

RICE

Characterizing the Environmental Response of a Gibberellic Acid-Deficient Rice for Use as a Model Crop

Jonathan M. Frantz,* Derek Pinnock, Steve Klassen, and Bruce Bugbee

ABSTRACT

Rice (*Oryza sativa* L.) is a useful model crop plant. Rice was the first crop plant to have its complete genome sequenced. Unfortunately, even semidwarf rice cultivars are 60 to 90 cm tall, and large plant populations cannot be grown in the confined volumes of greenhouses and growth chambers. We recently identified an extremely short (20 cm tall) rice line, which is an ideal model for larger rice cultivars. We called this line 'Super Dwarf' rice. Here we report the response of Super Dwarf to temperature, photoperiod, photosynthetic photon flux (PPF), and factors that can affect time to head emergence. Vegetative biomass increased 6% per degree Celsius, with increasing temperature from 27 to 31°C. Seed yield decreased by 2% per degree Celsius rise in temperature, and as a result, harvest index decreased from 60 to 54%. The time to heading increased by 2 d for every hour above a 12-h photoperiod. Yield increased with increasing PPF up to the highest level tested at 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (12-h photoperiod; 77.8 $\text{mol m}^{-2} \text{d}^{-1}$). Yield efficiency (grams per mole of photons) increased to 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and then slightly decreased at 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Heading was delayed by addition of gibberellic acid 3 (GA₃) to the root zone but was hastened under mild N stress. Overall, short stature, high yield, high harvest index, and no extraordinary environmental requirements make Super Dwarf rice an excellent model plant for yield studies in controlled environments.

MODEL SYSTEMS are simplified versions of the world (Rosenblueth and Wiener, 1945), and model organisms have long been used to provide simpler versions of more complex systems. Many biochemical pathways and physiological mechanisms are identical between small and large organisms, so small organisms are commonly used to predict responses in large organisms. Small organisms lend themselves to studies in laboratories. A recent supplement to *The Scientist* (Bahls et al., 2003) reviewed the contributions of the eight most widely used, and most valuable, model organisms in biology: *Escherichia coli*, yeast (*Saccharomyces cerevisiae*), the fly (*Drosophila melanogaster*), two fish (*Tetraodon nigroviridis* and *Fugu rubripes*), the nematode (*Caenorhabditis elegans*), the mouse (*Mus musculus*), and the plant *Arabidopsis thaliana*.

During the past two decades, there has been a major effort to sequence the genomes of these model organ-

isms and other species. This work has culminated in the published genomes of the nematode (The *C. elegans* Sequencing Consortium, 1998), fruit fly (Adams et al., 2000), the common mustard weed (The *Arabidopsis* Genome Initiative, 2000), and humans (*Homo sapiens*) (International Human Genome Sequencing Consortium, 2001) among a few others. The National Institutes of Health (NIH) has provided links to research using these model organisms (www.nih.gov/science/models/; verified 29 Apr. 2004). Rice is the first crop plant to have a complete genome sequence available for study (Yu et al., 2002; Goff et al., 2002). Sequencing these genomes are remarkable scientific achievements, but the next challenge is to relate form with function (Fields et al., 1999).

The study of functional genomics will benefit from model crops that facilitate laboratory studies. Small organisms lend themselves to study in small spaces. *Arabidopsis thaliana* is a prime example. Researchers have capitalized on its compact size (10 cm tall), rapid life cycle, and massive numbers of seeds produced from single plants (Walbot, 2000). Of course, the fact that it also has a relatively small genome makes *A. thaliana* ideal for genetic studies. *Arabidopsis thaliana* is an important model organism for the plant kingdom because of its small size and genetic simplicity, but its weedy characteristics do not make it a good model for the major food crops of the world. Rice also has a small genome and is a primary source of food in the world diet. The agronomic characteristics of rice facilitate genetic studies of seed set, tillering, C partitioning, seed size, and yield. However, even full dwarf rice lines are over half a meter tall, which makes them difficult to use in controlled environments.

We started to search for short rice lines in 1989 by contacting rice breeders and researchers around the world. We screened over 100 promising lines in a greenhouse to find extremely dwarf rice. This search identified the dwarf, early maturing rice cultivars '29-Lu-1' and 'Ai-Nan-Tsao', which were sent to us by the International Rice Research Institute (IRRI). Although these cultivars have an exceptionally short vegetative phase (34 d to panicle emergence in a 12-h photoperiod), they are still 60 to 70 cm tall. From 1993 to 1995, we studied a rice line called 'Dominant Dwarf', which we obtained from Dr. Tze-Xuan Wang and Dr. N. Iwata at Kyushu University, Japan. Dominant Dwarf is only 30 cm tall, but it had an extremely slow development rate. It continued to tiller without heading until it had produced over 50 tillers per plant. It headed in 90 d and required 120 d

J.M. Frantz, USDA-ARS, ATRU, Univ. of Toledo, Mail Stop 604, 2801 W. Bancroft, Toledo, OH 43606-0604; D. Pinnock and B. Bugbee, Crop Physiol. Lab., Dep. of Plants, Soils, and Biometeorol., Utah State Univ., Logan, UT 84322-4820; and S. Klassen, U.S. Fish and Wildlife Serv., Ecol. Serv. Office, 315 S. Allen St., State College, PA 16801. Approved by Utah Agric. Exp. Stn. as Journal Paper no. 7546. Received 14 Oct. 2003. *Corresponding author (jonathan.frantz@utoledo.edu).

Published in *Agron. J.* 96:1172-1181 (2004).
© American Society of Agronomy
677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: CWF, cool white fluorescent; GA, gibberellic acid; HPS, high-pressure sodium; PPF, photosynthetic photon flux.

to maturity, even in a 12-h photoperiod. The yield of Dominant Dwarf was also very low with over 80% sterile tillers, small panicles, and few small grains. In June 1998, we wrote to Dr. Toshiro Kinoshita, Hokkaido University, Japan, requesting seed samples of extremely short rice lines from his rice germplasm collection. Dr. Kinoshita sent samples of 20 lines, one of which was significantly shorter than any of the previous lines we had evaluated (Fig. 1). This rice line is a mutant selection from the japonica-type 'Shiokari' (Kinoshita and Shibashi, 1982) and lacks 3 β -hydroxylase, the enzyme that catalyzes the conversion of gibberellic acid (GA) 20 to GA₁, the final step in GA synthesis (Honda et al., 1996). This mutation results in a height of only 20 cm at maturity. This extremely dwarf stature makes Super Dwarf ideally suited for studies in confined spaces, but the lack of active GA could interfere with germination, plant development, earliness, and tiller development. Characterizing the environmental response of this cultivar, therefore, is an important prerequisite for its use as a model crop plant.

We evaluated the growth and development of Super Dwarf rice in a range of environments. Our objective was to determine environmental factors that resulted in high yield, high harvest index, and a short life cycle. The results of these studies may be used to establish optimal baseline growth conditions for laboratory studies and predict the response to experimental conditions that deviate from optimum.

MATERIALS AND METHODS

Methods to germinate Super Dwarf rice have been described previously (Frantz and Bugbee, 2002). These studies indicated that high germination percentages and uniformity can be achieved if seeds are germinated anaerobically. Anaerobic conditions can be created by completely submerging seeds in unstirred tap water. In all trials, seeds were germinated anaerobically and, after about 10 d, transplanted to obtain uniform stands.

