Journal of Experimental Botany, Vol. 51, GMP Special Issue,
pp. 459–473, February 2000

Journal of
Experimental
Botany

Avenues for genetic modification of radiation use efficiency in wheat

Matthew P. Reynolds[1]**, Maarten van Ginkel and Jean-Marcel Ribaut**

International Maize and Wheat Improvement Centre (CIMMYT), Mexico

Received 10 May 1999; Accepted 16 October 1999

Abstract

Radiation use efficiency (RUE) of a crop is a function of several interacting physiological phenomena, each of which can be tackled independently from the point of view of genetic improvement. Although wheat breeding has not raised RUE substantially, theoretical calculations suggest room for improvement. Selection for higher rates of leaf photosynthesis at saturating light intensities (A_{max}) has not resulted in improved RUE of crops, perhaps in part because most leaves in a canopy are not light-saturated. However, higher A_{max} may be observed as a pleiotropic effect of other yield-enhancing genes (e.g. genes for reduced height). Genetic transformation of Rubisco to double its specificity for CO_2 would theoretically increase A_{max} by perhaps 20%, and some evidence suggests that photosynthesis at sub-saturating light intensities would also be improved. However, photo-protection may be jeopardized if capacity for oxygenase activity is impaired. Photosynthetic rate of the whole canopy can be enhanced by manipulation of leaf angle, which is under relatively simple genetic control, and possibly by manipulating leaf-N distribution throughout the canopy. Genetic diversity for adaptation of lower canopy leaves (e.g. changes in chlorophyll *a*:*b* ratio) to reduced light intensity observed in some crops needs to be investigated in wheat. Improved RUE may be achieved by increasing sink demand (i.e. kernel number) if excess photosynthetic capacity exists during grain filling, as suggested by a number of studies in which source–sink balance was manipulated. Some evidence suggests that improved sink strength may be achieved by lengthening the duration of the period for juvenile spike growth. Balancing source- and sink-strength is a complex genetic challenge since a crop will change between source and sink limitation as conditions vary during the day, and with phenological stage. Improved RUE will be partly a function of a genotype's ability to buffer itself against changes in its environment to match the demand imposed by its development. Analysis of the physiological basis of genotype by environment interactions may indicate avenues for genetic improvement. The genetic control of photosynthetic regulation may be elucidated in the future through the application of genomics. However, given a lack of specific knowledge on the genetic basis of RUE, empirical selection is currently the most powerful tool for detecting favourable genetic interactions resulting from crosses between lines with superior photosynthetic traits and other high yielding characteristics. Selection for superior segregants can be accelerated using rapidly measured physiological selection traits, such as stomatal conductance or canopy temperature depression.

Key words: Radiation use efficiency, photosynthesis, genetic modification, wheat breeding, yield potential.

Introduction

Genetic yield potential of wheat has been increasing at an annual rate of approximately 0.9% over the last 30 years (Calderini *et al.*, 1999). However, global demand is predicted to increase by 1.6% per year over the next 20 years, from the current production of 550 million tonnes (Rosengrant *et al.*, 1995). Given the economic and environmental constraints related to increased land use or intensification of agricultural inputs, genetic

---

[1] Present address and to whom correspondence should be sent: CIMMYT Apartado No. 370, PO Box 60326, Houston, TX 77205, USA. Fax: +1 652 833 6656. E-mail: m.reynolds@cgiar.org

improvement in yield may be the most cost-effective way of meeting demand.

Improvement in wheat yield potential, both before and after the advent of semi-dwarf lines, has been shown to be most strongly associated with improved partitioning of assimilates to grain (Austin *et al.*, 1980; Kulshrestha and Jain, 1982; Waddington *et al.*, 1986, 1987; Calderini *et al.*, 1995; Sayre *et al.*, 1997). The generally non-significant association observed between yield and biomass (Slafer *et al.*, 1994; Calderini *et al.*, 1995, 1999) suggests that radiation use efficiency (RUE) has not been significantly improved. While photosynthetic capacity is the ultimate yield-limiting factor, few studies show clear associations between genetic improvements in yield and increased photosynthetic rate (Evans, 1993). Light interception by canopies and RUE have been examined in materials representing progress made in yield over time (Calderini *et al.*, 1999). For example, in irrigated environments, no differences in RUE or light interception were found (Slafer *et al.*, 1990), while more modern lines were shown to have higher RUE after anthesis (Calderini *et al.*, 1997), but not before, and it was concluded that the higher rates were sink driven. Reduced height (*Rht*) genes associated with the semi-dwarf growth habit have had the greatest impact on wheat yield of any other trait. However, studies in near isogenic backgrounds showed similar RUE and light interception characteristics for semi-dwarf (*Rht1* or *Rht2*) and tall (*rht*) lines (Miralles and Slafer, 1997). The fact that radiation use efficiency does not appear to have been improved significantly by plant breeding could be worrying, especially in the light of the theoretical limit to harvest index, estimated at approximately 60% (Austin *et al.*, 1980). However, recent examination of yield progress in irrigated spring wheat shows that biomass has been increased approximately 10% in the most recent cultivars (Reynolds *et al.*, 1999); in one case being associated with the introgression of alien chromatin for disease resistance from a wild grass species (Singh *et al.*, 1998).

If genetic gains in wheat yield potential are to be accelerated, an obvious approach is to attempt to modify crop photosynthesis genetically. Net photosynthetic capacity is a function of several interacting physiological phenomena, both in supply, photosynthesis *per se* and on sink strength, together with factors which determine the efficiency of these. Key areas include (i) photosynthetic metabolism, (ii) canopy photosynthesis, (iii) determination of grain number and size (sink strength), (iv) vascular transport of water, nutrients and assimilates, (v) respiratory costs, and (vi) buffering of these processes to environmental fluxes. In theory, RUE of a crop could be improved by genetic manipulation at any of these levels, since their interaction determines net assimilation. This review will consider the first three points (i–iii), and the last (vi). In addition, the use of *in situ* screening traits for photosynthetic capacity will also be discussed in the context of complementing conventional plant breeding methodologies.

Theoretical limits to RUE

Light absorption

The theoretical limits to RUE were revised recently (Loomis and Amthor, 1996). The energy contained in a mature crop represents only a small fraction (typically less than 5%) of incident radiation received. This apparent inefficiency is caused by a number of largely unavoidable factors. Chlorophyll has evolved to absorb approximately half of the sun energy, i.e. wavelengths between 400–700 nm, longer wavelengths having insufficient energy for photochemistry. Some photosynthetically active radiation (PAR) intercepted by closed canopies is not absorbed by chloroplasts, for example, light transmitted to non-photosynthetically active cell components, or structures such as dead leaves. A study with albino and green leaves (Seybold, 1933) suggested that 10% of light absorbed by leaves may not be used in photochemistry. For closed canopies about 6% of the light is reflected to the sky (Goudriaan and van Laar, 1994).

Quantum requirement and respiration

For the PAR absorbed, the relative efficiency of carbohydrate synthesis is determined by the quantum requirement (QR), which varies considerably with growing environment. Theoretically, the minimum quantum requirement is 9 mol photons mol^{-1} CO_2 fixed as CH_2O (Nobel, 1991). Assuming 2.07 mol photons MJ^{-1} of solar radiation absorbed by the canopy (which takes into account the 6% reflection from the canopy), and an energy content of 17 kJ mol^{-1} for crop dry matter, the maximum conversion efficiency, without any losses from respiration or any other cause, is approximately 12% of total solar radiation (approximately 25% of PAR). Quantum efficiency is reduced further when CO_2 concentrations are too low to match the availability of reductants produced by photochemistry. Low CO_2 availability inside the leaf promotes wasteful use of reductants in photorespiration, an effect exacerbated at higher temperatures in C_3 species. When exposed to excess light, the reaction centres of photosynthesis are down-regulated to protect them from damage, increasing QR (Weis and Berry, 1989). Irrespective of QR, there is a fixed energy cost for maintenance respiration, using approximately 20% of gross assimilates (calculated at $QR = 20$), plus the cost of growth respiration using approximately 30% of the remaining assimilates (Amthor, 1989).

Potential productivity

Based on these considerations, RUEs for values of QR between 10 to 30 mol photons mol^{-1} CO_2 have been presented (Loomis and Amthor, 1996) (Table 1). Such values can be used to calculate potential productivity for a given environment if radiation fluxes are known and the proportion of light absorbed by the crop for photosynthesis can be estimated. In NW Mexico, accumulated solar radiation for the spring wheat cycle averages 2250 MJ m^{-2}. Light interception is very close to 100% when the canopy is closed, but losses occur before canopy closure and towards physiological maturity due to senescence. A simple model for light intercepted (I) would therefore be represented by the following equation:

$$I = R(N - xF - yS) \qquad (1)$$

where I is the total radiation intercepted; R is the average daily radiation, N is the number of days from emergence to physiological maturity, F is the number of days from emergence to full canopy closure, and S is the number of days between initiation of senescence in flag leaves and physiological maturity. The fraction of radiation not intercepted during canopy closure, x, is a function of average amount of radiation not intercepted by the crop (assume 0.5) and incident radiation level during this period in comparison to the seasonal average (approximately 0.75 in NW Mexico); y represents the fraction of incident radiation not intercepted during senescence and is a function of the average amount of light not intercepted by green tissue (assume 0.5) and the incident radiation during this period in comparison to the seasonal average (approximately 1.25 in NW Mexico). For NW Mexico the following values can also be assumed $R = 16$ MJ m^{-2} d^{-1}, and $N = 140$, $F = 40$, $S = 10$ for a typical spring wheat genotype.

