, 1993; Berryman et al., 1994; Ferris and Taylor, 1994; Beerling and Woodward, 1995). But stomatal density also has been reported not to change with variation of carbon-dioxide level (Radoglou and Jarvis, 1992; Ryle and Stanley, 1992; Malone et al., 1993; Estiarte et al. 1994; Gay and Hauck, 1994; Pearson et al., 1995). Stomatal and leaf responses to elevated CO_2 are species specific (Knapp et al., 1994). Plants of different species might have different responses to variations in CO_2 concentration. Malone et al. (1993) even concluded that individual plants lack the plasticity to significantly alter stomatal density in response to increasing CO_2 in a single generation. In the reported documents, however, the highest CO_2 concentration was only 700 $\mu mol \cdot mol^{-1}$, which is lower than the optimum CO_2 level for wheat plants. In this project, we used a wide range of CO_2 concentrations from 360 to 7000 μmol/ mol. The results of this project showed that stomatal densities significantly changed with variation of photoperiod and CO_2 at 27 DAP on the abaxial side. The average data showed that abaxial stomatal density decreased with increasing photoperiod and CO_2 level at 27 DAP (Figs. 7 and 8). The results suggest that wheat plants do acclimate to increasing CO_2 concentration by changing stomatal density within a single generation.

Results indicated that there was a higher number of stomata on the adaxial surface compared to the abaxial surface. This phenomenon is closely related to wheat-leaf morphology. On cross-sectioned wheat leaves, there are much deeper grooves on the adaxial surface than on the abaxial surface. Thus there is actually a larger surface area on the adaxial surface than on the abaxial surface in a field of the microscope. This might bring about a

higher sensitivity for stomatal density on the abaxial surface in response to variations in CO_2 and photoperiod level.

At the 8 DAP stage, wheat leaves had just begun to develop. Stomatal density did not significantly change with variations of CO_2 and photoperiod on both the abaxial side and the adaxial side ($P>0.1$). At the 27 DAP stage, however, stomatal density acclimated to the change of photoperiod and CO_2 level, but the differences among treatments were significant only on the abaxial surface (Table 15), not on the adaxial surface ($P>0.1$). This might be due to less sensitivity for stomatal density on the adaxial surface in response to variations of photoperiod and CO_2 levels.

Cell-wall thickness of vessel elements

Carbon-dioxide level influences crop yield, but it does not have distinctive effects on plant vegetative growth. No significant differences in plant size under different CO_2 concentrations were apparent during plant growth preceding the experiment. Results of microscopic measurements indicated that there were no significant differences in cell-wall thickness of vessel elements for the plants with various CO_2 and photoperiod treatments at both seedling (8 DAP) stage and mature (55 DAP) stage. On space station Mir, however, plants do not need much support tissue under microgravity conditions. The stress conditions on Mir might reduce cell-wall thickness even though they do not affect vegetative growth. Thus, if plants from Mir show significant difference in cell-wall thickness of vessel elements from plants grown for my experiment, it would suggest that plants do develop thinner cell walls under microgravity.

Effects of long-term stored fixative on plant tissue

In space sampling, the plant tissue sample may be required to remain in the primary fixative for 90 d or longer. The primary fixative used by McDowell and Trump was shown to be a good fixative for animal tissue (McDowell and Trump, 1976). Our results have shown that McDowell fixative is also a suitable fixative for plant tissue in long-term storage for light microscopy. In the 180-d-stored wheat leaves, there were no significant changes in the shape of chloroplasts, leaf thickness, shape and size of mesophyll cells, and shape of bulliform cells under the light microscope. The size of bulliform cells showed no change in 90-d-stored wheat leaves in McDowell fixative, and only showed larger in 180-d-stored wheat leaves. Thus the results indicate that McDowell fixative is pretty good fixative for plant tissue. It can be used in space sampling for the use of the light microscope.

CONCLUSIONS AND FUTURE RESEARCH

Conclusions

1. Carbon-dioxide concentrations affected flower initiation rates of Super-Dwarf wheat. The optimum CO_2 level for flower initiation and development is 1200 $\mu mol \cdot mol^{-1}$. Super-optimum CO_2 levels delayed flower initiation, but did not decrease final flower bud number per head.

2. Longer photoperiods not only accelerateed flower initiation rates, but also decreased deleterious effects of super-optimum CO_2. Flower bud size and head length at the same developmental stage were larger under longer photoperiods. But final flower bud number was not affected by photoperiod.

3. Stomatal densities on the abaxial surface were more sensitive to the variation of photoperiod and CO_2 level than those on the adaxial surface for Super-Dwarf wheat. Stomatal density did not significantly change on the adaxial surface, but significantly changed at 27 DAP on the abaxial surface with variation of photoperiod and CO_2 level.