Carbon dioxide was elevated to 1200 $\mu\text{mol mol}^{-1}$ in all studies unless otherwise noted. Carbon dioxide was elevated to optimize growth and yield, to directly apply results to NASA space flight conditions, and to minimize temperature effects on photorespiration. Although elevated CO₂ increases yield, in previous studies in this lab, elevated CO₂ did not alter the development rate of rice (Bugbee et al., 1994). When tissue was analyzed, the tissue was weighed and dried at 80°C for 72 h. Dry biomass was subsequently weighed, ground, and sampled for analysis. Samples of 1.0 g were used for analysis of nutrients (ICP-ES analysis, Utah State University Plant and Soil Analysis Laboratory, Logan, UT), which measured total P, K, Ca, S, Mg, Fe, Cu, Mn, B, and Zn.

Temperature Sensitivity Studies

Temperature Study 1

Seedlings were transplanted into ten 3.8-L pots at a density of 100 plants m^{-2} in each of three reach-in growth chambers. Temperature treatments were maintained during the vegetative phase at 34/28, 32/26, and 30/24°C in the three growth chambers. Temperature of all treatments was decreased to 30/24°C at 3 d before heading to minimize possible heat-induced sterility (Mackill, 1981). Temperatures were further decreased to 28/22°C during grain fill to prolong the duration of grain fill and increase harvest index. Air temperatures were changed



Fig. 1. A comparison of rice cultivars 29-Lu-1 and Super Dwarf. 29-Lu-1, a semidwarf variety, is 80 cm tall; Super Dwarf is only 20 cm tall.

in a square-wave fashion from light and dark transitions and thus required only about 5 min to reach the set point. The PPF was 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ from cool white fluorescent (CWF) lamps, and the photoperiod was 12 h. Pots contained soilless media with a mixture of 1:1 (v/v) peat and perlite. Pots were irrigated three times daily with a nutrient solution (Peter's 20–10–20 with added chelated iron and sodium silicate; final concentrations: 7.0 mM N, 1.4 mM P, 2.0 mM K, 20 μM Fe-EDDHA, and 10 μM Si). At harvest, tissue was dried at 80°C for 72 h for determination of dry mass. Data from all 10 pots in each chamber were pooled to calculate means and standard errors for grain yield, vegetative biomass, and harvest index.

Temperature Study 2

A 10-chamber system was used in which rice canopies were grown until heading. The system was described by van Iersel and Bugbee (2000). Each chamber included a transparent acrylic chamber that was 0.5 by 0.4 by 0.9 m (length by width by height) and fully enclosed a hydroponic tub. Temperature treatments compared constant temperatures of 23, 26, 29, 32, and 35°C with day/night temperature cycling (square wave). The day/night temperature cycling treatments were 26/21, 27/23, 30/23, 32/26, and 35/29°C. Temperatures were measured with a shielded, aspirated, type-E (0.5 mm diam., 24 AWG) thermocouple and controlled to within $\pm 0.2^\circ\text{C}$ of set point by activating resistance heaters when the temperature fell below

the set point. Root zone temperature was also measured with a type-E thermocouple with silicon caulk on the tip to prevent corrosion in the nutrient solution. Root zone temperature was maintained at the daily mean of the chamber air by activating heaters wrapped around each hydroponic tub when the solution temperature dropped below the set point. Relative humidity was maintained between 60 and 85%. A PPF of $650 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($\pm 5\%$) was provided by water-filtered high-pressure sodium (HPS) lamps. The photoperiod was 16 h, for a daily integrated PPF of $37.4 \text{ mol photons m}^{-2} \text{ d}^{-1}$. Upon heading, the plants were harvested and separated into heads, remaining shoot biomass (leaves and tillers), and roots and then dried for 72 h at 80°C . The dried heads were used as an estimation of yield potential in the different temperature treatments. An ANOVA was performed to test for significant differences between treatments that had different day and night temperatures and constant temperatures by comparing the treatments with similar average daily temperatures (i.e., 26/21 vs. 23°C , 32/26 vs. 29°C , and 35/29 vs. 32°C). No significant difference was found, so in subsequent analyses, treatments were analyzed based on their mean daily temperatures. Regression analysis was performed using SigmaPlot (version 7.0, SPSS Inc., Chicago, IL).

Photoperiod Sensitivity Study

Ten 3.8-L pots were seeded at a density of 150 plants m^{-2} in each of three reach-in growth chambers. The mean PPF was $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ from CWF lamps. Each chamber had a different photoperiod: 12, 14, and 16 h. Temperature was $32/26^\circ\text{C}$ for the vegetative phase, $30/24^\circ\text{C}$ during heading, and $28/22^\circ\text{C}$ during grain fill for this and all subsequent trials unless otherwise noted. Data from all pots in each photoperiod were pooled and then divided by 10 to obtain mean harvest parameters for each photoperiod. Yield per day was calculated by dividing the grain yield by length of the life cycle. Yield efficiency, measured as grams per mole of photons, is a ratio of the dry grain biomass in grams per square meter per day, divided by the PPF in moles per square meter per day. Data from the 10 pots were pooled and used to calculate means for grain yield, yield per day, and yield efficiency. Linear regression was performed in SigmaPlot using data from all 10 pots at each treatment.

Photosynthetic Photon Flux Response Studies

High Photosynthetic Photon Flux Study

Four replications in each of three growth chambers were grown at a density of 250 plants m^{-2} in a recirculating hydroponic system under HPS lamps. Each chamber had a different PPF (at 600, 900, and $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$) in a 12-h photoperiod for a daily integrated PPF of 26, 39, and $78 \text{ mol m}^{-2} \text{ d}^{-1}$, respectively. The nutrient solution pH was maintained at 5.6. Heads were classified as fertile and infertile. Heads were considered fertile if about two-thirds of the seeds were filled. Data from the four plots were pooled to calculate means and standard errors for vegetative and grain yield, harvest index, and number of fertile heads. Values from each plot were used to generate regression lines for each parameter using SigmaPlot.

Low Photosynthetic Photon Flux Study

Ten 3.8-L pots were seeded at a density of 250 plants m^{-2} in a single growth chamber under CWF light. Four pots were shaded to maintain a mean PPF of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$, four

were shaded to maintain a PPF of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, and two were maintained at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Development Rate and Life Cycle Studies

Gibberellic Acid 3 Study

Since early heading and a short life cycle are important in a model crop, in addition to photoperiod studies on the life cycle, we investigated the influence of exogenous addition of GA_3 to the root zone. Individual seedlings were transplanted into 2-L, aerated hydroponic containers in a greenhouse (16-h photoperiod). The mean PPF was $600 \mu\text{mol m}^{-2} \text{s}^{-1}$ from sunlight supplemented with HPS lamps. Carbon dioxide was not elevated in this study. Gibberellic acid 3 was added at weekly intervals to the root zone to obtain the following five GA_3 concentrations: 0.0, 0.015, 0.15, 1.5, and $15 \mu\text{M}$ GA_3 . Each treatment was replicated three times. Plants were grown until heading and harvested. Data were used to generate a regression equation based on a logarithmic rise from a constant value (y offset) using SigmaPlot.