However, light interception by photosynthetically active tissue will be lower than the value of total light intercepted by the canopy (I), because a proportion of the light intercepted is absorbed by chlorotic and other non-photosynthetic tissue (C). Active absorption of light can therefore be represented by modifying equation 1 as follows:

$$A = R(N - xF - yS)(1 - C) \qquad (2)$$

where A is the absorbtion of light by photosynthetically active tissue and C is the proportion of light absorbed by chlorotic and other non-photosynthetic tissue. A value for C of 0.1 is assumed (Seybold, 1933). Using the stated parameters to calculate A, a value is obtained of 1748 MJ m^{-2} for an average crop cycle in NW Mexico.

The best current estimates of QR for a wheat crop are in the region of 20–24 mol photons mol^{-1} CO_2 fixed as CH_2O (Fischer, 1983). Smaller values of QR (15 photons mol^{-1} CO_2) have been reported for C_3 crop species at 25 °C and ambient CO_2 concentration in controlled situations (McCree, 1971). With values of QR from 15–24, RUE varies from 1.5 to 2.6 g CH_2O MJ^{-1} solar radiation (Table 1). Therefore, assuming 1748 MJ m^{-2} of absorbed radiation during the cycle, biomass ranges from 2620 and 4545 g m^{-2}. Irrigated wheat in this environment currently approaches an above-ground biomass of 2100 g m^{-2} (Reynolds *et al.*, 1999). This would suggest that if the cost of root growth and maintenance is not substantially higher than previously estimated (i.e. 10% of above-ground biomass; Weir *et al.*, 1984) improvements in RUE in the field are conceivable. Means for achieving this through modifications to photosynthetic metabolism, canopy architecture, and source–sink balance will be discussed in the following sections.

Leaf photosynthetic rate

A_{max} and productivity

Most research conducted with the aim of understanding the relationship between improving yields and crop photosynthesis has focused on measuring light saturated net carbon exchange rate ($A_{\max}$) of individual leaves. The

Table 1. *Estimates of potential radiation use efficiency (RUE) of a C_3 crop with various values of quantum requirement (from Loomis and Amthor, 1996)*

Quantum requirement (mol mol^{-1} CO_2)	CH_20 produced[a] (mmol)	CH_2O use in R_m (mmol)	CH_2O available for growth		Biomass produced at $Y_g = 0.72$		Total respiration/ gross photosynthesis
			(mmol)	(g)	(g MJ^{-1})	(g MJ^{-1} PAR)	
10	207	18	189	5.67	4.1	8.2	26
15	138	18	120	3.60	2.6	5.2	30
20	104	18	86	2.58	1.9	3.7	33
25	83	18	65	1.95	1.4	2.8	37
30	69	18	51	1.53	1.1	2.2	40

[a] Gross photosynthesis: CH_2O represents a mol of C as carbohydrate with mol weight of 30 g.
Y_g = growth yield factor, i.e. g biomass formed g^{-1} CH_2O consumed by growth respiration.
R_m = maintenance respiration.

relationship between grain yield and A_{max} is not straightforward for a number of reasons. Firstly, yield differences in wheat are generally better associated with the proportion of assimilates that are partitioned to the grain than with RUE (Slafer *et al.*, 1994; Calderini *et al.*, 1995, 1999). Even in situations where yield may be limited by photoassimilation, an association between A_{max} and yield cannot necessarily be anticipated. Several physiological factors reduce the amount of net carbon fixed which is available for growth, principally respiration of assimilates during the dark period (Amthor, 1989), as well as loss of carbon from root exudates, tissue necrosis, etc. Finally, because individual leaves generally operate well below light saturation, with incident radiation depending on leaf position in the canopy, and leaf angle, differences in A_{max} can be at best expected to make modest contributions to yield (Day and Chalabi, 1988; Nelson, 1988); in other words it is hard to extrapolate from spot readings of leaf A_{max} to whole canopy photosynthesis throughout the crop cycle.

Since direct measurement of canopy photosynthesis on multiple genotypes is highly impractical, crop physiologists have measured maximum leaf photosynthetic rate as a surrogate, perhaps encouraged by the fact that little genetic variation for photosynthetic rate at subsaturating light intensities has been reported (McCree, 1971; Charles-Edwards, 1978). While high values of A_{max} have been recorded, for example, in wheat's diploid progenitors (Austin *et al.*, 1982), such results and others are rarely associated with higher productivity (Evans, 1993). This lack of association is not surprising for many of the reasons already stated, not least of which is that yields were probably not limited by assimilation capacity.

At least where yield increases of cultivars are related to greater biomass, an association with higher leaf photosynthetic rate is more likely, assuming that differences are not entirely due to contrasts in light interception related to canopy development or senescence. An association between A_{max}, biomass and yield has been reported for different wheat cultivars under warm, irrigated conditions (Reynolds *et al.*, 1994, 2000). However, recent work also reported an association between yield and photosynthetic rate under temperate conditions, where above-ground biomass was not associated with yield (Fischer *et al.*, 1998). In this case, differences in A_{max} may have been pleiotropic effects of other genetic differences more causally related to yield. For example, higher A_{max} could be a feedback response caused by greater partitioning of assimilates to grain yield as indicated by the fact that differences in A_{max} among cultivars were greatest during grain filling. Further evidence that A_{max} can respond to higher partitioning of assimilates to grain yield is presented (Fig. 1) using isogenic lines contrasting in the *Rht* gene. The *Rht* gene is well documented to improve the partitioning of assimilates to grain yield in comparison to non-grain biomass (Gale and Youssefian, 1985; Fischer and Quail, 1990). In this study, a total of 16 pairs of spring wheat isolines were grown in replicated yield plots (Reynolds *et al.*, 1994, for details of growing environment), and canopy temperature depression (CTD) was measured approximately every 7 d between canopy closure and approximately 15 d after heading. CTD values were larger for the better yielding *Rht* isolines once grain filling had commenced (Fig. 1). Given that CTD has been shown to be associated with stomatal conductance and A_{max} (Reynolds *et al.*, 1994; Fischer *et al.*, 1998), the results suggest that greater sink demand during grain filling associated with the *Rht* allele led to increased rates of A_{max}.

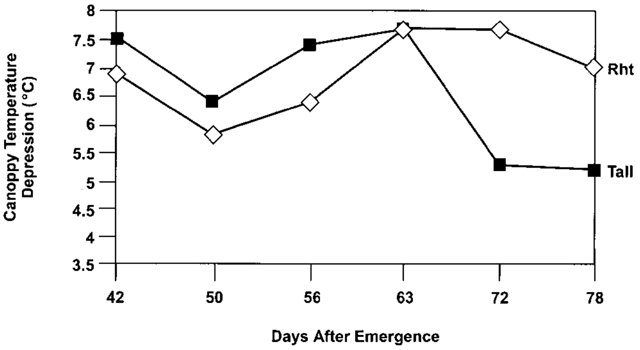

Fig. 1. Average canopy temperature depression (CTD) from canopy closure until approximately 2 weeks after heading for *Rht* and *rht* isolines in 16 high yielding spring wheat backgrounds, Tlaltizapán, Mexico, 1997. Final grain yields were approximately 5 versus 3 t ha^{-1} for *Rht* and *rht* lines, respectively, while differences in biomass were not apparent. (Pooled standard error for comparing *Rht* isolines = 0.11 °C.)

Selection for A_{max}

The idea of improving photosynthetic metabolism has been the subject of considerable research. Selection for A_{max} values higher than those of recombinant parents among their progeny, might be expected to increase crop productivity by at least some fraction of the genetic gains made for the trait (Nelson, 1988). However, despite attempts in several crops including wheat, practice does not seem to bear this out (Austin *et al.*, 1989). However, recent data (Gutierrez-Rodríguez *et al.*, 2000) show genetic gains in yield in response to selection for flag-leaf photosynthetic rate in F_5 sister lines (Fig. 2). The percentage increases in A_{max} and yield was of a similar magnitude, and increased A_{max} was associated with higher biomass, stomatal conductance and internal CO_2 concentration (Table 2). However, it is unlikely that genetic differences in A_{max} alone explained the differences in productivity.

