Yield, carbon isotope discrimination, canopy reflectance and cuticular conductance of barley isolines of differing glaucousness

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Abstract

The roles of wax bloom (glaucousness) in discrimination against stable isotope ^{13}C (Δ) and yield were studied for 2 years on a pair of near-isogenic lines of two-rowed barley (*Hordeum vulgare* L.) differing in their degree of glaucousness of the spike and the upper vegetative parts of the plant other than leaf blades. The effects of glaucousness on canopy reflectance and cuticular conductance were also assessed. Plants were grown under fully irrigated and rainfed Mediterranean conditions for 2 years. Grain yield for both years and total straw biomass measured in the second year were higher in glaucous than non-glaucous lines under both irrigated and rainfed conditions. Δ followed the same pattern in both grain and straw with the highest values in the glaucous line under irrigation and the lowest in the non-glaucous line under rainfed conditions. Differences in yield and Δ between the lines were greater under irrigated than rainfed conditions. The higher Δ of the glaucous line suggests that these plants had lower transpiration efficiency (net photosynthesis to transpiration ratio) than the non-glaucous line in the long term. Canopy reflectance at some of the wavelengths of the *PAR* region (400–700 nm) was significantly higher in the glaucous than in the non-glaucous line under both irrigated and rainfed conditions. Rainfed conditions also increased canopy reflectance in the *PAR* region. No significant effect of either glaucousness or water regime was observed in cuticular conductance or amount of cuticular waxes of the flag leaf blades or the ear.

Introduction

Glaucousness is the waxy covering (crystallized epicuticular waxes) that imparts a dull-white or bluish-green cast commonly referred to as bloom. This is one characteristic that has been considered a plant adaptation to drought (Blum, 1988). Glaucousness is not always related to wax quantity, but is associated with wax deposits that cause light dispersion (Juniper and Jeffree, 1983; Johnson *et al.*, 1983; Blum, 1986).

Breeding for increased water-use efficiency (*WUE*: dry matter produced/water transpired) has been limited by the lack of screening criteria that could be used to select desirable genotypes from large populations under field conditions. Glaucousness is believed to enhance yield of bread wheat (Richards, 1984), durum wheat (Chatterton *et al.*, 1975) in water-limited environments by increasing WU*E* or transpiration efficiency (*TE*: net photosynthesis/transpiration). Fischer and Wood (1979) found that the best prediction of yield under drought from traits measured in the absence of drought was given by a linear model containing an index for degree of leaf glaucousness.

An important role of epicuticular waxes (*EW*) related to *TE* seems to be the enhancement of leaf reflectance. Absorbed radiation (in the visible and near infra-red wavelengths) decreases, and thus leaf temperature may be reduced by the presence of *EW* (Blum, 1975*a, b*). This leads to a reduction in vapour pressure differences between the inside of the leaf and the ambient air which,

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Abbreviations: EW, epicuticular waxes; g_c, cuticular conductance; Δ, carbon isotope discrimination; *TE*, transpiration efficiency; IR, irrigated; R, rainfed.

in turn, may reduce transpirational losses and increase *TE*. Another important role of EW related to *TE* could be to increase the efficiency of stomatal control by reducing water loss through the cuticle after stomata closure (Blum, 1982; Clarke and Richards, 1988). Non-stomatally-controlled water loss through the leaf epidermis may account for up to 50% of total transpiration in drought-stressed wheat plants during the day and 100% during the night (Rawson and Clarke, 1988). Higher yielding lines of durum wheat show higher flag leaf water retention with a weak, but significant, correlation with yield (Clarke and McCaig, 1982). Similar results are reported for bread wheat under drought (Jaradat and Konzak, 1983), although the relationship under more favourable conditions is unclear (Clarke and Romagosa, 1991).

Within C$_3$ species discrimination against the stable ^{13}C isotope ($\varDelta$) of plant matter provides a time integrated measurement of the ratio of the intercellular to atmospheric partial pressures of CO$_2$ (p_i/p_a) (Farquhar and Richards, 1984; Farquhar *et al.*, 1989). The value of this ratio results from the interaction between net assimilation rate and leaf conductance through the leaf (basically the stomata). Not only has variation in $\varDelta$ been correlated with instantaneous measurements of TE, but also, when $\varDelta$ is analysed in dry matter (for plants grown under similar steady-state *VPD* conditions), it provides an integrated measurement of the *TE* (and thus of *WUE*) during the previous growth period (Farquhar and Richards, 1984; Farquhar *et al.*, 1989; Hubick and Farquhar, 1989).