Nitrogen Study

Mild N stress can shorten the duration of the vegetative stage. We investigated the effect of N stress on days to heading. Rice seedlings were direct-seeded and grown in twelve 0.2-m^2 hydroponic containers at a density of 200 plants m^{-2} . The N concentration was maintained at 0.07, 0.7, or 7.0 mM N. Each of the four replicate canopies was grown to heading. Cation concentration was kept constant, and anions were balanced with a combination of Cl^- and SO_4^{2-} salts. Canopies were grown under a 12-h photoperiod, a PPF of $600 \mu\text{mol m}^{-2} \text{s}^{-1}$ from HPS lamps, and in $32/28^\circ\text{C}$ day/night temperature.

Lamp Type Study: Trial 1

Light quality can alter phytochrome status and the development rate in some plants. Super Dwarf rice was grown at four plant densities (50, 100, 200, and 600 plants m^{-2}) in each of two growth chambers, one with HPS lamps and one with metal halide lamps. Individual plots (0.2 m^2) were separated with polished aluminum to minimize side lighting and guard row effects. Environmental conditions were $32/28^\circ\text{C}$ day/night, PPF of $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$, photoperiod of 12 h, relative humidity of 65%, and recirculating hydroponic culture.

Lamp Type Study: Trial 2

Super Dwarf rice was grown at four plant densities (50, 100, 200, and 600 plants m^{-2}) in each of three growth chambers. Individual plots were separated with polished aluminum. The environmental conditions were $32/28^\circ\text{C}$ day/night, preanthesis; $30/24^\circ\text{C}$ day/night, postanthesis; PPF of $1100 \mu\text{mol m}^{-2} \text{s}^{-1}$; photoperiod of 12 h; relative humidity of 65%; and recirculating hydroponic culture.

Drought Stress Study

Water stress can sometimes induce early heading. Super Dwarf rice was grown in hydroponic and soilless media culture to determine if slight water stress, caused by the soilless media, could cause earlier heading. Plants were grown at two plant densities (50 and 200 plants m^{-2}) in each of two reach-in growth chambers. Each chamber had four 30-L tubs; two of the tubs were recirculating hydroponic culture, and the other two were soilless media made of a 1:1 ratio of peat–perlite mix. The soilless media treatments were watered once a day by drip irrigation. Environmental conditions were $32/26^\circ\text{C}$ day/

night, preanthesis; 28/22°C day/night, postanthesis; PPF of 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$; photoperiod of 12 h; relative humidity of 65%; and recirculating hydroponic culture or soilless media.

RESULTS AND DISCUSSION

Temperature Sensitivity

Temperature strongly influenced development rate. Days to heading increased by 10 d at 30/24°C but was similar at both 32/26 and 34/28°C (Fig. 2). Color photographs of the plant response at each of the three temperature treatments are available at www.usu.edu/cpl/research_dwarf_rice6.htm (verified 29 Apr. 2004). Temperature effects on development rate are similar to previous studies with Ai-Nan-Tsao and 29-Lu-1 where days to heading was reduced by 15% as temperature increased from 29/22 to 35/28°C (Bugbee et al., 1994).

Models have been developed for most crops to predict leaf emergence rate or days to flower based on accumulated degree days (e.g., Volk and Bugbee, 1991). In grasses, the rate of leaf and tiller appearance is strongly correlated with growing degree days. Other factors, such as light and soil moisture, usually play less significant roles (Van Esbroeck et al., 1997; Bahmani et al., 2000). Rice is similar to other grasses in that the higher the temperature, generally, the faster the development rate (Yin and Kropff, 1996). The specific rate of development typically depends on the cultivar.

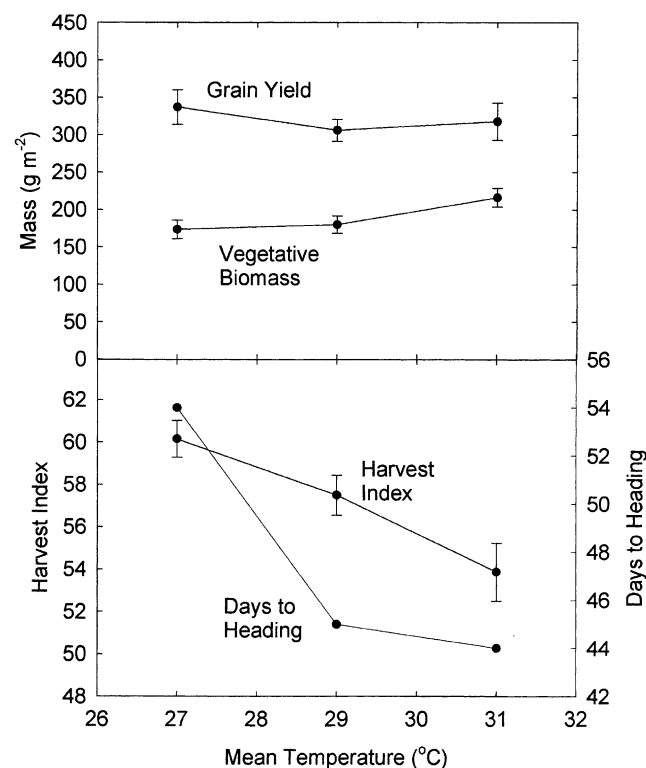


Fig. 2. Effect of temperature on yield and harvest index. Grain yield was similar across temperatures, but vegetative biomass increased as temperature increased ($P = 0.02$). As a consequence, harvest index declined significantly ($P = 0.0002$). Days to heading were longest at 27°C but were about 45 d at 29 and 31°C. Error bars indicate standard error of means ($n = 12$ for each point). There are no error bars on days to heading because all replicates headed on the same day.

Temperature had a small effect on growth rate. Vegetative biomass (leaves, stems, and sterile heads) increased only 6% per degree Celsius in the first trial (Fig. 2). There was a slight decrease in yield at 32/26°C, and therefore, the harvest index decreased from 60 to 54% from 30/24 to 34/28°C. It is not surprising that seed yields were similar since the temperature was only different during the vegetative phase. However, the higher biomass that was formed at 34/28°C did not produce correspondingly higher yields.

In the second temperature trial, there was no difference in days to heading, number of heads per square meter, and harvest index between the treatments that received an alternating day/night temperature vs. a constant temperature (data not shown), so analyses were based on mean daily temperature. The second trial showed a clear temperature optimum during the vegetative phase. At 29°C, the number of days to heading decreased to its lowest value while number of heads per square meter and mass of those heads were maximized (Fig. 3). This trial examined a larger temperature range than the initial trial and confirmed that time to heading was significantly delayed with temperatures lower than 29°C (average daily temperature). It also confirmed that days to