The F_5 lines were random-derived progeny and it is probable that more than one mechanism was influencing expression of yield potential in different segregating geno-

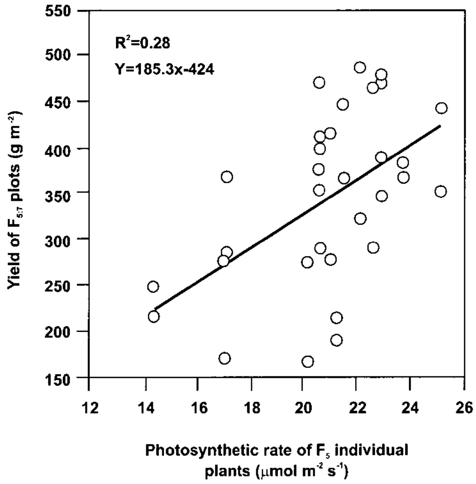

Fig. 2. Relationship between leaf photosynthesis rate of individual F_5 plants and $F_{5:7}$ grain yield, regression significant at $P = 0.01$ (adapted from Gutiérrez-Rodríguez *et al.*, 2000).

Table 2. *Correlation between photosynthetic traits measured of 16 individual* F_5 *plants and performance in* $F_{5:7}$ *yield plots, 1995–1996, Tlaltizapán, Mexico*

Individual F_5 plants (grain filling)	$F_{5:7}$ yield plots		
	Yield	Biomass	A_{max}
A_{max}	0.66**	0.67**	0.68**
Stomatal conductance	0.65**	0.68**	0.68**
Intercellular CO_2 concentration	0.70**	0.65**	0.55*

types. For example, the association of yield with internal CO_2 concentration (Table 2) suggests that stomatal conductance was a significant factor limiting photosynthesis. Reduced stomatal conductance may be related to vascular limitations (Ortelli *et al.*, 1996), for example, or to the sensitivity of stomata to high evaporative demand (Tardieu and Simmonneau, 1998). Root signalling may limit stomatal conductance as soil water deficit increases (Davies and Zhang, 1991) even under irrigated conditions. High stomatal conductance results in greater CTD when evaporative demand is high (Amani *et al.*, 1996) which may be a mechanism for avoiding heat stress as proposed for cotton (Lu *et al.*, 1998). On the other hand high, A_{max} and stomatal conductance may be a feedback mechanism related to an elevated demand for photoassimilates being driven by improved partitioning as already discussed.

In all probability, high expression of A_{max}, stomatal conductance or related traits like CTD especially during grain filling are indicative of a physiologically balanced genotype, with the potential to realize high yield, if not a superior RUE. The idea of using these traits to select for superior physiological ideotypes in wheat breeding programmes will be discussed in a subsequent section.

Genetic diversity for A_{max}

Scientists have been searching for genetic diversity of photosynthetic parameters with the view to finding sources of traits to help boost crop productivity. Superior A_{max} has been observed in some wheat ancestors, with rates up to 35% higher for *Triticum urartu* (Table 3) (Evans and Dunstone, 1970; Austin *et al.*, 1982). Unfortunately, this characteristic is only expressed during the first half of the growth cycle, does not confer superior RUE, and in most studies appear to be linked to small leaf area rendering the trait unsuitable for introgression into the cultivated species. Small leaf size is associated with smaller mesophyll cell size which itself is mechanistically involved in increasing A_{max} since it is associated with reduced resistance of the CO_2 diffusion pathway (Austin *et al.*, 1982). However, significant genetic variation in A_{max} has been reported in emmer wheat (*T. dicoccoides*) which was not associated with leaf anatomy (Carver and Nevo, 1990).

Rubisco specificity factor

Another way to increase RUE might be to reduce photorespiration. This could be achieved by increasing the affinity of Rubisco for CO_2, thereby decreasing its oxygenase activity. (Rubisco stands for ribulose *bis*phosphate carboxylase-oxygenase, the enzyme which fixes CO_2 into carbohydrates in photosynthetic tissue.) The ratio of carboxylase (V_c) to oxygenase (V_o) activity is known as the Rubisco specificity factor (expressed as relative units i.e. V_c/V_o). Modest variation for CO_2 specificity has been found in land plants (Parry *et al.*, 1989; Delgado *et al.*, 1995), with wheat having among the highest values for crop species. However, much higher values are reported in marine algae (Read and Tabita, 1994; Uemura *et al.*, 1997). Molecular techniques may offer the possibility of genetically transforming wheat Rubisco from its current specificity (i.e. $V_c : V_o$ ratio) of 95 to values of 195 corresponding to thermophilic alga *Galderia partita* (Uemura *et al.*, 1997). If this were achieved, increases in leaf photosynthetic rate of 20% at 20 °C and ambient CO_2 are predicted (Austin, 1999), using a biochemical model for CO_2 assimilation (presented by Farquhar *et al.*, 1980). Indirect evidence of the consequences of improving CO_2 specificity may be inferred from CO_2 enrichment studies.

In a recent review, evidence was presented of increased leaf and canopy photosynthetic rates in several species, including wheat, when exposed to elevated CO_2 concentrations (Drake and Gonzalez-Meler, 1997). This is a direct result of the shift in equilibrium of the carboxylase/oxygenase activity of Rubisco caused by the increased substrate (i.e. CO_2) concentration. Increased CO_2 concentration leads to a relatively larger benefit at higher temperatures by favouring carboxylase relative to oxygenase

Table 3. *Photosynthetic traits measured prior to anthesis and other traits for* Triticum *or* Aegilops *species averaged for ploidy groups (adapted from Austin* et al.*, 1982)*

	Net photosynthetic rate (mg CO_2 dm^{-2} h^{-1})	Net photosynthetic rate per unit chlorophyll content (mg CO_2 mg^{-1} h^{-1})	Leaf area (cm^2)	Stomatal frequency (number mm^{-2} abaxial surface)	Above-ground dry matter (g m^{-2})	Harvest Index (%)	Mean grain weight (mg)
Hexaploid ($n=5$)	28	5.7	18.4	71	1840	46	49
Tetraploid ($n=3$)	32	6.2	14.6	67	1640	33	30
Diploid ($n=7$)	38	8.8	8.3	100	1710	11	11

activity, thus reversing the effect of warmer temperatures. Experiments in several species have suggested that plants acclimate to elevated CO_2 with a reduction in Rubisco concentraion (Drake and Gonzalez-Meler, 1997). Evidence is starting to emerge that increased ambient CO_2 may also improve quantum yield, i.e. photosynthetic light use efficiency at subsaturating light (Kubiske and Pregitzer, 1996). There is some evidence that acclimation to higher ambient CO_2, with the subsequent reduced investment in Rubisco and associated enzymes may lead to a decline in dark respiration rate, which could further improve RUE. In addition to improved light use efficiency, Drake and Gonzalez-Meler's review presents considerable evidence for increases in water and nitrogen use efficiency in response to increased ambient CO_2 (Drake and Gonzalez-Meler, 1997).

The biochemical consequences of increasing Rubisco's specificity for CO_2 might be expected to be quite similar to those associated with increased CO_2 concentration. Since oxygenase activity reduces net photosynthesis at all light levels, increasing CO_2 specificity of Rubisco may also increase quantum yield, therefore stimulating net assimilation rate under light limitation, as well as light saturation. Such genetic manipulation might be expected to have significant impact on yields under high production, as well as in more marginal environments. It is suggested that natural variation in Rubisco specificity may be related to natural selection in hot dry environments (Delgado *et al.*, 1995).

One might question why higher CO_2 specificity has not evolved further in C_3 species. One theory is that since modern plants evolved when ambient CO_2 concentration was higher than at present, alleles controlling Rubisco regulation became genetically fixed in the absence of natural selection pressure. Alternatively, it may be related to the fact that oxygenase activity is a trade-off against the risk of irreversible photoinhibitory damage, for example, under severe water stress. Under such conditions, stomatal closure may reduce leaf internal CO_2 to such a low concentration that without oxygenase activity to use up reducing power, the photosynthetic apparatus would be permanently damaged. Even under irrigated conditions, when irradiance is very high, crops may show reversible photoinhibition (Murchie *et al.*, 1999), as a result of gas exchange limitations, which may themselves be related to vascular restrictions. This example serves to show that if CO_2 specificity of Rubisco is increased, other crop traits must be modified to match the increased demand for CO_2 if benefits are to be fully realized. In addition, Rubisco's protective role may have to be compensated for. In a recent review, it was stated that under field conditions 'there is no doubt that O_2 uptake in the light plays a significant role in preventing chronic photoinhibitions' (Osmond and Grace, 1995). If so, perhaps reducing Rubisco's oxygenase specificity would be risky, unless other means of using excess reducing power, for example, through the Mehler-ascorbate peroxidase reaction (Osmond and Grace, 1995) and by cycling carotenoid pigments (Gilmore, 1997), can also be genetically modified to compensate.