4. Cell walls of both stem and leaf tissues did not significantly change with variation of photoperiod and CO_2 levels at either seedling stage or mature stage.

5. McDowell fixative was basically suitable for long-term storage of plant tissue for use in light microscopy. When stored up to 180 d, there was no significant change in leaf thickness, shape and size of mesophyll cells, and shape of chloroplasts for wheat leaves under the light microscope.

Possible future research

1. In this project, the shortest photoperiod was 18 h. Since the photoperiod in the space station Mir was as short as 10 to 12 h in the 1995 experiment, there is a possibility that shorter photoperiods combined with low light intensity could inhibit flower initiation. Thus, in future simulation experiments, shorter photoperiods could be used to investigate plant responses.

2. Starch accumulation is a measurement of seed maturity for the plant. I-KI is only a color test. It is desirable to apply a quantitative analysis in future experiments.

3. For stomatal density, only the largest mature leaf in a sample plant was taken in the experiment. Flag leaves are more important in photosynthetic productivity with regard to seed filling. In future research, flag leaves and non-flag leaves may be taken as samples so that we can determine if stomatal densities have different responses in the two types of leaves.

REFERENCES

Abel, T. P.: A water and carbon dioxide gas analyzer. Master's degree thesis in Electrical Engineering, Merrill Library, Utah State University, Logan, 1997.

Baker, J., L. Allen, and K. Boote: Growth and yield responses of rice to carbon dioxide concentration. J. of Agri. Sci. *115*, 313-320 (1990).

Beerling, D. J. and W. G. Chaloner: Stomatal density responses of Egyptian Olea europaea L. leaves to CO_2 changes since 1327 BC. Anna. of Bot. *71*, 431-435 (1993).

Beerling, D. J. and F. I. Woodward: Stomatal responses of variegated leaves to CO_2 enrichment. Anna. of Bot. *75*, 507-511 (1995).

Berlyn, G. P. and J. P. Miksche : Botanical Microtechnique and Cytochemistry, p. 97. The Iowa State University Press, Ames, 1976.

Berryman, C. A., D. Eamus, and G. A. Duff: Stomatal responses to a range of variables in two tropical tree species grown with CO_2 enrichment. J. of Exp. Bot. *45* (274), 539-546 (1994).

Bingham, G. E., F. B. Salisbury, W. F. Campbell, J. G. Carman, D. L. Bubenheim, B. Yendler, V. N. Sytchev, Y. A. Berkovitch, M. A. Levinskikh, and I. G. Podolsky: The spacelab-Mir-1 "Greenhouse-2" experiment. Adv. in Spa. Res. *18* (4/5), 225-232 (1996).

Bugbee, B., B. Spanarkel, S. Johnson, S., O. Monje, and G. Koener: CO_2 crop growth enhancement and toxicity in wheat and rice. Adv. in Spa. Res. *14* (11), 257-267 (1994).

Clark, G.: Staining Procedures. 4th edition, pp. 325-326. William & Wilkins, Baltimore/London, 1981.

Craufurd, P.Q. and P. M. Cartwright: Effect of photoperiod and chlormequat on apical development and growth in spring wheat (*Triticum aestivum*) cultivar. Anna. of Bot. *63*, 515-525 (1989).

Ellis, R. H., R. J. Summerfield, E. H. Roberts, and J. P. Cooper: Environmental control of flowering in barley (Hordeum Vulgare). III. Analysis of potential vernalization responses, and methods of screening gerplasm for sensitivity to photoperiod and temperature. Anna. of Bot. *63* (6), 687-704 (1989).

Enoch, H.Z. and N. Zieslin: Growth and development of plants in response to carbon-dioxide concentrations. Appl. Agri. Res. *3* (5), 248-256 (1988) .

Estiarte, M., J. Penuela, B. A. Kimball, S. B. Idso, R. L. LaMorte, P. J. Pinter, P. J. Pinter Jr., G. W. Wall, and R. L. Garcia: Elevated CO_2 effects on stomatal density of wheat and sour orange trees. J. of Exp. Bot. *45* (280), 1665-1668 (1994).

Farina, E., C. Dalla-Guda, and E. Scored: Flowering and morphogenic responses of new Aster hybrids to photoperiod. Physio.-Planta. *91* (94), 312-16 (1994).

Ferris, R., and G. Taylor: Stomatal characteristics of four native herbs following exposure to elevated CO_2. Anna. of Bot. *73* (4), 447-453 (1994).

Gay, A. P. and B. Hauck: Acclimation of Lolium temulentum to enhanced carbon-dioxide concentration. J. of Exp. Bot. *45* (277), 1133-1141 (1994).

Geertsen, V.: Effect of photoperiod and temperature on the growth and flower production

of Heliconia psittacorum 'Tay'. Acta-Hortic. *252*, 117-122 (1989).