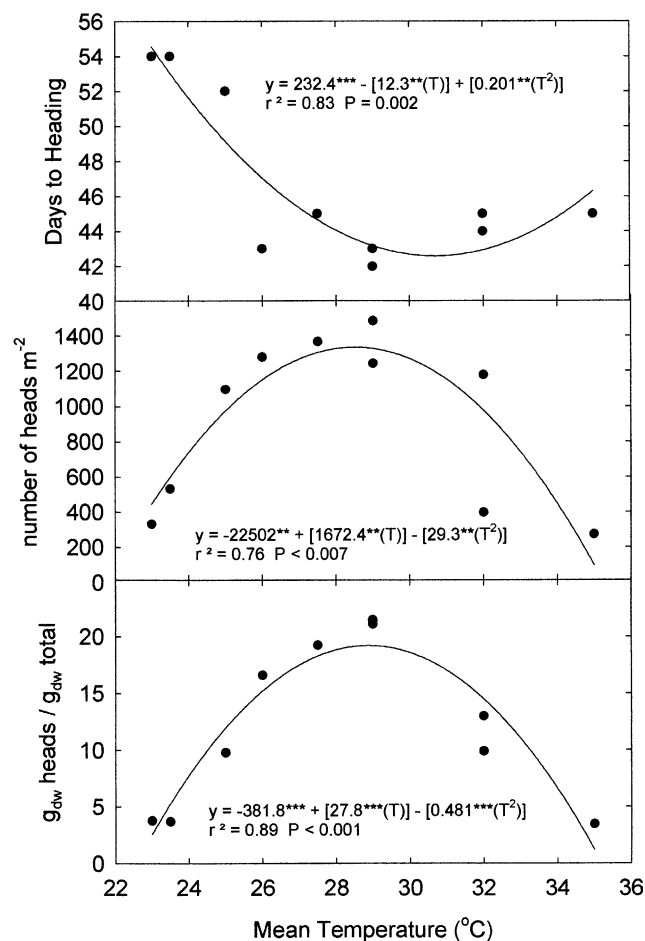


Fig. 3. Effect of temperature on days to heading, number of heads per square meter, and harvest index, as measured using the units grams dry weight (g_{dw}) of heads (seed plus rachis) and total biomass. The optimum temperature for time to heading and number of heads per square meter was about 29°C.

heading was not further decreased by temperatures higher than 29°C. In contrast to the first temperature trial, as temperatures increased from 29 to 35°C, the number of tillers and head number decreased.

Seed set in rice is extremely sensitive to high temperatures during heading (Yoshida, 1981). In a study screening 17 rice cultivars for CO₂ and temperature sensitivity, Ziska et al. (1996) found that grain yield at 37/29°C was less than 1% of the control treatment of 29/21°C due to a lack of adequate pollination. Mackill et al. (1982) found that the reduced yield was a result of poor pollen shedding as well as inadequate pollen growth in temperatures above about 34°C. The daytime temperature at which sterility results is about 32 to 38°C, depending on the cultivar. The temperature sensitivity for pollination of Super Dwarf is not yet well characterized.

Mackill (1981) found that the detrimental effects of temperature on seed set were greater in growth chambers when the temperature increased rapidly (minutes) after the start of the light period. Rice pollination occurs in the early morning, and high-temperature-induced sterility can be significantly reduced if the temperature increases slowly after the lights come on (Mackill, 1981). The possible advantage of temperature ramping on seed set in other crops has not been well studied.

Phasic temperature control is an underutilized factor in achieving high yield and high harvest index in controlled environments. Phasic temperature control is useful because the optimum temperature decreases over the life cycle. Temperatures above 28°C during vegetative growth reduces the days to heading and shortens the life cycle. Temperatures cooler than 28°C during grain fill increase its duration and increase seed size (Tashiro and Wardlaw, 1989). Tashiro and Wardlaw (1989) reported that rice was less sensitive during the grain-filling phase to elevated temperature during grain fill than wheat (*Triticum aestivum* L.) because it is better able to compensate for shorter duration of grain fill by increased rate of dry matter accumulation. Super-optimum temperature during grain filling can reduce seed mass because of a lower rate of starch accumulation (Bhullar and Jenner, 1986). Our studies did not examine specific optimal temperatures during flowering, pollination, and grain-fill stages. Additional studies are necessary to optimize phasic temperatures for each growth stage.

Grasses form tillers during vegetative growth. This is an advantage in the field where a variable number of tillers allows the plant to adapt to variable environmental conditions. Super Dwarf, however, has excessive tillering, which may be due to the lack of apical dominance caused by a lack of active GA. Additionally, elevated CO₂ typically increases tillering. At the relatively low plant density used in this study (100 plants m⁻²), many late tillers were formed, which resulted in 25 to 30% sterile heads.

Photoperiod Sensitivity

Rice is a short-day plant (Vergara and Chang, 1985), but rice cultivars differ in photoperiod sensitivity. Some rice cultivars (e.g., 'Fortuna', 'Habiganj', and 'Pate Blanc

MN3') do not respond to an increase in photoperiod from 10 to 16 h (Vergara and Chang, 1985). Super Dwarf appears to be a facultative short-day plant because days to heading increased by 1 d per hour increase in photoperiod (Fig. 4), but heading occurred in all photoperiods.

The daily PPF decreases with shorter photoperiods. Daily PPF is a strong determinant of yield, but production efficiency (grams per mole of photons) typically decreases with increasing PPF (Bugbee and Salisbury, 1988). As expected, yield increased ($r^2 = 0.79$) but yield efficiency decreased ($r^2 = 0.75$) with increasing PPF in the longer photoperiods (Fig. 4). Due to the delay in time to heading, yield per day was only slightly improved.

It should be possible to induce early flowering in short-day plants with short photoperiods and then switch to longer photoperiods to increase daily PPF and thus increase mass per seed and harvest index. However, Volk and Mitchell (1995) reported a decrease in harvest index with higher daily PPF from longer photoperiods due to excessive tillering and an increase in sterile tillers in the rice cultivar Ai-Nan-Tsao. Furthermore, no increase in grain yield was seen with photoperiods longer than 12 h during the grain-filling stage. It is interesting to note that time to panicle emergence for Ai-Nan-Tsao was almost twice as long (60 d) in the Volk and Mitchell (1995) studies than in similar studies with this cultivar in our laboratory (34 d; Bugbee et al., 1994). In a later study, Goldman and Mitchell (1999) had the highest yield and harvest index with Ai-Nan-Tsao in a constant 12-h photoperiod.

It is apparent that several factors in addition to photoperiod affect the length of the life cycle in rice. The photoperiod-sensitive phase occurs during vegetative growth and typically varies from 10 to 35 d. This is the development phase in which the plant responds to daylength and floral initiation begins (Vergara and Chang, 1985). Temperatures above 28°C can shorten the photoperiod-sensitive stage, but we did not observe a significant decrease in days to heading when the temperature was increased above 29°C. It is not known if GA has an effect on the photoperiod-sensitive stage through its role in apical dominance.

Photosynthetic Photon Flux Response

Both vegetative biomass and grain yield increased almost linearly with increasing PPF up to the highest PPF level of 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (90% of the intensity of summer sunlight at solar noon). Harvest index remained similar at about 50% for all PPF levels (Fig. 5). Heads per square meter increased with increasing PPF. Color photographs of the plant response at each of the three PPF levels are available at www.usu.edu/cpl/research_dwarf_rice5.htm (verified 29 Apr. 2004). Vertical-leafed crops like rice and wheat can utilize surprisingly high PPF levels, particularly in controlled environments with diffuse light (Bugbee and Salisbury, 1988). Crops with horizontal leaves, like potato (*Solanum tuberosum* L.) and soybean [*Glycine max* (L.) Merr.], light saturate at much lower PPF levels than grasses.