The effect of glaucousness on $\varDelta$ and yield of barley under irrigated and rainfed Mediterranean conditions is reported here. Canopy reflectance, cuticular conductance and amount of epicuticular waxes were also evaluated to gain insight into the ecophysiological mechanisms underlying the effect of glaucousness on $\varDelta$ and yield. Although glaucousness is commonly reported to appear on vegetative parts of cereals (Dakheel *et al.*, 1994) and there is evidence that it increases yield in barley (Baenziger *et al.*, 1983), there is little information concerning the role of glaucousness on the ear of barley. Indeed under Mediterranean conditions photosynthesis by the ear contributes more to yield than the leaf blades (Bort *et al.*, 1994).

Materials and methods

Plant material and growth conditions

A pair of near-isogenic lines (isolines) of two-row spring barley (*Hordeum vulgare* L.) was used, showing either the presence or absence of glaucousness in the spike, peduncle and leaf sheath (Febrero and Araus, 1994). They were derived from cv. Troubadour following treatment with sodium azide (Molina-Cano *et al.*, 1989) and have similar phenology and growth pattern under field conditions. Plants were grown under rainfed and well-irrigated conditions in two successive years at the

Experimental Fields of the University of Barcelona. Planting was on 4 January 1991 for the 1991 study and late December 1991 for the 1992 study. Two 1.5 × 2 m plots, consisting of six rows 25 cm apart, were assayed per genotype and growth condition. Accumulated rainfall during the plant cycle (January–May) was 328.3 mm for 1991 and 161.4 mm for 1992. After the beginning of stem elongation, plots from the irrigation treatment were watered three times a week (5 mm for each application) until maturity. Glaucousness developed, as has been observed in bread and durum wheat (Richards *et al.*, 1986; Dakheel *et al.*, 1994), at stem elongation and reached its maximum expression at heading and anthesis. Therefore, canopy reflectance was measured near anthesis, and flag leaves and ears were obtained at anthesis and 2 weeks after. Grain yield and straw biomass were recorded at maturity.

Carbon-isotope analysis

The ^{13}C/^{12}C ratios were determined on kernels and straw at maturity by mass spectrometry at Isotope Services Inc (Los Alamos, NM). Samples were sent to Isotope Services as dried powdered plant material. There they were weighed in tin capsules and processed by an NA 1500 Carlo-Erba elemental analyser by Dumas combustion with the carbon dioxide gas flowing to a VG Isomass mass spectrometer. Normally, analyses were performed in duplicate. If they did not agree then another duplicate pair was loaded. Reference gas was run with each analysis, and standards and reference materials were interspersed among the samples. Total carbon was measured both by the thermal conductivity detector of the NA 1500 and by the ion current intensities of the mass spectrometer. Values were expressed as carbon isotope composition (δ^{13}C) values, where:

$$\delta^{13}\text{C } (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}})-1] \times 1000$$

where R is the ^{13}C/^{12}C ratio of the sample and standard. The standard for comparison was a secondary standard calibrated against PeeDee belemnite (PDB) carbonate. Replicate samples differed by less than 0.10‰.

The value of $\varDelta$ was obtained from (Farquhar *et al.*, 1989):

$$\varDelta = \frac{\delta_a - \delta_p}{1 + \delta_p}$$

where δ_a and δ_p refer to air and plant, respectively. On the PDB scale, free atmospheric CO$_2$ has a current deviation, δ_a, of approximately -7.85‰ (Keeling *et al.*, 1989).

TE estimation

TE was calculated from the $\varDelta$ values as based on the model given by Farquhar *et al.* (1982), $\varDelta$ in C$_3$ plants is related to the ratio of the intercellular to atmospheric partial pressure of CO$_2$ (p_i/p_a) by:

$$\varDelta = a + (b-a) \ (p_i/p_a)$$

where a and b are the ^{13}C discriminations due to diffusion in air (4.4‰) and are caused by carboxylation (mainly) by the enzyme RuBP carboxylase (27‰), respectively. If leaf (or other photosynthetic organ) temperature is assumed to be close to ambient and ambient relative humidity during daylight is known, *TE* (measured as the ratio of CO$_2$ assimilation to transpiration) may be defined as a function of p_i/p_a:

$$TE = p_a(1-p_i/p_a)/V(1-RH)1.6$$

where V is the saturated partial water vapour pressure (mbar) at a given temperature and ambient relative humidity (Farquhar *et al.*, 1982). Thus, the following equation allows the estimation

Table 1. *Discrimination against* ^{13}C *(Δ) obtained from mature kernels harvested in 1991 and 1992 and from straw harvested in 1992*

p_i/p_a and *TE* were calculated using Δ equations (see Materials and methods). *TE* was calculated considering the same temperatures for glaucous and non-glaucous plants ($T_{NG} = T_G$), and also assuming that non-glaucous plant temperature was 1 °C higher than glaucous plants ($T_{NG} = T_G + 1$ °C). IR (irrigated), R (rainfed), G (glaucous line), NG (non-glaucous line).

