



Universitat de Lleida

**Barley Improvement and Yield
Constraints in Mediterranean
Environments: Interfacing Crop
Physiology with Plant Breeding**

Jordi Voltas Velasco

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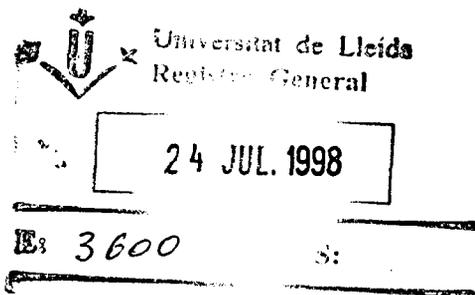
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UNIVERSITAT DE LLEIDA
ESCOLA TECNICA SUPERIOR D'ENGINYERIA AGRARIA

*Barley Improvement and Yield Constraints in Mediterranean Environments:
Interfacing Crop Physiology with Plant Breeding*



Tesi presentada per Jordi Voltas Velasco per optar al grau de Doctor per la Universitat de Lleida

El present treball ha estat realitzat en el Centre UdL-IRTA de Lleida, sota la direcció dels Drs. Ignacio Romagosa Clariana i Josep Lluís Araus Ortega.



Jordi Voltas Velasco

Vist-i-plau dels Directors de Tesi:

Ignacio Romagosa Clariana

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Agraïments

Voldria agrair d'una manera especial l'ajut i col.laboració de totes aquelles persones que, directament o indirectament, han fet possible que aquest document hagi pogut materialitzar-se. En primer lloc, a l'Ignacio Romagosa i a en Josep Lluís Araus per la direcció, dedicació i seguiment, i, encara més, per l'amistat. La feina de camp i les hores de sol van fer-se una mica més lleugeres gràcies a la companyia de la Paquita Santiveri i la Pilar Muñoz, i en Ricard Blanco va solucionar els meus dubtes estadístics en absència del 'jefe'. Aquesta tesi tampoc s'hauria pogut realitzar si no hagués estat per la inestimable col.laboració del personal administratiu, de camp i de laboratori de l'Àrea de Conreus Extensius del Centre UdL-IRTA, així com de tots els becaris de l'Àrea que no esmento aquí per por a l'oblit. També vull agrair d'una forma molt especial les facilitats proporcionades així com la realització d'assajos de camp per part de l'Alberto Lafarga i l'Ana Pilar Armesto del I.T.G.A. de Navarra, i de l'Aurora Sombrero i en Roberto Ontañón del Servicio de Investigaciones Agrarias de Valladolid. El Jordi, l'Anna, el Teo i la Judith de la Universitat de Barcelona han estat circumstancialment un ajut molt valuós, així com en Josep Matas i en Ricardo Simonneaux. Recordar també a en Roberto Tuberosa i al Departamento de Agronomía de la Universitat de Bolònia per la seva hospitalitat. Finalment, no puc deixar d'esmentar la meua família, els meus pares i la meua germana, que després de tots aquests anys veuen el fruit de tanta feina, i la Isabel, que ha sabut suportar-la i convida amb ella. Ah, i gràcies també *for improving the English*.

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Summary

Barley (*Hordeum vulgare* L.) is an important temperate cereal extensively cultivated in Mediterranean climates. It can be grown successfully where the average annual rainfall exceeds 250 mm. Yield improvement for Mediterranean areas during the last decades has been slow probably due to the limitation that drought and other abiotic stresses exert on plant growth. Future increases in productivity may be accelerated by a better understanding of processes that control growth and development and limit genotypic performance of barley provided water is scarce. Thus, physiological research should have a considerable impact in the near future in increasing the efficiency of traditional breeding programs. This thesis focusses on widening current physiological knowledge of factors that curtail growth, productivity and quality of barley in Mediterranean environments. To that end, a set of ten genetically diverse barley cultivars, which includes two- and six-rowed types differing in adaptation to semiarid environments, has been extensively evaluated in rainfed environments located in the province of Lleida (Northeastern Spain) and, occasionally, in the provinces of Navarra (Northern Spain) and Valladolid (Central Spain).

A subgroup of three high yielding, modern six-rowed genotypes (Barberousse, Orria and Plaisant) was used initially to examine the effect of a decrease in the reproductive sink (i.e., number of grains per spike) on individual grain weight and growth, carbohydrate accumulation and N uptake under semiarid conditions (Chapters I and II). Grain weight increases in response to a 50% sink-reduction were progressively greater in environments with smaller control grains. On the contrary, N accumulated uniformly across environments in response to sink manipulation. These results suggest that grain yield is largely limited by carbohydrate supply (i.e., source limited) during grain filling in poor rainfed environments, whereas protein accumulation into growing grains seems independent of the environmental conditions in which grain filling develops. The degree of such limitation to grain growth was consistently higher for those grains placed in lateral spikelets of the barley ear, irrespective of the availability of assimilates for grain filling. Such disadvantage of lateral grains could be ascribed mainly to lower dry matter accumulation rates during grain filling.

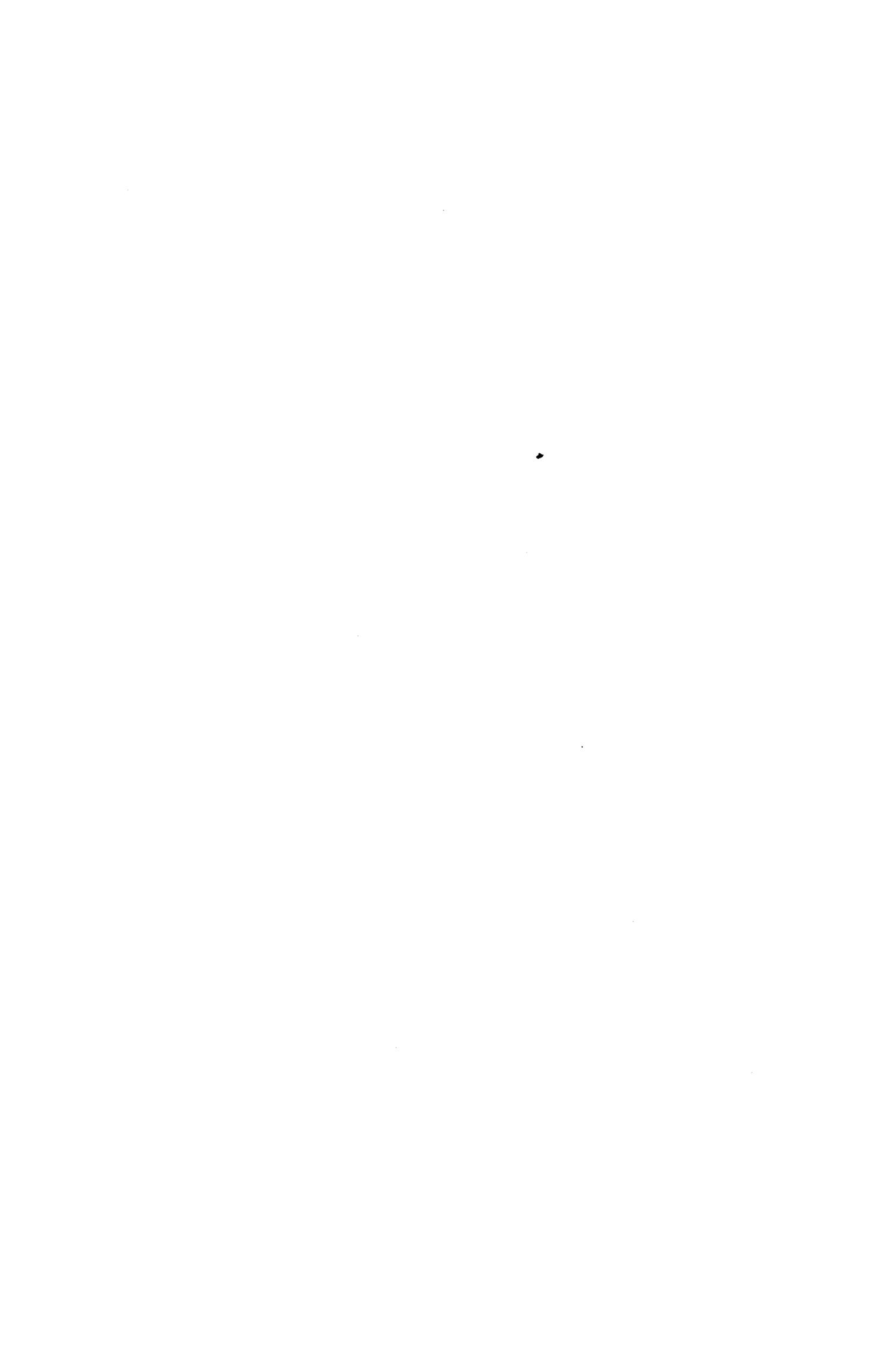
The influence of abiotic stresses such as drought or high temperature in the context of the grain filling process was further examined for the complete set of ten genotypes grown in 12

environments (Chapters III and IV). The final objective was to detect genetic variability and to determine possible morphophysiological mechanisms for tolerance to these abiotic constraints. Possible factors underlying genotype by environment interaction (G×E) for individual grain weight (IGW), grain filling rate (GFR) and grain filling duration (GFD) were explored by means of biadditive models. Differential genotypic sensitivities for IGW were found with respect to post-anthesis drought and elevated temperatures, which could be partially attributed to the difference between two- and six-rowed barleys. G×E for GFR could be partially explained by the joint effect of pre-anthesis climatic variables, suggesting that variation in genotypic behaviour for this trait may be caused by differences in source/sink balance between two- and six-rowed genotypes at anthesis. In addition, G×E for GFD seemed to be driven mainly by differences in anthesis date among genotypes, indicating the existence of an escape strategy lengthening the grain filling period of selected cultivars at the end of the crop cycle.

The relationship between grain yield and carbon isotope discrimination (Δ) of mature grains was thoroughly evaluated in a large set of 22 environments (Chapter V), and the feasibility of using ash concentration in aboveground tissues as a surrogate of Δ explored (Chapter VI). The genotypic expression for grain yield was considerably more affected by the environment than that for Δ . G×E for grain yield suggested the existence of a crossover point at below 3 t ha⁻¹, whereas genotypic ranking for Δ did not change substantially across environments. Overall, genotypes with lower Δ and, thus, with higher transpiration efficiency (TE), performed better in low-yielding environments, i.e., those below the crossover point, while a high genotypic Δ was advantageous in medium and high-yielding environments. It may be possible that, under moderate drought, a large reproductive sink (typical of modern cultivars) force the plant to increase its stomatal conductance and, consequently, its total water use. This phenomenon probably overrides the expected negative relation between Δ and biomass or yield when water is limiting. On the other hand, mineral concentration in mature grains was often negatively related to Δ , and mineral accumulation in vegetative tissues was unrelated to Δ . Both results suggest that mineral accumulation in aboveground tissues, sampled at maturity, is independent of the plant TE during grain filling. Ash concentration in mature grains could be used as a complementary criterion to Δ in semiarid environments, though a more accurate physiological understanding of the mechanisms underlying mineral accumulation in grains is still needed.

Drought arises as the most limiting factor to barley growth and productivity in rainfed Mediterranean environments. In the present study, differences in productivity in a set of 22

environments could be attributed largely to concomitant differences in water availability for growth from sowing to anthesis, a period in which the number of grains m^{-2} is determined. Presence of a crossover G×E interaction for grain yield, as well as changing relationships between productivity and Δ depending on the intensity of water stress, suggest that drought tolerance and yield potential are rather antagonistic concepts in barley.



Resum

L'ordi (*Hordeum vulgare* L.) és un cereal de zones temperades conreat extensament en climes mediterranis. Es desenvolupa favorablement en àrees de pluviometria mitjana anual superior a 250 mm. Durant les darreres dècades, els increments en rendiment deguts a activitats de millora genètica han estat poc importants a causa, probablement, de la limitació que la sequera i altres estressos abiòtics exerceixen sobre el creixement. Futurs increments poden veure's accelerats per un millor coneixement dels processos que controlen el creixement i desenvolupament i que limiten la productivitat dels genotips en situacions de manca d'aigua. Dins d'aquest contexte, les activitats d'investigació en fisiologia vegetal haurien de tenir un fort impacte, en un futur proper, en l'increment de l'eficiència dels programes de millora tradicionals. Aquesta tesi pretèn ampliar el coneixement actual d'aquells factors que redueixen el creixement, la productivitat i la qualitat de l'ordi en ambients mediterranis. Amb aquesta finalitat, s'han avaluat en assajos localitzats a la província de Lleida (nordest d'Espanya) i, ocasionalment, a les províncies de Navarra (nord d'Espanya) i Valladolid (centre d'Espanya), un conjunt de deu genotips d'ordi (incloent-hi dos i sis carreres) que difereixen en adaptació a ambients semiàrids.

Inicialment, un conjunt de tres genotips moderns i altament productius (Barberousse, Orria i Plaisant) va ésser utilitzat per examinar l'efecte que una reducció de l'embornal reproductiu (nombre de grans per espiga) provocava sobre el pes i el creixement del gra, l'acumulació de carbohidrats i el transport de nitrogen en condicions semiàrides (Capítols I i II). Els increments en pes de gra obtinguts en resposta a una reducció del 50% de l'embornal van ser progressivament superiors en aquells ambients amb grans testimoni de menor pes. Pel contrari, el nitrogen es va acumular uniformement en tots els ambients en resposta a una reducció de l'embornal. Aquests resultats suggereixen que el rendiment final es troba fortament limitat, en ambients productivament pobres, per la disponibilitat de carbohidrats durant l'omplenat del gra, en tant que l'acumulació de proteïnes en el gra sembla independent de les condicions ambientals en que té lloc l'omplenat del gra. El grau de limitació exercit per la font es va manifestar més elevat en els grans situats en espiguetes laterals de l'espiga, amb independència de la disponibilitat d'assimilats per l'omplenat del gra. Aquest desavantatge dels grans laterals de l'espiga es va poder atribuir principalment a taxes d'acumulació de matèria seca inferiors durant l'omplenat.

La influència d'estressos abiòtics com ara la sequera o les altes temperatures en el procés d'omplenat dels grans es va examinar en detall utilitzant el conjunt dels deu genotips assajats en dotze ambients (Capítols III i IV). L'objectiu final va consistir a detectar variabilitat genètica així com determinar possibles mecanismes morfofisiològics de tolerància als esmentats estressos. Els possibles factors causants d'interacció genotip-ambient (G×E) en el pes del gra, tasa i duració d'omplenat es van estudiar mitjançant l'ús de models estadístics biadditius. Es van detectar sensibilitats genotípiques diferencials en tolerància a sequera i a elevades temperatures de post-antesi pel pes final del gra, que varen atribuir-se parcialment a diferències entre els grups d'ordis de dos i sis carreres. La presència de G×E per a la taxa d'omplenat es va explicar per l'efecte conjunt de variables climàtiques de pre-antesi, la qual cosa va suggerir que les diferències genotípiques podrien ser degudes parcialment a diferències en el balanç font/embornal entre ordis de dos i sis carreres en antesi. L'existència de G×E per a la duració d'omplenat va poder-se atribuir principalment a diferències en data d'antesi entre genotips, indicant l'existència d'una estratègia d'escapament causant d'un allargament del període d'omplenat d'alguns genotips a finals del cicle de cultiu.

La relació entre rendiment i discriminació isotòpica del carboni (Δ) en grans va avaluar-se extensament en un grup de 22 ambients (Capítol VI), i també va examinar-se la possibilitat d'utilització de la concentració de cendres en teixits aeris com a substitut de Δ (Capítol VII). L'expressió genotípica del rendiment va estar condicionada per l'ambient d'una forma més important que la de Δ . L'existència de G×E pel rendiment va suggerir la presència d'una interacció qualitativa amb un punt de creuament aproximat situat en productivitats mitjanes inferiors a 3 t ha⁻¹. Pel contrari, la classificació de genotips per a Δ no va variar substancialment amb l'ambient. En general, aquells genotips amb valors baixos de Δ i, per tant, amb elevades eficiències de transpiració, van ésser superiors en ambients poc productius (ambients per sota de 3 t ha⁻¹), en tant que valors genotípics de Δ elevats van mostrar-se com avantatjosos en ambients de rendiment mig i alt. És probable que, quan la sequera sigui moderada, un important embornal reproductiu forci la planta a incrementar la seva conductància estomàtica i, com a conseqüència, l'aigua total utilitzada. Aquest fenomen probablement capgira la relació negativa esperada entre Δ i biomassa o rendiment quan la disponibilitat d'aigua és factor limitant. Per altra banda, la concentració mineral en grans va trobar-se relacionada freqüentment i de forma negativa amb Δ , en tant que no va trobar-se relació entre la concentració mineral en palla i Δ . Aquests resultats suggereixen que l'acumulació mineral en teixits aeris mostrejats a finals del cicle de cultiu és

independent de l'eficiència de transpiració durant l'omplenat del gra. La concentració de cendres en grans podria emprar-se com a criteri de selecció complementari a Δ en ambient semiàrids, si bé es fa necessari un coneixement fisiològic més profund dels mecanismes que afecten l'acumulació de minerals en el gra.

La sequera esdevé el principal factor limitant del creixement i la productivitat de l'ordi en els secans semiàrids mediterranis. En el present estudi, les diferències en productivitat en un conjunt de 22 ambients van poder atribuir-se, en gran part, a diferències paral·leles en disponibilitat hídrica des de sembra fins a antesi, període en el qual es determina el nombre de grans per m². La presència d'una interacció G×E de tipus qualitatiu pel rendiment, així com les relacions fluctuants entre rendiment i Δ , depenent de la intensitat de l'estrés hídric, suggereixen que la tolerància a la sequera i l'elevat potencial de rendiment són conceptes antagònics en ordi.

Resumen

La cebada (*Hordeum vulgare* L.) es un cereal de zonas templadas ampliamente cultivado en climas mediterráneos. Se desarrolla favorablemente en zonas de pluviometría media anual superior a 250 mm. Durante las últimas décadas, los incrementos en rendimiento debidos a actividades de mejora genética han sido poco importantes probablemente a causa de la limitación que la sequía y otros estreses abióticos ejercen sobre el crecimiento. Futuros incrementos pueden verse acelerados por un mejor conocimiento de los procesos que controlan el crecimiento y desarrollo y que limitan la productividad de los genotipos en situaciones caracterizadas por la falta de agua. En este contexto, las actividades de investigación en fisiología vegetal deberían tener un fuerte impacto, ya en un futuro próximo, en el incremento de la eficiencia de los programas de mejora tradicionales. La presente tesis pretende ampliar el conocimiento actual de aquellos factores que reducen el crecimiento, la productividad y la calidad de la cebada en ambientes mediterráneos. Con este fin se ha evaluado en ensayos situados en la provincia de Lérida (nordeste de España) y, ocasionalmente, en las provincias de Navarra (norte de España) y Valladolid (centro de España), un conjunto de diez genotipos de cebada (incluyendo dos y seis carreras) que difieren en adaptación a ambientes semiáridos.

Inicialmente, un conjunto de tres genotipos modernos y altamente productivos (Barberousse, Orria y Plaisant) fue utilizado para examinar el efecto que una reducción del sumidero reproductivo (número de granos por espiga) provocaba sobre el peso y el crecimiento del grano, la acumulación de carbohidratos y el transporte de nitrógeno en condiciones semiáridas (Capítulos I y II). Los incrementos en peso del grano obtenidos en respuesta a una reducción del sumidero del 50% fueron progresivamente superiores en aquellos ambientes con granos testigo de menor peso. Por el contrario, el nitrógeno se acumuló uniformemente en todos los ambientes en respuesta a una reducción del sumidero. Estos resultados sugieren que el rendimiento final se encuentra fuertemente limitado, en ambientes productivamente pobres, por la disponibilidad de carbohidratos durante el llenado del grano, mientras que la acumulación de proteínas en el grano parece independiente de las condiciones ambientales en las que el llenado del grano tiene lugar. El grado de limitación ejercido por la fuente fue más elevado para los granos situados en espiguillas laterales de la espiga, con independencia de la disponibilidad de asimilados durante el llenado del grano. Esta desventaja de los granos laterales de la espiga pudo atribuirse

principalmente a tasas inferiores de acumulación de materia seca durante el llenado.

La influencia de estreses abióticos tales como la sequía o las altas temperaturas en el proceso de llenado de los granos se examinó en detalle utilizando el conjunto de los diez genotipos ensayados en doce ambientes (Capítulos III y IV). El objetivo final perseguido consistió en detectar variabilidad genética así como en determinar posibles mecanismos morfofisiológicos de tolerancia a dichos estreses. Los posibles factores causantes de interacción genotipo-ambiente (G×E) en el peso del grano, la tasa y la duración de llenado se estudiaron mediante el uso de modelos estadísticos biaditivos. Se detectaron sensibilidades genotípicas diferenciales en la tolerancia a la sequía y a las elevadas temperaturas de post-antesis para el peso final del grano, que se atribuyeron parcialmente a diferencias entre los grupos de cebadas de dos y seis carreras. La presencia de G×E para la tasa de llenado se explicó por el efecto conjunto de variables climáticas de pre-antesis, lo que sugirió que las diferencias genotípicas pudieran deberse parcialmente a diferencias en el balance fuente/sumidero entre cebadas de dos y seis carreras en antesis. La existencia de G×E para la duración del llenado pudo atribuirse principalmente a diferencias en fecha de antesis entre genotipos, indicando la existencia de cierta estrategia de escape causante de un alargamiento del periodo de llenado de algunos genotipos al final del ciclo de cultivo.

La relación entre rendimiento y discriminación isotópica del carbono (Δ) en granos se evaluó extensamente en un grupo de 22 ambientes (Capítulo V), y también se examinó la posibilidad de utilizar la concentración de cenizas en tejidos aéreos como sustituto de Δ (Capítulo VI). La expresión genotípica del rendimiento fue condicionada por el ambiente de una forma más acusada que la de Δ . La existencia de G×E para el rendimiento sugirió la presencia de una interacción cualitativa cuyo punto de cruce cabría situarlo aproximadamente en productividades medias inferiores a 3 t ha⁻¹. Por el contrario, la clasificación de genotipos para Δ no cambió substancialmente con el ambiente. En general, aquellos genotipos con bajos valores de Δ y, por tanto, con elevadas eficiencias de transpiración, fueron superiores en ambientes poco productivos (ambientes por debajo de 3 t ha⁻¹), mientras que valores genotípicos de Δ elevados se revelaron como ventajosos en ambientes de rendimientos medios y altos. Es probable que, cuando la sequía es moderada, un importante sumidero reproductivo, típico de cultivares modernos, fuerce la planta a incrementar su conductancia estomática y, en consecuencia, el agua total utilizada. Este fenómeno probablemente invierte la relación negativa esperada entre Δ y biomasa o rendimiento cuando la disponibilidad de agua es un factor limitante. Por otra parte,

la concentración mineral en granos estuvo relacionada frecuentemente y de forma negativa con Δ , mientras que no se encontró relación entre la concentración mineral en paja y Δ . Estos resultados sugieren que la acumulación mineral en tejidos aéreos muestreados al final del ciclo de cultivo es independiente de la eficiencia de transpiración durante el llenado del grano. La concentración de cenizas en granos podría utilizarse como criterio de selección complementario a Δ en ambientes semiáridos, si bien es necesario un conocimiento fisiológico más profundo de los mecanismos que afectan a la acumulación de minerales en el grano.