At the lowest PPF (200 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 12-h photo-

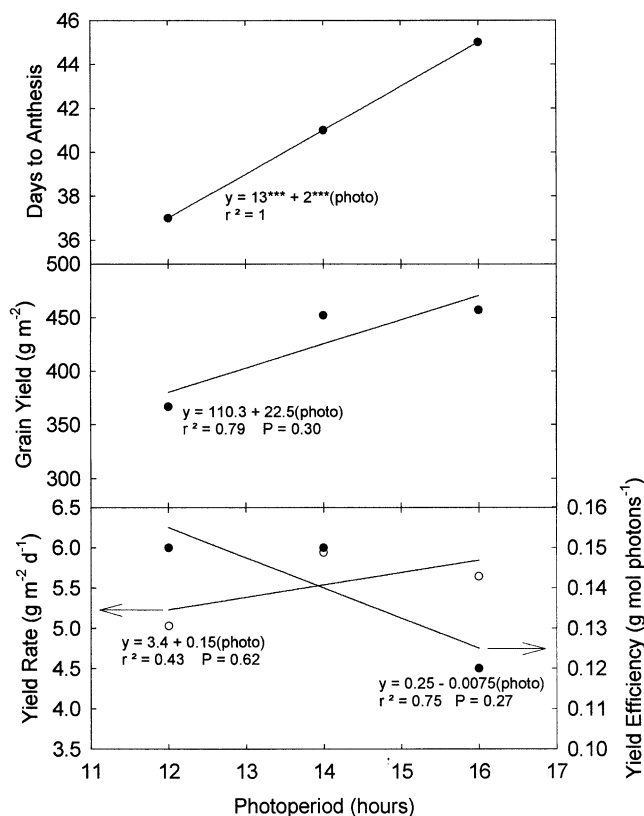


Fig. 4. Effect of photoperiod on development and yield. Increasing the photoperiod increased days to heading by 1 d for each additional hour ($P < 0.0001$). No other harvest parameters were statistically significant at the $P = 0.05$ level. Data from 10 pots were pooled and then divided by 10, and yield was calculated on a per square meter of ground area basis.

period; $8.6 \text{ mol m}^{-2} \text{ d}^{-1}$), plants produced only a few small heads containing less than 10 seeds per head. Tiller number was reduced, and canopy closure was never obtained. Field studies indicate a direct relationship between the amount of shade and the decrease in yield up to 50% shade (Venkateswarlu, 1977). These results are similar to those for wheat, except wheat can utilize continuous light (24 h) and thus gets the same daily integrated PPF with only half of the instantaneous PPF (Bugbee and Salisbury, 1988).

Development Rate and Life Cycle Studies

Days to panicle emergence in Super Dwarf ranged from 35 to 54 d, and it is apparent that development rate is affected by factors in addition to photoperiod. Root zone additions of GA promote early flowering in some plants. Increased GA concentrations caused earlier flowering in sorghum [*Sorghum bicolor* (L.) Moench] (Beall et al., 1991; Foster et al., 1997). Furthermore, exogenous applications of GA or overexpression of GA synthesis proteins in corn (*Zea mays* L.) and sorghum reduced tillering (Rood, 1985; Beall et al., 1991; Isbell and Morgan, 1982). Isbell and Morgan (1982) suggested that low concentrations of GA could be used to prevent tiller development without excessive shoot elongation.

The lack of active GA in Super Dwarf rice may increase tillering and delay heading. Addition of GA_3 to

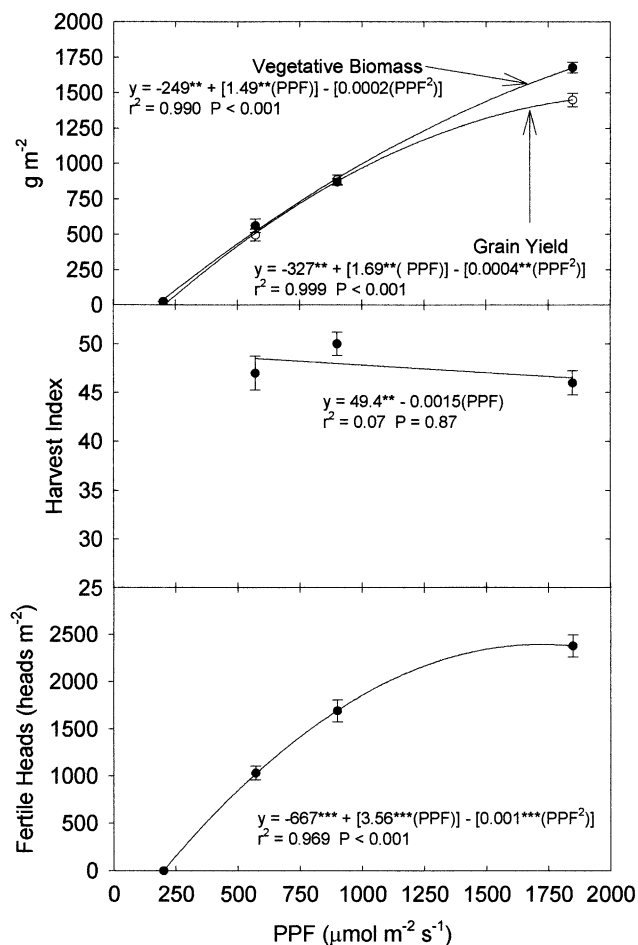


Fig. 5. The effect of photosynthetic photon flux (PPF) on yield. Both grain yield (open circles, $r^2 = 0.986$) and vegetative biomass (including roots, closed circles, $r^2 = 0.990$) increased with higher PPF, so harvest index remained relatively constant ($r^2 = 0.07$) at slightly below 50%. Increase in yield was likely due to higher number of fertile heads at higher PPF ($r^2 = 0.969$). No fertile heads were observed at PPF of $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ or less. Error bars indicate standard error of means ($n = 4$ for each point).

hydroponic solutions slightly decreased the number of tillers at very high application rates ($15 \mu\text{M}$) but had no effect at lower concentrations. Unfortunately, high application rates increased the days to heading while low rates had no effect (Fig. 6). As expected, adding GA_3 to the nutrient solution increased height. At the highest application rates, the plants were 60 cm tall.

Mild N stress can promote early flowering. Stress can either accelerate or delay flowering (Ali and Lovatt, 1995; Adams et al., 1998). The physiological mechanism of some flowering-inducing stress effects can be traced to phytochrome states (Adams et al., 1998) and hormonal status (Kinet et al., 1978; Yokoyama et al., 2000). This may be the result of an ecological push to reproduce when the environment becomes less favorable or in response to a seasonal change as in low-temperature-induced flowering (Ali and Lovatt, 1995). Excess N may result in either no effect (Schultheis and Dufault, 1994) or in a delay of duration, magnitude, and time to flowering (Spurway and Thomas, 2001). Others have reported decreased time to flowering under N deficiency