Gifford, R.: Growth pattern, carbon-dioxide exchange, and dry weight distribution in wheat growing under differing photosynthetic environments. Austr. J. of Plant Physio. *4*, 99-110 (1977).

Hart, J.W.: Light and Plant Growth, p. 173. Unwin Hyman Ltd., London (1988).

Havelka, U. D., R. C. Ackerson, M. G. Boyle, and V. A. Wittenbach: CO_2 enrichment effects on soybean physiology. I. Effects of long-term CO_2 exposure. Crop Sci. *24*, 1146-1154 (1984).

Imai, K., D. Coleman, and T. Yanagisa: Increase of atmospheric partial pressure of carbon-dioxide and growth and yield of rice. Jap. J. of Crop Sci. *54*, 413-418 (1985).

Jensen, W. A: Botanical Histochemistry, p. 205. W. H. Freeman and Company, San Francisco and London, 1962.

Kenworthy, W. J., A. H. Brown, and G. A. Thibou: Variation in flowering response to photoperiod in perennial Glycine species. Crop Sci. *29* (3), May/June, 678-682 (1989).

Kimball, B.A., and S. B. Idso: Increasing atmospheric CO_2: Effects on crop yield, water use, and climate. Agri. Water Manag. *7*, 55-72 (1983).

Knapp, A., K. M. Cocker, E. P. Hamerlynck, and C. E. Owensby: Effects of elevated CO_2 on stomatal density and distribution in a C4 grass and a C3 forb under field conditions. Anna. of Bot. *72* (6), 595-599 (1994).

Koerner, C: Does global increase of CO_2 alter stomatal density? Flora. *181*, 253-25 (1988).

Lawlor, D.W. and R. A. C. Mitchell: The effects of increasing CO_2 on crop photosynthesis and productivity: A review of field studies. Plant, Cell, and Environ. *14*, 807-818 (1991).

Malone, S. R., S. Herman, H. Mayeux, B. Johnson, and W. H. Polley: Stomatal density and aperture length in four plant species grown across a subambient CO_2 gradient. Ameri. J. of Bot. *80* (12), 1413-1418 (1993).

Mashinsky, A. L., I Ivanova, T. Derendyaeva, G. S. Nechitailo, and F. B. Salisbury: From seed to seed experiment with wheat plants under space-flight conditions. Adv. in Spa. Res. *14* (11), 13-19 (1994).

McDowell, E. M. and B. F. Trump: Histology fixatives suitable for diagnostic light and electromicroscopy. Arch. Pathol. Med.. *100*, 405-419 (1976).

Muchow, R. C. and T. R. Sinclair: Epidermal conductance, stomatal size among genotypes of Sorghum bicolor (L.) Moench. Plant, Cell, Environ. *12* (4), 425-431 (1987).

Norman, W. and T. Stubbs: Plants, Agriculture & Human Society. p. 104. W. A. Benjamin, Inc., Menlo Park, 1978.

Olson, R.L., M. W. Oleson, and T. J. Slavin: CELSS for advanced manned mission. Extraterrestrial Crop Production, Proceedings of the Symposium. pp. 275-386 (1988).

Oosterhuis, D. M. and P. M. Cartwright: Spike differentiation and floret survival in semidwarf spring wheat as affected by water and photoperiod. Crop Sci. *23*, July/Aug., 711-17 (1983).

Pearson, M., W. J. Davies, and T. A. Mansfield: Asymmetric response of adaxial and abaxial stomata to elevated CO_2 impacts on the control of gas exchange by leaves. Plants, Cells, Environ. *18* (8), 837-843 (1995).

Penuelas, J. and R. Matamala: Changes in N and S leaf content., stomatal density and specific leaf area of 14 plant species during the last three centuries of CO_2 increase. J. of Exp. Bot. *41*, 1119-1124 (1990).

Pinthus, M. J: Triticum. In: Halevy, A. H. (eds.): Handbook of Flowering, Volume IV, p. 431. CRC Press, Boca Raton, 1985.

Radoglou, K. M. and P. G. Jarvis: The effects of CO_2 enrichment and nutrient supply on growth morphology and anatomy of Phaseolus vulgaris L. Seedlings. Anna. of Bot. *70* (3), 245-256 (1992).

Rawson, H.M. and R. A. Richards: Effects of high temperature and photoperiod on floral development in wheat isolines differing in vernalization and photoperiod genes. Field Crop Res. *32*, 181-192 (1993).

Rowland-Bamford, A. J., C. Nordenbrock, J. T. Baker, G. Bowes, and L. H. J. Allen: Changes in stomatal density in rice grown under various CO_2 regimes with natural solar irradiance. Environ. and Exp. Bot. *30*, No.2, 175-180 (1990).

