ABSTRACT

The conversion efficiency of radiation into biomass and yield has steadily increased for centuries because of continued improvement in both plant genetics and environmental control. Considerable effort has gone into improving the environment for plant growth in space, but work has only begun to engineer plants for spaceflight. Genetic manipulation offers tremendous potential to improve our ability to study gravitational effects. Genetic manipulation will also be necessary to build an efficient regenerative life support system. We cannot fully characterize plant response to the spaceflight environment without understanding and manipulating their genetic composition. Identification and selection of the existing germplasm is the first step. There are thousands of cultivars of each of our major crop plants, each specifically adapted to a unique environment on our planet. Thousands of additional lines are held in national germplasm collections to maintain genetic diversity. Spaceflight imposes the need to tap this diversity. Existing lines need to be evaluated in the environment that is characteristic of closed-system spaceflight conditions. Many of the plant growth challenges we confront in space can be better solved through genetic change than by hardware engineering. Ten thousand years of plant breeding has demonstrated the value of matching genetics with the environment. For example, providing continuous light can increase plant growth in space, but this often induces calcium deficiencies because Ca is not supplied by guttation during a dark period. This deficiency cannot be eliminated through increased root-zone and foliar Ca applications. It can be solved, in wheat, through genetic selection of lines that do not have the deficiency. Subsequent comparison of lines with and without the Ca deficiency has also helped us understand the nature of the problem.

INTRODUCTION

Shakespeare wrote, "The Past is Prologue" (The Tempest, act II scene 1). This aphorism provides incentive to look well into the past to see far into the future. Homo sapiens have been on Earth for about 2,000,000 years. For the first 1,990,000 of these years we lived as hunter-gatherers. About 10,000 years ago our civilization became dependent on a continuous food supply, which could only be provided by agriculture. Before the development of agriculture, nomadic groups followed the seasonal availability of foods derived from wild plants. Agriculture led to permanent settlements and an increasing population. This transition has been considered the most significant cultural change in human history. Leaving our planet may be the second most significant change.

The shift from gathering to farming required selection for two crucial characteristics: loss of seed shattering, shown in Figure 1, and loss of seed dormancy (Harlan, 1992). Selection for these mutant characteristics (thought to be conducted mostly by women) made the plants less able to survive in the wild but much better suited for agriculture. This plant breeding effort revolutionized our existence. We are on the threshold of a similar genetic effort to facilitate spaceflight. The selection of

Figure 1. The shift from seed gathering to farming required selection for the loss of seed shattering. This made the crops less able to survive in the wild but better suited for agriculture. This plant breeding effort revolutionized our existence.

domesticated crops from wild varieties occurred over hundreds of years - without any knowledge of Mendel's laws or gene-splicing techniques. Our modern genetic tools should allow our next effort to progress at a dramatically accelerated rate.

There are two central reasons to genetically modify plants for spaceflight:

1. to efficiently provide life support
2. to efficiently study plant response to gravity.

The environment of space imposes unique constraints to plant productivity. These constraints affect our ability to study microgravity in an optimal environment and they affect our ability to produce food, oxygen, and water for life support. Here I discuss four unique spaceflight factors and the potential to genetically modify plants to minimize their impact.

1. Production per unit time and volume
2. Elevated ethylene
3. Light pollution
4. Light quality

PRODUCTION PER UNIT TIME AND VOLUME

The need for spaceflight efficiency demands smaller, quicker, cheaper satellites and we similarly need shorter, quicker plants for space research. These plants must be more than semi-dwarf, and more than even full-dwarf; they must be super-dwarf. Taller cultivars are needed on Earth to compete with weeds and to facilitate mechanical harvest, but these characteristics are not useful in space. Full size cultivars of many crop plants are 2-meters tall. Semi-dwarf and dwarf cultivars are 0.7 to 1-meter tall. Super-dwarf
cultivars (less than 0.3-m tall) are needed. These ‘super-dwarf’ cultivars have been identified in a few crop species but ‘super-dwarf’ genes probably exist in all plants. Mutant plants that block gibberelllic acid pathways can be created, but the first step should be selection among the existing plant diversity on Earth.

All breeding programs begin by categorizing the available genetic diversity (Shands and Wiesner, 1991). Because genetics interact with environment, the diversity must be categorized in situ. Some short cultivars become tall with ample water, CO₂, and nitrogen. Other cultivars significantly delay reproductive growth in luxuriant conditions. The most basic of all plant breeding principles should be used: apply selection pressure and select the fittest.