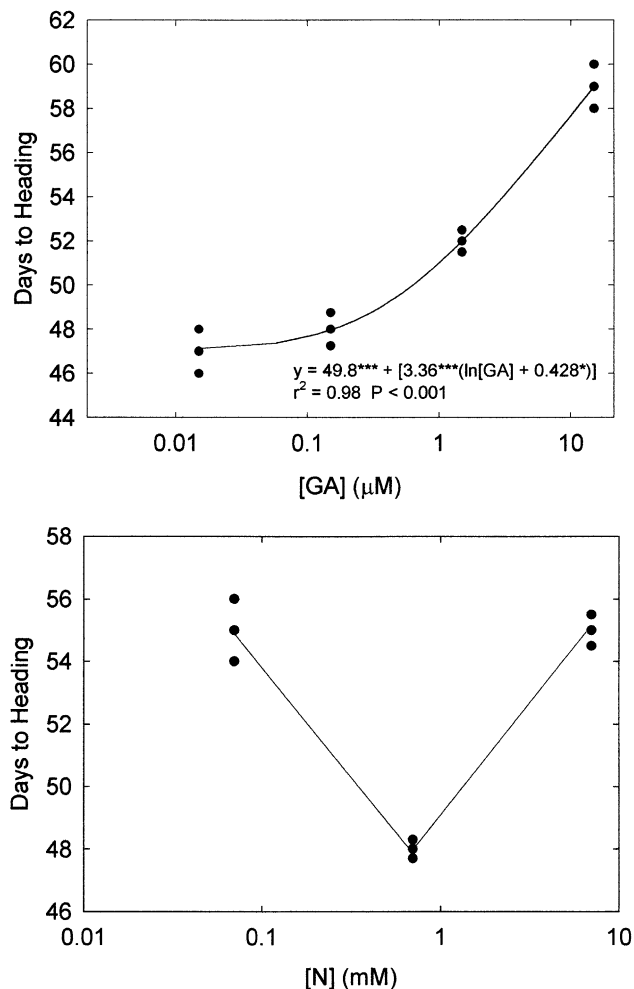


Fig. 6. The effect of (top graph) gibberellic acid 3 (GA₃) added to the root zone on days to heading in Super Dwarf rice and (bottom graph) N concentration in hydroponic solution on days to heading. Without GA₃, Super Dwarf headed in 47 d, but the rate of development decreased with additional GA₃. Super Dwarf headed in 48 d under intermediate N stress but required longer time at both high and low N stress.

(Xu et al., 2001; Kostenyuk et al., 1999). The response to N stress may depend on species. Kaczperski et al. (1996) reported the fastest time to flower in intermediate N treatments. Our results suggest that intermediate N stress in Super Dwarf rice can reduce time to flow-

ering, with the shortest days to heading in the 0.7 mM treatment (48 d) and delayed heading in both the 7 mM N and 0.07 mM N treatments (54 d). However, all treatments headed later than the 45-d mean in most other studies.

Water stress can also shorten the duration of the vegetative phase. However, the plants in the hydroponic treatments headed 1 d earlier than the plants in the soilless media. Yield, harvest index, and mass per seed were not altered by the root zone treatment. Yield parameters were similar at both plant densities. Additional details are available at www.usu.edu/cpl/onepager.pdf (verified 29 Apr. 2004).

Radiation quality can alter phytochrome status and thus alter the duration of the vegetative phase; however, the duration of the life cycle was not affected by radiation quality from metal halide or HPS lamps. Yield parameters were not statistically different under either metal halide or HPS lamps. There was a tendency for grain yield and harvest index to decrease at the highest planting density (600 plants m⁻²). Additional details and seed are available at www.usu.edu/cpl/one-pagerlamps.pdf (verified 29 Apr. 2004) and www.usu.edu/cpl/research_dwarf_rice7.htm (verified 29 Apr. 2004).

Nutritional Responses

Leaf-tip necrosis was observed in all trials. Nutritional analysis indicated slightly elevated levels of Cu in the leaf tips but adequate levels in the whole leaf (Table 1). Elevated tissue levels of Mn were observed in some trials, but rice tolerates a wide range of both Mn and Zn before toxicity symptoms appear (Wells et al., 1993). Visual symptoms appear similar to those reported for Fe toxicity by Yoshida (1981). Rice has a narrow range of Fe requirements, with more than 70 mg kg⁻¹ required for adequate growth and concentrations of 300 mg kg⁻¹ associated with toxicity symptoms (Yoshida, 1981). However, Fe concentrations were 110 to 115 mg kg⁻¹, which is well within the acceptable range. Phosphorus was lower in the leaf tip than in the leaf blade and was adequate based on recommendations from Wells et al. (1993) but was slightly below adequate based on Yoshida (1981). Potassium, Ca, Mg, Mn, B, and Zn differed significantly between the leaf tip and the leaf blade but were within the normal range for adequate growth.

Table 1. Elemental analysis of 'Super Dwarf' rice leaf tips and blades. Samples were measured by ICP-ES analysis at the Utah State University Plant and Soil Analysis Laboratory. There were significant differences between the concentrations of some nutrients in the leaf and leaf tip, but values of each remained within the range for all but Cu concentrations.†

Tissue type and recommendations	P	K	Ca	S	Mg	Fe	Cu	Mn	B	Zn
	g kg ⁻¹					mg kg ⁻¹				
Leaf	0.45	4.73	1.13	0.44	0.48	115.6	16.6	62.8	14.3	51.2
Leaf tip	0.29	2.49	3.05	0.51	1.20	111.2	35.0	150.0	47.3	17.0
Recom. from Yoshida (1981)	>0.4	>1.0	>0.15	>0.16 veg.‡ >0.06 repr.§	>0.4	70 to 300	6 to 30	20 to 7000	3.5 to 100	20 to 1500
Recom. from Wells et al. (1993)	>0.1	>1.0	N/A¶	>0.25 veg. >0.1 repr.	N/A	N/A	N/A	20 to 2500	N/A	15 to 1500

† Values are based on the mean of three samples for each biomass type.

‡ "veg." denotes recommendations during vegetative growth.

§ "repr." denotes recommendations during reproductive growth.

¶ N/A indicates that values were not available for those nutrients.

Silicon is generally considered to be a particularly beneficial element in rice because it provides increased insect and disease resistance and helps plants regulate excessive uptake of trace elements (Yoshida, 1981). Ma and Takahashi (1990) found that addition of 0.2 mM silica to nutrient solutions increased rice dry mass, photosynthesis, and grain yield and reduced the incidence of pathogen attack. Calcium uptake of rice can decrease with the addition of silica (Ma and Takahashi, 1993). Yoshida (1981) stated that the level of silica in the plant at which yield is improved is much higher in the field than in hydroponic culture. In other studies in our lab, increasing silica in the nutrient solutions delayed the onset and extent of tip necrosis but did not eliminate it (unpublished data, 2002). The necrotic leaf-tip symptoms were different from those caused by elevated ethylene (50 ppb or 50 nmol mol⁻¹; Klassen and Bugbee, 2002). Elevated ethylene caused dark brown to black spots while leaf-tip symptoms in these studies were uniformly light brown to tan.