Canopy photosynthesis

Direct measurement and simulation

The measurement of whole canopy carbon exchange rate is highly resource-use intensive (Harrison *et al.*, 1981), and even where measurements of canopy photosynthesis integrated over the crop cycle have been attempted (Puckridge, 1971) yield differences among cultivars were not always explained (Gent and Kiyomoto, 1985). Another approach for estimating differences in canopy photosynthesis would be by using simulation. Models of canopy photosynthesis may produce reasonable estimates if total canopy light interception and light extinction are estimated well, if standard responses of photosynthesis to light intensity and temperature are known, and when factors such as water and nutrition are assumed to be non-limiting. To model differences among genotypes would require a considerable *a priori* knowledge about each genotype's unique characteristics including (i) rate of canopy establishment and leaf area index development, (ii) canopy architecture, (iii) nitrogen distribution in the canopy, and (iv) senescence patterns of photosynthetic tissue. The possibility for unique genotypic responses of photosynthetic metabolism to light and temperature should not be ruled out. The fact that leaf metabolism can adapt to different light intensities according to their position in the canopy (Evans, 1993) may even necessitate multiple light-response relationships. Furthermore, the various mechanisms which dissipate excess light energy and thereby influence RUE through modifying photorespiration and photoinhibition (Björkman and Demmig-Adams, 1994) could also be subject to genetic variation. Finally, values for growth and maintenance respiration would be needed to calculate RUE.

Given the impracticality of measuring or accurately simulating canopy photosynthesis for individual cultivars, traits which could potentially modify RUE can only be tested empirically by measuring their effect on crop performance (preferably in near isogenic materials). Simulation models can provide an initial basis for testing the effect of any given trait, a good example being that of leaf angle.

Leaf angle

Since leaves at the top of a canopy experience supra-saturating light intensity for a significant portion of the time that they are photosynthetically active, an erectophile leaf canopy could, theoretically, increase crop assimilation rates, especially in high radiation environments (Duncan, 1971). Several lines of evidence support the hypothesis, for example, in wheat under fully irrigated conditions in the UK, more erect leaf lines were associated with up to 11% more biomass and 4% more yield when comparing sister lines contrasting in the trait (Innes and Blackwell, 1983). Similarly, in a comparison of two barley cultivars contrasting in this trait, net photosynthesis measured at different heights within the canopy was more evenly distributed in the more erect leaf cultivar, and leaf sheath photosynthesis was also greater than in the lax leaf canopy (Angus *et al.*, 1972). Work with more recent spring wheat lines showed the erect leaf trait was associated with more grains, and measurements of CTD and carbon isotope discrimination of grain suggested higher rates of stomatal conductance on an individual leaf basis. Yield superiority was not expressed, perhaps because leaf erectness in this material was associated with small leaf size resulting in suboptimal leaf area index (Araus *et al.*, 1993). Germplasm collections were screened for erect leaves at the International Maize and Wheat Improvement Centre (CIMMYT) in the early 1970s, and the trait introgressed into the wheat germplasm base. More erect leaf canopy types are characteristic of many of CIMMYT's best yielding wheat lines (Fischer, 1996).

Genetic manipulation of leaf angle is not complex and is thought to be controlled by only two to three genes (Carvalho and Qualset, 1978). However, an important question is whether manipulation of leaf angle will permit further gains in RUE over current high yielding agronomic types. Indirect evidence suggests that the possibility may exist. For example, when comparing two of the highest yielding CIMMYT cultivars, Bacanora 88 and Baviacora, the former has a partially erectophile leaf canopy, while the latter which has a higher biomass has lax leaves, suggesting that the current RUE threshold might be increased if the erect leaf trait could be introgressed into this cultivar. Data are not available to determine whether current high yielding agronomic types with partially erect leaf canopies represent a leaf angle 'ideotype' or if further improvement is possible. The fact that sources of the erect leaf trait that were introgressed into wheat (e.g. *T. aestivum* subsp. *sphaerococcum*) showed extreme expression of the trait and that segregants have been selected empirically for yield over the last 25 years would suggest the former. Simulation work in rice suggests that the benefits of very erect leaf angle in high radiation environments could only be realized at leaf areas indices over 8 (van Keulen, 1976), which is higher than for most wheat cultivars.

Adaptation to low light

Another way of improving canopy photosynthesis is to optimize the composition of the photosynthetic apparatus, as well as N distribution, throughout the canopy, so that leaf photosynthesis is equally efficient at different light intensities. This phenomenon was investigated in lucerne (JR Evans, 1993). Leaves showed a clear trend for reduced total leaf N at greater depth in the canopy. In addition, chlorophyll a : b ratios declined with depth (Fig. 3A), indicating an increased ability to capture scarce light by increasing investment in chlorophyll associated with the light harvesting antennae, relative to the reaction centres. This was consistent with a lower total N to chlorophyll N ratio (Fig. 3C), reflecting a smaller investment in soluble protein associated with CO_2 fixation. Consequently, lower leaves had a reduced overall photo-

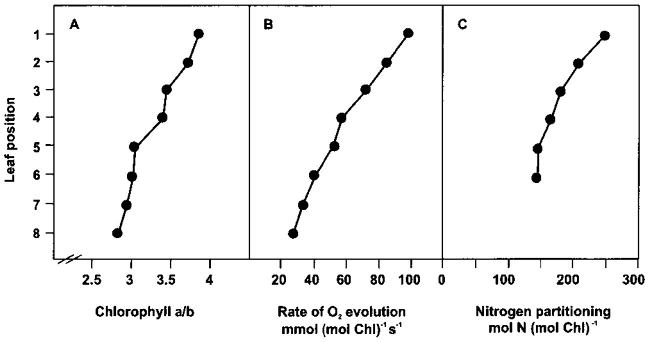

Fig. 3. Profiles of characteristics of photosynthetic acclimation with position in the canopy. Chlorophyll *a*:*b* ratio (A), photosynthetic capacity per unit of chlorophyll (B) and nitrogen content per unit of chlorophyll (C). (Adapted from JR Evans, 1993.)

synthetic capacity in normal light (Fig. 3B), but equally efficient RUE per unit of N at the light intensities experienced towards the bottom of the canopy. Crop models (Dreccer *et al.*, 1998) support the advantage of optimizing vertical distribution of canopy nitrogen in wheat.

It has been argued that the majority of photosynthesis in the field occurs at non-saturating light (Ort and Baker, 1988), and these authors suggested that it would be more profitable to improve genetically photosynthetic efficiency at low intensities, rather than focusing on improvement of $A_{\max}$. This conclusion is based on a review of a number of constitutive subcellular photosynthetic mechanisms that ensure efficient photosynthesis at low light. However, a survey of 22 crop species has indicated no genetic variability for quantum yield among them (McCree, 1971). In tobacco, antisense Rubisco transformants exhibit lower $A_{\max}$, but invest assimilates more efficiently in leaf area development (Stitt and Schulze, 1994), lending indirect support to the idea that selection for higher $A_{\max}$ is not necessarily the best way to achieve higher RUE.

Solar tracking

Another way to increase RUE of canopies might be to exploit heliotropic leaf movements, reported for many species including some dicot crops such as bean, cotton, sunflower and lucerne (Ehleringer and Forseth, 1980). However, in crop species, solar tracking declines within canopies, and interacts with row arrangement and plant density. In grasses, solar track has not been observed. However, at certain stages of development, leaves of some wheat genotypes adopt an exposed leaf posture, protruding upright from the rest of the leaf canopy, thereby maximizing their exposure to radiation. The phenomenon may have implications for improving assimilate supply to spike growth since the exposed leaf-posture is observed on flag-leaves, and coincides with the onset of rapid spike-growth. (MP Reynolds, unpublished data.)

Genetic manipulation of the source–sink balance to raise RUE

It can be a matter of choice in the literature as to whether source or sink is more limiting to yield. In wheat, a fall in leaf photosynthetic rate was shown in response to ear removal and an increased rate for remaining leaves when total leaf area was reduced (King *et al.*, 1967), supporting the sink limitation school, but evidence can be found both ways (Evans, 1993). A quantitative analysis has been undertaken to establish a relationship between assimilate supply and response of grain mass in wheat using data from 15 studies where source–sink balance was manipulated by degraining or shading treatments (Slafer and Savin, 1994). When comparing the relative change in assimilate supply with the relative change in grain mass a 1 : 1 response was not apparent. The relationship suggested that yield was either entirely sink limited (i.e. no response to assimilate supply) or co-limited by sink and source (i.e. proportionally lower response of grain weight in comparison to change in assimilate supply). Studies on materials representing historical yield gains indicate that while modern cultivars are still largely sink limited, they appear to have less excess assimilate than older cultivars. For example, degraining experiments on spring wheats from Russia indicated that while sink capacity has been improved, modern lines are now more assimilate limited than those in previous eras (Koshkin and Tararina, 1989). A similar result was shown for a set of Argentine wheats released between 1920 and 1990 which were subjected to post-anthesis defoliation (Kruck *et al.*, 1997), who stated ‘if the source–sink ratio is further reduced the grain yield of modern wheats will be simultaneously limited by source and sink’. In conclusion, the almost universal success of improving yield (sink) without changing biomass (source) clearly indicates that older wheat cultivars were severely sink limited, however, future progress is likely to require improvement in assimilation capacity.