Water treatment	Lines	Δ (‰)	p_i/p_a	*TE* (μmol CO$_2$ mmol^{-1} H$_2$O)	
				$T_{NG} = T_G$	$T_{NG} = T_G + 1$ °C
1991 Grain					
IR	G	19.7	0.68	2.70	2.70
	NG	18.2	0.61	3.27	3.08
R	G	18.2	0.61	3.26	3.26
	NG	18.0	0.60	3.34	3.14
1992 Grain					
IR	G	19.2	0.66	2.68	2.68
	NG	18.2	0.61	3.01	2.84
R	G	18.3	0.62	2.97	2.97
	NG	17.5	0.58	3.25	3.06
1992 Straw					
IR	G	19.0	0.65	2.88	2.88
	NG	18.9	0.64	2.91	2.74
R	G	17.9	0.60	3.29	3.29
	NG	17.7	0.59	3.34	3.15

of *TE* from Δ values:

$$TE = [p_a]\{1 - (\Delta - 4.4)/22.6\}/V(1 - RH)1.6$$

Current p_a was assumed to be 355 μbar (Keeling and Whorf, 1992). Carbon discrimination of grain and straw was used for *TE* calculations. *TE* was calculated considering the same temperatures for glaucous and non-glaucous plant (Table 1: $T_{NG} = T_G$) with *T* being the mean of daily maximal temperature during grain filling period for grain (23.3 °C and 26.4 °C for 1991 and 1992, respectively), and during the two months before grain filling for straw (23.1 °C for 1992). *TE* was also calculated assuming that non-glaucous plants were 1 °C higher than glaucous plants (Table 1: $T_{NG} = T_G + 1$ °C) (Richards *et al.*, 1986). Noon relative humidity (*RH*) during grain filling was 58% (1991) and 53% (1992); for the previous two months it was 59.5% (1992).

Canopy reflectance

During 1992, a Barringer field spectroradiometer (Model Mark II), with nine filters between 400 and 2200 nm wave-lengths, was used for radiometric measurements. The centre wavelength of the filters was 485, 545, 560, 635, 660, 842, 910, 1667, and 2210 nm. The radiometer was held on a tripod about 1.5 m above the soil surface with a field of vision of about 0.25 m^2. A BaSO$_4$ panel was used to calibrate the reflectance of each spectral channel before and after the measurements at each of the target wavelengths. A minimum of six readings per plot was made at noon to reduce the effects of sun angle on reflectance. Mean reflectivities of the bare dry soil for each of the wavelengths assayed are reported elsewhere (Fernández *et al.*, 1994).

Cuticular conductance

Cuticular conductance of flag leaf blades and ears from the different genotypes and growth conditions were studied using the water loss rate of excised leaves, as reported earlier (Araus *et al.*, 1991). For 1992 three leaf blades and ears were excised per replicate, and six replicates were studied per isoline and growth condition. After organ excision, leaf blade area was quickly measured, leaves and ears were hydrated by placing the cut end into a beaker of water, and the plant parts and beaker were placed inside a plastic bag in the dark overnight at 4 °C. Leaves and ears were weighed to determine their saturated fresh weight. They were then placed horizontally on Petri dishes and wilted in a darkened growth cabinet mantained at 25–26 °C and 40% relative humidity. Leaves and ears were weighed with digital balances (0.1 mg precision) about every 20 min for 120 min to establish the pattern of water loss through time. For each replicate the cuticular transpiration was estimated as the slope of the post-20 min regression between water loss and time (Rawson and Clarke, 1988). The mean r^2 value for the regression was 0.97 or higher. After the wilting experiment, leaves and ears were oven-dried (24 h at 80 °C) and weighed again to determine dry weight. Cuticular conductance (g_c) was then calculated using standard diffusion equations (Coombs *et al.*, 1985), from the cuticular transpiration measured under defined evaporative conditions. Values of ears were expressed per unit dry weight.

*Quantification of epicuticular waxes*

In the 1992 study, epicuticular waxes were removed by stirring flag leaf blades, sheaths and ears in 30 ml of chloroform for 15 s at room temperature (Mayeux and Jordan, 1987), which provides almost complete dissolution of EW (Mayeux *et al.*, 1981). The chloroform extract was transferred to preweighed beakers, dried at 50 °C to near dryness and allowed to stand at room temperature for several days until stable weight (Blakeman and Atkinson, 1976). Non-volatile residues from 30 ml of chloroform were weighed and subtracted from wax weights. Wax weights were expressed per unit leaf (blade or sheath) or ear dry weight.