Elevated Carbon Dioxide

Most of our studies were done at elevated CO₂, usually at 1200 μmol mol⁻¹. Elevated CO₂ increases tillering (Grotenhuis and Bugbee, 1997) and improves canopy quantum yield (Monje and Bugbee, 1998) and total yield in wheat (Reuveni and Bugbee, 1997). Most, if not all, other C₃ plant species have increased productivity in elevated CO₂. As a result of decreased photorespiration, an increase in the temperature optimum of about 2 to 3°C is predicted by photosynthesis models of C₃ species grown under elevated CO₂ (Long, 1991; Brooks and Farquar, 1985). Studies in rice with elevated CO₂ indicate an enhancement in grain yield likely due to increased grain number and mass per seed (Yoshida, 1973). Baker et al. (1990) reported faster development of rice with increased CO₂, but this may have been caused by an increased canopy temperature, which was caused by stomatal closure and decreased transpiration rather than a direct effect of elevated CO₂. Previous studies in this lab found that the days to panicle emergence and the length of the life cycle were identical in ambient and elevated CO₂ (340 and 680 μmol mol⁻¹; Bugbee et al., 1994). No recent detailed data are available on the influence of CO₂ and the development rate of rice, but two recent studies on common bean (*Phaseolus vulgaris* L.) (Prasad et al., 2002) and peanut (*Arachis hypogaea* L.) (Prasad et al., 2003) in elevated and non-elevated CO₂ indicate that CO₂ has no effect on time to flowering or harvest. Only one of the current studies was performed in ambient CO₂ (GA₃ addition), but all of the developmental data should apply to ambient CO₂ environments.

Tillering

Super Dwarf rice tillers excessively, probably because of the lack of GA and the subsequent lack of apical dominance. We studied a wide range of plant densities

from 50 to 600 plants m⁻². Higher plant densities decreased tillers per plant but increased total tillers and heads per area due to the additional plants. Because of these trade-offs, there were no consistent increases in yield, harvest index, height, or tiller appearance with increasing plant density.

Application to Food Production in Controlled Environments

In addition to its use as a model crop plant, Super Dwarf rice is directly useful to NASA for studies of food production on the International Space Station where volume is extremely limited. Efficient food production in the microgravity environment of space, and on planetary surfaces, is a prerequisite to the development of a bioregenerative life support system for long-term manned space travel.

CONCLUSIONS

Baseline growth characteristics are important to establish for model crops. The unique biochemistry of Super Dwarf rice results in cultural considerations that differ from more common and taller rice cultivars, especially in stand establishment. Days to panicle emergence was the most variable developmental parameter. Days to panicle emergence ranged from 35 to 54 d, but heading occurred on about Day 45 in most studies.

Applying GA to root zones did not shorten time to heading and significantly increased plant height. Photoperiod, N, low temperature, low light, and GA affect the time to heading. Additional studies are needed to determine potential interactions among these parameters.

In spite of these problems, Super Dwarf rice is extremely useful in controlled environments where space is limited. We recommend growing Super Dwarf rice in a 12-h photoperiod with a PPF of at least 500 μmol m⁻² s⁻¹. Like other C₃ species, rice benefits from elevated CO₂. Rice may especially benefit from elevated CO₂ given the high temperature optimums where photorespiration is significantly increased. A phasic temperature control regime would reduce the likelihood of high-temperature-induced sterility and prolong the duration of grain fill, which would increase the harvest index. More studies are needed to address the temperature sensitivity of the flowering and grain-filling stages for this cultivar. Super Dwarf rice complements *A. thaliana* for use as a model crop for studies involving grain yield, harvest index, seed maturation, and many other developmental processes that are difficult to study because of the small seed size and yield of *A. thaliana*.

ACKNOWLEDGMENTS

This research was supported by the National Aeronautics and Space Administration Advanced Life Support Program and by the Utah Agric. Exp. Stn., Utah State Univ. We thank Catherine Billings and Jayne Silvester for hydroponic system maintenance and Julie Chard and two anonymous reviewers for helpful comments.