**A Case Study: the Development of ‘USU-Apogee’ Wheat.** Hundreds of dwarf wheat lines, personally selected from the International Center for Wheat and Maize Improvement (CIMMYT; Oregom, Mexico) were evaluated under the selection pressures of elevated CO₂, 24-h photoperiod from high pressure sodium lamps, and constant day/night temperatures, and a hydroponic, ample-nutrient, root-zone environment. Selection criteria were short height, early maturity, reduced tillering, small leaves, and finally, yield. These trials resulted in the selection of two dwarf wheat cultivars with excellent yield potential in optimal conditions (Veery-10 and Yecora Rojo). Among these lines we also discovered ‘Super-dwarf’ wheat.

Hybrid crosses between ‘super-dwarf’ and other parent lines with desirable characteristics produced ‘USU-Apogee’ (Bugbee et al., 1997), a shorter, higher yielding alternative to ‘Yecora Rojo’ and ‘Veery-10’. The yield advantage of USU-Apogee is 10 to 30% over these other cultivars, depending on environmental conditions. USU-Apogee (45-50 cm tall, depending on temperature) is 10 to 15 cm shorter than Yecora Rojo and 1 to 4 cm shorter than Veery-10. USU-Apogee was also selected for resistance to the calcium-induced leaf tip chlorosis that occurs in controlled-environments.

Reduced tillering is desirable in high yield conditions because the late tillers are less productive (Donald, 1968; 1979). Small leaves are often more photosynthetically efficient than large leaves, and two small leaves may be better than one large leaf (Morgan et al., 1990a; LeCain et al., 1989; Bhagsari and Brown, 1986; Bishop and Bugbee, 1998). Continuous cultivation made it possible to evaluate 3 to 4 generations per year. Yields in this environment (about 16 Mg ha⁻¹; 240 bushels per acre) are double the best irrigated field yields when the greenhouse temperature is set at a low temperature and the life cycle is extended to about 100 days (Bugbee and Salisbury, 1988). Mice got into the greenhouse prior to harvest in the F₈ generation and damaged all six replicate plots of USU-Apogee. No other plots were damaged. We speculate that the mice may have sensed a volatile compound coming from the ‘USU-Apogee’ wheat.

**Resistance to Calcium-deficiency Induced “Tipburn”** USU-Apogee is resistant to leaf tip chlorosis that occurs in wheat under rapid growth conditions, particularly in continuous light. This chlorosis (caused by a calcium deficiency) can kill the top 30% of the flag leaf. The chlorosis is severe in Veery-10 and also occurs in Yecora Rojo. Calcium deficiencies, such as tipburn in lettuce (Lactuca sativa L.) and blossom end rot in tomatoes (Lycopersicum esculentum L.) are common in controlled-environment crop production because Ca has low phloem mobility and is thus not sufficiently translocated to growing meristems. Foliar Ca applications and increased root-zone Ca are not effective because they do not reach the meristematic tissue (Marschner, 1995). USU-Apogee has significant rates of guttation during dark periods and guttation occurs even during the light period when the stomates are partly closed by elevated CO₂. Significant amounts of Ca can be translocated by guttation (Marschner, 1995). The segregating lines with the smallest leaves had the least chlorosis. Tissue analysis indicated adequate Ca in the top 30% of small leaves (0.4 % Ca), but inadequate amounts (0.05 % Ca) in large leaves. USU-Apogee has smaller flag leaves (11 to 20 cm long, depending on temperature) than Yecora Rojo and Veery-10 (20 to 30 cm long).

**Developmental Characteristics and Yield Studies.** USU-Apogee has an extremely rapid development rate, which helps reduce leaf size and excessive vegetative growth. Heads emerge 23 days after seeding emergence in continuous light with a constant 25°C temperature. Heads of Yecora Rojo and Veery-10 emerge about 6 days later under these conditions.

Table 1 summarizes the results of 20 yield studies (Bugbee and Koerner, 1997). Most studies compared USU-Apogee to Veery-10 because these cultivars are similar in height.

**Breadmaking Quality.** The USDA-ARS Western Quality Wheat Laboratory at Pullman, WA, evaluated Breadmaking quality. Milling and baking tests indicated that USU-Apogee has similar quality to Veery-10 and slightly poorer quality than Yecora Rojo, which is the best bread wheats grown in the United States.