Results and discussion

Effect of epicuticular waxes on canopy reflectance

Reflectance in the photosynthetic active radiation region (*PAR*: 400–700 nm) was about 20% higher in the glaucous than non-glaucous line (Fig. 1b); the differences were greater at the shortest (485 nm) and longest (660 nm) wavelength measured. The effect of water regime during growth was greater than that of glaucousness. Thus *PAR* reflectance of both lines under rainfed conditions was 30–50% higher (depending on the wavelength) than that under irrigation. Differences in reflectance between lines and growth conditions within the near far infra-red region (700–2200 nm) were much lower (Fig. 1a). Indeed glaucousness, similar to pubescence and trichoma, increases reflectance within the *PAR* region (Blum, 1988).

Previous studies showed that these two lines differed in the presence of filamentous epicuticular waxes (EW) on the vertical parts of the plant (Febrero and Araus, 1994). Apart from thickening the boundary layer, wax filaments (tubes, rows, etc.) lower the incident radiation by increasing reflectance more than, for example, plates (Juniper and Jeffree, 1983; Blum, 1988), which are the typical EW
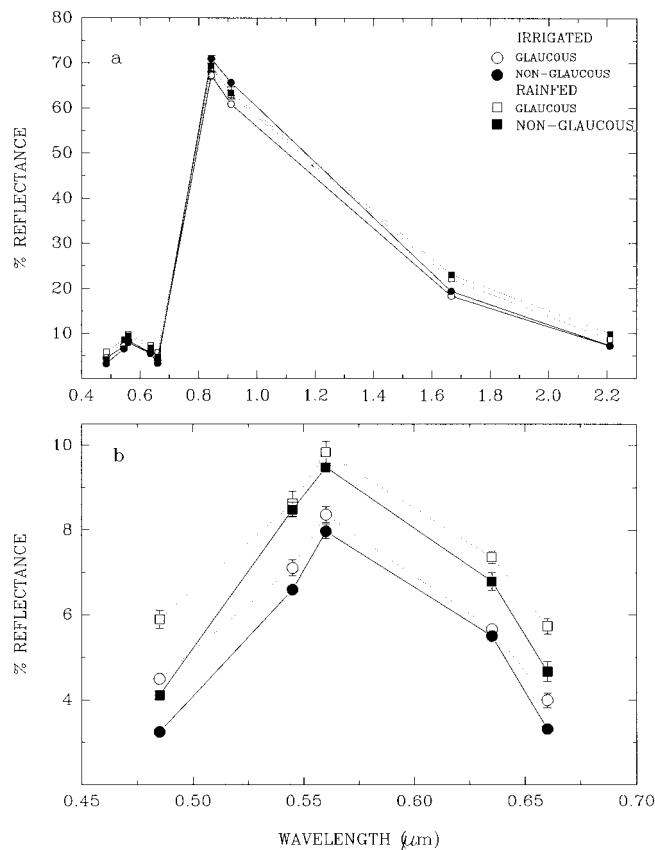
Fig. 1. (a) Percentage of incoming *PAR* plus near far infra-red (400–2200 nm) reflected from glaucous and non-glaucous barley isolines grown under irrigated and rainfed conditions. (b) Reflectance detailed in the *PAR* region. Values are means ±SE of two plots per treatment and isoline (four replicates per plot). Measurements were performed at anthesis during 1992. For 485, 660 and 635 nm wavelengths differences were significant ($P < 0.05$) for both treatment and glaucousness factors. For 545 nm there were significant differences for glaucousness factor only under irrigation. For 560 nm wavelengths differences were significant only for treatment factor.

Table 2. *Cuticular conductance for water vapour of flag leaves (mmol m^2 s^{-1}) and ears (mmol g^{-1} dw s^{-1}) from glaucous and non-glaucous isolines grown under irrigated and rainfed conditions*