La sequía representa el principal factor limitante del crecimiento y la productividad de la cebada en los secanos semiáridos mediterráneos. En el presente estudio, las diferencias en productividad en un conjunto de 22 ambientes pudieron atribuirse en gran medida a diferencias paralelas en disponibilidad hídrica desde siembra hasta antesis, período en el cual se determina el número de granos por m^2 . La presencia de una interacción $G \times E$ de tipo cualitativo para el rendimiento, así como las relaciones fluctuantes entre rendimiento y Δ , dependiendo de la intensidad del estrés hídrico, sugieren que la tolerancia a la sequía y el elevado potencial de rendimiento son conceptos antagónicos en cebada.

Account

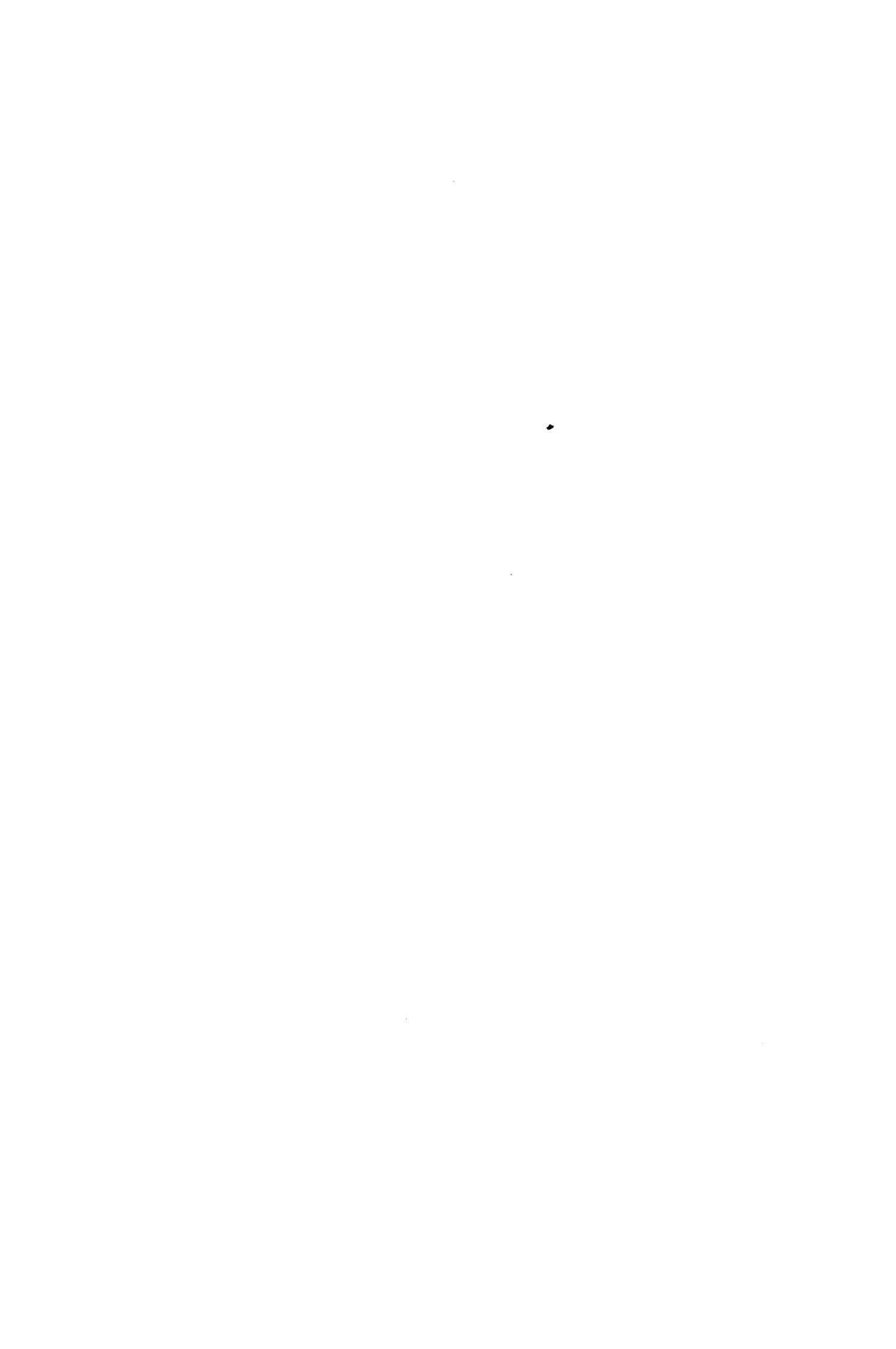
Part of this thesis has been included in or submitted to the following publications:

- Chapter I Voltas, J., Romagosa, I., and Araus, J.L., 1997. Grain size and nitrogen accumulation in sink reduced barley under Mediterranean conditions. *Field Crops Research* 52, 117-116.
- Chapter II Voltas, J., Romagosa, I., and Araus, J.L., 1998. Growth and final weight of central and lateral barley grains under Mediterranean conditions as influenced by sink strength. *Crop Science* 38, 84-89.
- Chapter III Voltas, J., van Eeuwijk, F.A., Sombrero, A., Lafarga, A., Igartua, E., and Romagosa, I. Integrating statistical and ecophysiological analyses of genotype by environment interaction for grain filling of barley in Mediterranean areas. I. Individual grain weight. *Field Crops Research* (submitted).
- Chapter IV Voltas, J., van Eeuwijk, F.A., Araus, J.L., and Romagosa, I. Integrating statistical and ecophysiological analyses of genotype by environment interaction for grain filling of barley in Mediterranean areas. II. Grain growth. *Field Crops Research* (submitted).
- Chapter V Voltas, J., Romagosa, I., Lafarga, A., Armesto, A.P., Sombrero, A., and Araus, J.L. Genotype by environment interaction for grain yield and carbon isotope discrimination of barley in Mediterranean Spain. *Australian Journal of Agricultural Research* (submitted).
- Chapter VI Voltas, J., Romagosa, I., Muñoz, P., and Araus, J. L., 1998. Mineral accumulation, carbon isotope discrimination and indirect selection for grain yield in two-rowed barley grown under semiarid conditions. *European Journal of Agronomy* (in press).

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INTRODUCTION



1. OPENING CONSIDERATIONS

This thesis aims to broaden, to a greater or lesser extent, current physiological understanding of those factors limiting grain yield of barley in Mediterranean environments. During the last two decades, it has been claimed repeatedly that physiological research should have a major impact in increasing the efficiency of traditional breeding programs. So far, however, crop physiology has contributed only modestly. Empirical breeding has been directly responsible for the increases in yield over the last century due to exploitation of genetic variability in crop species. Actually, most plant breeders still rely on the renowned principle *cross the best with the best, and hope for the best*. Provided this approach is being successfully applied, which are the reasons pushing conventional breeding towards the adoption of additional or different approaches to improve yield? Two possible answers has been suggested (Richards, 1996). First, it has been argued that empirically-based selection efficiency has been improved considerably due to, basically, better machinery for handling small plots, new experimental designs for a better identification of the best entries, and computers for increasing the speed of data handling. Benefits derived from use of this technology may have reached a plateau. Second, any increase in yield must have a physiological basis that, if targeted, should permit a significant advance in the identification of superior genotypes. Unfortunately, this level of understanding does not exist yet.

In a very informative paper, Jackson *et al.* (1996) surveyed the role of physiological cognizance from the perspective of conventional breeding programs. Both plant breeders and physiologists agreed in that a better knowledge of the physiological processes affecting grain yield should substantially assist plant breeding activities over the next 20 years. However, some constraints perceived by some breeders and physiologists were emphasized. Among some others, it was pointed out that, whereas physiological understanding should play a key role in identifying constraints to higher yields in target environments, most physiological research is so far not going further than making suggestions about possible indirect selection criteria to use in active breeding programs. If so, this approach seems somewhat contradictory since breeders complain of a quite restricted or superficial level of understanding of the constraints to better performance for the genetic material and environments being used. In contrast, other physiologists argue that such an unjustified bias towards research concerned with traits for selection does not exist because, on one hand, a proper formulation of indirect selection criteria needs *per se* a profound perception

of those factors limiting crop growth and productivity; and, on the other hand, such a formulation may help to delimitate, in turn, major constraints to achieve higher yields. Jackson *et al.* (1996) suggested, based on the cited survey, a sequential approach aimed at improving the efficiency of breeding programs through physiological research. First, a proper knowledge of the environmental factors and/or associated physiological processes limiting genotypic performance in targeted environments is needed. Second, genetic variation and genotype by environment (G×E) interaction for a particular constraint or a set of constraints should be demonstrated. Third, trials in which to perform selection based on the identification of key constraints (i.e., target environments) must be determined. Finally, traits useful for indirect selection should be identified.

Empirical breeding, relying on generating and widely evaluating large numbers of genotypes for productivity, will continue being used more or less successfully. Physiological research may have a major impact on plant breeding in the next future, but up to now it has proved to be mostly fruitless. The work presented here intends to increase our physiological knowledge of those factors applicable to plant breeding limiting grain yield of a temperate cereal, namely barley, grown in Mediterranean environments. Existence of genetic variation to some of these constraints to yield is studied, G×E addressed and, finally, traits for indirect selection are proposed.

2. BARLEY AND ITS ENVIRONMENT

Barley (*Hordeum vulgare* L.) is, after wheat, the second most important cool temperate cereal in the world. It is cultivated from near the Equator in Ethiopia to beyond the Arctic Circle at 70°N latitude in Norway, from below sea level in the Netherlands to the limits of cultivation at 5,000 m in Tibet. In the North hemisphere it is mainly grown between latitudes 30° and 60°. Other cereals are commonly preferred to barley in specific situations, although economic considerations must be also taken into account. Rye is a frequent substitute for barley in cold regions with sandy soils; oats in regions with acid soils where the climate tends to be cool and wet; maize in warm areas with sufficient rainfall or under irrigation where stock feed is needed; wheat on the deeper, finer textured soils where water is non-limiting; sorghum in hot, dry regions.

2.1. Importance and distribution in the European Community and Spain

The amount of total arable land sown to barley has increased steadily during the last decades until reaching 970,000 km² in 1980. This quantity has declined to about 800,000 km² in 1990, which represents approximately 11% of the total land dedicated to cereals all over the world (FAO, 1991). The countries in the former Soviet Union are the first barley producers with a total of about 270,000 km² cultivated. Barley is grown in virtually all EC countries, but more than 80% of the area occurs in just five countries, Denmark, France, Germany, Spain and the United Kingdom. The same countries are responsible for almost 90% of total EC barley production. Since 1940, there have been large changes in the relative importance of barley compared to other cereals (Fig. 1). Up to now, the area dedicated to barley in the countries which now constitute the EC has more than doubled from 55,680 km² to 122,340 km² (1988). In the same period, the wheat area has fallen slightly (185,510 to 155,340 km²) and oats and rye have declined drastically (109,890 to 16,820 km² and 70,490 to 9,190 km², respectively).

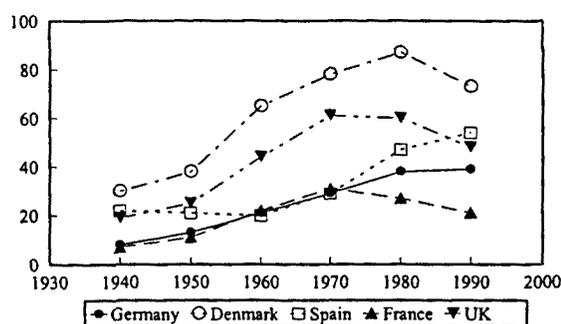


Figure 1. Barley as a percentage of the total cereal area (FAO, 1991).

Most of the arable land where barley is grown in Spain is located at the inner part of the country. In these areas, temperatures are extreme, rainfall is scarce, and soils are frequently shallow. Yields are affected by various abiotic stresses at a time, although drought uses to be the most common and harmful limitation to growth. Nevertheless, Spain is the first European country in total area dedicated to this cereal, and the fourth in the world after Russia, USA, and Canada. More than 50% of the total cereal area cultivated in Spain, about 35,000 km², is barley-producing

land. Castilla-León is the first barley-producer region of Spain, followed by Castilla-La Mancha and Aragon. Together, these regions are responsible for more than 75% of the Spanish barley production, an activity that occupies an important part of their total land area (Fig. 2).

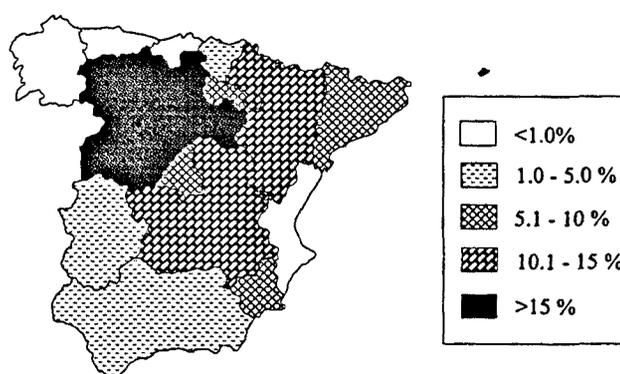


Figure 2. Harvested barley Spanish area as a percentage of total land area (adapted from Russell, 1990)

2.2. A brief history

The beginnings of agriculture in the Ancient World, which occurred sometime during the period of transition from hunter/gathering to farming communities, seem to be associated with cereal domestication (Harlan, 1979). The oldest reported archaeological sites where domesticated crops have been reported date from the tenth (Jordan valley; Garrard *et al.*, 1996) and ninth millennium (Tell Halula, Syria; Wilcox, 1996) before the present. These Neolithic sites witness the appearance of domesticated crops such as naked wheat (*Triticum aestivum/durum*), emmer (*Triticum dicoccum*) and barley (*Hordeum vulgare*). In this scenery, barley was one of the basic crops of early irrigated agriculture in Mesopotamia and Egypt. The geographical origin of cultivated barley in the Iberian Peninsula is a subject of debate. Evidence exists that suggests the relevance of *in situ* domestication from an ancestral *Hordeum spontaneum* (Moralejo *et al.*, 1994). Other possibilities arise from the eastern Mediterranean in either Neolithic or historical times. By Roman times, agronomy of barley and its ecological requirements in the Mediterranean region were apparently well understood as judging by the writings of Columela (Columela, publ.

1988) and Varro (Varro, publ. 1979). Together with the gradual substitution of the naked barley (*Hordeum vulgare* var. *nudum* L.) by the currently grown hulled barley, wheat began to replace barley as a primary human food. Nowadays, barley is mainly used to feed cattle or in the brewing and distilling industries.

2.3. Types of barley

Barley (*Hordeum vulgare* L.) is a grass of the family Poaceae, the sub-family Pooideae and the tribe Triticeae. Barley cultivars are usually divided into winter and spring, and six- and two-row types. 'Winter' and 'spring' allude to particular genotypic characteristics rather than to the time of sowing, although autumn sowings are usual for winter types in Central Europe. In fact, unless extremely low temperatures are a feature of the region, the date of sowing is a more important agronomic consideration than whether a cultivar is a winter or spring type. Spring cultivars tend to have shorter development cycles than winter ones and are sometimes preferred in Mediterranean environments where barley is sown in winter (November to February) to allow the crop to mature before the onset of the summer drought. In Spain, 41% of the barley cultivated is of spring type, whereas the rest (59%) is of winter type (Russell, 1990).

Barley ears are formed by spikelets in groups of three at each node of the rachis. The lateral spikelets may be sterile or fertile, and this feature is of taxonomic relevance for distinction between subspecies. In two-row types (*Hordeum vulgare* ssp. *vulgare distichon*), the lateral spikelets are sterile and do not bear grains, whereas they are fertile in six-row types (*Hordeum vulgare* ssp. *vulgare hexastichon*). Six-row types tend to be more frost-hardy and drought tolerant, and most of the six-row cultivars currently cultivated in Spain are winter types. Morphologically, six-row types support fewer ears m^{-2} but have more grains per ear, giving a greater number of grains m^{-2} than two-row types. On the contrary, the latter tend to present heavier individual grains.

2.4. Climatic constraints in Mediterranean environments. Yields and yield variability

Barley can be grown in many regions of the world. Thermal time from sowing to harvest ranges from 1,800 °C at latitude 67°N to 3,750 °C at latitude 38°N (base temperature 0°C). Winter barley cultivars have a sufficient frost hardiness to allow their use under mean air

temperatures greater than -2°C , but sowing must be completed early enough in autumn to permit the development of an adequate root system. Spring barley is normally sown after the last lethal frosts, making the risk of frost damage a minor factor in its distribution. However, in individual years frosts can cause disastrous yield reductions to barley crops that reach anthesis early.

Climatic constraints are the main cause of yield variation over seasons and locations. Average European barley yields are the highest of the world, with an average of 3.8 t ha^{-1} in 1994. However, mean country yields averaged over nine years 1980-1988 ranged from 1.36 t ha^{-1} for Portugal to 6.43 for the Netherlands. Growing conditions are not, by far, uniform among different countries and even not within countries. The Mediterranean countries, Spain, Portugal, Greece and Italy, show mean yields less than 4.0 t ha^{-1} , whereas the other countries exceed this value. In addition, spatial and seasonal fluctuations in grain yield, mainly caused by water availability, are of greater relevance than those in Central Europe (Fig. 3). The Mediterranean climates are basically characterized by long, hot, dry summers, and short, mild, wet winters. Cereals are mainly grown under dryland conditions and inadequate rainfall is usually the most limiting factor to yield. Barley is relatively more tolerant to thermic stress than wheat, although damage can be caused at mean temperatures above 20°C . Drought constraints to growth are complicated to define because the effects of evapotranspiration and soil type must be taken into account. Usually, barley can be cultivated where the mean annual rainfall exceeds 250 mm . Rainfall becomes less frequent and temperature increases during spring. This phenomenon gives origin to what is designated as terminal drought, and its timing and intensity varies from year to year also according to the crop growth. Ludlow (1989) distinguishes three different strategies of plant adaptation to water-stressed conditions. In many environments, the availability of varieties allows the completion of the growth cycle before severe drought occurs. This strategy is termed *escape*. Maximization of water uptake, as well as minimization of water loss from the soil, are together referred to as *avoidance*. Finally, the mechanisms that enable a plant to cope with cell desiccation are termed drought *tolerance*. These strategies are useful for barley plants to overcome the effects of drought.

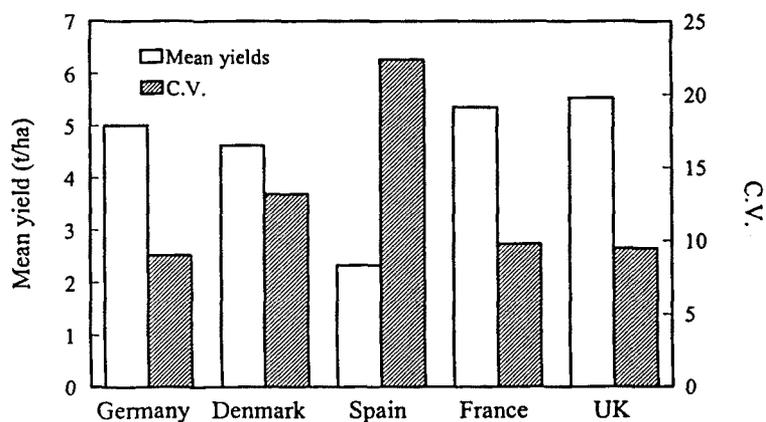


Figure 3. Mean yields and coefficients of variation (C.V.) for the five most productive barley countries of the EC (adapted from Russell, 1990)

3. BIOMASS PRODUCTION AND PARTITIONING IN WATER-LIMITED ENVIRONMENTS

The erratic yields of barley in Mediterranean-type climates bring about economic and social implications derived from the instability of the production and the low quality of the grain harvested. To face this problem, plant breeding must offer alternatives to the old cultivars and landraces still cultivated, since the high yielding varieties currently used in non-limiting environments and imported from northern countries often perform inadequately under harsh conditions. For areas like the Mediterranean region, where rainfall arises as the most limiting factor in cereal production, Passioura (1977) proposed a framework for examining ways to improve crop yields. In this model, grain yield (GY) is a function of the water used by the crop (WU), the efficiency with which it is converted into biomass (water use efficiency, WUE), and the harvest index (HI) of the crop.

$$GY = WU \times WUE \times HI$$

WU refers to soil evaporation plus transpiration. In barley grown in Mediterranean environments, all the soil water is depleted frequently by maturity (Cantero-Martínez *et al.* 1995).

WUE is the amount of dry matter produced per unit of WU. The components in this identity are relatively independent of each other, so an increase in any of them is likely to increase yield. Richards (1996) points out that considerable opportunities exist to increase both the total amount of biomass produced by the plant (the product of WU and WUE in the Passioura's identity), and the partitioning of this carbon to the growing ears and grains (HI).

3.1. Biomass production

Changes in total biomass production due to breeding practices over time show contradictory results in cereals. Most authors conclude that biomass production has varied little in the last decades (e.g., Jedel and Helm, 1994), though some studies have found an increase in total biomass with time (e.g., Martiniello *et al.*, 1987). There are two ways by which breeding may increase WU (Richards, 1987). One is to increase the total water extracted from the soil by, for example, increasing the depth of rooting. The other is to increase the ratio of water transpired to water evaporated from the soil surface by maximizing the early growth of leaf area (early vigour). The expected consequence of increasing early vigour is the greater yield of biomass and, hence, of grain due to increased transpiration relative to evaporation from the soil surface (Richards, 1996). Improving WUE seems now feasible through increased transpiration efficiency (TE, ratio of net assimilation to water transpired), a measure of WUE at the plant level. TE may be indirectly inferred by measuring the carbon isotope discrimination (Δ) in plant parts. For C_3 species such as barley, Δ of mature kernels constitutes an integrated record of the ratio of intercellular to atmospheric partial pressure of CO_2 (p/p_a) and, thus, of the TE during grain filling (Farquhar and Richards, 1984). The negative relationship between TE and Δ has been confirmed within several species in all field studies where TE has been determined (Condon *et al.*, 1993). For a limited amount of transpirable water, the association between TE and Δ should translate to low Δ genotypes having greater biomass and also yield (Richards, 1996).