If yield gains are to be achieved through increasing RUE, one route may be through simultaneously increasing capacity for both photoassimilation capacity as well as sink strength (Slafer *et al.*, 1996; Richards, 1996; Kruck *et al.*, 1997). One way to achieve this may be to focus on improving assimilate supply during spike development thereby increasing sink capacity, which itself may drive higher assimilation rates during grain filling. Most experiments indicate that yield, as determined by grain number, is limited by growth factors during the period of juvenile spike development prior to anthesis.

Modifying juvenile spike growth phase

The juvenile spike development period, and especially the rapid spike growth phase (RSGP) which has a duration of approximately 20 d in irrigated spring wheat, is critical in determining wheat yield potential (Fischer, 1985). It is when final grain number is set, not only determining the partitioning of photoassimilates to yield, but also influencing photosynthetic assimilation rate during seed filling. The relative duration of the juvenile spike growth period shows genetic variability (Slafer and Rawson, 1994), which is determined by the different alleles present for *Ppd* and *Vrn* genes (conferring photoperiod and vernalization sensitivity, respectively) as well as those influencing earliness per se, and how they interact with photoperiod and temperature. Slafer has hypothesized that final grain number and yield potential may be improved by manipulating these genes so as to increase the relative duration of RSGP (Slafer *et al.*, 1996). The idea stems from the notion that increased partitioning of photoassimilates to spike growth will increase floret survival (Bingham, 1969). Comparison of different radiation regimes during juvenile spike growth lend support to the hypothesis (Fischer, 1985; Slafer *et al.*, 1994; Abbate *et al.*, 1997). In addition, it is supported by the observation that increased partitioning of biomass to spike growth is observed in response to the presence of the Rht dwarfing genes, which are also associated with higher grain number and improved partitioning to yield (Miralles *et al.*, 1998). Recently, the duration of RSGP has been manipulated using short day photoperiod treatment on wheat and barley, showing a highly significant relationship between its duration and number of fertile florets per spike (Miralles and Richards, 1999).

Modifying spike anatomy

Wheat breeders have succeeded in increasing the crop's sink capacity by manipulation of spike morphology. Genotypes with branched tetrastichon (two spikelets per node of the rachis) were crossed with high yielding lines that contained other desirable traits(Dencic, 1994). After 10 years of breeding and selection, four lines were developed with 13% higher yields (approximately 1 t ha^{-1}) than the standards. This was related to improvement in the following traits, spike length (16%), number of spikelet/spike (10%), grains/spikelet (9%), and grains m^{-2} (18%). The fact that the original tetrastichon donor lines had severe problems of sterility and low kernel weight, indicates that through exhaustive recombination and selection, the high sink potential of the tetrastichon trait was complemented by other (unknown) traits, permitting it to be expressed through higher yield potential. The multi-ovary trait is another which could be exploited in a similar fashion (Chen *et al.*, 1998). Attempts to raise yield by modifying spike anatomy may be facilitated if the issue of vascular supply of assimilates to the spike is addressed, as shrivelled grains is a common symptom in extreme high fertility lines.

Grain weight potential

While increased grain number has been strongly associated with genetic progress in yield potential, mass per grain has generally not (Slafer *et al.*, 1994), though there are a few exceptions in irrigated wheat (Calderini *et al.*, 1995, 1999). An unresolved issue is whether grain weight potential can be increased independently of increases in grain number. While there have been arguments for and against this (Slafer *et al.*, 1994, 1996), recent research with synthetic wheats has indicated that increased sink demand may be achieved through this avenue. Synthetic wheats, which are a hybrid of tetraploid (*Triticum durum*) and diploid (*Aegilops squarrosa*) lines where the chromosome number has been doubled (recreating the original sequence of events which led to the evolution of *T. aestivum*), have generally larger kernel weights than conventional hexaploid cultivars. In experiments where assimilate supply was increased by degraining treatments within the spikelet during rapid spike-growth, significant increases in grain weight potential of up to 12% were shown, while no effect on grain size was apparent when degraining occurred a week after anthesis (Calderini and Reynolds, 2000). The authors suggest that increasing the duration of juvenile spike growth phase may also be a means to increasing assimilate availability for grain formation permitting larger grain weight potential.

Genetic improvement in adaptation to diurnal and seasonal fluxes

Manipulation of source–sink balance is a likely avenue to achieve gains in RUE given that even modern cultivars still seem to be more sink than source limited. However, the critical and difficult task will be to optimize the balance of source and sink throughout the life cycle of the plant. As suggested earlier (Evans, 1993) 'a variety may veer from source to sink limitation as the weather changes, as well as diurnally between day and night'. Like any crop, wheat experiences a wide range of environmental conditions, which almost by definition it cannot be equally well adapted to. Hence, realization of yield improvement to some extent must be due to buffering photosynthesis and other metabolic processes to light, temperature, water availability etc. It was argued that modern maize owes some of its improved yield potential to genetic improvement in the tolerance of photosynthesis to low temperatures during the early part of the day and soil moisture deficits during grain filling, as well as the adaptation to high planting density (Tollenaar and Wu, 1999). In cotton, genetic improvement is associated with higher stomatal frequency and it is suggested that the trait confers the ability to buffer foliar temperature during periods of heat stress (Lu *et al.*, 1998). The fact that genotypes can be sensitive to relatively small fluxes in environmental conditions is indicated by the genotype by environment interaction for yield that are frequently observed within similar cropping environments.

Analysis of genotype by environment interaction

Statistical analyses were performed to help elucidate the physiological basis of genotype by environment interaction in spring wheat by partitioning the interaction term to environmental data (i.e. meteorological measurements collected during the growth cycle) using partial least squares analysis (Vargas *et al.*, 1998). For varieties grown under irrigation, either in the same environment over years (temperate environment), or in similar environments within a defined agroclimatic region (warm environment), genotype by environment interaction for yield in both cases was best associated with variation in night temperatures during the spike growth phase. This result confirmed the critical nature of spike growth in determining yield. One might speculate further that the differential genetic sensitivity to higher night temperatures is related to acceleration of developmental rate and/or high dark respiration rates, either of which could prejudice spike growth at high temperature. For genotypes grown under temperate conditions, interaction in the performance was also explained by differences in radiation, while in the warmer regions maximum temperature had a more significant effect. This makes sense, since radiation is the principal yield-limiting factor in temperate sites, while in warmer regions temperature is more likely to limit photosynthetic rates. Such analyses provide important clues as to which mechanisms are sensitive to environment and may therefore be restricting genetic yield potential. They also suggest that interaction of genotype with environment can be related to both source and sink constraints, supporting the notion that 'spare on both sides (i.e. excess source and sink) is probably the best buffer against environmental fluxes, so the crop is not too sensitive to fluctuations' (Evans, 1993).

Genetic manipulation of complex systems

The interaction of genotypes with environment discussed above illustrates how complex the issue of crop improvement is. Even the modification of a single process such as photosynthetic rate is not a simple genetic exercise due to its metabolic complexity, involving many enzymes. According to enzymological theory (Kacser and Burns, 1981), the probability of any single gene modification significantly altering the flux of the system is extremely low, since the flux (e.g. photosynthetic assimilation) is a systematic property and variation at any single locus is reflected and buffered by the whole system. The response, nonetheless, can be quantified as the fractional change in the flux (F) over the fractional change in the enzyme activity (E), and is known as the sensitivity coefficient Z (i.e. $Z=[dF/F]/[dE/E]$). Since the sum total of all Zs is equal to one for any defined flux, the more genes involved in a system the lower the probability of any one having a high value of Z. This theoretical view is borne out by much work in transgenic plants (reviewed by Stitt and Sonnewald, 1995), where components of metabolism have been modified by insertion of antisense genes. Even the sensitivity (or *flux* control) coefficient of Rubisco was shown to be relatively low (0.2–0.3) at saturating light intensities, and lower still (0.1–0.2) under non-saturating light conditions (Lauerer *et al.*, 1993).

The difficulties inherent in improving complex systems, because they involve many genes, is why an integrated approach to plant improvement is crucial to its success. Improvement of photosynthetic and other traits must be complementary so that potential benefits of individual genetic manipulations can be realized through their synergy, in the absence of which they may well be partially or completely masked. This is almost certainly why empirical plant selection (which allows superior phenotypes to emerge experimentally) has been successful, and ideotype breeding not so (Rasmusson, 1991). The power of empirical selection for raising yield potential is that by crossing diverse high yielding parents with good combining ability, there is a good probability of creating new synergistic gene combinations. After selecting progeny in early generations based on a knowledge of high yielding 'agronomic types', favourable epistatic or additive gene action can then be identified in subsequent yield trials. Since good agronomic performance in a genotype requires complementarity among many different traits, recombinant lines are usually inferior to both parents (traits conferring superior agronomic performance not having been fixed during the evolution of the genome). However, there is a small chance that new allelic combinations at target genes will be identified which outperform the parents. That is the aim of the breeding/selection process; driven by number of crosses made and population size it permits low frequency events to be observed and selected for. As additive effects are fixed in a mature breeding programme, progress for complex traits like yield and RUE will depend more on epistasis or background effects, therefore making genetic progress more difficult to attain. Simulation tools for modelling complex genotype–environment systems are being developed to help improve the probability of making genetic gains for complex traits and trait combinations within a target population of environments (Podlich and Cooper, 1998). The use of physiological tools to help improve the efficiency of empirical breeding will be discussed later.