Ryle, G. J. A. and J. Stanley: Effect of elevated CO_2 on stomatal size and distribution in perennial ryegrass. Anna. of Bot. *69*, 1992 (1992).

Salisbury, F. B.: The Flowering Process, p. 210. Pergamon Press, Oxford 1963.

Salisbury, F. B., G. E. Bingham, W. F. Campbell, J. G. Carman, D. L. Bubenhein, B. Yendler,

and G. Johns: Growing super-dwarf wheat in Svet on Mir. Life Support & Biosph. Sci. *2*, 31-39 (1995).

Salisbury, F. B. and B. Bugbee: Plant productivity in controlled environments. HortScience *23* (2), April, 293-299 (1988).

Wanser, H. M.: Photoperiodism of wheat, a determining factor in acclimatization. Science. *56*, 313-315 (1922).

Woodward, F. I.: Stomatal numbers are sensitive to increases in CO_2 from pre-industrial levels. Nature. *327*, 617-618 (1987).

Woodward, F. I. and F. A. Bazzaz: The responses of stomatal density to CO_2 partial pressure. J. of Bot. *39*, 1771 (1988).

APPENDICES

Appendix I. Make-up McDowell fixative

Phosphate buffer:

Add 17 mL of 2.52 % sodium hydroxide (NaOH) to 83 mL of 2.26 % monobasic sodium phosphate (NaH_2PO_4), pH=7.2

McDowell fixative:

10.8 mL formaldehyde (37 % biological-grade) + 4 mL glutaraldehyde (25 % biological-grade) + 85.2 mL phosphate buffer = 100 mL McDowell fixative

Appendix II. Standard paraffin technique

Step 1: The tissue is killed and fixed in a chemical fluid designed to preserve cell structure.

Step 2: Water is removed from the cell by gradual replacement with alcohol -- Dehydration

Step 3: The tissue is infiltrated with paraffin and is then embedded in a block of the same material.

Step 4: The infiltrated and embedded tissue is then sectioned on a microtome, and the sections are affixed to slides.

Step 5: After the paraffin is removed, the tissue is stained.

Step 6: Finally, the tissue is covered with a cover slip held in place with a permanent mounting medium.

Fixation

Plant tissues were fixed in McDowell fixative for 24 h, using a vacuum pump to reduce pressure so that the tissue sinks to the bottom of the vial. The fixed tissue was stored in the same fixative in a 4 °C cool room.

Dehydration

5% alcohol → 10 % alcohol → 30 % alcohol

(2 h) (2 h) (2 h) ↓

50 mL H_2O, 40 mL 95 % alcohol, 10 mL TBA (Tertiary Butyl Alcohol) (3 h)

↓

30 mL H_2O, 50 mL 95 % alcohol, 20 mL TBA (3 h)

↓

15 mL H_2O, 50 mL 95 % alcohol, 35 mL TBA (3 h)

↓

45 mL 95 % alcohol, 55 mL TBA (3 h)

↓

25 mL absolute alcohol, 75 mL TBA (+Eosin) (20 min)

↓

25 mL absolute alcohol, 75 mL TBA (8 h)

↓

100 % TBA (8 h), three changes

Paraffin Infiltration and Embedding

Transfer the tissues to a vial → TBA + solid paraffin at 56 °C oven 6 h

↓

add more solid paraffin, 61 °C oven 4 h

↓

pour off 1/4 of the paraffin, add more solid paraffin 4 h

↓

pour off ½ of the paraffin, add more solid paraffin 4 h

↓

replace it with fresh molten paraffin overnight

↓

repeat the fresh molten paraffin two more times

↓

Embedding

Sectioning (Rotary Microtome)

chill the paraffin by placing it in a refrigerator, place the block in the microtome, set the microtome to 10 μm thickness, cut sections in desired direction of plant material.

Affixing sections to slides

cut the paraffin ribbon → cover the slide with Haupt's adhesive (containing gelatin and glycerine) → add 4 % formalin → floating the segments → slide warmer, add more 4 % formalin to extend the segments → drain the formalin → slide warmer (overnight)

Staining

xylene 20 min

↓

1:1 of xylene and absolute alcohol 5 min

↓

absolute alcohol 5 min

↓

95 % alcohol 5 min

↓

70 % alcohol 5 min

↓

50 % alcohol 5 min

↓

stain in safranin 24 h

↓

wash in double distilled water

↓

70 % acidified alcohol briefly (10 sec)

↓

95 % alcohol 5 min

↓

absolute alcohol 5 min

↓

counterstain with fast green 3 -- 5 s, wash in double distilled water

↓

clove oil + xylene 10 min, two changes

↓

xylene 15 min, three changes

↓

mount cover slip with balsam → slide warmer (overnight)