3.2. Biomass partitioning

There is ample evidence indicating that genetic improvement of barley yields during this century has been associated mainly with reduced plant height and increased HI (Riggs *et al.*, 1981). This activity has involved an unconscious change in dry matter partitioning through an

increase of the grain sink size at the expense of shoot and root size. However, although HI of modern cultivars grown in non-limiting conditions is about 0.5, their HI usually diminish to a value around 0.35 when grown in droughted environments. Since the time of maximum stem, root, and ear growth coincide, ear growth is limited by competition of assimilates at this stage (Loss and Siddique, 1994). Thus HI is lowered and a considerable amount of the potential yield is not achieved (Richards, 1987). It has been proposed that such an amount depends critically on how well the phenology of the crop is suited to the environment (Passioura, 1996). Thus, if anthesis is delayed there is too much vegetative growth, little water remains stored in the soil to fill the grains, and HI will be marginally low. But if anthesis is too early in relation to the pattern of WU, yield is also reduced because the crop fails to establish a sufficiently large number of grains to be filled and also of photosynthetic tissue to benefit from the substantial water supply during grain filling. A proper balance between the availability of assimilates for grain filling (i.e., source strength) and the number of grains (i.e., reproductive sink) determines the achievement of a satisfactory crop HI in both non-limiting and water-limited environments. The route to an increased grain yield can be from either the carbon source (photosynthesis) or the carbon sink (grain number and/or grain weight), but preferably both simultaneously (Gifford *et al.*, 1984).

In terms of yield components, cereal improvement has mostly involved the number of grains m^{-2} rather than the individual grain weight. A high yield potential implicates the establishment of a large number of grains (sink size) before grain filling begins (Slafer *et al.*, 1996). The average weight per grain is a more conservative property of a cultivar than is grain number m^{-2} . Grain weight can nevertheless vary depending on the particular growing conditions, and certainly differs among genotypes. The determination of grain number m^{-2} and individual grain weight throughout the crop development do not overlap in time, but many studies indicate a negative relationship between these two yield components (e.g., García del Moral *et al.*, 1991). Thus, increases in grain number determine reductions, although relatively smaller, in average grain weight. Slafer *et al.*, (1996) suggest that future attempts at increasing grain yield through increasing grain number would be unwise if they are to be compensated by reductions in individual grain weight. Current evidence indicates that, under near-optimum growing conditions, grain yield is mostly limited by potential sink size, although there is small but significant source limitation (i.e., lack of photoassimilates) for grain filling (Slafer and Savin, 1994).

A large sink related to grain number is an inherent characteristic of the high-yielding genotype. On the other hand, the capacity of the source to fill the potential sink is also an

essential component of yield potential (Blum, 1996). Both interact with each other: a large number of grains promotes high leaf conductance, gas exchange and transpiration, but also hastens drought-induced leaf senescence (Blum *et al.*, 1988). In water-limited environments, a high yielding genotype may be penalized *a priori* due to its inherently high stomatal conductance and, probably, low TE. In these conditions, a large sink may be disadvantageous during grain filling. It is necessary to assess whether such a large sink is still counterbalanced by a concomitant load on the source. Blum (1996) suggests that, as water stress increases, high yield potential and drought tolerance may become mutually exclusive. Understanding of these associations is very limited and extensive research is needed for improvement of barley in Mediterranean-type environments.

4. OBJECTIVES

The general objective of this thesis is to understand, in the context of breeding programs, the environmental factors and associated physiological processes limiting genotypic performance of barley in Mediterranean environments. This general objective can be split into the following specific objectives:

- (i) To examine source-sink relationships in modern, high yielding, six-rowed barley genotypes, in order to determine to which degree individual grain weight of barley in Mediterranean environments is limited by sink size or source supply.
- (ii) To evaluate the differential incidence of abiotic stresses on grain growth and final weight of two- and six-rowed barleys grown in Mediterranean conditions, and to identify possible genotypic mechanisms that could serve to overcome the detrimental influence of such abiotic factors on grain development.
- (iii) To determine possible genotypic associations between grain yield and carbon isotope discrimination (Δ) in a large set of rainfed Mediterranean environments, and to establish the value of ash concentration in aboveground tissues as a surrogate of Δ under semiarid conditions.

The studies performed to fulfill these objectives are described in six independent chapters, in addition to an introduction and a general discussion. The chapters are written as scientific papers and are, therefore, self-contained. Some of them are already published whereas the rest are in press or have been submitted recently for evaluation. Chapters I and II attempt to answer those questions raised in the first objective of this thesis. Chapters III and IV face those aspects of grain development encountered in the second objective. Finally, chapters V and VI address the opportunity of selecting for carbon isotope discrimination and/or ash concentration looking for an indirect correlated response in grain yield.

In addition, other studies have been performed during the same postgraduate period that are partially related to the document presented here. They have contributed to a deeper knowledge of some experimental aspects developed in extent in this thesis, although dealing with somewhat different genetic, physiological and/or methodological backgrounds. For this reason, they have not been incorporated in the present memory. These studies include a classical genetic analysis of yield and yield components of barley (Voltas *et al.*, 1993), as well as an evaluation of changes in adaptation of the same species during the last decades in northern Spain (Muñoz *et al.*, in press); the analysis of the relationship between grain yield and ash content in mature kernels of barley under irrigated and rainfed conditions (Febrero *et al.*, 1994); the assessment of genetic and environmental variation in malting and feed quality (Molina-Cano *et al.*, 1997) and the effect of grain filling conditions in malting quality of barley (Swanston *et al.*, 1998); the assessment of the performance of fast-chlorophyll fluorescence kinetics, measured under field conditions, in assessing genotypes differences in yield of durum wheat under Mediterranean conditions (Araus *et al.*, 1998); the evaluation of crop water availability in early agriculture based on the carbon isotope discrimination of plant seeds from the south-east Iberian Peninsula (Araus *et al.*, 1997) and the Middle Euphrates (Araus *et al.*, in press); and, finally, the contribution of pre-anthesis assimilates to grain yield in winter and spring triticales under semiarid conditions (Royo *et al.*, submitted).

The references of these publications are:

Araus, J.L., Febrero, A., Buxó, R., Rodríguez-Ariza, M.O., Molina, F., Camalich, M.D., Martín, D., and Voltas, J., 1997. Identification of ancient irrigation practices based on the carbon isotope discrimination of plant seeds: a case study from the south-east Iberian Peninsula. *Journal of Archaeological Science* 24, 729-740.

Araus, J.L., Amaro, T., Voltas, J., Nakkoul, H., and Nachit, M.M., 1998. Chlorophyll fluorescence as a selection criterion for grain yield in durum wheat under Mediterranean conditions. *Field Crops Research* 55, 209-223.

Araus, J.L., Febrero, A., Catala, M., Molist, M., Voltas, J., and Romagosa, I., 1998. Crop water availability in early agriculture: evidence from carbon isotope discrimination of seeds from a tenth millennium BP site on the Euphrates. *Global Change Biology* (in press).

Febrero, A., Bort, J., Català, J., Marzabal, P., Voltas, J., and Araus, J.L., 1994. Grain yield, carbon isotope discrimination and mineral content in mature kernels of barley under irrigated and rainfed conditions. *Agronomie* 2, 127-132.

Molina-Cano, J.L., Francesch, M., Pérez-Vendrell, A.M., Ramo, T., Voltas, J., and Brufau, J., 1997. Genetic and environmental variation in malting and feed quality of barley. *Journal of Cereal Science* 25, 37-47.

Muñoz, P., Voltas, J., Araus, J.L., Igartua, E., and Romagosa, I., 1998. Changes in adaptation of barley releases over time in northeastern Spain. *Plant Breeding* (in press).

Royo, C., Voltas, J., and Romagosa, I. Remobilisation of pre-anthesis assimilates in winter and spring triticales for grain and for dual purpose (forage+grain). Submitted.

Swanston, J.S., Ellis, R.P., Pérez-Vendrell, A.M., Voltas, J., and Molina-Cano, J.L. Patterns of barley grain development in Spain and Scotland and their implications for malting quality. *Cereal Chemistry* 74, 456-461.

Voltas, J., Lafarga, A., Armesto, A.P., Sombrero, A., Ontañón, R., Montoya, J.L., and Romagosa, I., 1993. A preliminary genetic analysis of barley adaptation to drought-prone environments in northern Spain. In: Monneveux, P., Ben Salem, M. (Eds.), *Tolérance à la sécheresse des céréales en zone méditerranéenne. Diversité génétique et amélioration variétale. Les Colloques*, 64. INRA, France, pp. 265-271.

REFERENCES

Blum, A., Mayer, J., and Golan, G., 1988. The effect of grain number per ear (sink size) on source activity and its water-relations in wheat. *Journal of Experimental Botany* 39, 106-114.

Blum, A., 1996. Crop responses to drought and the interpretation of adaptation. *Plant Growth Regulation* 20, 135-148.

- Cantero-Martinez, C., Villar, J.M., Romagosa, I., and Fereres, E., 1995. Growth and yield responses of two contrasting barley cultivars in a Mediterranean environment. *Eur. J. Agron.* 4, 317-326.
- Columela, L.M. De los trabajos de campo. M.A.P.A. Siglo XXI. Madrid, 1988.
- Condon, A.G., Richards, R.A., and Farquhar, G.D., 1993. Relationships between carbon isotope discrimination, water use efficiency and transpiration efficiency for dryland wheat. *Aust. J. Agric. Res.* 44, 1693-1711.
- F.A.O., 1991. F.A.O. production yearbook. F.A.O. Statistics Series, Roma.
- Farquhar, G.D., and Richards, R.A., 1984. Isotopic composition of plant carbon correlates with water use efficiency of wheat genotypes. *Aust. J. Plant Physiol.* 11, 539-552.
- García del Moral, L.F., Ramos, J.M., García del Moral, M.B., Jiménez-Tejada, M.P., 1991. Ontogenic approach to grain production in spring barley based on path-coefficient analysis. *Crop Sci.* 31, 1179-1185.
- Garrard, A., Colledge, S., Martin, L., 1996. The emergence of crop cultivation and caprine herding in the 'Marginal Zone' of the southern Levant. In: Harris, D.R. (Ed.), *The Origins and Spread of Agriculture and Pastoralism in Eurasia*. University College London Press, London, UK, pp. 204-226.
- Gifford, R.M., Thorne, J.H., Hitz, W.D., and Giaquinta, R.T., 1984. Crop productivity and photoassimilate partitioning. *Science* 225, 801-808.
- Harlan, J.R., 1979. Barley. In: Simmonds, N.W. (Ed.), *Evolution of crop plants*. Longman, London, pp. 93-98.
- Jackson, P., Robertson, M., Cooper, M., and Hammer, G., 1996. The role of physiological understanding in plant breeding; from a breeding perspective. *Field Crops Res.* 49, 11-37.
- Jedel, P.E., and Helm, J.H., 1994. Assessment of western canadian barleys of historical interest. I. Yield and agronomic traits. *Crop Sci.* 34, 922-927.
- Loss, S.P., and Siddique, K.H.M., 1994. Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. *Advances in Agronomy* 52, 229-276.
- Ludlow, M.M., 1989. Strategies of response to water stress. In: Krieb, K.H., Ritcher, H., Hinckley, T.M. (Eds.), *Structural and Functional Responses to Environmental Stresses*. SPB Academic Publishing, Gravenhage, The Netherlands, pp. 269-281.

Martiniello, P., Delogu, G., Odoardi, M., Boggini, G., and Stanca, A.M., 1987. Breeding progress in grain yield and selected agronomic characters of winter barley (*Hordeum vulgare* L.) over the last quarter of a century. *Plant Breeding* 99, 289-294.

Moralejo, M., Romagosa, I., Salcedo, G., Sánchez-Monge, R., and Molina-Cano, J.L., 1994. On the origin of Spanish two-rowed barleys. *Theor. Appl. Genet.* 87, 829-836.

Passioura, J.B., 1977. Grain yield, harvest index, and water use of wheat. *J. Aust. Inst. Agric. Sci.* 43, 117-121.

Passioura, J.B., 1996. Drought and drought tolerance. *Plant Growth Regulation* 20, 79-83.

Richards, R.A., 1987. Physiology and the breeding of winter-grown cereals for dry areas. In: Srivastava, J.P., Porceddu, E., Acevedo, E., Varma, S. (Eds.), *Drought tolerance in winter cereals*. John Wiley & Sons Ltd., pp. 133-149.

Richards, R.A., 1996. Defining selection criteria to improve yield under drought. *Plant Growth Regulation* 20, 157-166.

Riggs, T.J., Hanson, P.R., Start, N.D., Miles, D.M., Morgan, C.L., and Ford, M.A., 1981. Comparison of spring barley varieties grown in England and Wales between 1880 and 1980. *J. Agric. Sci.* 97, 599-610.

Russell, G., 1990. Barley knowledge base. European Communities - Commission, Luxembourg, 142 pp.

Slafer, G.A., and Savin, R., 1994. Source-sink relationship and grain mass at different positions within the spike in wheat. *Field Crops Res.* 37, 39-49.

Slafer, G.A., Calderini, D.F., and Miralles, D.J., 1996. Yield components and compensation in wheat: opportunities for further increasing yield potential. In: Reynolds, M.P., Rajaram, S., McNab, A. (Eds.), *Increasing Yield Potential in Wheat: Breaking the Barriers*. CIMMYT. Mexico D.F., pp. 101-133.

Varro, M.T. *De re rustica*. Cambridge (Mass.). Harvard Univ. Press, 1979.

Wilcox, G., 1996. Evidence for plant exploitation and vegetation history from three Early Neolithic pre-pottery sites on the Euphrates (Syria). *Vegetation History and Archaeobotany* 5, 143-152.

CHAPTER I

Grain size and nitrogen accumulation in sink-reduced barley under Mediterranean conditions

ABSTRACT

In rainfed Mediterranean environments, grain growth of barley is often hindered by the occurrence of unpredictable terminal stresses such as drought or high temperatures. To study the effect of an enhancement in the assimilate availability on final grain size, a 50% sink-reduction was done in the mainstems of three six-row barley cultivars (*Hordeum vulgare* L.). The study was conducted in 11 field trials during four years in northeast Spain. The average grain size of control spikes varied significantly among trials between 26.4 mg and 49.1 mg. The average increase on grain size in response to a 50% sink-reduction was 20%. These increments were progressively greater in trials with small grains of control spikes, indicating a major degree of source limitation in low-yielding rainfed environments. Total N content per grain showed a larger average increase (47.6%) in sink-reduced spikes. N accumulated uniformly across trials in response to sink manipulation, indicating that environmental conditions did not limit protein synthesis during grain filling. Dry matter increments in response to sink halving were mainly due to protein accumulation when environmental conditions determined large grains, suggesting that a sink limitation for carbohydrate accumulation may also occur under Mediterranean conditions.

1. INTRODUCTION

Winter cereals are frequently able to produce reasonably sized grains despite the occurrence of terminal stresses such as drought or high temperatures (Austin *et al.*, 1980; Kobata *et al.*, 1992). Under these conditions, characterized by a decline in net photosynthetic rates and N uptake in the plant during the post-anthesis period, grain filling is supported by mobilisation of dry matter and N stored in vegetative organs of the plant before flowering (Bidinger *et al.*, 1977; Shepherd *et al.*, 1987; Papakosta and Gagianas, 1991). Consequently, there is little variation in grain size as compared with other yield components (Gallagher *et al.*, 1975). Nevertheless, under more severe stressful situations, grain growth can finally suffer from lack of assimilates, causing a significant reduction in grain size.

In Mediterranean environments, seasonal variations in rainfall and temperatures are rather unpredictable in terms of economic yield (Ceccarelli *et al.*, 1991), and these fluctuations ultimately bring about changes in source/sink relationships during grain filling. In breeding for such zones, the attainment of an adequate grain size remains important to guarantee good nutritional properties and milling quality (Wrigley *et al.*, 1994). Few attempts have been made, however, to determine the relative importance of drought and high temperatures and their interaction patterns in the field.

The environmental conditions during growth determine the availability of assimilates for grain filling (i.e. source strength) as well as the potential sink size. The assessment of source/sink relationships on the grain size of cereals is commonly done by decreasing the reproductive sink through partial elimination of developing grains. Reproductive-sink reduction is practiced either, by partial or complete spikelet removal, or by degrading, leaving the glumes intact. In Mediterranean conditions, the relative photosynthetic contribution of the spike to the grain filling cannot be neglected (Blum, 1985) and, therefore, sink manipulation must be carried out keeping intact the photosynthetic structure of the spike.

Sink manipulation experiments performed in wheat suggest that grain size is mostly sink-limited under non-limiting growing conditions (Jenner *et al.*, 1991; Nátrová and Nátr, 1993; Slafer and Savin, 1994). However, in rainfed environments grain size often increases in response to a sink reduction treatment (Blum *et al.*, 1988; Blade and Baker, 1991), which can be interpreted as the result of a source limitation for grain growth. For barley, a crop that clearly outyields other temperate cereals in poor rainfed environments (López-Castañeda and Richards,

1994), there is a lack of information on the source-sink relationships affecting grain size. In northern Spain, high yielding, widely adapted genotypes are gradually replacing landraces or highly stable but less productive barleys. However, when water and high temperature stresses become severe, grain growth is specially reduced in these high yielding cultivars. A proper characterisation of the plant source/sink relationships is relevant to understand if grain yield of such cultivars can be limited by source supply during grain filling. Since growing grains are mainly dependent on processes regulating the accumulation of C and N supplied by other vegetative organs, the assessment of responses to sink manipulation may provide more insights about the regulation of carbohydrates and protein deposition in the grain.

The aim of this study was to examine the effect of decreasing the reproductive sink on grain size across a large number of semiarid Mediterranean environments, in order to determine to what degree grain size of currently grown barleys in northern Spain is source-limited under non-optimal growing conditions. An examination of dry matter and N uptake responses to reduced sink size may help understand how carbohydrates accumulate in growing grains and grain protein concentration is determined.

2. MATERIALS AND METHODS

2.1. Experimental sites

11 trials (including fall and winter sowings) were conducted near Lleida, northeastern Spain, in a 4 year study. Three contrasting locations about 75 km apart from each other were chosen: Gimenells (41° 36' N., 0° 13' E.), Bell-lloc (41° 40' N., 0° 41' E.), and Artesa de Segre (42° 11' N., 0° 32' E.). The soil at Gimenells was highly fertile, classified as Xerollic Paleorthid (SSS, 1990), and a crop rotation with forage corn was practiced for the three year study at this site (1991, 1992 and 1993). Plots were provided with 50 kg N ha⁻¹ at sowing and 60 kg N ha⁻¹ at jointing stage. The soil at Bell-lloc was Xeric Torriorthent (SSS, 1990) of medium fertility, and experimental plots at this location had been fallowed in rotation with barley during two years (1991 and 1992). Each plot received at this site only 40 kg N ha⁻¹ at sowing due to the high initial soil N levels. Plots at Artesa de Segre were situated on a low-fertility Fluventic Xerochrept soil (SSS, 1990) during two years (1993 and 1994). Continuous barley cropping was practiced at this

site and fertilizer was applied at rates of 50 kg N ha⁻¹ at sowing and 60 kg N ha⁻¹ at jointing.

At each location, preplant N was broadcast as urea immediately before sowing. Fertilizer was also applied to supply 75 kg P₂O₅ ha⁻¹ and 75 kg K₂O ha⁻¹. Nitrogen applied at jointing was broadcast as NH₄NO₃. Details on the trials used in this study are given in Table 1. Weather data were collected from meteorological stations located few km apart from the experimental sites.

2.2. Treatments and plot design

Three six-rowed, winter type barley (*Hordeum vulgare* L.) cultivars were used in this study: Barberousse and Plaisant are high-yielding genotypes of French origin widely cultivated in northern Spain; Orria is a recent, highly productive Spanish variety of CIMMYT origin well adapted to fertile rainfed environments. Plots consisted of six 12-m rows, spaced 20 cm, and seeded at rates of 350 plants m⁻². The experimental design at each trial was a randomized complete block with four replications.

Anthesis was recorded as the time when 50% of spikes extruded anthers. Barberousse was usually the earliest heading cultivar, followed by Plaisant and Orria. Anthesis dates of the cultivars differed from two to five days depending on the trial. At this stage, plant samples composed of two 1-m row segments from each plot were taken in one block of all trials, except in 1991. The samples were separated into leaf plus culm and chaff, dried at 60°C to constant weight. For each genotype, 120 mainstems were tagged and lateral florets on one-side together with the opposing central florets of 60 spikes were sterilized by piercing the carpel with a sharp needle, consequently halving the reproductive sink. The other 60 labeled spikes remained as controls. Samples of five half sink-reduced (treated) and control tagged spikes were taken every 5-7 days from anthesis to maturity. Grains from control and treated spikes were collected separately from four central and four lateral spikelets belonging to mid-spike nodes, resulting in 20 grains per sample. In 1991, however, only three grains per spike, one for each different position within a particular rachis-node, were collected and bulked up to a total of 15 grains per sample. Grain dry weight was measured after drying to constant weight at 60°C. Grain filling was determined by changes in the dry weight of individual grains and fitted to a logistic curve as proposed by Darroch and Baker (1990). Except in 1991, the curve fitting was carried out separately for central and lateral grains. Maximum grain size was derived from the fitted curves.

Table 1. Description of the 11 trials used in the present study. Anthesis and maturity (days after sowing, DAS), grain yield, grain number per spike, and grain size averaged over cultivars at each trial.

Trial code ¹	Sowing date	Anthesis (DAS)	Maturity (DAS)	Average temperatures and rainfall (grain filling period)			Grain yield (t ha ⁻¹)	Grain no. per spike	Grain size (mg)
				T _{max}	T _{mean}	T _{min}			
BEL ₉₁	4 Dec 1990	152	180	23.5	15.6	7.8	55	58.3	38.7
GIM ₉₁	20 Nov 1990	161	196	22.9	15.9	9	10	55.8	35.8
BEL ₉₂	28 Nov 1991	149	179	25.5	18.1	10.6	76	47.8	41.9
BEL _{w92}	9 Jan 1992	122	149	25.6	18.8	12.1	114	48.6	44.8
GIM ₉₂	8 Nov 1991	165	199	25.2	18.1	11	63	39.1	45.6
GIM _{f92}	1 Feb 1992	105	136	24	18.4	12.9	112	40.6	49.2
ART ₉₃	18 Nov 1992	173	202	24.7	18.1	11.5	72	36.9	33.7
ART _{w93}	8 Jan 1993	129	154	26	19.2	12.4	30	39.5	26.4
GIM ₉₃	23 Nov 1992	153	189	23	16.4	9.9	84	46.6	46.6
GIM _{w93}	10 Jan 1993	110	142	24	17.2	10.5	39	33.1	46.1
ART ₉₄	9 Dec 1993	147	176	26	18.3	10.6	36	44.4	40.4

¹ ART= Artesa de Segre; BEL= Bell-lloc; GIM= Gimenez; f= fall sowing; w= winter sowing.

A spike mean grain size was obtained by weighting the ratio of central to lateral grains (1:2) within the barley spike. Grain yield was derived by harvesting at maturity all plots of each trial, and yield values were corrected for grain moisture of 12%.

2.3. Nitrogen analysis

Oven-dried samples of vegetative plant parts and bulked central and lateral grains were ground through a 0.5 mm screen with a Cyclotec 1093 sample mill (Tecator AB, Höganäs, Sweden). Total N concentration of vegetative samples at anthesis and grain at maturity were then measured using an automatic nitrogen analyser (Carlo Erba 1500, Milan, Italy). N content of vegetative samples was expressed as the amount of N accumulated per unit area. N content per grain was calculated using grain N concentration and spike mean grain weight. Grain protein concentration was estimated by multiplying grain N concentration by 5.71. The carbohydrate content was further inferred by subtracting the estimated protein content from the total dry weight of grains.