Cuticular conductance values were estimated from the slope of the post-20 min regression data of excised organ water loss. Values represent the mean ±SE of six replicates. For each plant part means sharing different letters are significantly different ($P < 0.05$) by Duncan's comparison test.
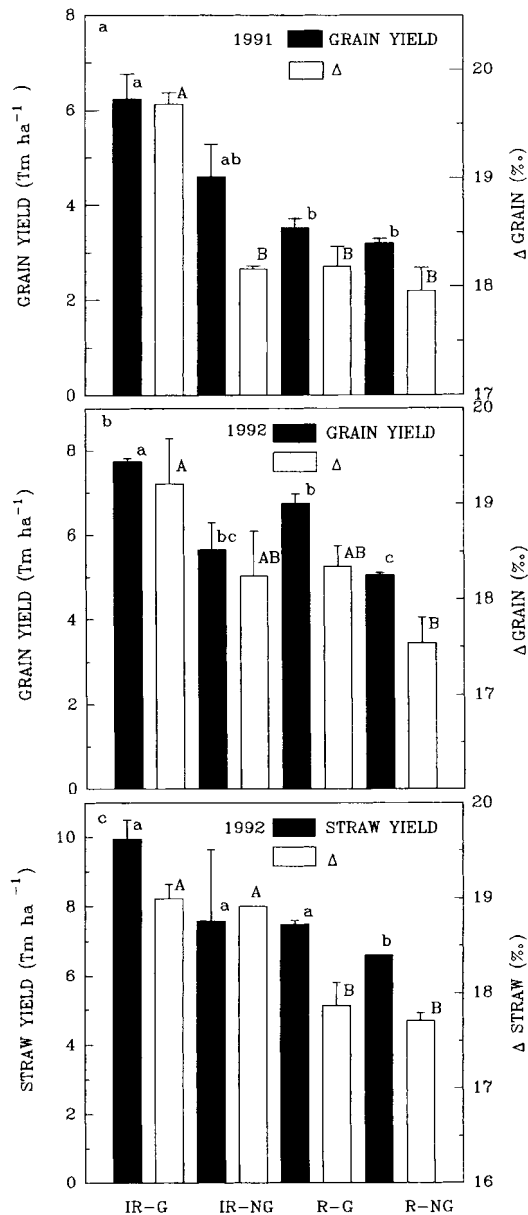
	Irrigated		Rainfed	
	Glaucous	Non-glaucous	Glaucous	Non-glaucous
Flag blade	17.26 ± 1.40^a	21.92 ± 1.80^a	16.56 ± 3.05^a	18.74 ± 1.29^a
Ear	1.05 ± 0.08^{ab}	0.87 ± 0.04^b	1.14 ± 0.07^a	0.93 ± 0.08^{ab}

on leaf blades of cereals (Araus *et al.*, 1991; Febrero and Araus, 1994). In this regard Johnson *et al.* (1983) found that reflectance of the ear and flag leaf in wheat increased linearly with the amount of EW, but reflectance was much greater in the ear. Increasing reflectance is an adaptation of the upper vertical parts of cereal plants (spike, peduncle, and flag leaf sheath), which are more exposed to incident radiation during grain filling.

Cuticular conductance and epicuticular wax content

No differences were observed in cuticular conductance (g_c) for the flag leaf between lines or treatments (Table 2). Glaucous lines tended to have a higher ear g_c than non-glaucous lines (Table 2). In addition, ear g_c was more than nine times higher than that of flag leaves when g_c was expressed per unit dry weight. Similar results were reported in durum wheat (Araus *et al.*, 1991). These differences in g_c between leaves and ears may be attribut-

able to differences in the surface to volume ratios, which are higer in leaves than in ears.

The amount of epicuticular waxes may be relevant to the capacity of the wax to reduce cuticular conductance (Bengtson *et al.*, 1978), as evidenced by a negative correlation between g_c and EW load in sorghum (Jordan *et al.*, 1984). This is not the case in our growth conditions, where differences across lines and treatments in the amount of EW on a dry weight basis of either ears or leaves were not significantly correlated with differences observed in g_c. Moreover, whereas in the glaucous line EW tended to increase in the flag leaf (blade and sheath) and decrease in the ear from irrigated to rainfed conditions, the opposite pattern occurred on the non-glaucous line. No correlation between amount of EW and g_c has been reported for cereals such as barley (Larsson and Svenningsson, 1986), oat (Svenningsson and Liljenberg, 1986) or durum wheat (Araus *et al.*, 1991). Thus, the presence of glaucousness is not related to higher amounts of EW, which agrees with previous reports (see references in Araus *et al.*, 1991).

Grain yield

Grain yield was higher in glaucous than non-glaucous lines under both irrigated and rainfed conditions. In 1991 grain yield was 6.2 t ha^{-1} for the glaucous line under irrigated conditions, whereas it decreased by 26% for the non-glaucous line under the same conditions, and was 43% and 48% lower for glaucous and non-glaucous lines under rainfed conditions, respectively (Fig. 2a). Marked differences in grain and straw yield were also observed between lines in 1992 (Fig. 2b, c), particularly under irrigation. For both years differences between glaucous and non-glaucous lines were higher under irrigated than rainfed conditions, as reported earlier for grain yield in wheat (Johnson *et al.*, 1983). Thus, while glaucous lines of wheat outyielded their non-glaucous counterparts under conditions of full irrigation, the isolines did not differ significantly in yield in the very dry treatment (Johnson *et al.*, 1983). In contrast, although there were