**Future Breeding Efforts.** There is a need for an even shorter cultivar, especially to facilitate spaceflight research. We have reselected from segregating lines of the same hybrid cross that produced USU-Apogee and now have a series of lines ranging from 25 to 40 cm tall that appear to have most of the desirable characteristics of USU-Apogee. These lines are shown in Figure 2. Yield evaluations indicate that their yield per unit area will be less than USU-Apogee, but their yield per unit volume is higher.

All of our work has been done with hard-red wheat, which is most useful for making bread. Soft-white wheat is used on Earth to make all purpose flour for cookies and pastries, and durum wheat is used for noodles. Will we need different types of wheat for long-term dietary palatability in space? Possibly, but it is rapidly becoming possible to insert genes into a common wheat type to achieve specific flour quality characteristics (Blechl and Anderson, 1996).
Table 1. Results of yield studies in 3 environments.

<table>
<thead>
<tr>
<th>Cultivar Name</th>
<th>Feb.-May '94</th>
<th>Mar.-June '95</th>
<th>July-Sept. '95</th>
<th>Nov. 95 to Feb. '96</th>
<th>Aug.-Dec. '96</th>
<th>12 studies PPF=700</th>
<th>1 study PPF=1500</th>
<th>4 Reps. '94</th>
<th>6 Reps. '94</th>
</tr>
</thead>
<tbody>
<tr>
<td>USU-Apogee</td>
<td>129</td>
<td>101</td>
<td>130</td>
<td>130</td>
<td>116</td>
<td>111</td>
<td>108</td>
<td>118</td>
<td>112</td>
</tr>
<tr>
<td>USU-Line 56</td>
<td>128</td>
<td>99</td>
<td>127</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>98</td>
<td>114</td>
<td>111</td>
</tr>
<tr>
<td>USU-Line 1</td>
<td>111</td>
<td>106</td>
<td>115</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>108</td>
<td>104</td>
<td>96</td>
</tr>
<tr>
<td>USU-Line 10</td>
<td>108</td>
<td>104</td>
<td>112</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>98</td>
<td>107</td>
<td></td>
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<tr>
<td>Veery 10</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Yecora Rojo</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>141</td>
<td>101</td>
<td>--</td>
<td>--</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>Statistical Significance</td>
<td>0.05</td>
<td>n.s.</td>
<td>0.01</td>
<td>0.01</td>
<td>0.05</td>
<td>--</td>
<td>0.08</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Data are indicated as % yield relative to Veery-10 to facilitate comparisons among the diverse studies. USU lines 1, 10, and 56 are from the same hybrid cross that produced USU-Apogee. The 12 growth-chamber studies at PPF=700 are described in detail elsewhere (Graetnhus and Bugbee, 1997; Graetnhus et al., 1998). The greenhouse studies included 4 to 6 replicate plots per genotype. A dashed line indicates that the genotype was not included in the study. Yield, measured as g of dry seed per mole of photosynthetic photons, ranged from about 0.3 g mol⁻¹ in greenhouse studies to 0.15 g mol⁻¹ in field studies. The primary cause of the increased yield of USU-Apogee is increased harvest index, which is 5 to 15% higher than that of Veery-10. Using USU-Apogee, we achieved harvest indexes of 56 and 60% (not including roots) in two greenhouse studies with phasic environmental control (23 °C decreasing to 17 °C after anthesis).

Case Study Number 2: Super-dwarf Rice. We have also used our CO₂-enriched greenhouse to screen for shorter, quicker rice cultivars. We obtained and evaluated many lines from the International Rice Research Institute (IRRI) in the Philippines, along with lines from Japan, China, India, California, Texas, and Louisiana, and Arkansas. Over a hundred lines were evaluated. Two full-dwarf lines emerged from these trials: Ai-Nan-Tsao and 29-Lu-1 (both from southern China). Both lines have early maturity (43 days to heading and about 73 days to harvest in a 12-hour photoperiod at 30/25°C day/night temperatures). They are 70 to 80 cm tall, but this is 20 to 40 cm shorter than standard, semi-dwarf rice lines. Although these were the shortest lines in our studies, they are still too tall for the confined volumes of most controlled environments.