Traits taken into account during visual selection apart from disease resistance, include the potential to tiller when needed, desired plant height, elevation of spikes above the foliage, similar spike height, lack of excessive leaf tip necrosis, certain level of 'stay green' of flag-leaf, spikelet and spike fertility, lack of tip- or base spike sterility, lack of awn malformations, lodging tolerance, and adaptation to targeted planting method. These traits represent the minimum requirements of a 'desirable agronomic type'. Within these restrictions a breeding programme may pursue several different ideotypes that yield consistently well.

Application of genomics to identify the role of the individual genes for photosynthesis

As outlined above, the effect of altering a single gene of photosynthesis cannot be predicted by studying the effect of the enzyme activity in isolation. The net change in flux of this modification as expressed in the phenotype can only be effectively measured *in vivo* and, because individual genes have limited impact on the plant phenotype, they are difficult to identify. The relationship between genotype and phenotype is a major conceptual bottleneck in modern genetics, especially for the improvement of very polygenic trait like photosynthesis. However, technology is starting to emerge which may improve the study of gene regulation and identify key physiological pathways in complex systems.

Using new hybridization technologies on microarrays or DNA chips (Brownstein *et al.*, 1998), it may be possible to determine the relative importance of each gene involved in photosynthesis at different phenological stages, and under different environmental conditions. RNA is extracted at a certain plant stage from the relevant tissue of the organism being studied and labelled cDNA or cRNA is generated from the RNA extracted. Probes are hybridized with the cDNA immobilized on the microarray, which is then optically scanned. The pattern of hybridization reveals the precise spectrum of gene expression that was being realized when the RNA was extracted. Such information, when analysed at different crop stages, under a range of environmental conditions and with a number of phenotypes expressing genetic variability in photosynthetic capacity, should permit a more quantitative understanding of the control of photosynthesis. They are also several other technologies based on mRNA-profiling analysis, for example, CuraGen technology, to quantify gene expression under various conditions.

In this approach mRNA is harvested from target plant tissue under different conditions, and/or at different timing. The cDNA obtained from the mRNA is then digested with several frequent cutter enzymes producing a typical profile, revealed on gel-based analysis. Based on the restriction sites of the endonucleases and the size of the fragments, a virtual mRNA can be constructed and the gene encoding for this mRNA identified using the EST database. This technique was successfully reported in a recent test case (Bruce *et al.*, 1999), when it was possible to identify the complete pathway of flavanoid synthesis in maize after it had been elaborated biochemically. To complement the genetic dissection of complex pathways, other approaches, like the candidate gene, might be relevant. This approach involves the identification of candidate genes based on their function and expression, in relation to their genomic location (De Vienne *et al.*, 1996). This approach is especially relevant as quantitative trait loci (QTL) for traits related to RUE (e.g. biomass or canopy traits) have already been identified in segregating populations. This set of new molecular tools may provide insights into how combinations of genetic modifications at a number of loci could improve photosynthetic efficiency. However, it should be remembered that such processes, being the interaction of many genes which are expressed at multiple levels (i.e. in time, tissue and dosage), will require considerable investment before they are well understood at the level of genetic control.

Using surrogates of photosynthetic rate to select for performance

Measurement of leaf photosynthetic rate in field plots is a slow, painstaking process, even with modern apparatus, permitting no more than a few dozen plots to be evaluated in a single day. Clearly, direct measurement of photosynthetic rate of a plot is not a practical consideration in plant selection. Evidence is accumulating that certain physiological traits related to stomatal aperture do have potential application to breeding. For example, genetic differences in leaf canopy temperature can be observed among wheat lines and the trait is associated with differences in stomatal conductance and leaf photosynthetic rate (Amani *et al.*, 1996; Fischer *et al.*, 1998).

Canopy temperature depression

Leaf temperatures are cooled when water evaporates from their surface, a trait affected directly by stomatal conductance, which is affected by feedback mechanisms of other processes such as carbon fixation and vascular transport of water. Canopy temperature depression, therefore, is a good indicator of a genotype's physiological fitness, since a high value will be indicative of good expression for all of those traits under a given set of environment conditions. The trait can be measured in a few seconds with an infrared thermometer, which measures the surface temperature of a field plot. Since the reading integrates the temperatures of plant leaves and spikes over a small area of the canopy, error associated with plant to plant variability is reduced. CTD measured on irrigated yield trials showed a good association with plot performance, but in addition to being a good predictor of yield *in situ*, CTD showed a significant association with performance of the same lines grown at a number of target breeding locations (Reynolds *et al.*, 1994). Further work confirmed the potential for making genetic gains in response to selection for CTD in recombinant inbred lines (Reynolds *et al.*, 1998). Recently, CIMMYT breeders successfully used CTD measured on small plots in their heat tolerance nurseries to identify the highest yielding entries. Genetic correlation coefficients of 0.6–0.8 were observed between final yield and CTD measured during grain filling, indicating the potential of this technique to pre-screen for physiological potential, prior to the execution of expensive yield trials (M van Ginkel *et al.*, unpublished data).

Table 4. *Comparison of canopy temperature depression (CTD) data from aerial infrared (IR) imagery with hand-held IR thermometers, Obregon, 1996–1997 (adapted from Reynolds* et al.*, 1999)*

Trial	*n*	Correlation of CTD with yield			
		Aerial		Hand-held	
		Phenotypic	Genetic	Phenotypic	Genetic
RILs (Seri82*7C66) random derived sisters	81	0.40**	0.63**	0.50**	0.78**
Advanced lines bread wheat	58	0.34**	–	0.44**	–

** Denotes statistical significance at 0.01 level of probability; – genetic correlations not calculated due to design restrictions.

Aerial infrared imagery

Recent results from NW Mexico showed that aerial infrared (IR) images collected at a height of 800 m had sufficient resolution to detect CTD differences on relatively small plots (1.6 m wide). The data, collected from an IR radiation sensor mounted on a light aircraft, showed positive correlations with final grain yield for a set of random derived recombinant inbred lines as well as a set of advanced breeding lines (Table 4). The results indicate the potential of aerial IR imagery as a means of screening thousands of breeding lines in a few hours for CTD (Reynolds *et al.*, 1999).

Spectral reflectance

Another technique which may have application in screening for physiologically superior progeny is spectral reflectance (SR) which can be used to estimate a range of physiological characteristics including canopy chlorophyll content, absorbed PAR, leaf area index, and plant water status (Araus, 1996). These traits are associated with absorption of very specific wavelengths of radiation, for example, water absorbs energy at 970 nm. Solar radiation reflected by the crop is measured, and calibrated against light reflected from a white surface. Different coefficients can be calculated from specific bands of the crop's absorption spectrum, giving a semi-quantitative estimate (or index) of a number of such characteristics. Preliminary studies showed significant associations between crop performance and a number of SR indexes. (Reynolds *et al.*, 1999).

Conclusion: an integrated approach to plant breeding

Given the nature of empirical breeding, the fact that genetic progress in wheat yield potential to date has come mostly from improved partitioning suggests two things: (i) sink limitation has been more limiting to yield than assimilation capacity; (ii) RUE is genetically much more complex to improve than sink capacity and is, therefore, a less frequent outcome of genetic recombination. This trend will need to change as partitioning approaches its theoretical limits. By combining information coming to light on the genetic limitations to crop photosynthetic rate with new physiological selection tools, the probability of improving the RUE of the wheat crop through plant breeding should be significantly increased. Parents can be selected for improved biochemical, physiological and anatomical traits and crossed to high yielding agronomically élite materials. Good performance in a genotype requires complementarity among a number of different traits so that higher RUE can be expressed. By promoting large numbers of progeny in the breeding process there is a chance of identifying phenotypes where favourable interactions among genes permit the expression of higher RUE. The probability of selecting these lines can be enhanced by (i) eliminating inferior agronomic phenotypes visually in early generations, (ii) selecting superior physiological phenotypes using rapid detection techniques such as IR- thermometry in intermediate generations, and (iii) selecting for higher performance in yield trials in advanced generations.

Acknowledgements

The authors would like to thank Gustavo Slafer for critically reviewing the manuscript, and Ravi Singh for providing the *Rht* isolines used to produce data for Fig. 1.