2.4. Analysis of data

Data on grain yield, dry matter at anthesis, grain size, as well as N content and N concentration in vegetative samples and mature grain, were analyzed by analysis of variance using SAS/STAT (SAS Inst., 1987). Linear and decay regressions were performed using TableCurve 2D v.2.03 (Jandel Corporation, San Rafael, California, 1994).

3. RESULTS

Table 1 describes the trials used in this study and the climatic conditions during grain filling at each particular site. Winter sowings delayed heading dates (in calendar days) about five to 15 days as compared with fall seedings, thus exposing genotypes to higher temperature. Mean temperatures during the post-anthesis period were always higher than 15°C, and rainfall was extremely variable, even for trials only differing in sowing date.

The environmental conditions before and during grain development resulted in a wide

range of responses in terms of grain size. Thus, average grain size of control spikes for all genotypes across trials varied from 26.4 mg to 49.2 mg (Table 1). Such responses were partially due to climatic constraints taking place from anthesis to maturity. For instance, the low average grain size of ART_w93 (26.4 mg) or GIM_p91 (35.8 mg) were related to high mean temperature and/or low rainfall, whereas the highest average grain size (49.2 mg) was achieved under the high rainfall conditions of GIM_w92.

The average grain yields produced in these experiments ranged from 1.5 to 5.6 t ha⁻¹, showing a larger variation in comparison with grain size (Table 1). The average grain number of control spikes varied from 33.0 to 58.3 (Table 1). Mean grain yield and grain size were positively correlated across trials ($r=+0.63, p<0.05$). This significant correlation was particularly determined by the lowest values of grain size in the poorest yielding environments. Mean grain yield was unrelated to grain number per spike ($r=+0.05, p>0.05$). No significant relationship across trials appeared between mean grain size and grain number per spike ($r = -0.12, p>0.05$).

Total vegetative dry matter accumulated by anthesis significantly differed among environments (Table 2). Differences in dry matter at anthesis were also observed among genotypes. Grain yield was significantly related to total dry matter at anthesis ($r=+0.46, p<0.05$). N content at anthesis was significantly affected by the environment (Table 2). In Bell-lloc 1992, less N accumulation was determined in plant tissues. This was probably related to the fact that less N fertilizer was applied at this site. Orria significantly accumulated more N in vegetative parts than Barberousse and Plaisant, but this was caused by higher accumulation of dry matter by anthesis rather than an absolute increase in N concentration in plant parts.

Grain yield did not show significant differences among cultivars, although there was a significant genotype×environment interaction (Table 3). Number of grains per spike did not statistically differ among genotypes (data not shown). Grain size, N content per grain, and N concentration showed significant differences among genotypes (Table 3). Barberousse significantly showed the lowest grain size, N content per grain, and N concentration. The effect of the partial sterilization on the remaining grains was always highly significant ($p<0.001$) for the set of characters under study. Mean relative increments in grain size from treated spikes was 20%, whereas N content per grain and N concentration increased by 47.6% and 22.6%, respectively. Grain size was positively related to total vegetative dry matter at anthesis in control ($r=+0.49, p<0.01$) and half sink-reduced spikes ($r=+0.52, p<0.01$). Grain N content was also correlated positively with total vegetative N content at anthesis, both in intact ($r=+0.47, p<0.05$) and half

Table 2. Dry matter, N content and N concentration at anthesis in vegetative parts of three six-rowed barley cultivars grown in nine environments.

Genotype	Dry matter (t ha ⁻¹)			N content (kg ha ⁻¹)			N conc. (g kg ⁻¹)		
	Leaf + culm	Chaff	Total	Leaf + culm	Chaff	Total	Leaf + culm	Chaff	Total
Barberousse	3.1	0.7	3.8	55	12	67	18	18	18
Oria	4.0	0.8	4.8	74	15	91	19	19	19
Plaisant	2.9	0.6	3.5	55	13	68	19	19	22
LSD _{0.05}	0.7	ns	0.8	13	2	15	ns	1	1
Environment									
BEL ₉₂	3.6	0.7	4.3	47	11	58	13	17	17
BEL ₉₂	2.9	0.8	3.7	48	12	60	16	16	16
GIM ₉₂	4.6	0.7	5.3	71	12	83	16	19	19
GIM ₉₂	3.4	0.8	4.2	82	18	100	24	22	22
ART ₉₃	2.3	0.6	2.9	46	13	59	21	24	24
ART ₉₃	2.9	0.6	3.5	61	13	74	21	22	22
GIM ₉₃	3.5	0.8	4.3	61	14	75	16	17	17
GIM ₉₃	4.4	0.9	5.3	82	17	99	18	18	18
ART ₉₄	2.4	0.5	2.9	51	9	60	21	20	20
LSD _{0.05}	1.2	ns	1.4	23	3	22	4	3	3
Level of significance									
Genotype	0.009	0.079	0.008	0.006	0.031	0.006	0.089	0.001	0.001
Environment	0.010	0.166	0.012	0.007	0.001	0.006	0.001	0.001	0.001

LSD_{0.05}, least significant difference at the 0.05 level of significance

Table 3. Genotype and treatment effects on grain yield ($t\ ha^{-1}$), grain weight (mg), N content per grain (mg) and grain N concentration ($mg\ g^{-1}$) of control and half sink-reduced spikes of three six-rowed barley cultivars grown in 11 environments.

	Yield ($t\ ha^{-1}$)	Grain weight (mg)	Grain N content (mg)	Grain N concentration ($mg\ g^{-1}$)
Genotype				
Barberousse	3.890	42.7	0.90	21.2
Orria	3.671	46.3	1.10	23.5
Plaisant	3.574	45.8	1.04	23.0
LSD _{0.05}	ns	2.3	0.07	1.1
Treatment				
Control spikes	-	40.8	0.82	20.3
½ sink-reduced spikes	-	49.0	1.21	24.8
Level of significance				
Genotype (G)	0.285	0.001	0.001	0.001
Environment (E)	0.001	0.001	0.001	0.001
G×E	0.004	0.026	0.315	0.049
Treatment (T)	-	0.001	0.001	0.001
G×T	-	0.087	0.750	0.064
E×T	-	0.088	0.713	0.094

LSD_{0.05}, least significant difference at the 0.05 level of significance

sink-reduced spikes ($r=+0.49, p<0.01$). Genotypes with smaller grains seemed to exhibit larger increments in grain size in response to sink halving (25.1, 21.3 and 14.6% for Barberousse, Plaisant and Orria, respectively). However, statistically cultivars responded similarly to the treatment, as shown by the non significant genotype \times treatment interaction.

When the environmental conditions determined small grain sizes of control spikes, the grain size of the half sink-reduced spikes increased proportionally more than when grains of control spikes were large (Fig. 1). In fact, the slope of the regression line shown in this figure (0.64 mg mg^{-1}) was significantly lower than one. Larger increments in grain size due to sink halving tended to be negatively related to total dry matter accumulated by anthesis ($r=-0.29, p>0.05$), suggesting that less assimilates were initially available for grain filling in low-yielding environments. In contrast, a similar pattern of increase in N content in grains from sink-reduced spikes was observed in all trials, regardless of N level of grains from control spikes (Fig. 2a). This nearly constant level was given by the intercept of the linear regression ($0.43 \text{ mg grain}^{-1}$). Although there was no significant relationship between the responses in grain size and grain N content due to sink manipulation, the positive correlation between grain size and grain N content in control spikes ($r=+0.60, p<0.001$) was maintained in half sterilized spikes ($r=+0.55, p<0.001$).

Grain N concentration (Fig. 2b) mimicked the pattern of response of grain size to sink halving (Fig. 1). This was due to the fact that grain N content increased similarly, in absolute terms, throughout the range of genotype-environment combinations (Fig. 2a). Thus, the corresponding linear regression with a slope of 0.69 was statistically lower than unity (Fig. 2b) and closely approached the value of 0.64 for grain size response. Grain N concentration was moderately related to grain size in control spikes ($r=-0.36, p<0.05$), but unrelated when sink halving was practiced ($r=-0.10, p>0.05$). N content per grain and N concentration were positively related in both control ($r=+0.49, p<0.01$) and treated spikes ($r=+0.77, p<0.001$).

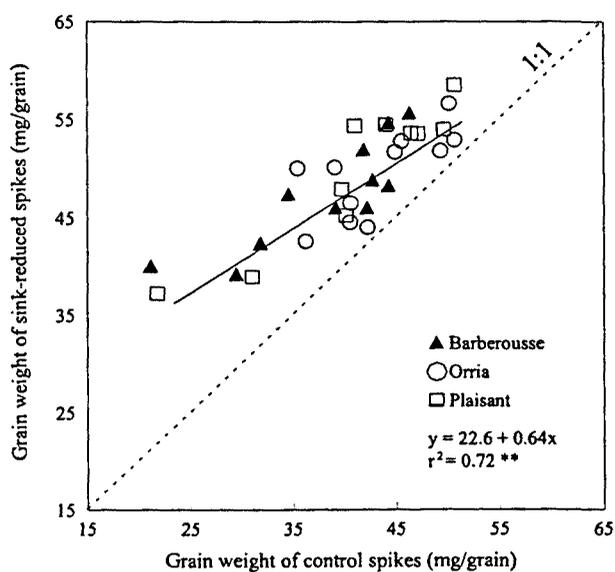


Figure 1. Grain weight of sink-reduced spikes in relation to that of control spikes for three six-rowed barley genotypes in 11 environments.

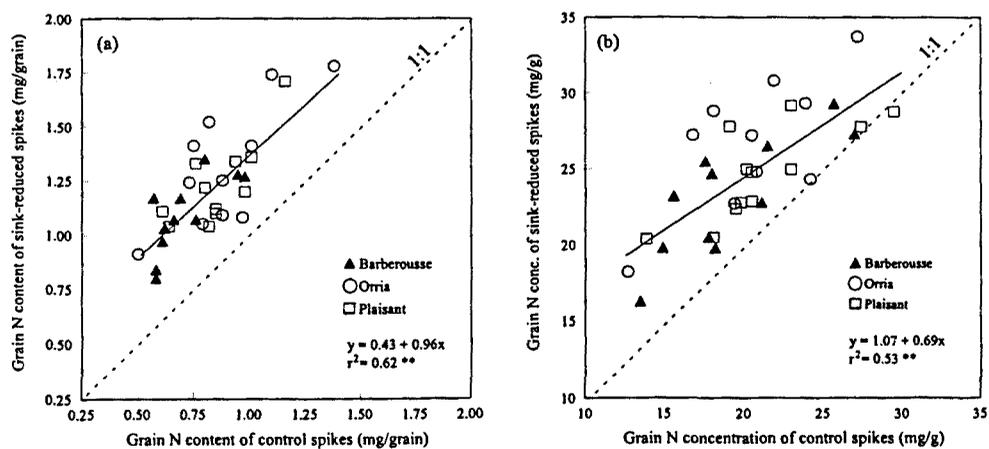


Figure 2. Grain N content (a), and grain N concentration (b) of sink-reduced spikes in relation to that of control spikes for three six-rowed barley genotypes grown in 11 environments.

The dry matter transported through phloem to the developing barley caryopsis is comprised basically of sucrose and amino acids. Sucrose is mostly converted to starch, which represents between 60% and 75% of final grain dry weight (Duffus and Cochrane, 1992). Considering the multicompositional nature of the barley grain, a simplification to the main two components (carbohydrates and proteins) may help understand how dry matter accumulates and grain protein concentration is determined in response to changing source-sink relationships during grain filling. Based upon total grain size and N accumulation per grain in sink-reduced spikes, the corresponding amounts of newly synthesized protein and carbohydrates were estimated for each genotype-environment combination. Since the protein accumulation pattern seemed constant in absolute values and independent of the grain size of control spikes, proteins and carbohydrates accumulated differentially in growing grains as a consequence of greater assimilate availability during grain filling (Fig. 3a).

The relative increase (%) in grain size as a consequence of sink halving showed a typical and highly significant decay response when plotted against the grain size of control spikes (curve A, Fig. 3b). Sink halving, thus, seemed to stimulate growth of remaining grains exponentially as grains of control spikes decreased in size. In comparison with the relative dry matter increase in grains of control spikes, carbohydrate accumulation (curve B, Fig. 3b) showed a slightly shifted response when corrected for protein content. Parameters of the non-linear relationship ($y=a+b\exp(-cx)$) have a biological meaning: a (asymptotic response to an increasingly larger grain size), b (initial response to near-zero grain size) and c (decay response rate). Decay rates were very similar in both equations, so the shifted behaviour was mainly driven by differences in asymptotic (a constant) and initial (b constant) responses. An approximate relative value of 6.5% dry matter increment in the largest grains could be assigned to protein accumulation as a result of subtracting the a value of carbohydrate response from the global response asymptote. It is noteworthy that the asymptotic value of carbohydrate accumulation was not statistically different from zero (2.33 ± 4.61 s.e.), implying a sink limitation in those situations favouring large grains. In such cases, positive responses to enhanced supply of assimilates were mostly related to protein accumulation.

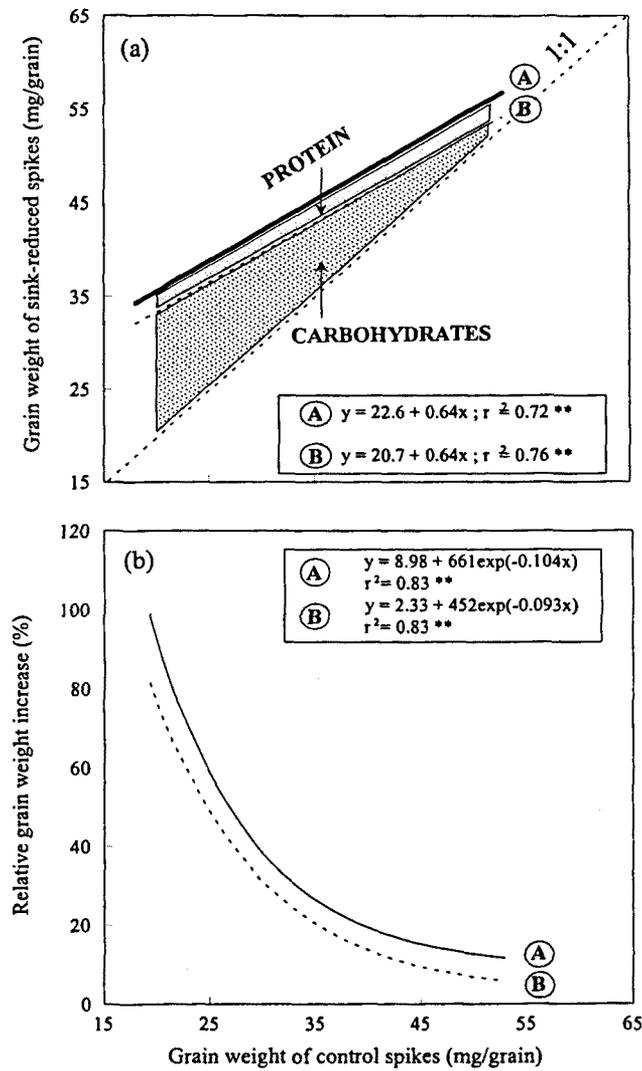


Figure 3. (a) Linear regressions of total grain weight (thick line, A) and grain carbohydrate deposition (dashed line, B) of sink-reduced spikes in relation to the grain weight of control spikes for three six-rowed barley genotypes in 11 environments. (b) Decay relationships for relative grain weight increase (Eq. A) and relative grain carbohydrate deposition (Eq. B) of sink-reduced spikes in relation to grain weight of control spikes.

4. DISCUSSION

In the present study, water and high temperature stresses played a major role in determining grain size. In fact, post-anthesis rainfall and mean temperatures together explained 44% of variability among environments in grain size. In medium- and high-yielding trials, remobilisation of assimilates possibly coped with the eventual reduction in photosynthesis caused by drought and high temperatures. Current evidence suggests that vigorous early growth is important in providing assimilates for grain filling when drought occurs during post-anthesis (Siddique *et al.*, 1989). The positive relationship found between dry matter accumulated at anthesis and grain size of control spikes supports this hypothesis. Furthermore, remobilisation of assimilates was probably more limited under severe pre-anthesis growing conditions, since greater increments in grain size to reduced sink strength were detected in environments with small dry matter production at anthesis. In these cases, high temperatures and drought caused a major penalty in final grain size.

Average grain size responses to sink halving were slightly greater for those genotypes with small grains, such as Barberousse and Plaisant. Similar responses were reported in wheat by other authors (Blum *et al.*, 1988; Ma *et al.*, 1990). In agreement with our results, the relative increments found in grain size (well under 100%) were not large enough to account for the greater source/sink ratio (2:1). Ma *et al.* (1990) suggested that grain growth was partly source-limited, but a sink limitation in grains of control spikes probably developed in later stages of grain filling. Because the endosperm cell number is regulated by assimilate supply during the early phases of grain development (Brocklehurst, 1977), pre-anthesis stresses could reduce potential grain size in this study by decreasing source availability. Therefore, only a complete lack of response to an increase in assimilate availability can be effectively interpreted as the result of a sink limitation to grain development. Otherwise, grain growth would be primarily limited by source supply.

The degree of a source limitation for grain growth (i.e. the increase in grain size in response to sink halving as compared with the grain size of control spikes) is suggested by the regression slope in Fig. 1. Under favourable growing conditions, large grains barely increased in size in response to a 50%-reduced sink but, when the environment determined smaller grains, grain growth became progressively more source-limited. Indeed, a reduction of 1 mg in the grain weight of control spikes resulted into 0.64 mg weight decrease of grains from sink-reduced spikes. If both treated and control mainstems produce similar grain yields per spike, grain yield

would be completely source-limited during grain filling. Based on the regression slope, a complete source limitation would theoretically appear if grains of sink-reduced spikes double the size of control grains (i.e. about $16.6 \text{ mg grain}^{-1}$). Inversely, a lack of response to sink halving would indicate a complete sink limitation to grain yield. This would be attained with a theoretical grain size of $62.7 \text{ mg grain}^{-1}$, derived from the regression line. The grain size of control spikes fell between these two extreme responses, suggesting a partial source limitation to total dry matter accumulation (greater when grain size of control spikes was smaller). In fact, the particular responses found to reduced sink strength were induced by the differential accumulation of carbohydrates and proteins in growing grains, as discussed below.

N content in grains of control spikes was partially related to N stored in vegetative organs at anthesis. Particularly, less N fertilizer applied at Bell-lloc was expressed in smaller amounts of N accumulated in vegetative organs as well as in mature grains. An examination of the moderate, but negative, relationship found between grain size of control spikes and N concentration suggests that the increased grain N percentage was mainly a dilution effect caused by the reduction in carbohydrate accumulation in stressed environments. This is supported by the higher grain N content found in larger control grains, indicating a greater N uptake in more favourable environments. The average increment in grain N content due to sink manipulation (47.6%) was greater than that found for grain size (20%), indicating that N uptake was largely regulated by the level of N supply. Consequently, average grain N concentration also increased in response to sink halving (22.6%), but to a lesser extent than N content per grain. This type of response is well documented elsewhere for wheat (e.g., Radley and Thorne, 1981; Pérez *et al.*, 1989; MacKown *et al.*, 1992).

The uniformity of grain N accumulation responses to reduced sink strength throughout all genotype-environment combinations has not been clearly reported previously, suggesting that environmental constraints do not affect N accumulation and protein synthesis during grain filling. Palta *et al.* (1994) found similar amounts of total grain N when comparing one wheat variety grown under two different post-anthesis water deficits, and Stone and Nicolas (1994) showed a relative insensitivity of N accumulation to post-anthesis heat stress in wheat. In the present study, the similarity in N responses to sink halving confirms that the pattern of dry matter accumulation was mainly driven by differential amounts of carbohydrate deposition in the endosperm.

Jenner *et al.* (1991) proposed a transport mechanism with saturable characteristics in which carbohydrate deposition in growing grains would lie often on an asymptotic region of an

accumulation versus supply relationship. Hence, under optimal growing conditions, grains fail to respond positively to an increase in carbohydrate availability, indicating a clear sink limitation to grain growth (see e.g., Martinez-Carrasco and Thorne, 1979; Slafer and Savin, 1994). In our conditions, where pre- and post-anthesis stresses cause a source limitation to grain growth of six-rowed barleys, carbohydrate deposition would often lie on the linear region of such relationship rather than towards its asymptotic end. This would explain the wide range of relative carbohydrate responses to source enhancement (from 75% to a level non significantly different from zero) and the decreased contribution of carbohydrates as grain size of control spikes increased. Conversely, the same authors (Jenner *et al.*, 1991) proposed protein deposition to lie on the linear region of an identical relationship for amino acid supply. This would suggest a greater dependency of grain protein content on N availability. Indeed, McKown *et al.* (1992) showed that wheat plants with 50%-reduced sink strength redistributed vegetative N to the spike almost as effectively as control plants. In our study, protein deposition may have taken place in the linear region of the amino acid supply, with relative increments always beyond 25%. Protein did not accumulate more in favourable conditions probably due to the existence of an upper biological limit to protein deposition within the grain itself (MacKown *et al.*, 1989; Ma *et al.*, 1995). In fact, the relative decrease in carbohydrate accumulation when control grains increased in size changed protein contribution to total dry matter increment from about 14% to 58%.

Under near-optimal growing conditions, expressed in large-seeded grains, carbohydrates hardly accumulated beyond a given grain size, indicating that potential grain size was being approached. Further responses were mainly due to protein deposition. Even though source availability is the main factor limiting grain size of high yielding six-rowed barley cultivars, the present study also shows that a sink limitation to carbohydrate deposition can be involved in Mediterranean conditions.

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REFERENCES

- Austin, R.B., Morgan, C.L., Ford, M.A. and Blackwell, R.D., 1980. Contribution to grain yield from pre-anthesis assimilation in tall and dwarf barley phenotypes in two contrasting seasons. *Ann. Bot.*, 45: 309-319.
- Bidinger, F., Musgrave, R.B. and Fischer, R.A., 1977. Contribution of stored pre-anthesis assimilate to grain yield in wheat and barley. *Nature*, 270: 431-433.
- Blade, S.F. and Baker, R.J., 1991. Kernel weight response to source-sink changes in spring wheat. *Crop Sci.*, 31: 1117-1120.
- Blum, A., 1985. Photosynthesis and transpiration in leaves and ears of wheat and barley varieties. *J. Exp. Bot.*, 36: 432-440.
- Blum, A., Mayer, J. and Golan, G., 1988. The effect of grain number per ear (sink size) on source activity and its water-relations in wheat. *J. Exp. Bot.*, 39: 106-114.
- Brocklehurst, P.A., 1977. Factors controlling grain weight in wheat. *Nature*, 266: 348-349.
- Ceccarelli, S., Acevedo, E. and Grando, S., 1991. Breeding for yield stability in unpredictable environments: single traits, interaction between traits, and architecture of genotypes. *Euphytica* 56: 169-186.
- Darroch, B.A. and Baker, R.J., 1990. Grain filling in three spring wheat genotypes: statistical analysis. *Crop Sci.*, 30: 525-529.
- Duffus, C.M. and Cochrane M.P., 1992. Grain structure and composition. In: P.R. Shewry (Editor), *Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology*. C.A.B. International, pp. 291-317.
- Gallagher, J.N., Biscoe, P.V. and Scott, R.K., 1975. Barley and its environment. V. Stability of grain weight. *J. Appl. Ecol.*, 12: 319-336.
- Jenner, C.F., Ugalde, T.D. and Aspinall, D., 1991. The physiology of starch and protein deposition in the endosperm of wheat. *Aust. J. Plant Physiol.*, 18: 211-226.
- Kobata, T., Palta, J.A. and Turner, N.C., 1992. Rate of development of postanthesis water deficits and grain filling of spring wheat. *Crop Sci.*, 32: 1238-1242.