REFERENCES

- Adams, M.D., S.E. Celniker, R.A. Holt, C.A. Evans, J.D. Gocayne, P.G. Amanatides et al. 2000. The genome sequence of *Drosophila melanogaster*. *Science* 287:2185–2195.
- Adams, S.R., P. Hadley, and S. Pearson. 1998. The effects of temperature, photoperiod, and photosynthetic flux on the time to flowering of petunia 'Express Blush Pink'. *J. Am. Soc. Hortic. Sci.* 123: 577–580.
- Ali, A.G., and C.J. Lovatt. 1995. Relationship of polyamines to low-temperature stress-induced flowering of the 'Washington' navel orange (*Citrus sinensis* L. Osbeck). *J. Hortic. Sci.* 70:491–498.
- The *Arabidopsis* Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408: 796–815.
- Bahls, C., J. Weitzman, and R. Gallagher. 2003. Model organisms. *The Scientist* 17(Suppl. 1):1–40.
- Bahmani, I., L. Hazard, C. Varlet-Grancher, M. Betin, G. Lemaire, C. Matthew, and E.R. Thom. 2000. Differences in tillering of long- and short-leaved perennial ryegrass genetic lines under full light and shade treatments. *Crop Sci.* 40:1095–1102.
- Baker, J.T., L.H. Allen, Jr., K.J. Boote, P. Jones, and J.W. Jones. 1990. Developmental responses of rice to photoperiod and carbon dioxide concentration. *Agric. For. Meteorol.* 50:201–210.
- Beall, F.D., P.W. Morgan, L.N. Mander, F.R. Miller, and K.H. Babb. 1991. Genetic regulation of development of *Sorghum bicolor*: V. The *ma⁵* allele results in gibberellin enrichment. *Plant Physiol.* 95:116–125.
- Bhullar, S.S., and C.F. Jenner. 1986. Effects of temperature on the conversion of sucrose to starch in the developing wheat endosperm. *Aust. J. Plant Physiol.* 13:605–615.
- Brooks, A., and G.D. Farquar. 1985. Effect of temperature on the CO₂/O₂ specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. *Planta* 165:397–406.
- Bugbee, B.G., and F.B. Salisbury. 1988. Exploring the limits of crop productivity: I. Photosynthetic efficiency of wheat in high irradiance environments. *Plant Physiol.* 88:869–878.
- Bugbee, B., B. Spanarkel, S. Johnson, O. Monje, and G. Koerner. 1994. CO₂ crop growth enhancement and toxicity in wheat and rice. *Adv. Space Res.* 14:257–267.
- The *C. elegans* Sequencing Consortium. 1998. Genome sequence of the nematode *C. elegans*: A platform for investigating biology. *Science* 282:2012–2018.
- Fields, S., Y. Kohara, and D.J. Lockhart. 1999. Functional genomics. *Proc. Natl. Acad. Sci. USA* 96:8825–8826.
- Foster, K.R., I.J. Lee, R.P. Pharis, and P.W. Morgan. 1997. Effects of ring D-modified gibberellins on gibberellin levels and development in selected *Sorghum bicolor* maturity genotypes. *J. Plant Growth Regul.* 16:79–87.
- Frantz, J.M., and B. Bugbee. 2002. Anaerobic conditions improve germination of a gibberellic acid deficient rice. *Crop Sci.* 42: 651–654.
- Goff, S.A., D. Ricke, T. Lan, G. Presting, R. Wang, M. Dunn et al. 2002. A draft sequence of the rice genome (*Oryza sativa* L. sp. *japonica*). *Science* 296:92–100.
- Goldman, K.R., and C.A. Mitchell. 1999. Transfer from long to short photoperiods affects production efficiency of day-neutral rice. *HortScience* 34:875–877.
- Grotenhuis, T.P., and B. Bugbee. 1997. Super-optimal CO₂ reduces seed yield but not vegetative growth in wheat. *Crop Sci.* 37: 1215–1221.
- Honda, I., K. Sudo, S. Iwasaki, T. Yanagisawa, I. Yamagichi, H. Kato, H. Ikeda, and N. Takahashi. 1996. Characterization of endogenous gibberellins in dwarf rice mutants. *Biosci. Biotechnol. Biochem.* 60:2073–2075.
- International Human Genome Sequencing Consortium. 2001. Initial sequencing and analysis of the human genome. *Nature* 409:860–921.
- Isbell, V.R., and P.W. Morgan. 1982. Manipulation of apical dominance in sorghum with growth regulators. *Crop Sci.* 22:30–35.
- Kaczperski, M.P., A.M. Armitage, and P.M. Lewis. 1996. Performance of plug-grown geranium seedlings preconditioned with nitrogen fertilizer or low-temperature storage. *HortScience* 31:361–363.
- Kinet, J.M., D. Hurdebise, A. Parmentier, and R. Stainier. 1978. Pro- motion of inflorescence development by growth substance treatments to tomato plants grown in insufficient light conditions. *J. Am. Soc. Hortic. Sci.* 103:724–729.
- Kinoshita, T., and N. Shinbashi. 1982. Identification of dwarf genes and their character expression in the isogenic background. *Jpn. J. Breed.* 32:219–231.
- Klassen, S.P., and B. Bugbee. 2002. Sensitivity of wheat and rice to low levels of atmospheric ethylene. *Crop Sci.* 42:746–753.
- Kostenyuk, I., B.J. Oh, and I.S. So. 1999. Induction of early flowering in *Cymbidium niveo-marginatu*, Mak in vitro. *Plant Cell Rep.* 19:1–5.
- Long, S.P. 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO₂ concentrations: Has its importance been underestimated? *Plant Cell Environ.* 14:729–739.
- Ma, J., and E. Takahashi. 1990. The effect of silicic acid on rice in a P-deficient soil. *Plant Soil* 126:121–125.
- Ma, J., and E. Takahashi. 1993. Interaction between calcium and silicon in water-cultured rice plants. *Plant Soil* 148:107–113.
- Mackill, D.J. 1981. Studies on the mechanism and genetics of high temperature tolerance in rice. Ph.D. thesis. Univ. of California, Davis (Publ. no. AAT8200524).
- Mackill, D.J., W.R. Coffman, and J.N. Rutger. 1982. Pollen shedding and combining ability for high temperature tolerance in rice. *Crop Sci.* 22:730–733.
- Monje, O., and B. Bugbee. 1998. Adaptation to high CO₂ concentration in an optimal environment: Radiation capture, canopy quantum yield, and carbon use efficiency. *Plant Cell Environ.* 21:315–324.
- Prasad, P.V., K.J. Boote, L.H. Allen, Jr., and J.M.G. Thomas. 2002. Effects of elevated temperature and carbon on seed-set yield of kidney bean (*Phaseolus vulgaris* L.). *Global Change Biol.* 8:710–721.
- Prasad, P.V., K.J. Boote, L.H. Allen, Jr., and J.M.G. Thomas. 2003. Super-optimal temperatures are detrimental to peanut (*Arachis hypogaea* L.) reproductive processes and yield at both ambient and elevated carbon dioxide. *Global Change Biol.* 9:1775–1787.
- Reuveni, J., and B. Bugbee. 1997. Very high CO₂ reduces photosynthesis, dark respiration, and yield in wheat. *Ann. Bot. (London)* 80: 539–546.
- Rood, S.B. 1985. Application of gibberellic acid to control tillering in early-maturing maize. *Can. J. Plant Sci.* 65:901–911.
- Rosenbluth, A., and N. Wiener. 1945. The role of models in science. *Philos. Sci.* 12:316–321.
- Schultheis, J.R., and R.J. Dufault. 1994. Watermelon seedling growth, fruit yield, and quality following pretransplant nutritional conditioning. *HortScience* 29:1264–1268.
- Spurway, M.I., and M.B. Thomas. 2001. Nutrition of container-grown Christmas cacti. *J. Plant Nutr.* 24:767–778.
- Tashiro, T., and I.F. Wardlaw. 1989. A comparison of the effect of high temperature on grain development in wheat and rice. *Ann. Bot. (London)* 64:59–65.
- Van Esbroeck, G.A., M.A. Hussey, and M.A. Sanderson. 1997. Leaf appearance rate and final leaf number of switchgrass cultivars. *Crop Sci.* 37:864–870.
- van Iersel, M.W., and B. Bugbee. 2000. A multiple chamber, semicontinuous, crop carbon dioxide exchange system: Design, calibration, and data interpretation. *J. Am. Soc. Hortic. Sci.* 125:86–92.
- Venkateswarlu, B. 1977. Influence of low light intensity on growth and productivity of rice, *Oryza sativa* L. *Plant Soil* 47:713–719.
- Vergara, B.S., and T.T. Chang. 1985. The flowering response of the rice plant to photoperiod: A review of the literature. IRRI, Los Banos, Laguna, Philippines.
- Volk, T., and B. Bugbee. 1991. Modeling light and temperature effects on leaf emergence in wheat and barley. *Crop Sci.* 31:1218–1224.
- Volk, G.M., and C.A. Mitchell. 1995. Photoperiod shift effects on yield characteristics of rice. *Crop Sci.* 35:1631–1635.
- Walbot, V. 2000. A green chapter in the book of life. *Nature* 408: 794–795.
- Wells, B.R., B.A. Huey, R.J. Norman, and R.S. Helms. 1993. Rice, p. 15–19. In W.F. Bennett (ed.) *Nutrient deficiencies and toxicities in crop plants*. APS Press, Am. Phytopathological Soc., St. Paul, MN.
- Xu, G., S. Wolf, and U. Kafkafi. 2001. Interactive effect of nutrient

- concentration and container volume on flowering, fruiting, and nutrient uptake of sweet pepper. *J. Plant Nutr.* 24:479–501.
- Yin, X., and M.T. Kropff. 1996. The effect of temperature on leaf appearance in rice. *Ann. Bot. (London)* 77:215–221.
- Yokoyama, M., S. Yamaguchi, S. Inomata, K. Komatsu, S. Yoshida, T. Iida, Y. Yokokawa, M. Yamaguchi, S. Kaihara, and A. Takimoto. 2000. Stress-induced factor involved in flower formation of *Lemna* is an α -ketol derivative of linolenic acid. *Plant Cell Physiol.* 41: 110–113.
- Yoshida, S. 1973. Effects of CO₂ enrichment at different stages of panicle development on yield components and yield of rice (*Oryza sativa* L.). *Soil Sci. Plant Nutr. (Tokyo)* 19:311–316.
- Yoshida, S. 1981. *Fundamentals of rice crop science*. IRRI, Los Banos, Laguna, Philippines.
- Yu, J., S. Hu, J. Wang, G.K. Wong, S. Li, B. Liu et al. 2002. A draft of the rice genome (*Oryza sativa* L. ssp. *indica*). *Science* 296:79–92.
- Ziska, L.H., P.A. Manalo, and R.A. Ordonez. 1996. Intraspecific variation in the response of rice (*Oryza sativa* L.) to increased CO₂ and temperature: Growth and yield response of 17 cultivars. *J. Exp. Bot.* 47:1353–1359.