We continued to search for shorter rice cultivars. In 1998, rice breeders at IRRI referred us to Dr. Tashiro Kinoshita, Professor Emeritus at Hokkaido University in Japan. Dr Kinoshita has maintained a collection of dwarf rice cultivars. He sent 27 rice lines for our evaluation (Kinoshita and Shinbashi, 1982). As shown in Figure 3, one of these lines (line N71) was only 20 cm tall in our studies and had a harvest index of over 60%. This line is dwarf because it lacks the 3β-hydroxylase enzyme that is responsible for the conversion of gibberellic acid 20 (GA-20) to GA-1. This line represents a breakthrough in the use of rice for advanced life support and also in rice research in controlled environments. We are currently characterizing the response of this line to temperature, photoperiod, and light intensity. We have increased the seed supply and seed samples are available on request to other research groups.

Case Study Number 3: the Making of Wisconsin Fast Plants. Organisms with short life cycles and small size are particularly valuable in laboratory research. Drosophila and mice have long been important laboratory organisms for this reason. Research on Arabidopsis thaliana has exploded in the past decade. Species in the genus Brassica, better known as Wisconsin Fast Plants, are also unique as model organisms (Williams and Hill, 1986).

In the 1970’s Paul Williams and Curtis Hill grew brassicas from the world collection of over 2000 accessions obtained from the USDA’s national Plant Germplasm System. They found enormous differences in time to flower among the lines. They hybridized the early flowering types and selected among segregating lines for reduced
reproductive time, absence of seed dormancy, and small size. They made their selections in a typical laboratory environment with a shallow soil-less hydroponic root-zone and low level fluorescent lighting. It should not be surprising that selection in this laboratory environment turned out to be important. The selections responded strongly to the environment in which they were grown. Many lines with short phenotypes were many times larger when given more space for root growth.

The rest is history. Rapid cycling brassicas are now genetically well characterized and are widely used in laboratory research. They have been an important plant for spaceflight research (Musgrave et al., 1998). Wisconsin Fast Plants have become a mainstay in plant biology courses from kindergarten to graduate school. They are smaller, quicker, and cheaper.

**ELEVATED ETHYLENE**

Ethylene is a potent plant hormone. Actively growing crop plants produce about 0.2 nmol of ethylene kg\(^{-1}\) s\(^{-1}\) (Wheeler et al., 1996; Morgan, 1990b; Petruzelli et al., 1994) and this accumulates in the air of closed plant growth chambers. Ethylene levels in the field rarely rise above 1 ppb, but levels well above 1000 ppb have been measured on Mir and the ethylene level rose to 800 ppb in a study at NASA-JSC. Ethylene levels in closed plant growth chambers at NASA-KSC routinely increase to 50 to 100 ppb.

Wheat plants from the long-term study on space station Mir in 1997 failed to set seed. Analysis of the floral parts at Utah State University indicated that the anthers did not shed pollen. Reduced seed set and even complete sterility has been associated with elevated ethylene levels, but no controlled studies have been done to clearly implicate ethylene in the poor seed set. However, these findings are consistent with the ethylene mediated sterility, associated with Ethephon, that has been well studied in wheat. Ethephon, a commercial product used to generate ethylene, is widely used to reduce plant height and lodging in wheat and barley (Abeles et al., 1992). Unfortunately, Ethephon is also a potent inhibitor of pollen formation and can cause full male sterility (Rowell and Miller, 1971; Bennett and Hughes, 1972; Simmons et al., 1988). Moss and Stobbe (1991) reported that Ethephon reduced seeds per head by up to 26% when used to prevent lodging in barley (*Hordeum vulgare* L.). Unfortunately, the quantitative relationship between direct Ethephon application to plants and the effect of ethylene gas concentration in the atmosphere is unknown.
We recently examined ethylene effects in two studies. In the first study, levels from 1 to 20 ppm on Super-dwarf wheat in replicate growth chambers were performed and the effects were compared to control chambers without ethylene. Plants grew well at even 20 ppm ethylene but plant height decreased as ethylene increased. There was no effect on the rate of development as indicated by the time of heading. Seed set and yield were excellent in the control chambers, but plants in all the ethylene chambers were completely sterile as shown in Figure 4. In the second study, effects of 250, 500, 750, & 1000 ppm on USU-Apopge were compared to a control chamber. As in the previous study, there was a linear decrease in plant height with increasing ethylene. Longitudinal leaf rolling (into cylinders, Figure 5) was visually apparent at all ethylene levels as compared to flat leaves on control plants. Leaf rolling is a sensitive indicator of ethylene pollution in closed chambers. Seed set was reduced by 50% at 250 ppm ethylene and by 90% at 500 ppm ethylene. We are now configuring a gas chromatograph for automated ethylene monitoring of 16 chambers and plan to use this capability to quantify short- and long-term ethylene effects at different stages of the life cycle. Ethylene efflux appears to be correlated with growth rate and may peak during anthesis. These studies are an essential prerequisite to the design and sizing of ethylene scrubbing equipment for bioregenerative life support. Passing the air through a filter (typically potassium permanganate) can remove ethylene, but it is extremely difficult to scrub it to below about 1000 ppb.