- López-Castañeda, C. and Richards, R.A., 1994. Variation in temperate cereals in rainfed environments. I. Grain yield, biomass and agronomic characteristics. *Field Crops Res.*, 37: 51-62.
- Ma, Y.Z., MacKown, C.T. and Van Sanford, D.A., 1990. Sink manipulation in wheat: compensatory changes in kernel size. *Crop Sci.*, 30: 1099-1105.
- Ma, Y.Z., MacKown, C.T. and Van Sanford D.A., 1995. Kernel mass and assimilate accumulation of wheat: cultivar responses to 50% spikelet removal at anthesis. *Field Crops Res.*, 42: 93-99.
- MacKown, C.T., Van Sanford, D.A. and Ma, Y.Z., 1989. Main stem sink manipulation in wheat. Effects on nitrogen allocation to tillers. *Plant Physiol.*, 89: 597-601.
- MacKown, C.T., Van Sanford, D.A. and Zhang, N., 1992. Wheat vegetative nitrogen compositional changes in response to reduced reproductive sink strength. *Plant Physiol.*, 99: 1469-1474.
- Martinez-Carrasco, R. and Thorne, G.N., 1979. Physiological factors limiting grain size in wheat. *J. Exp. Bot.*, 30: 669-679.
- Nátrová, Z. and Nátr, L., 1993. Limitation of kernel yield by the size of conducting tissue in winter wheat varieties. *Field Crops Res.*, 31: 121-130.
- Palta, J.A., Kobata, T., Turner, N.C. and Fillery, I.R., 1994. Remobilization of carbon and nitrogen in wheat as influenced by postanthesis water deficits. *Crop Sci.*, 34: 118-124.
- Papakosta, D.K. and Gagianas, A.A., 1991. Nitrogen and dry matter accumulation, remobilization, and losses for Mediterranean wheat during grain filling. *Agron. J.*, 83: 864-870.
- Pérez, P., Martínez-Carrasco, R., Martín del Molino, I.M., Rojo, B. and Ulloa, M., 1989. Nitrogen uptake and accumulation in grains of three winter wheat varieties with altered source-sink ratios. *J. Exp. Bot.*, 40: 707-710.
- Radley, M.E. and Thorne, G.H., 1981. Effects of decreasing the number of grains in ears of cvs Hobbit and Maris Huntsman winter wheat. *Ann. Appl. Biol.*, 98: 149-156.
- SAS Institute, 1987. *SAS/STAT Guide for Personal Computers, Version 6*. SAS Institute, Inc., Cary, NC.
- Shepherd, K.D., Cooper, P.J.M., Allan, A.Y., Drennan, D.S.H. and Keatinge, J.D.H., 1987. Growth, water use and yield of barley in Mediterranean-type environments. *J. Agric. Sci. (Camb.)*, 108: 365-378.

Siddique, K.H.M., Belford, R.K., Perry, M.W. and Tennant, D., 1989. Growth, development and light interception of old and modern wheat cultivars in a Mediterranean-type environment. *Aust. J. Agric. Res.*, 40: 473-487.

Slafer, G.A. and Savin, R., 1994. Source-sink relationships and grain mass at different positions within the spike in wheat. *Field Crops Res.*, 37: 39-49.

SSS, 1990. Keys to Soil Taxonomy. SMSS Technical Monograph No. 6, Blacksburg, VA.

Stone, P.J. and Nicolas, M.E., 1994. Wheat cultivars vary widely in their responses of grain yield and quality to short periods of post-anthesis heat stress. *Aust. J. Plant Physiol.*, 21: 887-900.

Wrigley, C.W., Blumenthal, C., Gras, P.W. and Barlow, E.W.R., 1994. Temperature variation during grain filling and changes in wheat-grain quality. *Aust. J. Plant Physiol.*, 21: 875-885.

CHAPTER II

Growth and final weight of central and lateral barley grains under Mediterranean conditions as influenced by sink strength

ABSTRACT

Grain weight largely determines the end-use quality of barley (*Hordeum vulgare* L.). In six-rowed types, central spikelets commonly bear heavier grains than lateral spikelets. However, the extent to which such differences in weight are influenced by source availability remains unclear. Field experiments were carried out in nine rainfed trials of northeastern Spain showing a large variability in grain weight. Three six-rowed genotypes were used: Barberousse, Orria and Plaisant. Sink strength of mainstems was decreased by half at anthesis by sterilizing 50% of fertile central and lateral florets. Variation among spikelets in grain growth parameters [maximum grain filling rate (GFR) and grain filling duration (GFD)] and in final grain weight was evaluated in control and sink-reduced spikes. Central grains outweighed lateral ones in control spikes from 7.3 mg to 14.8 mg across trials. Absolute differences in weight increased progressively in those trials favoring larger grains, although lateral to central grain weight ratios (L/C) remained fairly constant, with an average value of 0.77. This advantage of central grains was mostly caused by higher GFR values. A 50%-reduced sink increased grain weight of both central and lateral spikelets similarly and did not diminish the absolute differences in weight found in control spikes. This result suggests that central grains have a greater intrinsic potential weight. While grain yield was partially source-limited in all environments, lateral grains exhibited on average a major degree of source limitation (24%) than central grains (19%). Sink reduction increased grain weight of both central and lateral grains by stimulating GFR in all trials, and also by lengthening GFD in poor rainfed environments.

1. INTRODUCTION

Kinetics of photosynthate incorporation into growing grains of cereals is commonly defined by two parameters: dry matter accumulation rate and effective period of assimilate deposition. The relative importance of both parameters on determining grain weight has been extensively studied, especially in wheat (*Triticum aestivum* L.) (Nass and Reiser, 1975; Sofield *et al.*, 1977), and the influence of environmental constraints has been well established (Wardlaw *et al.*, 1980; Wiegand and Cuellar, 1981). Grain filling rate and duration are known to display genetic and environmental variation, although grain filling rate seems more genotype-dependent and grain filling duration is influenced mainly by post-anthesis temperature.

Grain size, as influences test weight, is an important factor determining nutritional and malting quality in barley (Molina-Cano *et al.*, 1997). Six-rowed cultivars clearly produce smaller grains than do two-rowed types when grown in low-rainfall areas (Acevedo *et al.*, 1991). This is partially related to large differences in grain weight between central and lateral spikelets of the spike in six-rowed cultivars. Possible factors underlying such differences have not been completely established. One possible explanation is that central grains may physically restrain the growth of lateral spikelets, especially when sufficient assimilates are available for grain filling. Alternatively, central grains may constitute a greater sink for assimilates. Kirby and Rymer (1975) observed that vascular bundles feeding the lateral spikelets of barley were smaller compared with those of the central spikelets. Studies made on pedicelled lateral spikelets (Gilbertson and Hockett, 1986) found that lateral grains were about half the weight of central grains on average in fifteen near isogenic six-rowed barley lines. Scott *et al.* (1983) demonstrated that the grain weight of six-rowed barley was closely related to the carpel size before anthesis, with central carpels being heavier than lateral ones. This relationship was assessed under near-optimal growing conditions when grain weight approaches its maximum potential size, but might not be applicable in situations characterized by a shortage of assimilates during grain filling.

In wheat, differences in grain weight were found within a particular spikelet (Bremmer and Rawson, 1978) and were mostly due to differences in grain filling rates (Rawson and Ruwali, 1972; Simmons and Crookston, 1979). After a sink reduction treatment, similar weight increases in individual grains were found regardless of their position within the spikelet (Simmons *et al.*, 1982). In barley, Scott *et al.* (1983) found lateral grains to have about 25% slower growth rates than central grains of six six-rowed cultivars grown under near-optimal conditions. Conversely,

grain filling duration seldom varied and, therefore, grain filling rate explained most of the variation in grain weight. However, the role of grain filling parameters in determining grain growth and final grain weight of central and lateral spikelets of barley under Mediterranean conditions remains to be elucidated. In this regard, the responses to a reduced sink strength may help determine whether differences in grain weight between central and lateral spikelets are related to source availability during grain filling.

This study was designed to: (i) define possible variation in patterns of grain growth between central and lateral grains of six-rowed barleys in rainfed Mediterranean environments, and (ii) examine the consequences of enhanced assimilate availability (i.e., 50%-reduced sink strength) on grain filling rate and duration and on final grain weight.

2. MATERIALS AND METHODS

2.1. Experimental design and plant material

Field trials, summarized in Table 1, were sown in the Fall (November-December) and Winter (January-February) for three consecutive years (1992, 1993, and 1994) at three locations: Artesa de Segre, Bell-lloc d'Urgell and Gimènells, northeastern Spain. Winter sowings were included in an attempt to delay anthesis and, thus, expose grain filling to drier and hotter conditions compared with the fall planted trials. The experimental design at each trial was a randomized complete block with four replicates. For the specific purpose of this study, only one replicate was used. Plots were six 12-m rows, spaced 20 cm apart, and seeded at rates of 350 seeds m^{-2} . Plots in Artesa de Segre and Gimènells were fertilized with 50 kg N ha^{-1} , 20 kg P ha^{-1} , and 35 kg K ha^{-1} at sowing, and 60 kg N ha^{-1} at the jointing stage (ZCK scale 31; Zadocks *et al.*, 1974). Plots in Bell-lloc d'Urgell were fertilized with 40 kg N ha^{-1} , 20 kg P ha^{-1} , and 35 kg K ha^{-1} at sowing. Three six-rowed, highly-productive barley genotypes were used: Barberousse and Plaisant are two widely cultivated varieties in northern Spain of French origin, and Orria is a new Spanish release of CIMMYT origin.

Table 1. Trial characterization and mean climatic data during the grain filling period. Anthesis date represents the average for all cultivars at each trial.

Location	Coordinates	Soil classification	Sowing period	Trial code	Sowing date	Anthesis date	Average temperatures† and total rainfall (grain filling period)			
							T_{max}	T_{mean}	T_{min}	R
							°C			mm
Artesa de Segre	42°11'N 0°32'E	Fluventic Xerochrept	Fall	ART _p 93	18 Nov	10 May	24.7	18.1	11.5	72
				ART _p 94	9 Dec	5 May	26.0	18.3	10.6	36
			Winter	ART _w 93	8 Jan	17 May	26.0	19.2	12.4	30
Bell-lloc d'Urgell	41°40'N 0°41'E	Xeric Torriorthent	Fall	BEL _p 92	28 Nov	25 Apr	25.5	18.1	10.6	76
			Winter	BEL _w 92	9 Jan	10 May	25.6	18.8	12.1	114
Gimenells	41°36'N 0°13'E	Xerollic Paleorthid	Fall	GIM _p 92	8 Nov	21 Apr	25.2	18.1	11.0	63
				GIM _p 93	23 Nov	25 Apr	23.0	16.4	9.9	84
			Winter	GIM _w 92	1 Feb	15 May	24.0	18.4	12.9	112
				GIM _w 93	10 Jan	30 Apr	24.0	17.2	10.5	39

† Average maximum (T_{max}), mean (T_{mean}) and minimum (T_{min}) temperatures

2.2. Sink reduction treatment and grain filling characterization

Anthesis date was recorded when 50% of spikes extruded anthers. For each genotype, 120 mainstems were tagged at anthesis in one replicate of each trial. A 50% sink reduction treatment was carried out at this stage in 60 tagged mainstem spikes per genotype by hindering the growth of 50% selected spikelets per spike. Briefly, the carpels of lateral florets along the entire length of one spike side and of central florets along the entire length of the opposite side of the same spike were pierced with a sharp needle. The remaining 60 labeled spikes were used as controls. Every 5-7 days from anthesis until maturity, five control and five sink-reduced spikes were collected per genotype. Four central and four lateral grains per spike were collected separately from mid-spike nodes to produce samples of 20 grains each. Dry weight of samples was obtained after drying to constant weight in a forced-air oven at 60°C, and used to calculate average grain weight at sampling.

Final grain weight, maximum grain filling rate and grain filling duration of control and sink-reduced spikes were calculated with the logistic regression equation suggested by Darroch and Baker (1990):

$$y = W/[1 + \exp(B - Cx)] \quad [1]$$

where:

y = grain weight (mg)

x = accumulated growing degree-days (GDD) from anthesis

W = fitted final grain weight (mg)

B and C = empirical values related to grain filling rate (GFR, mg GDD⁻¹) and grain filling duration (GFD, GDD)

Maximum GFR was obtained by finding the first derivative of Eq. [1]; GFD was calculated when 95% of the final grain weight was achieved by replacing y with $0.95W$ in Eq. [1].

Growing degree-days were calculated using a base temperature of 0°C (Bauer *et al.*, 1984). Curve fitting was performed using the NLIN procedure of SAS/STAT for nonlinear regression (SAS Inst., 1987). The coefficients of determination (r^2) obtained were always greater than 95%. Relative responses (%) in GFR, GFD and final grain weight due to the sink reduction

treatment were calculated as $100 \times [(\text{sink-reduced} - \text{control}) / \text{control}]$ and used to characterize the effect of 50%-reduced sink strength on grain filling. GFR, GFD and final grain weight were independently analyzed for control and sink-reduced spikes by analyses of variance combining genotypes, grain position in the spike and environment effects. Three-way interactions were used as experimental errors. Separate analyses were also calculated for relative responses to 50%-reduced sink strength in GFR, GFD and final grain weight.

3. RESULTS

The analyses of variance for grain weight, GFR and GFD, independently performed for control and sink-reduced spikes, are shown in Table 2. In control spikes, the effects of genotype, environment and genotype by environment interaction were all significant ($p < 0.01$), except GFD when comparing genotypes. On average, Orria had the heaviest control grains (46.4 mg), followed by Plaisant (43.6 mg) and Barberrouse (40.9 mg); Orria showed the highest GFR ($0.1076 \text{ mg GDD}^{-1}$), compared with Plaisant ($0.0993 \text{ mg GDD}^{-1}$), and Barberrouse ($0.0893 \text{ mg GDD}^{-1}$). The effect of grain position (lateral vs. central) was also significant ($p < 0.01$) for grain weight and GFR in control spikes. Lateral grains were on average 23% smaller than central grains and showed a significantly lower (22.8%) GFR, but no significant difference in GFD. The effect of grain position on grain weight, GFR and GFD was similar for all genotypes, as there was no significant genotype \times position interaction.

In spite of the large variability in grain weight of control spikes found among environments (Fig. 1a), relative differences in weight between positions remained constant. This is indicated by similar lateral (L) to central (C) grain weight ratios (L/C) (Fig. 1a), with an average value of 0.77. Accounting for these similar ratios, greater absolute differences in weight between central and lateral grains were found in more favorable environments, which produced heavier grains. Thus, central grains outweighed lateral ones from 7.3 mg (ART_w93) to 14.8 mg (GIM_r92) on an environmental mean basis. This difference between positions is underscored by a highly significant ($p < 0.01$) interaction between environment and grain position for grain weight of control spikes (Table 2). When examining grain filling parameters, GFR of central grains was consistently higher than GFR of lateral grains across environments (Fig. 1b). This difference was not evident for GFD (Fig. 1c).

Table 2. Mean squares for grain weight, grain filling rate (GFR), and grain filling duration (GFD) for control and sink-reduced spikes, and for their relative responses to sink reduction. Analysis includes three six-rowed barley cultivars evaluated in nine environments and two grain positions in the spike.

Source of variation	df	Control spikes			Sink-reduced spikes			Relative responses†		
		Grain weight (mg)	GFR (mg GDD ⁻¹) (x10 ⁻⁵)	GFD (GDD) (x10 ²)	Grain weight (mg)	GFR (mg GDD ⁻¹) (x10 ⁻⁵)	GFD (GDD) (x10 ²)	Grain weight (%)	GFR (%)	GFD (%)
Genotype (G)	2	136.8**	151.0**	15.1	29.0*	19.4**	85.2**	973.6**	1294.6**	96.2
Environment (E)	8	354.1**	161.2**	238.4**	205.1**	156.9**	147.5**	1462.7**	628.8**	695.1**
G×E	16	27.3**	27.9**	45.2**	13.6*	27.9**	60.9**	311.7**	389.6**	323.3**
Grain Position (P)‡	1	1766.1**	863.1**	2.3	1801.6**	575.4**	15.8	315.1*	1331.6**	82.1
G×P	2	2.2	3.5	1.1	5.2	17.7*	6.3	49.8	200.1	3.9
E×P	8	6.8**	6.2	12.1	3.2	7.3	14.6*	17.7	45.5	36.6
G×E×P §	16	0.8	8.3	6.3	5.4	2.9	5.3	38.9	73.6	42.2

***: Significant at the 0.05 and 0.01 probability levels, respectively

† 100×[(sink-reduced-control)/control]

‡ Central versus lateral grains

§ 3-way interaction used as experimental error

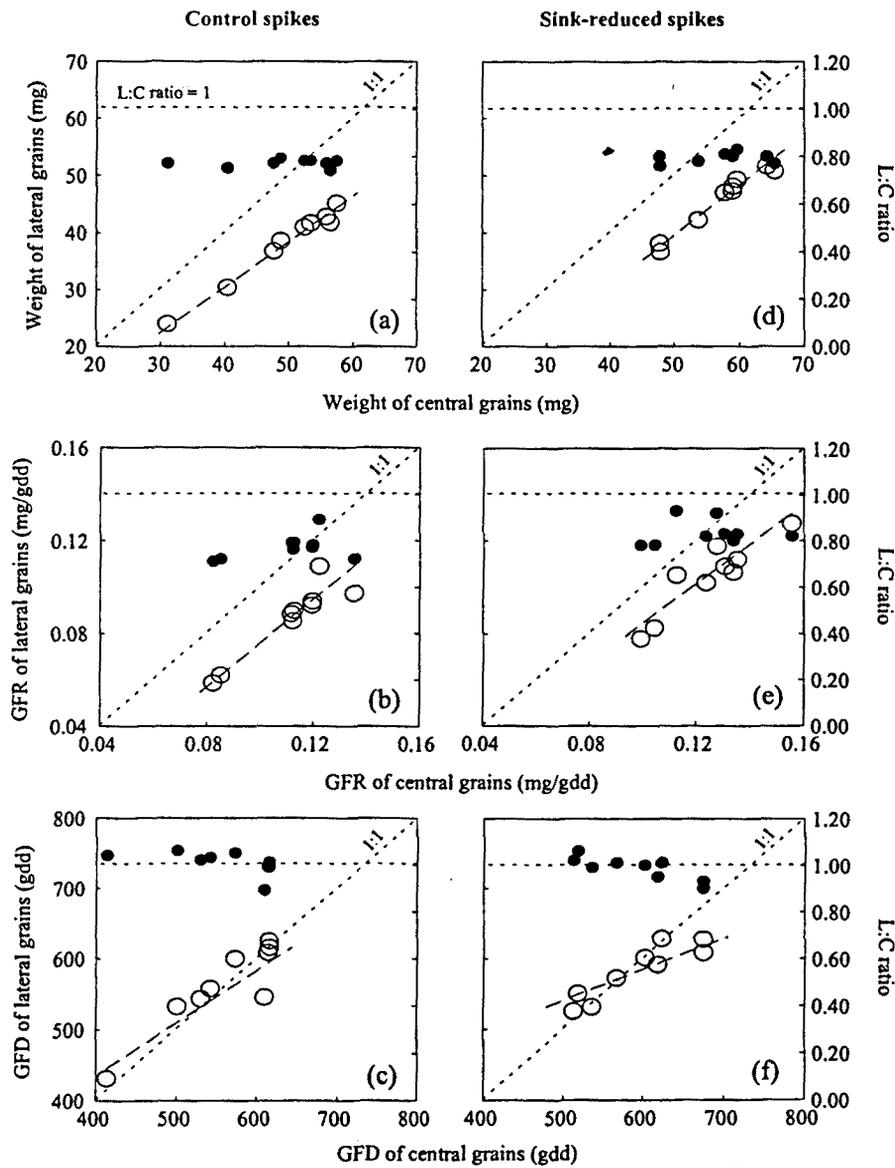


Figure 1. Relationships between final grain weight (a, d), grain filling rate (b, e), and grain filling duration (c, f) of central (x-axis) and lateral (y-axis) grains on control (a, b, c) and sink-reduced (d, e, f) spikes, averaged over cultivars at each trial (open circles). Filled circles represent lateral to central ratios (L/C) for grain weight, GFR and GFD and are displayed in secondary y-axes. Horizontal lines indicate L/C ratios = 1.

Mainstems with 50%-reduced sink showed significant differences among genotypes for grain weight and GFR, but also for GFD (Table 2). There were also significant differences ($p < 0.01$) between positions for grain weight and GFR, but not for GFD. Lateral grains were on average 20.1% smaller than central grains (Fig. 1d) and showed a significant 16.5% decrease in GFR (Fig. 1e). In contrast, GFD was on average only 2% smaller in lateral grains (Fig. 1f). The range of grain weights found among environments in both central and lateral grains decreased compared with that of control spikes (Fig. 1d vs Fig. 1a). In addition, the range of differences in weight between central and lateral grains of sink-reduced spikes was also smaller (from 9.7 mg (ART_w93) to 14.7 mg (GIM_w92)). Hence the environment \times grain position interaction for grain weight was not significant (Table 2). Lateral to central grain weight ratios (L/C) remained fairly constant, although greater ratios were achieved under sink-reduced conditions, with an average value of 0.80 (Fig. 1d).

Relative responses in GFR, GFD and grain weight due to the sink reduction treatment varied significantly among genotypes and environments (Table 2). Grain weight increased on average by 26.7%, 23.9% and 12.8% for Barberrouse, Plaisant and Orria, respectively. Sink reduction also increased GFR (27.4%, 19.6%, and 10.4% for Barberrouse, Plaisant and Orria, respectively), and lengthened GFD by 5.6%, 6.8% and 2% for Barberrouse, Plaisant and Orria, respectively. A similar overall increase in weight was observed in central (7.8 mg grain⁻¹) and lateral grains (7.7 mg grain⁻¹). However, lateral grains responded significantly more to the treatment when expressed as percentage (Table 2): 24% compared with a 19% increase of central grains. On average, GFR increased by 14.1% and 24% in central and lateral grains, respectively. GFD increased less in response to sink reduction: 7% and 5% for central and lateral grains, respectively.