References

Abbate PE, Andrade FH, Culot JP, Bindraban PS. 1997. Grain yield in wheat: effects of radiation during spike growth period. *Field Crops Research* **54,** 245–257.

Amani I, Fischer RA, Reynolds MP. 1996. Canopy temperature depression association with yield of irrigated spring wheat cultivars in hot climate. *Journal of Agronomy and Crop Science* **176,** 119–129.

Amthor JS. 1989. *Respiration and crop productivity*. Berlin: Springer-Verlag.

Angus JF, Jones R, Wilson JH. 1972. A comparison of barley cultivars with different leaf inclinations. *Australian Journal of Agricultural Research* **23,** 945–957.

Araus JL, Reynolds MP, Acevedo E. 1993. Leaf posture, grain yield, growth, leaf structure, and carbon isotope discrimination in wheat. *Crop Science* **33,** 1273–1279.

Araus JL. 1996. Integrative physiological criteria associated with yield potential. In: Reynolds MP, Rajaram S, McNab A, eds. *Increasing yield potential in wheat: breaking the barriers*. Mexico: CIMMYT, 150–160.

Austin RB, Bingham J, Blackwell RD, Evans LT, Ford MA, Morgan CL, Taylor M. 1980. Genetic improvement in winter wheat yields since 1900 and associated physiological changes. *Journal of Agricultural Science* **94,** 675–689.

Austin RB, Ford MA, Morgan CL. 1989. Genetic improvement in the yield of winter wheat: a further evaluation. *Journal of Agricultural Science* **112,** 295–301.

Austin RB, Morgan CL, Ford MA, Bhagwat SG. 1982. Flag leaf photosynthesis of *Triticum aestivum* and related diploid and tetrapolid species. *Annals of Botany* **49,** 177–189.

Austin RB. 1999. Yield of wheat in the UK: recent advances and prospects. *Crop Science* (in press).

Bingham J. 1969. The physiological determinants of grain yield in cereals. *Agricultural Progress* **44,** 30–42.

Björkman O, Demmig-Adams B. 1994. Regulation of photosynthetic light energy capture, conversion, and dissipation in leaves of higher plants. In: Schulze E-D, Caldwell MM, eds. *Ecophysiology of photosynthesis*. Ecology Studies 100. Berlin: Springer-Verlag, 17–70.

Brownstein MJ, Trent JM, Boguski MS. 1998. Functional genomics. *Trends Guide to Bioinformatics* Supplement, 1998.

Bruce W, Folkerts O, Garnaat C, Roth B, Solan FP, Brouwer C, Bowen B. 1999. mRNA profiling of the flavanoid genes controlled by inducible transcriptional factors, CRC and P. A*bstract from the 41st Annual Maize Genetics Conference*, 11–14 March 1999, Lake Geneva, Wisconsin, 39.

Calderini DF, Dreccer MF, Slafer GA. 1995. Genetic improvement in wheat yield and associated traits. a re-examination of previous results and the latest trends. *Plant Breeding* **114,** 108–112.

Calderini DF, Dreccer MF, Slafer GA. 1997. Consequences of plant breeding on biomass growth, radiation interception and radiation use efficiency in wheat. *Field Crops Research* **52,** 271–281.

Calderini DF, Reynolds MP, Slafer GA. 1999. Genetic gains in wheat yield and main physiological changes associated with them during the 20th century. In: Satorre EH, Slafer GA, eds. *Wheat: ecology and physiology of yield determination*. New York: Food Products Press.

Calderini DF, Reynolds MP. 2000. Changes in grain weight as a consequence of de-graining treatments at pre- and post-anthesis in synthetic hexaploid lines of wheat (*Triticum durum* × *T. tauschii*). *Australian Journal of Plant Physiology* **27,** (in press).

Carvalho FIF, Qualset CO. 1978. Genetic variation for canopy architecture and its use in wheat breeding. *Crop Science* **18,** 561–567.

Carver BF, Nevo E. 1990. Genetic diversity of photosynthetic characters in native populations of *Triticum dicoccoides*. *Photosynthesis Research* **25,** 119–128.

Charles-Edwards DA. 1978. An analysis of the photosynthesis and productivity of vegetative crops in the United Kingdom. *Annals of Botany* **42,** 717–731.

Chen, T.Y., Skovmand, B.S., Rajaram, S., Reynolds, M.P. 1998. Novel source of increased spike fertility in wheat multi-seeded flowers. *Agronomy Abstracts*, 1998.

Davies WJ, Zhang J. 1991. Root signals and the regulation of growth and development in drying soil. *Annual Review of Plant Physiology and Plant Molecular Biology* **42,** 55–76.

Day W, Chalabi ZS. 1988. Use of models to investigate the link between the modification of photosynthetic characteristics and improved crop yields. *Plant Physiology and Biochemistry* **26,** 511–517.

De Vienne D, Burstin J, Gerber S, Leonardi A, Le Guilloux M, Murigneux A, Beckert M, Bahrman N, Damerval C, Zivy M. 1996. Two-dimensional electrphoresis of proteins as a source of monogenic and codominant markers for population genetics and mapping the expressed genome. *Heredity* **76,** 166–177.

Delgado E, Medrano H, Keys AJ, Parry MAJ. 1995. Species variation in Rubisco specificity factor. *Journal of Experimental Botany* **292,** 1775–1777.

Dencic S. 1994. Designing a wheat ideotype with increased sink capacity. *Plant Breeding* **112,** 311–317.

Drake BG, Gonzalez-Meler MA. 1997. More efficient plants: a consequence of rising atmospheric CO_2? *Annual Review of Plant Physiology and Plant Molecular Biology* **48,** 609–639.

Dreccer MF, Slafer GA, Rabbinge R. 1998. Optimization of vertical distribution of canopy nitrogen: an alternative trait to increase yield potential in winter cereals. *Journal of Crop Production* **1,** 47–77.

Duncan WG. 1971. Leaf angles, leaf area, and canopy photosynthesis. *Crop Science* **11,** 482–485.

Ehleringer J, Forseth I. 1980. Solar tracking by plants. *Science* **210,** 1094–1098.

Evans JR. 1993. Photosynthetic acclimation and nitrogen partitioning within a lucerne canopy. I. Canopy characteristics. *Australian Journal of Plant Physiology* **20,** 55–67.

Evans LT. 1993. *Crop evolution: adaptation and yield.* Cambridge: Cambridge University Press.

Evans LT, Dunstone RL. 1970. Some physiological aspects of evolution in wheat. *Australian Journal of Biological Sciences* **23,** 725–741.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* **149,** 78–90.

Fischer RA. 1983. Wheat. In: Smith WH, Banta JJ, eds. *Potential productivity of field crops under different growth conditions*. Los Baños, Philippines; IRRI, 129–154.

Fischer RA. 1985. Number of kernels in wheat crops and the influence of solar radiation and temperature. *Journal of Agricultural Science* **108,** 447–461.

Fischer RA. 1996. Wheat physiology at CIMMYT and raising the yield plateau. In: Reynolds MP, Rajaram S, McNab A, eds. *Increasing yield potential in wheat: breaking the barriers*. Mexico: CIMMYT, 195–203.

Fischer RA, Quail KJ. 1990. The effect of major dwarfing genes on yield potential in spring wheats. *Euphytica* **46,** 51–56.

Fischer RA, Rees D, Sayre KD, Lu Z-M, Condon AG, Larque-Saavedra A. 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. *Crop Science* **38,** 1467–1475.

Gale MD, Youssefian S. 1985. Dwarfing genes in wheat. In: Russel E, ed. *Progress in plant breeding*. London: Butterworth and Co., 1–35.

Gent MPN, Kiyomoto, RK. 1998. Physiological and agronomic consequences of *Rht* genes in wheat. *Crop Science: Recent Advances* 27–46.

Gilmore AM. 1997. Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplasts and leaves. *Physiologia Plantarum* **99,** 197–209.

Goudriaan J, van Laar HH. 1994. Modelling potential crop growth processes: A textbook with exercises. *Current issues in production ecology*, Vol. 2. The Netherlands: Kluwer Academic Publishers, 238.

Gutiérrez-Rodríguez M, Reynolds MP, Larqué-Saavedra A. 2000. Photosynthesis of wheat in a warm, irrigated environment. II. Traits associated with genetic gains in yield. *Field Crop Research* (in press).

Harrison SA, Boerma HR, Ashley DA. 1981. Heritability of canopy apparent photosynthesis and its relationship to seed yield in soybeans. *Crop Science* **21,** 222–226.

Innes P, Blackwell RD. 1983. Some effects of leaf posture on the yield and water economy of winter wheat. *Journal of Agricultural Science* **101,** 367–376.

Kacser H, Burns JA. 1981. The molecular basis of dominance. *Genetics* **97,** 639–666.

King RW, Wardlaw IF, Evans LT. 1967. Effect of assimilate utilization on photosynthetic rate in wheat. *Planta* **77,** 261–276.

Koshkin EI, Tararina VV. 1989. Yield and source/sink relations of spring wheat cultivars. *Field Crops Research* **22,** 297–306.