Fortunately, plants with mutations that affect the synthesis or perception of ethylene have been identified (Ecker, 1995) and the mutant ETR1 gene has been used to transform tomato and petunia plants, making them insensitive to ethylene (Wilkinson et al., 1997). Engineering genetic insensitivity to ethylene may be a highly cost effective alternative to large ethylene scrubbers in spaceflight. In addition to ethylene insensitivity, the genetics controlling ethylene synthesis have been characterized and it should be possible to significantly reduce ethylene synthesis in plants (Klee et al. 1991).

LIGHT POLLUTION

Light pollution is becoming increasingly common around the world. It affects astronomers, sea turtle nesting habits, and bird migration (see). Short-day plants require an 8 to 12 hour period of darkness to become reproductive. Light pollution may be a significant problem with multiple crops sharing a common space in life support systems. How dark is dark? NASA sponsored research may provide the incentive to define a level for reagent grade darkness.
Frank Salisbury (1981) determined that light from the full moon is insufficient to trigger photoperiod responses in *Xanthium*, even when it is in its most sensitive stage, but we know little about other light levels and other plants. There is considerable potential to develop and select normally short-day plants that are photoperiod insensitive. For example, soybeans are a short-day plant but we discovered a cultivar that flowers in continuous light (unpublished data. Plant Introduction Number PI 494.525, seed available on request). Similar photoperiod insensitivity has also been discovered in potatoes (Wheeler et al., 1991a).

LIGHT QUALITY

Plants have evolved over millions of years to utilize sunlight. Sunlight has almost equal amounts of each color of light from violet radiation at 400 nm to far-red radiation at 750 nm. The most energetically efficient electric lamps deviate dramatically from these equal ratios. Radiation at short wavelengths is more energetically expensive to produce (following Planck's Law: $E = h \nu / \lambda$), so the most efficient lamps within a technology have the least blue light. Blue light reduces cell expansion (Cosgrove, 1981) and there is considerable evidence that plants grow better with less blue light than is in sunlight (Dougher and Bugbee, 1998; Yorio et al. 1995). However, some blue light appears to be essential to the growth of some species (Brown et al. 1995; Wheeler et al., 1991b; Goins et al. 1998). Furthermore, since the energy in 'far-red' photons is not high enough to excite electrons in photosynthesis, this radiation is useless for photosynthesis and plant dry mass gain and it adds considerably to the heat load in spaceflight.

There is considerable potential to genetically modify plants to change their morphogenic response to unusual light qualities. Changing the concentration of photoreceptors for red, far-red, and blue light may be useful in increasing or decreasing plant sensitivity to specific wavelengths (Tennessen and Ciolkosz, 1998). Increasing the amount of the Phy-B phytochrome photoreceptor can result in a dwarf phenotype that may be useful in making dwarf cultivars for life support (Smith, 1994a). Increasing the phytochrome photoreceptor Phy-A might be useful to make plants hypersensitive to trace amounts of far-red light (Smith, 1994b). Over-expression of an *Arabidopsis thaliana* phytochrome gene in transgenic tobacco has resulted in hypersensitivity to blue, UV-A and green light (Lin et al., 1995). Selecting a wheat line with reduced tillering (branching) represents another useful approach to matching genetics with environment.

Basic research to improve lighting efficiency for plant growth needs to be continued, but in the long run, it may be far cheaper to change the plant genetics than to change the lighting technology.

CONCLUSIONS

Genetic selection and modification should parallel efforts to improve the growth chamber environment for spaceflight. Some environmental factors are extremely difficult to modify in space. Plant selection and modification can make many of these environmental modifications unnecessary. Basic plant breeding principles should be followed: collect genetic diversity, apply selection pressure, and select the fittest.

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