Sink reduction did not modify differentially (relative to control conditions) the growth pattern of central and lateral grains across environments. This is indicated by the lack of significant environment \times position interactions for relative responses in GFR, GFD and grain weight (Table 2). Grain weight responses to sink reduction ranged from 5.7% to 63.1% across environments (Fig. 2a). To verify whether such increases were caused by changes in either GFR or GFD, or both, correlations between responses in grain weight, GFR and GFD, expressed relative to control spikes, were computed separately in central and lateral grains of each genotype (Table 3). In most cases, greater increases in grain weight in response to sink reduction were related to longer GFD (except central grains of Plaisant). In contrast, relative responses for grain

weight and GFR were positively and significantly correlated only in central grains of Plaisant, though GFR was always enhanced by sink reduction, except in one case (Fig. 2b). In fact, relative responses in GFD were often negative (Fig. 2c), despite the increase in grain weight indicated by positive percent responses. To account for such increases, relative responses in GFR and GFD often compensated each other in Orria and Plaisant (Table 3), with correlation coefficients ranging from -0.22 (central grains of Plaisant) to -0.84 (central grains of Orria).

Table 3. Simple correlations between relative responses to 50%-reduced sink strength for grain weight, grain filling rate (GFR) and grain filling duration (GFD). Calculations performed separately for central (above diagonal) and lateral grains (below diagonal) on each genotype.

	Barberousse			Orria			Plaisant		
	Grain weight	GFR	GFD	Grain weight	GFR	GFD	Grain weight	GFR	GFD
Grain weight	-	0.60	0.89**	-	-0.84**	0.85**	-	0.74*	0.42
GFR	0.61	-	0.18	-0.48	-	-0.84**	0.31	-	-0.22
GFD	0.91**	0.24	-	0.86**	-0.75*	-	0.64*	-0.53	-

*, **: Significant at the 0.05 and 0.01 probability levels, respectively, df=7

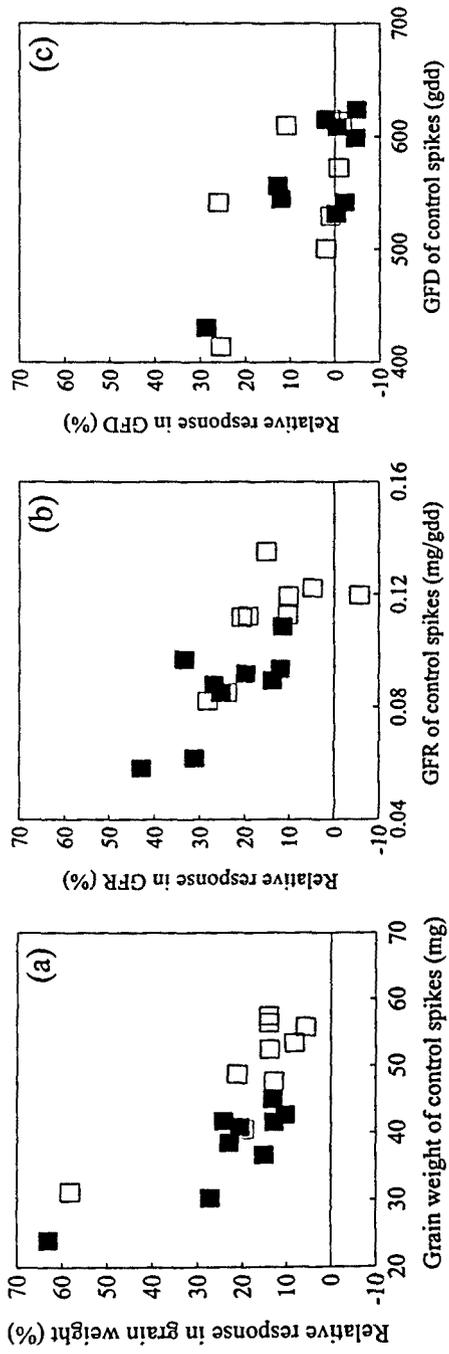


Figure 2. Relative responses in grain weight (a), grain filling rate (b), and grain filling duration (c) to 50%-reduced sink strength compared to control conditions (x-axis). Relative response was calculated as follows: $100 \times [(\text{sink-reduced} - \text{control}) / \text{control}]$. Each point is the average of all cultivars at each trial for central (open squares) and lateral (filled squares) grains.



4. DISCUSSION

Although environmental factors strongly influence grain growth of cereals, many studies have concluded that grain development is also genetically controlled (Metzger *et al.*, 1984; Bruckner and Frohberg, 1987). Genotypes used in this experiment showed significant variation in grain weight under control and sink-reduced conditions. While the average genotypic values for grain weight were directly related to GFR in control spikes, GFD did not differ significantly among genotypes. This observation suggests that post-anthesis stresses possibly limited the duration of transport and deposition of assimilates during the grain filling process. Indeed, mean maximum temperature and GFD were negatively correlated in this study across environments ($r = -0.71, p < 0.05$), which agrees with a previous study in wheat (Wiegand and Cuellar, 1981). This result implies a large genotypic dependence of grain weight on GFR in rainfed environments. However, when more assimilates were available (i.e., after 50% sink reduction) to fulfill grain requirements for growth (Voltas *et al.*, 1997), GFD also displayed significant genotypic variation.

Greater differences in weight between central and lateral grains of control spikes were observed in those conditions favoring heavier grains. Sink reduction not only decreased the competition for assimilates among growing grains, but eliminated, if existing, physical size constraints affecting the development of lateral florets. Since lateral grains did not reach, by far, the weight of central grains in sink-reduced spikes, the existence of a crowding effect along the six-row spike limiting grain growth of lateral florets cannot be accepted, at least as the only valid explanation. Other factors can be involved: for instance, a physical restriction on growth by the lemma and palea of lateral florets may have limited final grain weight. Probably, anatomical differences in the vascular bundles serving the florets were also involved (Kirby and Rymer, 1975), although they were not evaluated. Furthermore, under increased source availability, mature grains would approach their potential weight as determined by the carpel size at meiosis, the stage at which differences in weight between central and lateral grains are already established (Scott *et al.*, 1983). However, estimates of potential grain weight, as obtained by doubling the source/sink ratio (Voltas *et al.*, 1997), were clearly influenced by the environment (see Fig. 1d). This could be due to the fact that growing conditions by the time of meiosis were not uniform among trials, thus determining different carpel sizes. At maturity, differences in weight between central and lateral grains of sink-reduced spikes ranged from 9.7 mg to 14.7 mg, depending on the environment. Therefore, an increased assimilate supply did not decrease the absolute

differences in weight between grains observed under control conditions. These results clearly indicate that the advantage of central grains in final weight must be under strong genetic control and caused either by physical or anatomical factors. Nevertheless, a reduced sink slightly increased the relative contribution of lateral grains to total spike weight, as suggested by larger L/C ratios in comparison with those found in control spikes. This may be relevant in terms of feeding and malting quality, in which the overall grain weight is an important trait.

Increases in grain weight in response to sink reduction (from 5.7% to 63.1% across environments) indicate that grain yield is partially source-limited under semiarid Mediterranean conditions. Nevertheless, the extent of such responses differed between grain positions: lateral grains displayed greater relative increases than central grains, suggesting a major degree of source limitation for grain growth. This fact suggests that central grains may act as a greater sink for assimilates under reduced source availability. In general, a reduced sink enhanced growth rates of both central and lateral grains, while increased only slightly the duration of grain filling. Nevertheless, it is noteworthy that changes in grain weight due to sink reduction, expressed relative to control conditions, were positively related to those for GFD, which often displayed negative values. This phenomenon was not unexpected since it has been shown for wheat (Simmons *et al.*, 1982) that grain growth rates always increased in response to increased source availability, but the grain filling period was often shortened compared with controls. In our study, sink reduction lengthened GFD in those environments showing larger responses in weight (i.e., more source-limited). Blum *et al.* (1988) inferred that a 50%-reduced sink strength in wheat improved source resistance to drought. Indeed, it has been reported that sink reduction may delay leaf senescence in the plant (Patterson and Brun, 1980; Gwathmey *et al.*, 1992; Martín del Molino *et al.*, 1995), although this is not always obvious (Labraña and Araus, 1991). In this study, longer GFD in response to sink reduction could be related to a delay in the plant senescence pattern.

In summary, this study showed that central grains of six-rowed barley genotypes consistently outweighed lateral grains in both control and sink-reduced spikes. This advantage in weight was caused primarily by higher dry matter accumulation rates in central grains. While grain yield was found partially source-limited in all environments, lateral grains exhibited a major degree of source limitation than central grains did. A 50%-reduced sink at anthesis increased grain weight not only by stimulating GFR, but also by lengthening GFD in strongly source-limited environments.

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REFERENCES

- Acevedo, E., Craufurd, P.Q., Austin, R.B., and Pérez-Marco, P., 1991. Traits associated with high yield in barley in low-rainfall environments. *J. Agric. Sci., Camb.* 116:23-36.
- Bauer, A.C., Fanning, C., Enz, J.W., and Eberlein, C.V., 1984. Use of growing degree-days to determine spring wheat growth stages. *North Dakota Agricultural Experimental Station Bulletin* 37.
- Blum, A., Mayer, J., and Golan, G., 1988. The effect of grain number per ear (sink size) on source activity and its water-relations in wheat. *J.Exp. Bot.* 39:106-114.
- Bremmer, P.M., and Rawson, H.M., 1978. The size of individual grains of the wheat ear in relation to their growth potential, the supply of assimilate and interaction between grains. *Aust. J. Plant Physiol.* 5:61-72.
- Bruckner, P.L., and Frohberg, R.C., 1987. Rate and duration of grain fill in spring wheat. *Crop Sci.* 27:451-455.
- Cottrell, J.E., and Dale, J.E., 1984. Variation in size and development of spikelets within the ear of barley. *New Phytol.* 97:565-573.
- Darroch, B.A., and Baker, R.J., 1990. Grain filling in three spring wheat genotypes: statistical analysis. *Crop Sci.* 30:525-529.
- Gilbertson, K.M., and Hockett, E.A., 1986. The effect of pedicel length on lateral kernel weight in two-six rowed near isogenic lines of barley (*Hordeum vulgare* L.). *Euphytica* 35:363-368.
- Gwathmey, C.O., Hall, A.E., and Madore, M.A., 1992. Pod removal effects on cowpea genotypes contrasting in monocarpic senescence traits. *Crop Sci.* 32:1003-1009.
- Kirby, E.J.M., and Rymer, J.L., 1975. The vascular anatomy of the barley spikelet. *Ann. Bot.* 39:205-211.

- Labraña, X., and Araus, J.L., 1991. Effect of foliar application of silver nitrate and ear removal on carbon dioxide assimilation in wheat flag leaves during grain filling. *Field Crops Res.* 28:149-162.
- Martín del Molino, I.M., Martínez-Carrasco, R., Pérez, P., Hernández, L., and Morcuende, R., 1995. Influence of nitrogen supply and sink strength on changes in leaf nitrogen compounds during senescence in two wheat cultivars. *Physiol. Plant.* 95:51-58.
- Metzger, D.D., Czaplowski, S.J., and Rasmusson, D.C., 1984. Grain-filling duration in spring barley. *Crop Sci.* 24:1101-1105.
- Molina-Cano, J.L., Francesch, M., Perez-Vendrell, A.M., Ramo, T., Voltas, J., and Brufau, J., 1997. Genetic and environmental variation in malting and feed quality of barley. *J. Cereal Sci.* 25:37-47.
- Nass, H.G., and Reiser, B., 1975. Grain filling period and grain yield relationships in spring wheat. *Can. J. Plant Sci.* 55:673-678.
- Patterson, T.G., and Brun, W.A., 1980. Influence of sink removal in the senescence pattern of wheat. *Crop Sci.* 20:19-23.
- Rawson, H.M., and Ruwali, K.N., 1972. Ear branching as a means of increasing grain uniformity in wheat. *Aust. J. Agric. Res.* 23:551-559.
- SAS Institute Inc. 1987. SAS/STAT guide for personal computers. Version 6 Edition. Cary, NC.
- Scott, W.R., Appleyard, M., Fellowes, G., and Kirby, E.J.M., 1983. Effect of genotype and position in the ear on carpel and grain growth and mature grain weight of spring barley. *J. Agric. Sci., Camb.* 100:383-391.
- Simmons, S.R., and Crookston, R.K., 1979. Nitrogen and dry matter accumulation by kernels formed at specific florets in spikelets of spring wheat. *Crop Sci.* 18:139-143.
- Simmons, S.R., Crookston, R.K., and Kurle, J.E., 1982. Growth of spring wheat kernels as influenced by reduced kernel number per spike and defoliation. *Crop Sci.* 22:983-988.
- Sofield, I., Evans, L.T., Cook, M.G., and Wardlaw, I.A., 1977. Factors influencing the rate and duration of grain filling in wheat. *Aust. J. Plant Physiol.* 4:785-797.
- Voltas, J., Romagosa, I., and Araus, J.L., 1997. Grain size and nitrogen accumulation in sink-reduced barley under Mediterranean conditions. *Field Crops Res.* 52, 117-126.

Wardlaw, I.F., Sofield, I., and Cartwright, P.M., 1980. Factors limiting the rate of dry matter accumulation in the grain of wheat grown at high temperature. *Aust. J. Plant Physiol.* 7:387-400.

Wiegand, C.L., and Cuellar, J.A., 1981. Duration of grain filling and kernel weight of wheat as affected by temperature. *Crop Sci.* 21:95-101.

Zadocks, J.C., Chang, T.T., and Konzak, C.F., 1974. A decimal code for the growth stages of cereals. *Weed Res.* 14:415-421.

CHAPTER III



Integrating statistical and ecophysiological analyses of genotype by environment interaction for grain filling of barley in Mediterranean areas. I.

Individual grain weight

ABSTRACT

Genotype by environment interaction (G×E) for grain weight has often been reported in small grain crops. In Mediterranean-type environments, grain weight of barley (*Hordeum vulgare* L.) is greatly influenced by the presence of terminal abiotic stresses such as drought or elevated temperatures. However, the relative importance of these climatic constraints acting together prior to and after anthesis, as well as the specificity of genotypic responses, have not been clearly documented. Field experiments were carried out in 12 rainfed environments of Northern Spain in which a large variability in grain weight was recorded. Five six-rowed and five two-rowed cultivars were used. An analysis of variance revealed existence of G×E interaction for grain weight. Environments could be characterized by climatic covariables (temperature, rainfall and potential evapotranspiration) measured during three consecutive stages of plant development: jointing, heading and grain filling. Genotypes could be described by covariables related to differences in several morphological, developmental and physiological traits. Possible factors underlying G×E were examined by AMMI (Additive Main Effects and Multiplicative Interaction) and factorial regression models. Genotypic and environmental descriptors were used as concomitant variables at the levels of the genotypic and environmental factor to partition G×E. A factorial regression model for G×E includes the ratio of rainfall to total evapotranspirative demand during grain filling, the mean maximum temperature during the same stage, and the environmental mean grain weight, as environmental covariables. Thus, differential genotypic sensitivities were found with respect to drought and thermic stress. These sensitivities could be partially ascribed to the difference between two- and six-row barleys. For breeding purposes there are opportunities to exploit the apparent genetic variability for tolerance to these terminal abiotic stresses .

1. INTRODUCTION

In rainfed Mediterranean environments, the incidence of drought and high temperatures at the end of the growing cycle reduces the grain growth of temperate cereals such as wheat or barley (Loss and Siddique, 1994). Several physiological processes are differently affected by these phenomena but, as a result, grain size is always decreased. Water stress diminishes leaf conductance and net photosynthesis and, therefore, the availability of current assimilates for grain filling (Nicolas *et al.*, 1985; Kobata *et al.*, 1992). Grain growth is then supported to a greater or lesser extent by remobilisation of pre-anthesis assimilates stored in vegetative organs (Bidingger *et al.*, 1977). High temperature (above 20°C) hinders the conversion of sucrose to starch in the endosperm (McLeod and Duffus, 1988), irrespective of the supply of assimilates to the grain (Denyer *et al.* 1994).

Cultivars have proved to respond differentially to changing environmental conditions during grain filling, and several studies report existence of genotype by environment interaction (G×E) for grain weight (Wych *et al.*, 1982; Van Sanford, 1985). Genotypic differences in remobilisation of vegetative reserves seem responsible for keeping grain weight stable under drought (Rawson *et al.*, 1977; Blum *et al.*, 1983). Furthermore, genetic variation in grain growth sensitivity to high temperature has been detected (Hunt *et al.*, 1991; Wardlaw and Moncur, 1995). It has been also pointed out that growing conditions prior to anthesis may modify the grain growth response to terminal stresses (Wardlaw, 1994). All these circumstances should be addressed when examining the differential behaviour of genotypes to the environment during grain filling.

Although G×E on grain weight is of great relevance in breeding for Mediterranean areas, few attempts have been made to assess the relative effects of drought and thermic stress in the field. A comprehensive approach to the nature of G×E requires more sophisticated statistical methods than standard analysis of variance (ANOVA). A popular extension of ANOVA for studying G×E is the additive main effects and multiplicative interaction (AMMI) model (Gauch, 1992). Applications of AMMI have led to more insight in the complicated patterns of genotypic responses to changing environmental conditions. These patterns have been successfully related to biotic and abiotic factors (Royo *et al.*, 1993). In other applications, AMMI unveiled plant processes that contributed to better performance in some environments (Romagosa *et al.*, 1993; Romagosa *et al.*, 1996). Alternatively, factorial regression models can be used that permit a direct

interpretation of G×E in terms of differential genotypic sensitivities to explicit environmental variables (Hardwick and Wood, 1972; Denis, 1988; van Eeuwijk, 1995; van Eeuwijk *et al.*, 1996).

In the present study, we have evaluated weather variables at different stages of plant development with possible influence in the determination of grain weight. Genotypic variables studied are related to morphological and developmental differences (spike type, crop phenology), physiological measurements (carbon isotope discrimination), and proposed screening methods for stable grain size i.e., chemical desiccation (Blum *et al.*, 1983). This study was designed to (i) assess the nature and timing of abiotic factors that limit differential grain weight of genotypes in Mediterranean-type climates, and (ii) determine possible morphophysiological causes underlying genotypic responses to such abiotic factors. To accomplish these objectives, AMMI and factorial regression models have been extensively applied to our data.

2. MATERIALS AND METHODS

2.1. Experimental design and plant material

Field experiments were conducted for three consecutive years (1992, 1993 and 1994) at various locations in Northern Spain. Since not all locations were represented at each of the three years of this study, the term 'trial' will be used herein to define the combination of a particular location in a given year. At all trials, Fall and Winter sowings were carried out giving a total of 12 'year-location-sowing period' combinations (hereafter referred to as environments) (Table 1). The experimental layout at each environment was a completely randomised block design with four replicates. For the specific aim of this study, only one replicate per environment was used. Plots consisted of six 12-m rows, spaced 20 cm, and seeded at rates of 350 seeds m⁻², and were fertilized according to standard cultural practices. Ten genetically diverse barley genotypes differing in adaptation to rainfed environments were used: Alpha, Beka, Kym, PC4 and Tipper (two-rowed types), and Albacete, Barberousse, Monlon, Orria and Plaisant (six-rowed types).

Table 1. Characterization of the 12 environments used in this study.

Location	Coordinates	Soil classification	Year	Sowing period	Environment code	Sowing date	First node detectable ¹	First visible awns ¹	Anthesis ¹	Individual Grain Weight ¹ (mg)
Artesa de Segre	42°11'N 0°32'E	Fluventic Xerochrept	1993	Autumn	ART93A	18 Nov	1 Apr	30 Apr	6 May	42.9
Bell-lloc d'Urgell	41°40'N 0°41'E	Xeric Torriorthent	1992	Winter	ART93W	8 Jan	15 Apr	6 May	15 May	34.8
			1992	Autumn	BEL92A	28 Nov	22 Mar	15 Apr	25 Apr	51.0
Gimenells	41°36'N 0°13'E	Xerollic Paleorthid	1992	Winter	BEL92W	9 Jan	15 Apr	7 May	14 May	53.5
			1992	Autumn	GIM92A	8 Nov	17 Mar	13 Apr	21 Apr	53.5
			1993	Winter	GIM92W	1 Feb	22 Apr	9 May	15 May	56.7
Valladolid	41°42'N 4°42'W	Typic Xerofluvent	1993	Autumn	GIM93A	23 Nov	21 Mar	16 Apr	22 Apr	57.8
			1993	Winter	GIM93W	10 Jan	6 Apr	21 Apr	30 Apr	59.3
			1993	Autumn	VAL93A	23 Nov	15 Mar	15 Apr	25 Apr	45.0
1994	Autumn	VAL94A	1994	Winter	VAL93W	12 Jan	10 Apr	1 May	12 May	38.3
			1994	Winter	VAL94W	25 Jan	5 Apr	1 May	28 Apr	48.5
										53.4

¹Averaged over genotypes at each environment

2.2. Plant development and grain filling characterization

At each environment, the following stages of the crop growing cycle were recorded (Zadocks decimal code, ZDC) (Zadocks *et al.*, 1974): first node detectable (ZDC scale 31), first visible awns (ZDC scale 49) and mid-anthesis (ZDC scale 65) (Table 1). At mid-anthesis (65), plant samples composed of two 1-m row segments were taken from each plot of one replicate per environment, and total dry matter accumulated at anthesis was obtained. In addition, about 60 mainstems were tagged in the same plots to characterize the grain filling process. Every 5 to 7 days from mid-anthesis until maturity, five spikes were collected per genotype. Four grains per spike were taken from mid-spike nodes to produce samples of 20 grains each (grains of six-rowed genotypes were collected from central rows of the spike). Dry weight of samples was obtained after drying to constant weight in a forced-air oven at 60°C, and used to calculate average grain weight at sampling. Final individual grain weight (IGW) was calculated using the logistic regression equation proposed by Darroch and Baker (1990).

2.3. Definition of environmental and genotypic covariables

Maximum, mean, and minimum temperatures, total rainfall and water deficit experienced by the crop (by means of the ratio of rainfall to total evapotranspirative demand obtained according to the Hargreaves method, R/ETP), calculated at three growth periods, were evaluated as possible environmental covariables (Table 2). The three growth periods were defined as follows:

- from first node detectable (ZCK 31) to first visible awns (ZCK 49) (jointing stage, J).
- from first visible awns to mid-anthesis (ZCK 65) (heading, H).
- from mid-anthesis to maturity (ZCK 91) (grain filling, GF).

Besides weather observations, we also evaluated as environmental covariable the total biomass accumulated at mid-anthesis (Biomass), averaged over genotypes, in an attempt to account for the differential contribution of soil fertility or cultural practices to G×E variation.

Table 2. Summary of weather data for particular stages of plant development in 12 rainfed environments.