Kruck BC, Calderini DF, Slafer GA. 1997. Grain weight in wheat cultivars released from 1920 to 1990 as affected by post-anthesis defoliation. *Journal of Agricultural Science* **128,** 273–281.

Kubiske ME, Pregitzer KS. 1996. Effects of elevated CO_2 and light availability on the photosynthetic light response of trees of contrasting shade tolerance. *Tree Physiology* **16,** 351–358.

Kulshrestha VP, Jain HK. 1982. Eighty years of wheat breeding in India: past selection pressures and future prospects. *Zeitschrift für Pflanzenzüchtung* **89,** 19–30.

Lauerer M, Saftic D, Quick WP, Fichtner K, Schulze ED *et al.* 1993. Decreased ribulose-1.5-bisphosphate in transgenic tobacco transformed with 'antisense' rbcS. VI. Effects on photosynthesis in plants grown at different irradiance. *Planta* **190,** 332–345.

Loomis RS, Amthor JS. 1996. Limits to yield revisited. In: Reynolds MP, Rajaram S, McNab A, eds. *Increasing yield potential in wheat: breaking the barriers*. Mexico: CIMMYT, 76–89.

Lu Z, Percy RG, Qualset CO, Zeiger E. 1998. Stomatal conductance predicts yields in irrigated Pima cotton and bread wheat grown at high temperatures. *Journal of Experimental Botany* **49,** 453–460.

McCree KJ. 1971. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. *Agricultural Meteorology* **9,** 191–216.

Miralles DF, Katz SD, Colloca A, Slafer GA. 1998. Floral development in near isogenic wheat lines differing in plant height. *Field Crops Research* **59,** 21–30.

Miralles DF, Slafer GA. 1997. Radiation interception and radiation use efficiency of near isogenic wheat lines with different height. *Euphytica* **97,** 201–208.

Miralles DJ, Richards RA. 1999. Sensitivity to photoperiod during the reproductive phase changes grain number in wheat and barley. *Proceedings of The Australian Society of Plant Physiology*, 28 September–3 October, 1998. Adelaide, Australia.

Murchie EH, Chen Y, Hubbart S, Peng S, Horton P. 1999. Interactions between senescence and leaf orientation determine in situ patterns of photosynthesis and photoinhibition in field-crown rice. *Plant Physiology* **119,** 553–564.

Nelson CJ. 1988. Genetic associations between photosynthetic characteristics and yield: review of the evidence. *Plant Physiology and Biochemistry* **26,** 543–554.

Nobel P. 1991. Biochemical and environmental plant physiology. San Diego:Academic Press.

Ort DR, Baker NR. 1988. Consideration of photosynthetic efficiency at low light as a major determinant of crop photosynthetic performance. *Plant Physiology and Biochemistry* **26,** 555–565.

Ortelli S, Winzeler H, Winzeler M, Fried PM, Nosberger J. 1996. Leaf rust resistance gene Lr9 and winter wheat yield reduction. II. Leaf gas exchange and root activity. *Crop Science* **36,** 1595–1601.

Osmond CB, Grace SC. 1995. Perspectives on photoinhibition and photorespiration in the field. Quintessential inefficiencies of the light and dark reactions of photosynthesis? Journal of Experimental Botany **46,** 1351–1362.

Parry MAJ, Keys AJ, Gutteridge S. 1989. Variation in the specificity factor of C_3 higher plant Rubiscos determined by the total consumption of ribulose-P_2. *Journal of Experimental Botany* **40,** 317–320.

Podlich DW, Cooper M. 1998. QU-GENE: a simulation platform for quantitative analysis of genetic models. *Bioinformatics* **14,** 632–653.

Puckridge DW. 1971. Photosynthesis of wheat under field conditions. III. Seasonal trends in carbon dioxide uptake of crop communities. *Australian Journal of Agricultural Research* **22,** 1–9.

Rasmusson DC. 1991. A plant breeder's experience with ideotype breeding. *Field Crops Research* **26,** 191–200.

Read BA, Tabita FR. 1994. High substrate specificity factor ribulose bisphosphate carboxylase/oxygenase from eukaryotic marine algae and properties of recombinant cyanobacterial Rubisco containing 'Algal' residue modifications. Archives of Biochemistry and Biophysics **312,** 210–218.

Reynolds MP, Balota M, Delgado MIB, Amani I, Fischer RA. 1994. Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. Australian Journal of Plant Physiology **21,** 717–730.

Reynolds MP, Delgado MI, Gutierrez-Rodríguez M, Larqué-Saavedra A. 2000. Photosynthesis of wheat in a worm irrigated environment. I. Genetic diversity and crop productivity. *Field Crops Research* (in press).

Reynolds MP, Sayre KD, Rajaram S. 1999. Physiological and genetic changes of irrigated wheat in the post-green revolution period and approaches for meeting projected global demand. *Crop Science* **39,** 1611–1621.

Reynolds MP, Singh RP, Ibrahim A, Ageeb OAA, Larque Saavedra A, Quick JS. 1998. Evaluating physiological traits to complement empirical selection for wheat in warm environments. *Euphytica* **100,** 84–95.

Richards RA. 1996. Defining selection criteria to improve yield under drought. *Plant Growth Regulation* **20,** 57–166.

Rosengrant MW, Agcaoili-Sombilla M, Perez ND. 1995. *Global food projections to 2020: implications for investment*. Washington, DC: IFPRI.

Sayre KD, Rajaram S, Fischer RA. 1997. Yield potential progress in short bread wheats in northwest Mexico. *Crop Science* **37,** 36–42.

Seybold A. 1933. Über die optischen Eigenschaften der Läubblatter IV. *Planta* **21,** 251.

Singh RP, Huerta-Espino J, Rajaram S, Crossa J. 1998. Agronomic effects from chromosome translocations 7DL.7Ag and 1BL.1RS in spring wheat. *Crop Science* **38,** 27–33.

Slafer GA, Andrade FH, Storre EH. 1990. Genetic improvement effects on pre-anthesis physiological attributes related to wheat grain yield. *Field Crops Research* **23,** 225–263.

Slafer GA, Calderini DF, Miralles DJ. 1996. Yield components and compensation in wheat: Opportunities for further increasing yield potential. In: Reynolds MP, Rajaram S, McNab A, eds. *Increasing yield potential in wheat: breaking the barriers*. Mexico: CIMMYT, 101–134.

Slafer GA, Rawson HM. 1994. Sensitivity of wheat phasic development to major environmental factors: a re-examination of some assumptions made by physiologists and modelers. *Australian Journal of Plant Physiology* **21,** 393–426.

Slafer GA, Satorre EH, Andrade FH. 1994. Increases in grain yield in bread wheat from breeding and associated physiological changes. In: Slafer GA, ed. *Genetic improvement of field crops*. New York: Marcel Dekker, Inc., 1–68.

Slafer GA, Savin R. 1994. Sink–source relationships and grain mass at different positions within the spike in wheat. *Field Crops Research* **37,** 39–49.

Stitt M, Schulze E-D. 1994. Does Rubisco control the rate of photosynthesis and plant growth? An exercise in molecular eco-physiology. *Plant Cell Environment* **17,** 465–488.

Stitt M, Sonnewald U. 1995. Regulation of metabolism in transgenic plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **46,** 341–368.

Tardieu F, Simmonneau T. 1998. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviour. *Journal of Experimental Botany* **49,** 419–432.

Tollenaar M, Wu J. 1999. Yield improvement in temperate maize is attributable to greater stress tolerance. *Crop Science* **39,** 1597–1604.

Uemura K, Anwaruzzaman S, Miyachi S, Yokota A. 1997. Ribulose-1,5-bisphosphate carboxylase/oxygenase from thermophilic red algae with a strong specificity for CO_2 fixation. *Biochemistry and Biophysics Research Communications* **233,** 568–571.

Van Keulen H, 1976. A calculation method for potential rice production. *Contributions of the Centre for Research, Institute for Agriculture, Bogorra* **21,** 26.

Vargas M, Crossa J, Sayre K, Reynolds MP, Ramírez ME, Talbot M. 1998. Interpreting genotypes by environment interaction in wheat by partial least square regression. *Crop Science* **38,** 679–689.

Waddington SR, Osmanzai M, Yoshida M, Ransom JK. 1987. The yield of durum wheats released in Mexico between 1960 and 1984. *Journal of Agricultural Science* **108,** 469–477.

Waddington SR, Ransom JK, Osmanzai M, Saunders DA. 1986. Improvement in the yield potential of bread wheat adapted to northwest Mexico. *Crop Science* **26,** 698–703.

Weir AH, Bragg PL, Porter JR, Rayner JH. 1984. A winter wheat crop simulation model without water or nutrient limitations. *Journal of Agricultural Science, Cambridge* **102,** 371–382.

Weis E, Berry J. 1989. Quantum efficiency of PSII in relation to energy dependent quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **894,** 198–208.