Fig. 2. (a) Grain yield and carbon isotope discrimination ($\varDelta$) for glaucous (G) and non-glaucous (NG) isolines of barley grown under irrigated (IR) and rainfed (R) Mediterranean conditions during 1991. (b) Grain and (c) straw yield and carbon isotope discrimination ($\varDelta$) of a pair of glaucous (G) and non-glaucous (NG) barley isolines grown under irrigated (IR) and rainfed (R) conditions during 1992. Values represent the mean $\pm$ SE of two plots. Bars sharing different letters are significantly different ($P < 0.05$) by Duncan's comparison test.

no negative effects of glaucousness on yield potential in well-watered treatments for barley (Baenziger *et al.*, 1983), durum wheat (Dakheel *et al.*, 1994) and sorghum (Jordan *et al.*, 1984), the better performance of glaucous lines was magnified under water-limited conditions. In the same way, among several physiological traits measured on 34 wheat cultivars, only leaf waxiness correlated significantly with grain yield under drought (Fischer and Wood, 1979).

Carbon isotope discrimination

The pattern among isolines and treatments of discrimination against stable isotope ^{13}C ($\varDelta$) in mature kernels paralleled that of grain yield. The mean value of $\varDelta$ in 1991 was 19.7‰ for the glaucous isoline under irrigated conditions, whereas it was 1.5‰ lower for the non-glaucous isoline under the same growth conditions, and 1.5‰ and 1.7‰ lower, respectively, for the glaucous and non-glaucous isolines under rainfed conditions (Fig. 2a). A similar pattern was attained for the $\varDelta$ of mature kernels in the 1992 (Fig. 2b). Values of $\varDelta$ for the straw were markedly higher under irrigation than rainfed conditions (Fig. 2c), whereas differences between lines were negligible. By the time differences in glaucousness between isolines were fully established (heading-anthesis), most of the biomass contributing to the straw yield at maturity may have already formed.

Glaucousness may reduce photosynthesis rates because of increased reflectance in the *PAR* region (Richards *et al.*, 1986), although other studies did not show any difference in rate of photosynthesis between glaucous and non-glaucous isolines (Dakheel *et al.*, 1994). Under irrigated conditions barley isolines did not differ in rate of field photosynthesis for either the flag leaf blade or spike (data not shown). However, even if some reduction in photosynthesis occurs due to glaucousness, the reduction in transpiration rate is reportedly greater, resulting in increased (instantaneous or short-term) measurements of transpiration efficiency (*TE*) for the glaucous plants (Chatterton *et al.*, 1975; Johnson *et al.*, 1983; Richards *et al.*, 1986; Dakheel *et al.*, 1994). The situation for long-term *TE*, however, may be different. From the present results the higher $\varDelta$ of kernels for the glaucous line suggested higher intercellular to atmospheric partial pressure of CO_2 (p_i/p_a) and lower *TE* in the glaucous than non-glaucous line during grain filling (Farquhar and Richards, 1984; Hubick and Farquhar, 1989; Farquhar *et al.*, 1989), when kernels are formed either under irrigated or rainfed conditions (Table 1). Even when a higher vapour pressure difference in non-glaucous plants due to their higher temperature (Richards *et al.*, 1986) would actually decrease the transpiration efficiency of non-glaucous plants, *TE* values probably would be still higher than those of glaucous plants. Thus, for a tissue temperature 1 °C higher, twice that reported in non-glaucous durum wheat plants compared with glaucous plants during grain filling (Richards *et al.*, 1986), calculated *TE* from the $\varDelta$ values of kernels was 11.4% (1991) and 5.3% (1992) higher in non-glaucous plants grown under irrigated conditions, and 2.9% (1992) higher in non-glaucous plants grown under rainfed conditions (Table 1).

Non-glaucous plants may be more susceptible to drought than glaucous plants. Drought-stressed plants

close their stomata, which in turn decreases Δ by decreasing p_i (Farquhar *et al.*, 1989). Under field conditions Δ has been positively correlated with yield in cereals such as wheat (Condon *et al.*, 1987; Araus *et al.*, 1993; Sayre *et al.*, 1995) and barley (Romagosa and Araus, 1991; Acevedo, 1993). This is probably because a higher p_i, due to a higher stomatal conductance, is associated with higher photosynthetic rates. Although glaucousness is traditionally associated with higher *TE*, these Δ data suggest that long-term (i.e. integrated through time) *TE* is probably higher in non-glaucous than glaucous lines, especially under irrigation.