	Jointing (J)			Heading (H)			Grain filling (GF)					
	Mean	Max	Min	SD ¹	Mean	Max	Min	SD ¹	Mean	Max	Min	SD ¹
T _{max} (°C)	18.9	23.4	14.9	2.9	22.8	27.5	16.1	3.4	24.5	27.2	21.3	1.7
T _{mean} (°C)	11.9	15.7	8.8	2.5	15.3	19.6	9.5	2.7	17.8	20.2	15.1	1.5
T _{min} (°C)	4.8	9	1.6	2.2	7.8	11.8	2.8	2.4	11.1	13.2	8.8	1.5
Rainfall (R) (mm)	25	51	1	17	15	59	0	20	83	133	31	31
R/E/P	0.31	0.78	0.01	0.24	0.57	2.61	0	0.83	0.84	1.37	0.25	0.33

¹Standard deviation

Five genotypic variables, which showed little or no G×E interaction, were used to characterize genotypes (Table 3). Three were obtained by estimation of the genotypic mean over environments: average anthesis date of cultivars, carbon isotope discrimination of mature grains (Δ_g), and carbon isotope discrimination of peduncles sampled at anthesis (Δ_p). Carbon isotope discrimination, a useful integrated indicator of water use efficiency of cultivars, was determined by mass spectrometry as described elsewhere (e.g. Farquhar *et al.*, 1989). The other two variables chosen, namely percentage decrease in grain weight (IGW_{decr}) and increase in dry matter translocated to grains (DM_{trans}), were obtained from a field experiment carried out in 1992 at Gimenezs involving the same cultivars of this study, to which a desiccation treatment with potassium iodide 0.5 % was applied ten days after anthesis (J. Voltas, unpublished data). IGW_{decr} refers to the percentage decrease in grain weight compared to an untreated control. DM_{trans} was obtained as dry weight decrease of the last stem internode between 15 days and maturity, expressed as percentage relative to an untreated control. The use of senescing agents has been proposed to simulate post-anthesis drought stress and assess drought tolerance of genotypes based on their ability to support grain filling from storage carbohydrates in the absence of photosynthesis (Blum *et al.*, 1983; Nicolas and Turner, 1993). In addition to these five covariables, the type of spike of each genotype (two- or six-rowed) was also included as qualitative genotypic covariable (Ear-type), and defined as +1 for six-rowed and -1 for two-rowed genotypes.

Relationships among these variables (either environmental or genotypic) were investigated by means of simple correlations and principal component analysis.

Table 3. Summary of selected phenological and physiological traits for ten barley genotypes grown at 12 environments

	Mean	Max	Min	SD ¹
Anthesis date (AD) ²	3.3	6	1	1.8
Δ grain (Δ_g)	16.3	17.1	15.6	0.4
Δ peduncle (Δ_p)	17.1	17.8	16.8	0.3
Decrease in grain weight (%) (GW_{decr}) ³	19.7	30.5	10.8	6.2
Increase in dry matter translocated to grains (%) (DM_{trans}) ³	22.1	84.7	0	26.6

¹Standard deviation

²Number of days from the earliest flowering genotype at each environment

³In response to a desiccation treatment with KI 0.6% 10 days after anthesis

2.4. Statistical analyses

Preliminary analysis

A fixed three-way ANOVA model was fitted. The factorial structure was: Genotype by Sowing Date by Trial ($G \times S \times T$). Inspection of the estimated three-way interaction effects in a halfnormal plot did not reveal obvious patterns. Therefore the three-way $G \times S \times T$ interaction mean square was chosen as an estimate for error in this and subsequent analyses. For further analyses, data were structured as a two-way genotype by environment table, with I rows (genotypes) and J columns (environments). Each environment referred to a particular Sowing Date by Trial combination. $G \times E$ interaction was quantified for genotypes and environments by ecovalences: the contribution of individual genotypes and environments to the interaction sum of squares (Wricke, 1962).

AMMI models

AMMI and factorial regression models were fitted to the two-way $G \times E$ table. AMMI models are special cases of biadditive models, models that are characterized by terms that consist of products of row and column parameters. The term 'AMMI model' was introduced by Gauch

(1988). 'Biadditive models' were introduced by Denis and Gower (1994). For more details on theory and application see Kempton (1984), Gauch (1988, 1992), Denis and Gower (1994, 1996), van Eeuwijk (1995), and van Eeuwijk *et al.* (1995).

The general formulation for the AMMI model for the expectation of genotype i in environment j is

$$E(Y_{ij}) = \mu + g_i + e_j + \sum_{k=1}^K c_{ik} d_{jk}$$

where μ is the overall mean, g_i and e_j are genotypic and environmental main effects, and c_{ik} and d_{jk} are the genotypic and environmental scores, respectively, for the k -th multiplicative term. In AMMI the usual GxE interaction term, ge_{ij} , of the standard two-way ANOVA model, $E(Y_{ij}) = \mu + g_i + e_j + ge_{ij}$, is replaced by a sum of products $c_{ik} d_{jk}$ and a random remainder, ϵ_{ij} . Often two multiplicative terms provide a reasonable description of the ANOVA interaction. Thus $K=2$ and $ge_{ij} \approx c_{i1} d_{j1} + c_{i2} d_{j2}$.

Estimates for the genotypic and environmental scores are obtained from the minimization of $\sum_{i=1}^I \sum_{j=1}^J \left(ge_{ij} - \sum_{k=1}^K c_{ik} d_{jk} \right)^2$. This minimization can be solved by the use of either latent root or singular value decompositions. A popular way of getting the estimates for genotypic and environmental scores is by applying principal components analysis to the ANOVA interactions, where the interactions ge_{ij} are included as either J genotypic variables of length I , or I genotypic variables of length J . For identification of the scores constraints must be imposed. Various types of constraints can be chosen. Convenient constraints are $\sum_{i=1}^I c_{ik}^2 = \sum_{j=1}^J d_{jk}^2$ for all k , and $\sum_{i=1}^I c_{ik} c_{ik'} = 0$, $\sum_{j=1}^J d_{jk} d_{jk'} = 0$, for all $k \dots k'$ (Denis and Gower, 1996).

The number of multiplicative terms necessary for adequate description of the interaction, K , was assessed by first transforming the eigenvalues (=sum of squares) corresponding to the individual terms to mean squares (Gollob, 1968). The mean squares were obtained by dividing the eigenvalues by degrees of freedom: $I+J-3$ for the first term, $I+J-5$ for the second, etc. Subsequently, significance for these mean squares was assessed by calculating variance ratios, with the mean squares to be tested in the numerator and an estimate for error in the denominator. The variance ratios were assumed to follow F-distributions.

The genotypic scores c_{ik} can be interpreted as genotypic sensitivities to hypothetical environmental variables, d_{jk} . These hypothetical environmental variables are constructed such as to maximally discriminate between genotypes. Genotypic scores can be related to genotypic

covariables and environmental scores to environmental covariables in an attempt to relate the results of the AMMI analysis to explicit information on genotypes and environments. It should be acknowledged that the results of AMMI analyses are arbitrary in the sense that the multiplicative terms jointly approximate the original ANOVA interaction, but that the approximation can be attained by different sets of scores, where these scores still follow the same identification constraints. This phenomenon is known under the name of rotational invariance. The practical consequence is that we should not try to interpret the genotypic and environmental scores of each multiplicative term individually, but that we should try to relate the complete set of genotypic scores to genotypic covariables and the complete set of environmental scores to environmental covariables. To that end individual genotypic covariables can be regressed on the set of genotypic scores, i.e. on c_{i1} and c_{i2} for the case of only two multiplicative terms, and environmental covariables on the set of environmental scores, for example, d_{j1} and d_{j2} .

Biplots

An essential visual aid in the interpretation of the results of multiplicative models, of which principal component analysis and AMMI models are special cases, is the biplot. For AMMI models the interactions are visualized, for principal components the deviations from the column means are visualized. For an AMMI model with interaction being described by two multiplicative terms, genotypes and environments are depicted as points on a plane. The position of the points for the genotypes is given by the estimates for the genotypic scores, the environmental point coordinates come from the estimates for the environmental scores. For example, genotype i has position (c_{i1}, c_{i2}) and environment j is at (d_{j1}, d_{j2}) (for convenience we ignore the difference between parameters and their estimates). Proximity of genotypes in a biplot indicates similar behaviour over environments, and little or no interaction between these genotypes. Distant genotypes are indicative of clear interaction. The origin, $(0,0)$, can be interpreted as an imaginary additive genotype, a genotype without adaptations or maladaptations. Therefore, the distance from the origin is an indication of the amount of interaction that is exhibited by a genotype (for environments similar relations are true).

Let the genotypic and environmental points in the biplot now determine lines whose directions are given by their coordinates with respect to the origin. Genotype i determines a line with direction vector (c_{i1}, c_{i2}) , or slope c_{i2} / c_{i1} , and environment j determines a line with direction vector (d_{j1}, d_{j2}) , or slope d_{j2} / d_{j1} . The angles between two genotypic direction vectors give

information about the correlation between the genotypes; the correlation is 1 for coinciding directions, positive for acute angles, 0 for orthogonal directions, negative for obtuse angles and -1 for opposing directions. Likewise for environments. Finally, projection of the environmental point for environment j ($=1 \dots J$) on the line determined by the direction vector for genotype i gives an approximation to the ANOVA interaction, ge_{ij} . Equivalently, projection of the genotypic point for genotype i ($=1 \dots I$) on the line determined by the direction vector for environment j also approximates the interaction ge_{ij} . Examples of the application of biplots will be given in the result section.

We used AMMI to get a first impression of the interaction and to make a preselection of sets of genotypic and environmental covariables that warranted further investigation by means of factorial regression models. To achieve this goal the standard biplot of AMMI was enriched with genotypic and environmental information: the coefficients of the regressions of the individual genotypic/ environmental covariables on the two genotypic/ environmental scores from AMMI immediately define directions of greatest change for these covariables in the biplot. The direction is given by the regression coefficients and the origin. For example, when the regression of the environmental covariable z_j on the scores d_{j1} and d_{j2} gives the estimated coefficient b_1 and b_2 , the line with direction vector (b_1, b_2) , or slope b_2/b_1 , will give upon projection of the environmental points on this line an ordering of the environments with respect to that covariable. Furthermore, the angles between the direction vectors again give information about correlations. A condition for the inclusion of this kind of information in the AMMI biplot is that enough variation in the covariables is explained by the regression on the scores.

Principal components analysis on genotypic and environmental covariables

The correlation structures of the sets of genotypic and environmental covariables were analysed by means of principal components analysis (PCA) and biplots. This analysis was done to get insight in the relations and redundancies in the covariable sets. In principle this analysis reveals similar information as the inclusion of covariable information in the AMMI biplot. The difference is that now the relationships between the covariables can be investigated directly, without the implicit filtering of information due to the use of the AMMI biplot.

Factorial regression models

In factorial regression models environmental covariables are introduced to describe interaction. Estimated regression coefficients receive an interpretation as genotypic sensitivities.

An example of a factorial regression model with the environmental covariables $z_1 \dots z_H$ is

$E(Y_{ij}) = \mu + g_j + e_j + \sum_{h=1}^H \beta_{jh} z_{jh}$. In this model all parameters, including the genotypic sensitivities β_{jh} , can be estimated by standard least squares techniques as the model is a linear model.

The function to be minimized is $\sum_{i=1}^I \sum_{j=1}^J \left(g e_{ij} - \sum_{h=1}^H \beta_{jh} z_{jh} \right)^2$. For details on theory and application of factorial regression models see Denis (1988, 1991), van Eeuwijk (1995), van Eeuwijk *et al.* (1995) and van Eeuwijk *et al.* (1996).

For variable selection in the factorial regression model we employed the following strategy. PCA and AMMI were used to screen the covariables at possible redundancies. Additional physiological knowledge then allowed us to define a number of covariable sets that could be tried in factorial regression models. Environmental data were available for the three consecutive stages of jointing (J), heading (H) and grain filling (GF). As grain development takes place during the last stage, it seemed sensible to first introduce covariables observed during GF, next those for H, to assess whether additional information was added after correction for the GF variables, and finally, those covariables related to the J stage. Biomass was also tried after GF covariables, since it was taken at anthesis, but the inclusion of true external environmental covariables, during both H and J, was preferred to the inclusion of Biomass (which is determined during H and J) in the model.

Factorial regression models were investigated for inclusion of environmental covariables. After having decided on a reasonable model containing only environmental covariables, the estimated genotypic coefficients were correlated to genotypic covariables to find out whether the sensitivities to particular environmental covariables were related to specific genotypic information.

For the analyses, programs in SAS (SAS Inst., 1987) and GENSTAT (GENSTAT 5 Committee, 1993) were used.

3. RESULTS

3.1. Relationships among variables

Principal component analyses were performed on the sets of standardized genotypic and environmental covariables. The first two PCA axes for the environmental variables accounted for 74% of the total variation. The biplot is given in Fig. 1a. An obvious conclusion is that within each of the three growth stages examined (J, H, GF), all temperature variables were highly correlated, i.e., they appear closely together in the biplot. Accordingly, only one variable (T_{max}) was chosen at each stage as indicator of thermic stress for subsequent analyses. The biplot shows that T_{max_J} , T_{max_H} and $T_{max_{GF}}$ were inversely related to Biomass, i.e. the higher the temperatures during jointing and heading, the lower the plant growth until anthesis.

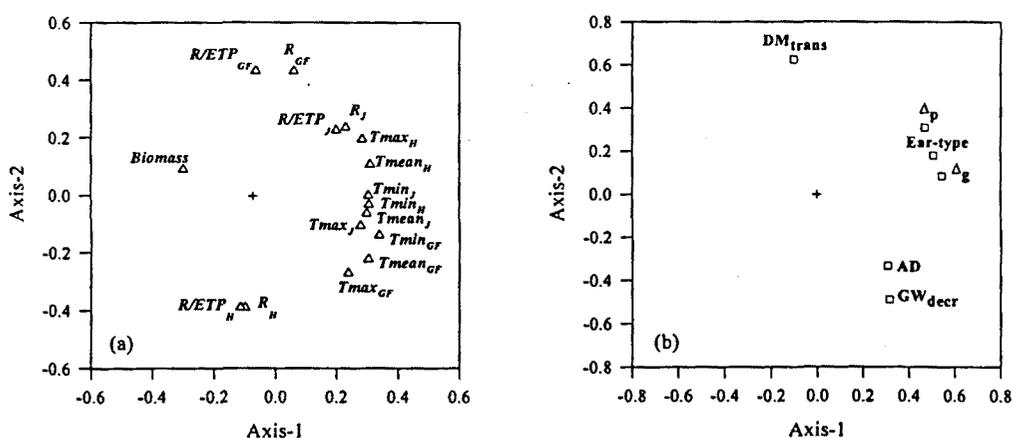


Figure 1. Principal component analysis biplot summarizing the relationships among environmental (a) and genotypic (b) descriptors initially proposed as explanatory variables for the analysis of G×E interaction in individual grain weight. Abbreviations are described in the Materials and Methods section.

The biplot for the genotypic variables, which represents 74% of the total variation in the standardized genotypic variables, is shown in Fig. 1b. Essentially, the genotypic information seems to be concentrated in three clusters: Ear-type/ Δ_g/Δ_p , $IGW_{decr}/Anthesis$, and DM_{trans} . The position of ear-type at the top of the biplot, giving the difference between six- and two-row types, indicates that six-rowed types tended to give higher Δ values than two-rowed types. As expected, the positions of DM_{trans} and IGW_{decr} in the biplot appear partially opposed, i.e. the larger the decrease for IGW in the desiccation treatment, the lower the amount of dry matter translocated to the grains.

3.2. Analysis of Variance

The growing conditions before and during grain filling caused a wide range of responses in IGW, varying among environments between 34.8 mg (A_w93) and 59.3 mg (G_w93) (Table 1). Though anthesis occurred 10-15 days later for winter than for autumn sowings (Table 1), ANOVA revealed that differences among trials rather than between sowing dates were important in accounting for environmental variation in IGW (Table 4). The interaction Trial \times Sowing Date, however, was highly significant, emphasizing the inherent unpredictability of Mediterranean climates in terms of grain weight. Variation in genotypic means for IGW was considerable, it ranged from 45.29 mg (Tipper) to 57.40 mg (Monlon). G \times E was mostly present as Genotype by Trial (G \times T) interaction, i.e. genotypes filled their grains differentially based on yearly and/or spatial fluctuations, but they were seldom influenced by the time of sowing. Two-way (G \times E) residuals from additivity were inspected by a half normal plot to check for possible outliers. Four genotype-environment combinations, which will be considered later, were found suspect. When fitting separate expectations for these four cells, it was found that these outliers accounted for 24% of the G \times E sum of squares.

Table 4. Analysis of variance for individual grain weight (mg) of ten barley genotypes grown at 12 environments

Source of variation	df	Sum of squares	Mean squares	Variance ratio	P-value
Trial (T)	5	5961.9	1192.38	196.75	0.001
Sowing Date (S)	1	5.7	5.71	0.95	0.335
T x S	5	760.0	152.00	25.08	0.001
Genotype (G)	9	1305.5	145.06	23.93	0.001
G x T	45	788.8	17.53	2.89	0.001
G x S	9	69.3	7.70	1.27	0.278
Error	45	272.7	6.06		

3.3. Model formulation of interaction

The first two multiplicative terms for interaction were found significant in AMMI. These terms explained 63% of the $G \times E$. The biplot with genotypic and environmental covariables superimposed, is given in Fig. 2. Six-rowed cultivars preferentially lie at the top, two-rowed types at the bottom, as indicated by the direction of Ear-type. Environments at Valladolid exhibited relatively low $T_{max_{GF}}$ and high Biomass. R/ETP_{GF} was especially low at ART93W. Following the direction of IGW_{mean} (mean environmental grain weight), above average locations for IGW (e.g. Bell-lloc) tend to group at the lower-right side of the biplot, and below average ones (e.g. Artesa) at the upper-left side. As mentioned, the influence of sowing period on $G \times E$ seems very limited. Only GIM93 and ART93 environments were partially separated by the sowing effect. The biplot representation provided analytical insight into $G \times E$. For instance, six-rowed cultivars like Plaisant, Barberousse and Monlon performed relatively better under the favourable grain filling conditions of Valladolid, i.e. high R/ETP_{GF} and low $T_{max_{GF}}$. Two-rowed Beka and Alpha behaved better when R/ETP_{GF} was low, while six-rowed Orria and Albacete did better in environments with poor IGW_{mean} .

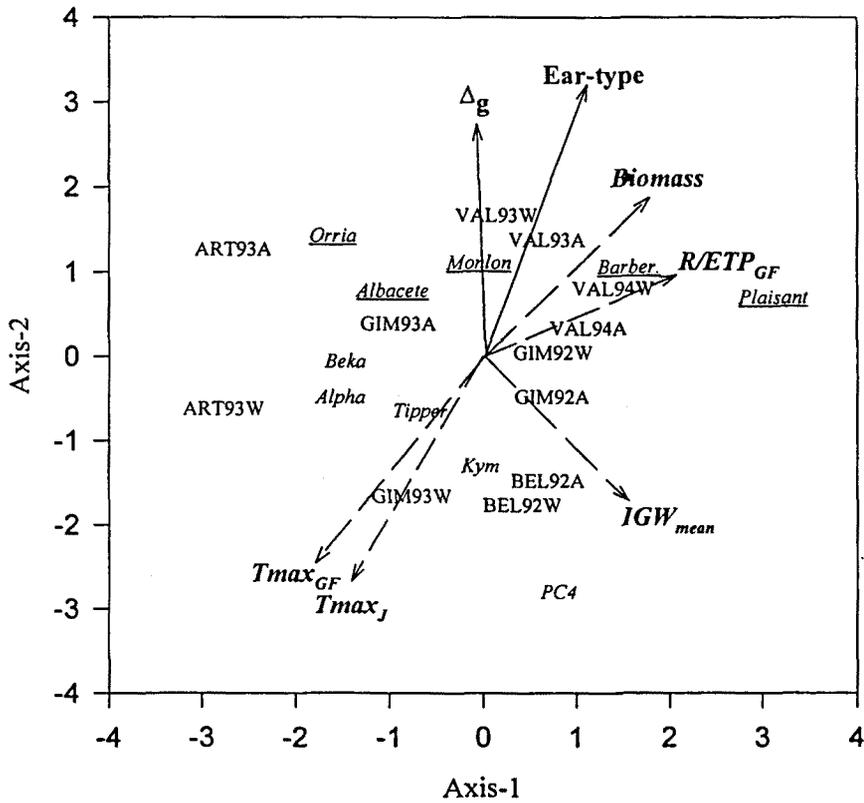


Figure 2. Biplot of the AMMI analysis for grain weight. Environments are represented in capitals, genotypes in italics. Six-rowed genotypes appear underlined. Relevant genotypic (in bold) and environmental (in italics) variables in the explanation of G×E have been included: Δ_g (carbon isotope discrimination of mature grains), Ear-type (two- or six-rows), Biomass (biomass accumulated by the crop at anthesis), IGW_{mean} (mean environmental grain weight), $Tmax_{GF}$ (average maximum temperature during grain filling), $Tmax_J$ (average maximum temperature during jointing), and R/ETP_{GF} (ratio of total rainfall to potential evapotranspiration during grain filling). The arrows indicate directions of greatest change for genotypic (solid lines) and environmental (dashed lines) variables.

The AMMI analysis gave clear indications for candidate factorial regression models. In addition, the information present in some variables seemed strongly overlapping (note, for instance, the positions in the biplot, Fig. 2, of $Tmax_{GF}$ and $Tmax_j$, with Biomass approximately at a 180° angle). As mentioned in the Materials and Methods section, the selection process of environmental variables first focussed on those measurements done at the GF stage. Therefore, only three environmental variables were considered worthwhile to test at this point: R/ETP_{GF} , $Tmax_{GF}$, and IGW_{mean} . Given the magnitude of the four putative outliers previously detected, we decided that any proposal for IGW should also take into account these deviating observations. The outliers were included in the model after correction of $G \times E$ for covariables, to check whether their initial importance could be partially or totally eliminated by the $G \times E$ explanation based on external measurements. The factorial regression model for IGW is shown in Table 5. The order of inclusion of R/ETP_{GF} and $Tmax_{GF}$ could be changed without much of a difference. IGW_{mean} was introduced after the true physical covariables, to check whether a significant amount of interaction left unexplained by R/ETP_{GF} and $Tmax_{GF}$ could be accounted for by the regression on the environmental mean. The inclusion of IGW_{main} in the model was not unexpected, since the information present in this term is rather orthogonal with that of the other two covariables, i.e. IGW_{main} appears at approximately a right angle with both earlier covariables (Fig. 2). The selected layout caught about 43% of $G \times E$ variation with 27% of its degrees of freedom. The remainder was not significant anymore. Remarkably, the four outliers discovered when no covariables were in the model, disappeared after the inclusion of the environmental covariables. A more detailed study (data not shown) revealed that the most deviating point was determined by a high negative sensitivity of Plaisant to a low R/ETP_{GF} environment, ART93W. This behaviour can also be directly inferred from the biplot (Fig. 2).

Genotypic sensitivities for especially R/ETP_{GF} and $Tmax_{GF}$ were clearly related to Ear-type. The squared correlation between the sensitivities for R/ETP_{GF} and Ear-type, or, the fraction explained in the sensitivities for R/ETP_{GF} by the contrast between six- and two-row types was 0.34. Six-row types were in general positively sensitive to this covariable, while two-row types were negatively sensitive. For $Tmax_{GF}$, 0.74 of the differences in sensitivity was due to the contrast six rows - two rows, with six-row types being negatively sensitive and two-row types positive. Δ_g did not add any significant information about the differences in genotypic sensitivities to R/ETP_{GF} and $Tmax_{GF}$ beyond that already accounted for by Ear-type.