Conclusions

Results suggest that neither g_c nor canopy reflectance is the main factor mediating the effect of glaucousness in Δ and yield. Thus, whereas g_c increased or decreased depending on the plant part studied, canopy reflectance in the *PAR* region was altered little by glaucousness.

Therefore other factors may be involved. A higher temperature in the non-glaucous lines could partially account for the lower discrimination and yield of this line compared with the glaucous line. Another factor is the possible effect of the glaucousness on the peristomatic transpiration. Such transpiration, through the guard cell cuticle, can determine the pattern of stomatal closure in conditions of high gradient of partial vapour pressure, which are typical at noon during barley grain filling in mediterranean conditions. Indeed, strong differences in the load pattern and degree of crystalization of epicuticular waxes on the guard cells has been reported between those two lines (Febrero and Araus, 1994).

Acknowledgements

References

Acevedo E. 1993. Potential of carbon isotope discrimination as a selection criterion in barley breeding. In: Ehleringer JR, Hall AE, Farquhar GD, eds. *Stable isotopes and plant carbon-water relations.* San Diego, CA, USA: Academic Press, 399–417.

Araus JL, Febrero A, Vendrell P. 1991. Epidermal conductance in different parts of durum wheat grown under Mediterranean conditions: the role of epicuticular waxes and stomata. *Plant, Cell and Environment* **14,** 545–58.

Araus JL, Reynolds MP, Acevedo E. 1993. Leaf posture, grain yield, growth, leaf structure and carbon isotope discrimination in wheat. *Crop Science* **33,** 1273–9.

Baenziger PS, Wesenberg DM, Sicher RC. 1983. The effects of genes controlling barley leaf and sheath waxes on agronomic performance in irrigated and dry land environments. *Crop Science* **23,** 116–20.

Bengtson C, Larsson S, Lilienberg C. 1978. Effects of water stress on cuticular transpiration rate and amount and composition of epicuticular wax in seedlings of six oat varieties. *Physiologia Plantarum* **44,** 319–24.

Blakeman JP, Atkinson P. 1976. Evidence for a spore germination inhibitor co-extracted with wax from leaves. In: Dickinson CH, Preece TF, eds. *Microbiology of aerial plant surfaces.* New York, USA: Academic Press, 441–9.

Blum A. 1975*a*. Effect of the *bm* gene on epicuticular wax and the water relations of *Sorghum bicolor. Israel Journal of Botany* **24,** 50-ss.

Blum A. 1975*b*. Effect of the *bm* gene on epicuticular wax deposition and the spectral characteristics of *Sorghum* leaves. *SABRAO Journal* **7,** 45–52.

Blum A. 1982. Evidence for genetic variability in drought resistance and its implications for plant breeding. In*: Drought resistance in crops with emphasis on rice.* Los Banos, Phillipines: International Rice Research Institute, 53–68.

Blum A. 1986. The effect of heat stress on wheat leaf and ear photosynthesis. *Journal of Experimental Botany* **37,** 111.

Blum A. 1988. *Plant breeding for stress environments.* Boca Raton, Florida: CRC Press, Inc.

Bort J, Febrero A, Amaro T, Araus JL. 1994. Role of awns in ear water-use efficiency and grain weight in barley. *Agronomie* **2,** 133–9.

Chatterton NJ, Hanna WW, Powell JB, Lee DR. 1975. Photosynthesis and transpiration of bloom and bloomless *Sorghum. Canadian Journal of Plant Science* **55,** 641–3.

Clarke JM, McCaig TN. 1982. Excised-leaf water retention capability as an indicator of drought resistance of *Triticum* genotypes. *Canadian Journal of Plant Science* **62,** 571–8.

Clarke JM, Richards RA. 1988. Effects of glaucousness, epicuticular wax, leaf age, plant height, and growth environments on water loss rates of excised wheat leaves. *Canadian Journal of Plant Science* **68,** 975–83.

Clarke JM, Romagosa I. 1991. Evaluation of excised-leaf water loss rate for selection of durum wheat for dry environments. In: Acevedo E, Conesa AP, Monneveux Ph, Srivastava JP, eds. *Proceedings of ICARDA-INRA-ENSA International Symposium on Physiology/Breeding of Winter Cereals for Stressed Mediterranean Environments* Le Colloque No. 55, INRA, Paris. 401–14.

Condon AG, Richards RA, Farquhar GD. 1987. Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat. *Crop Science* **27,** 996–1001.

Coombs J, Hall DO, Long SP, Scurlock JMO. (eds) 1985. *Techniques in bioproductivity and photosynthesis*, 2nd edn. Oxford: Pergamon Press.

Dakheel AJ, Makdis F, Safi S, Naji I, Peacock JM. 1994. Effects of glaucousness and awns on transpiration efficiency in durum wheat isolines. *Aspects of Applied Biology* **38,** 121–30.

Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40,** 503–37.

Farquhar GD, O'Leary MH, Berry JA. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* **9,** 121–37.

Farquhar GD, Richards RA. 1984. Isotopic composition of plant carbon correlates with water-use-efficiency of wheat genotypes. *Australian Journal of Plant Physiology* **11,** 539–52.

Febrero A, Araus JL. 1994. Epicuticular wax load of

near-isogenic barley lines differing in glaucousness. *Scanning Microscopy* **8,** 735–48.

Fernández S, Vidal D, Simon E, Sole-Sugrañes L. 1994. Radiometric characteristics of *Triticum aestivum* cv. Astral under water and nitrogen stress. *International Journal of Remote Sensing* **15,** 1867–84.

Fischer RA, Wood JT. 1979. Drought resistance in spring wheat cultivars. III. Yield associations with morphophysiological traits. *Australian Journal of Agricultural Research* **30,** 1001–20.

Hubick K, Farquhar GD. 1989. Carbon isotope discrimination and the ratio of carbon gained to water lost in barley cultivars. *Plant, Cell and Environment* **12,** 795–804.

Jaradat A, Konzak CF. 1983. Screening of wheat genotypes for drought tolerance. I. Excised-leaf water retention. *Cereal Research Communications* **11,** 3–4.

Johnson DA, Richards RA, Turner NC. 1983. Yield, water relations, gas exchange, and surface reflectance or near-isogenic wheat lines differing in glaucousness. *Crop Science* **23,** 318–25.

Jordan WR, Shouse PJ, Blum A, Miller FR, Monk RL. 1984. Environmental physiology of sorghum. II. Epicuticular wax load and cuticular transpiration. *Crop Science* **24,** 1168–73.

Juniper BE, Jeffree CE. 1983. *Plant surfaces.* London: Edward Arnold.

Keeling CD, Bacastow RB, Carter AF, Piper SC, Whorf TP, Heimann M, Mook WG, Roeloffzen H. 1989. A three-dimensional model of atmospheric CO_2 transport based on observed winds. 1. Analysis of observational data. In: Peterson DH. ed. *Aspects of climate variability in the Pacific and the Western Americas.* Geophysical Monograph **55,** 165–235.

Keeling CD, Whorf TP. 1992. Atmospheric CO_2 –modern record. In: Boden TA, Sepanski RJ, Stoss FW eds. *'Trends 91: A compendium of data on global change—highlights'.* ORNL/CDIAC–049. Oak Ridge, USA: Carbon Dioxide Information Analysis Center, 14–17.

Larsson S, Svenningsson M. 1986. Cuticular transpiration and epicuticular lipids of primary leaves of barley (*Hordeum vulgare*). *Physiologia Plantarum* **68,** 13–19.

Mayeux Jr HS, Jordan WR. 1987. Rainfall removes epicuticular waxes from *Isocoma* leaves. *Botanical Gazette* **148,** 420–5.

Mayeux Jr HS, Jordan WR, Meyer RE, Meola SM. 1981. Epicuticular wax on goldenweed (*Isocoma* spp.) leaves: variation with species and season. *Weed Science* **29,** 389–93.

Molina-Cano JL, Roca de Togores F, Royo C, Pérez A. 1989. Fast-germinating low β-glucan mutants induced in barley with improved malting quality and yield. *Theoretical and Applied Genetics* **78,** 748–54.

Rawson HM, Clarke JM. 1988. Nocturnal transpiration in wheat. *Australian Journal of Plant Physiology* **15,** 397–406.

Richards RA. 1984. Glaucousness in wheat, its effect on yield and related characteristics in dryland environments, and its control by minor genes. In: Sakanoto S, ed. *Proceedings of the 6th International Wheat Genetics Symposium.* Kyoto, Japan, 447–51.

Richards RA, Rawson HM, Johnson DA. 1986. Glaucousness in wheat: its development and effect on water-use efficiency, gas exchange and photosynthetic tissue temperatures. *Australian Journal of Plant Physiology* **13,** 465–73.

Romagosa I, Araus JL. 1991. Genotype–environment interaction for grain yield and ^{13}C discrimination in barley. *Barley Genetics* **VI,** 563–7.

Sayre KD, Acevedo E, Austin RB. 1995. Carbon isotope discrimination and grain yield for three bread wheat germ-plasm groups grown at different levels of water stress. *Field Crops Research* **41,** 45–54.

Svenningsson M, Liljenberg C. 1986. Changes in cuticular transpiration rate and cuticular lipids of oat (*Avena sativa*) seedlings induced by water stress. *Physiologia Plantarum* **66,** 9–14.