Table 5. Factorial regression model for the partition of G×E for individual grain weight

Source of variation	df	Sum of squares (SS)	% SS G×E	Mean squares	Variance ratio	P-value
G×E	99	1130.1				
G×R/ETP _{GF}	9	190.1	16.8	21.12	3.49	0.001
Ear-type×R/ETP _{GF}	1	66.1	5.8	66.06	10.91	0.002
Deviations	8	124.0	11.0	15.5	2.56	0.022
G×Tmax _{GF}	9	148.7	13.2	16.52	2.73	0.009
Ear-type×Tmax _{GF}	1	109.7	9.7	109.74	18.10	0.001
Deviations	8	38.3	3.5	4.78	0.79	0.614
G×IGW _{mean}	9	148.2	13.1	16.47	2.72	0.009
Model remainder	72	643.1	56.9	8.93	1.47	0.083
Error (G×S×T)	45	272.7		6.06		

Table 6 shows the relative contribution of each genotype to the total G×E variation. According to this criterion, the most sensitive genotype to environmental changes was, by far, Plaisant, followed by PC4 and Barberousse, whereas Tipper was an example of an additively behaving variety, i.e. with a low contribution to the total G×E variation. Such 'instability' may be partially explained by the responses of individual varieties to climatic covariables, as expressed by the regression coefficients shown in Table 6. Barberousse and Plaisant seemed negatively affected by high temperatures during GF and benefited from good water availability at the end of the growing cycle. Conversely, Beka performed better relative to Plaisant and Barberousse just under opposite climatic conditions. The behaviour of Orria is worth mentioning: it seemed negatively affected by the high temperatures during GF, but showed a prominent behaviour under drought conditions. Other cultivars showed specific responses to either R/ETP_{GF} (e.g. Tipper) or Tmax_{GF} (e.g. Monlon). The responses to IGW_{mean}, which may provide a rough idea of the genotypic sensitivities to the overall grain filling conditions (not already accounted

by R/ETP_{GF} or $Tmax_{GF}$), involved also several genotypes. For instance, Albacete was insensitive to the climatic covariables, but performed relatively badly in environments favouring higher IGW

Table 6. Average individual grain weight (IGW), ecovalence (W_i) values, contribution (%) of genotypes to G×E interaction sum of squares, and estimates of the regression coefficients of selected environmental covariables for each genotype according to a factorial regression model for individual grain weight (IGW).

Genotype	Ear type	Average IGW (mg)	W_i	%SS G×E	R/ETP_{GF}	$Tmax_{GF}$	IGW_{mean}
Albacete	Six-rowed	50.35	96.4	8.5	0.12	-0.1	-1.46 [*]
Alpha	Two-rowed	51.57	92.6	8.2	-1.03 [†]	1.25	0.57
Barberousse	Six-rowed	46.28	132	11.7	1.82 ^{**}	-1.49 [†]	-0.37
Beka	Two-rowed	46.84	100.6	8.9	-1.76 ^{**}	1.43 [†]	-1.47 [*]
Kym	Two-rowed	47.8	64.3	5.7	0.06	2.86 ^{**}	0.47
Monlon	Six-rowed	57.4	78.4	6.9	0.61	-2.69 ^{**}	0.69
Orria	Six-rowed	51.32	116.3	10.3	-1.29 [*]	-1.69 [*]	-1.52 [*]
PC4	Two-rowed	49.22	156.9	13.9	-0.23	1.49 [†]	2.41 ^{**}
Plaisant	Six-rowed	49.74	235.9	20.9	2.62 ^{**}	-1.2	1.05 [†]
Tipper	Two-rowed	45.29	56.8	5	-0.91 [†]	0.13	-0.37

†, *, **: Regression coefficients with confidence intervals that do not include zero at $\alpha=0.1$, 0.05, and 0.01, respectively

4. DISCUSSION

A large environmental variation constitutes a desirable starting point for studying G×E interaction. Thus differential responses may easily emerge when genotypes are exposed to a sufficiently wide range of growing conditions. Unfortunately, such extreme conditions are not rare to occur in Mediterranean areas and especially not at the end of the growing season, a period under the harmful influence of terminal abiotic stresses: high temperatures and drought. In this study, G×E on grain weight accounted for a percentage of variation (13 % SS) similar to that due

to differences among genotypes (15 % SS), suggesting the existence of contrasting genotypic responses to the environment during grain filling. Possible factors underlying such differential performance were examined by AMMI and factorial regression methods.

The inclusion of R/ETP_{GF} and $Tmax_{GF}$ as external covariables in the factorial regression model for IGW agrees with previous observations that water stress and high temperatures during grain filling do not affect genotypic performance identically (McLeod and Duffus, 1988; Kobata *et al.*, 1992). If genotypes had responded in the same manner to both constraints, redundancy would have occurred with respect to the explanation for G×E between thermic and water stresses, and only one of the two variables, R/ETP_{GF} and $Tmax_{GF}$, would have been kept in the model. It could have also occurred that only one environmental variable had effectively accounted for G×E, meaning that efforts to understand this phenomenon would have subscribed to the predominance of only one climatic constraint. The existence of differential genotypic responses to both water deficit and thermic stress emphasizes the need of investigating their joint effect under real field conditions, and this has important breeding implications for the attainment of cultivars with stable grain weight in Mediterranean areas.

Deviations from the genotypic average grain weight were lower for two-rowed cultivars than for six-rowed types in the presence of drought and/or high temperatures. The results from our study should be taken cautiously since it may be a consequence of the limited number of genotypes used in this study. For instance, most of the cultivars within the six-rowed group (Barberousse, Orria and Plaisant) are high yielding, widely adapted genotypes that may suffer to a larger extent the effect of terminal stresses on grain growth. Remarkably, six-rowed Albacete, a landrace still widely cultivated in Spain, showed insensitivity to these constraints, and Orria did relatively better in environments with low water availability at the end of the cycle. In this context, it is also noteworthy that some cultivars were affected (either positively or negatively) just by one abiotic stress: drought (e.g. Tipper) or high temperatures (e.g. Kym, Monlon). Therefore, it seems that substantial genetic variation can be found for a wide range of combined responses to such climatic variables. Existence of genetic variation has already been pointed out for the separate effects of drought (Rawson *et al.*, 1977; Nicolas and Turner, 1993) and thermic stress (Wardlaw *et al.*, 1989) on grain weight of wheat.

The last covariable entering the model for IGW was the environmental mean for this parameter (IGW_{mean}). The inclusion of this F-W-like (Finlay and Wilkinson, 1963) term after correction for all true covariables indicates that we were unable to account for the IGW

environmental main effect by using physical weather measurements alone. Many authors report a continuous reduction in grain weight at temperatures above 15°C for wheat (e.g., Wiegand and Cuellar, 1981; McDonald *et al.*, 1983), a phenomenon that should have influenced the average grain weight in each environment. However, the growing conditions among environments were not, by far, uniform at anthesis, and it is known that some of the environments assayed were partially source-limited during grain filling (Voltas *et al.*, 1997). In semiarid areas, average grain weight is likely to depend not solely on post-anthesis, but also on pre-anthesis growing conditions (Wardlaw, 1994). Undoubtedly, other site-specific factors existed, apart from weather variables, that might influence grain growth (e.g. soil fertility, presence of water table, etc.), but they were not included as external variables due to the difficulty associated with precise environmental determination. For instance, Artesa can be considered as a below-average fertility location and Bell-lloc as an above-average one (cf. Fig. 2). Regression on the environmental mean distinguished between old, well-adapted genotypes to semiarid areas (Albacete, Beka), with negative regression coefficients, and more demanding genotypes like Plaisant, which showed positive signs. Particularly, the bad performance of Albacete in above-average environments for IGW can be attributed to the lodging susceptibility of this cultivar, thus causing a poor grain filling.

It is noteworthy that the genotypic sensitivities to R/ETP_{GF} and $Tmax_{GF}$ were so highly related to Ear-type. Δ_g could have acted as a partial substitution for Ear-type, but had nothing to add to the explanation for the differences between the genotypic sensitivities after Ear-type was included already. There was no obvious relation between anthesis date (AD) and the sensitivities to the environmental covariables included in the final factorial regression model for G×E. Neither could such a relation be established for the variables obtained by using a desiccation treatment (IGW_{decr} and DM_{trans}). Both findings may seem unexpected, since the positive effect of these genotypic attributes during grain filling in Mediterranean-type climates has been widely recognized. For instance, flowering earliness is a well-known escape mechanism from terminal abiotic stresses (Blum, 1988; Loss and Siddique, 1994), but in this study such mechanism did not provide any additional genotypic advantage, especially at the winter sowings (theoretically more prone to intensive drought and elevated temperatures with time). However, the non-existence of G×S interaction, together with the lack of Sowing Date effect on grain weight, makes this result feasible. With regard to the chemical desiccation experiment, the failure of the two variables derived from it (IGW_{decr} and DM_{trans}) to account for a significant part of G×E may be ascribed to

strong environmental differences in plant growth conditions before anthesis. In fact, the use of senescing agents best simulates drought during grain filling in the absence of severe pre-anthesis drought (Blum, *et al.*, 1983; Nicolas and Turner, 1993). This circumstance does not always occur in rainfed Mediterranean environments and, therefore, the amount of pre-anthesis photosynthates susceptible to be remobilised to the grains may be very heterogeneous. For instance, Royo *et al.* (1996) did not find association between the reduction in grain weight caused by drought and by KI treatment under similar growing conditions to these reported here.

In summary, this field study showed that grain weight was significantly affected by both drought and elevated temperatures during grain filling. Responses to these constraints were genotype-dependent, as indicated by the fitted factorial regression model for G×E interaction. The differential genotypic sensitivities found for the separate effects of drought and thermic stress suggests that both constraints did not affect the same physiological mechanisms during the grain filling process. This finding indicates that sufficient genetic variability for tolerance to terminal abiotic stresses may exist, which can be further explored for breeding purposes in barley grown in Mediterranean climates.

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REFERENCES

- Bidinger, F., Musgrave, R.B., and Fischer, R.A., 1977. Contribution of stored preanthesis assimilate to grain yield in wheat and barley. *Nature* 270: 431-433.
- Blum, A., J. Mayer and Golan, G., 1983. Chemical desiccation of wheat plants as a simulator of post-anthesis stress. II. Relations to drought stress. *Field Crops Res.* 6:149-155.
- Blum, A., 1988. *Plant breeding for stress environments*. CRC Press Inc. Boca Raton, Florida.

Darroch, B.A., and Baker, R.J., 1990. Grain filling in three spring wheat genotypes: statistical analysis. *Crop Sci.* 30:525-529.

Denis, J.B., 1988. Two-way analysis using covariates. *Statistics* 19: 123-132.

Denis, J.B., 1991. Ajustement de modèles linéaires et bilinéaires sous contraintes linéaires avec données manquantes. *Revue de Statistique Appliquée* 39: 5-24.

Denis, J.B., and Gower, J.C., 1994. Biadditive models. Letter to the editor. *Biometrics* 50: 310-311.

Denis, J.B., and Gower, J.C., 1996. Asymptotic confidence regions for biadditive models, interpreting genotype-environment interaction. *Applied Statistics* 45: 479-493.

Denyer, K., Hilton, C.M., and Smith, A.M., 1994. The effect of high temperature on starch synthesis and the activity of starch synthase. *Aust. J. Plant. Physiol.*, 21: 783-789.

Farquhar, G.D., Ehleringer, J.R., and Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40: 503-537.

Finlay, K.W., and Wilkinson, G.N., 1963. The analysis of adaptation in a plant-breeding programme. *Aust. J. Agric. Res.* 14: 742-754.

Gauch, H.G., 1988. Model selection and validation for yield trials with interaction. *Biometrics* 44: 705-715.

Gauch, H.G., 1992. *Statistical analysis of regional yield trials*. Elsevier, Amsterdam.

GENSTAT 5 Committee, 1993. *GENSTAT 5 release 3 reference manual*. Clarendon Press, Oxford.

Gollob, H.F., 1968. A statistical model that combines features of factor analysis and analysis of variance techniques. *Psychometrika* 33: 73-115.

Hardwick, R.C. and Wood, J.T., 1972. Regression methods for studying genotype \times environment interaction. *Heredity* 28:209-222.

Hunt, L.A., van der Poorten, G., and Pararajasingham, S., 1991. Postanthesis temperature effects on duration and rate of grain filling in some winter and spring wheats. *Can. J. Plant Sci.* 71: 609-617.

- Kempton, R.A. 1984., The use of biplots in interpreting variety by environment interactions. *Journal of Agricultural Science, Cambridge* 103: 123-135.
- Kobata, T., Palta, J.A., and Turner, N.C., 1992. Rate of development of postanthesis water deficits and grain filling of spring wheat. *Crop Sci.* 32: 1238-1242.
- Loss, S.P., and Siddique, K.H.M. 1994., Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. *Advances in Agronomy* 52: 229-276.
- McDonald, G.K., Sutton, B.G., and Ellison, F.W., 1983. The effect of time of sowing on the grain yield of irrigated wheat in Namoi Valley, New South Wales. *Aust. J. Agric. Res.*, 34: 229-240.
- McLeod, L.C., and Duffus, C.M., 1988. Reduced starch content and sucrose synthase activity in developing endosperm of barley plants grown at elevated temperatures. *Aust. J. Plant. Physiol.* 15: 367-375.
- Nicolas, M.E., Lambers, H., Simpson, R.J., and Dalling, M.J., 1985. Effect of drought on metabolism and partitioning of carbon in two wheat varieties differing in drought-tolerance. *Ann. Bot. (London)* 51: 85-95.
- Nicolas, M.E., and Turner, N.C., 1993. Use of chemical desiccants and senescing agents to select wheat lines maintaining stable grain size during post-anthesis drought. *Field Crops Res.*, 31: 155-171.
- Rawson, H.M., Bagga, A.K., and Bremner, P.M., 1977. Aspects of adaptation by wheat and barley to soil moisture deficits. *Aust. J. Plant Physiol.*, 4: 389-401.
- Romagosa, I., Fox, P.N., García del Moral, L.F., Ramos, J.M., García del Moral, B., Roca de Togores, F., and Molina-Cano, J.M., 1993. Integration of statistical and physiological analyses of adaptation of near-isogenic barley lines. *Theor. Appl. Genet.*, 86: 822-826.
- Romagosa, I., Ullrich, S.E., Han, F., and Hayes, P.M., 1996. Use of the additive main effects and multiplicative interaction model in QTL mapping for adaptation in barley. *Theor. Appl. Genet.*, 93: 30-37.
- Royo, C., Rodríguez, A., and Romagosa, I., 1993. Adaptation of complete and substituted triticale to acid soils. *Plant Breeding* 111: 113-119.
- Royo, C., Abaza, M, Cantero, C, Calderó, A., Ramos, J.M., and García del Moral, L.F., (1996) Likening between the effect of drought and terminal water-stress simulated by a senescing agent in triticale. *J. Agronomy & Crop Science* 176: 31-38.

- SAS Institute Inc., 1987. SAS/STAT guide for personal computers. Version 6 Edition. Cary, NC.
- van Eeuwijk, F.A., 1995. Linear and bilinear models for the analysis of multi-environment trials: I. An inventory of models. *Euphytica* 84: 1-7.
- van Eeuwijk, F.A., L.C.P. Keizer, and J.J. Bakker., 1995. Linear and bilinear models for the analysis of multi-environment trials: II. An application to data from the Dutch Maize Variety Trials. *Euphytica* 84: 9-22.
- van Eeuwijk, F.A., Denis, J.B., and Kang, M.S., 1996. Incorporating additional information on genotypes and environments in models for two-way genotype by environment tables. In: M.S. Kang and H.G. Gauch Jr. (Editors), *Genotype by environment interaction: New perspectives*. CRC Press. Boca Raton, pp. 15-49.
- Van Sanford, D.A., 1985. Variation in kernel growth characters among soft red winter wheats. *Crop Sci.*, 25: 626-630.
- Voltas, J., I. Romagosa, and Araus, J.L., 1997. Grain size and nitrogen accumulation in sink-reduced barley under Mediterranean conditions. *Field Crops Res.*, 52: 117-126.
- Wardlaw, I.F., Dawson, I.A., and Munibi, P., 1989. The tolerance of wheat to high temperatures during reproductive growth. II. Grain development. *Aust. J. Agric. Res.*, 40: 15-24.
- Wardlaw, I.F., 1994. The effect of high temperature on kernel development in wheat: variability related to pre-heading and post-anthesis conditions. *Aust. J. Plant Physiol.*, 21: 731-739.
- Wardlaw, I.F., and Moncur, L., 1995. The response of wheat to high temperature following anthesis. I. The rate and duration of kernel filling. *Aust. J. Plant Physiol.*, 22: 391-397.
- Wiegand, C.L., and Cuellar, J.A., 1981. Duration of grain filling and kernel weight as affected by temperature. *Crop Sci.*, 21: 95-101.
- Wych, R.D., McGraw, R.L., and Stuthman, D.D., 1982. Genotype×year interaction for length and rate of grain filling in oats. *Crop Sci.*, 22: 1025-1028.
- Wricke, G., 1962. Über eine Methode zur Erfassung der ökologischen Streubreite in Feldversuchen. *Z. Pflanzzüchtg.*, 47: 92-93.
- Zadocks, J.C., Chang, T.T., and Konzak, C.F., 1974. A decimal code for the growth stages of cereals. *Weed Res.*, 14:415-421.

CHAPTER IV

Integrating statistical and ecophysiological analyses of genotype by environment interaction for grain filling of barley in Mediterranean areas. II. Grain growth

ABSTRACT

In Mediterranean areas, grain growth of temperate cereals often progresses under the harmful influence of abiotic stresses i.e., drought and high temperature. Genotypic responses are mediated by the specific occurrence of these constraints, thus causing genotype by environment interaction (G×E). Field experiments were carried out in 12 environments of Northern Spain to characterize G×E on grain growth of five six-rowed and five two-rowed barley (*Hordeum vulgare* L.) cultivars. Grain growth was defined as the result of two components: grain filling rate (GFR) and grain filling duration (GFD). Genotypic and environmental descriptors were used as concomitant variables at the levels of the genotypic and environmental factor to partition G×E. For a first exploration of the G×E structure AMMI (Additive Main Effects and Multiplicative Interaction) models were used. Subsequently, separate factorial regression models were fitted for GFR and GFD. G×E for GFR could be attributed to the joint effect of two pre-anthesis climatic variables (rainfall during heading, and average maximum temperature during jointing), in addition to the environmental mean GFR. The factorial regression model for GFR explained more than half of the analysis of variance G×E sum of squares with a quarter of its degrees of freedom (d.f.). Genotypic sensitivities to the climatic variables were partially explained by the ear type, two- or six-rows. A factorial regression model for G×E in GFD contained the pre-anthesis climatic variable, ratio of rainfall to total evapotranspirative demand during heading, and the genotypic covariable anthesis date. This factorial regression model retained about 35% of the analysis of variance interaction sum of squares with a fifth of its d.f. These results suggest that G×E for GFR may be caused largely by differences in source/sink balance between two- and six-rowed genotypes at anthesis. On the other hand, G×E for GFD seems to be brought about mainly by differences in phenology (i.e., anthesis date) among genotypes. Finally, the success of the integration of statistical and ecophysiological tools for the explanation of the grain filling process in barley is discussed.

1. INTRODUCTION

Substantial genetic variation has been detected in the kinetics of photosynthate accumulation into growing grains of cereals (Nass and Reiser, 1975; Van Sanford, 1985; Bruckner and Frohberg, 1987; Ho and Jui, 1989). This process is commonly described in the literature by two parameters: grain filling rate and grain filling duration (e.g. Darroch and Baker, 1990). In a previous paper, Voltas *et al.* (1998) reported existence of genotype by environment interaction (G×E) for individual grain weight of barley, a phenomenon that could be partially ascribed to differential genotypic sensitivities to terminal abiotic stresses. Other studies also describe G×E interaction for grain filling parameters (Wych *et al.*, 1982; Van Sanford, 1985). Efforts to understand the mechanisms by which grain filling rate and duration are modified by environmental constraints have been restricted mainly to controlled growth experiments (e.g., Kobata *et al.*, 1992, Wardlaw and Moncur, 1995). Differences among cultivars in tolerance to high temperature in either or both grain filling rate and duration have been reported in wheat (Hunt *et al.*, 1991; Wardlaw and Moncur, 1995). Responses monitored in growth chambers, however, have not often coincided with those described in the field (Jenner, 1994).

In Mediterranean environments, grain growth is affected by the joint occurrence of water and thermic stresses at the end of the crop cycle. Both constraints, acting together in an unpredictable manner, complicate a proper characterization of the phenomenon when assessed in controlled experiments. In addition, growing conditions prior to anthesis may generate different grain growth responses to terminal stresses (Jenner, 1994; Wardlaw, 1994). Some authors associate the influence of pre-heading conditions on grain growth to the interaction between photosynthate supply (source availability) and sink strength (grain number). For instance, a reduction in the source-sink ratio enhances sensitivity of wheat to high temperature, this being a genotype-dependent response (Wardlaw *et al.*, 1989). However, it is more common to read that under the non-limiting environmental conditions where wheat is usually cultivated, this crop appears largely sink-limited (see e.g., Slafer and Savin, 1994; Richards, 1996). Therefore, it is expected to be seldom influenced by pre-heading conditions during grain growth. This circumstance does not apply necessarily to barley, which is usually grown in substitution of wheat in poor, rainfed environments. Voltas *et al.* (1997) found six-rowed barleys to be clearly source-limited in Mediterranean environments. Hence, effects of post-anthesis abiotic stresses on grain growth are prone to be mediated by the availability of photoassimilates at anthesis,

which is, in turn, dependent on pre-anthesis growing conditions.

In this work, we have attempted to describe G×E interaction on grain growth parameters (grain filling rate and duration) by characterizing genotypic responses to a set of contrasting environmental conditions. To provide more insight into G×E, external genotypic and environmental information (including that related to pre- and post-anthesis stages of plant development, as described in the previous paper (Voltas *et al.*, 1998)) has been incorporated into statistical models that allow a more direct interpretation of G×E, factorial regression (Denis, 1988; van Eeuwijk *et al.*, 1996). As an preliminary exploratory tool AMMI (Additive Main Effects and Multiplicative Interaction) models (Gauch, 1992) were used. The specific aims of this study were (i) to evaluate the differential incidence of abiotic stresses on grain growth of ten barley cultivars grown in rainfed, Mediterranean conditions, and (ii) to identify possible genotypic mechanisms that could serve to overcome the detrimental influence of such abiotic factors on grain development.

2. MATERIALS AND METHODS

2.1. Experimental design and plant material

Field experiments, involving five two- and five six-rowed barley genotypes, were conducted for three consecutive years (1992, 1993 and 1994) at various locations in central and northeastern Spain. Details on the experimental layout and trial description, genotypes used, and plant development characterization, are provided in a preceding paper (Voltas *et al.*, 1998).

2.2. Grain filling characterization

To characterize the grain filling process, about 60 main stems per genotype were tagged in one replicate of each trial when 50% of spikes extruded anthers. Every 5 to 7 days from mid-anthesis until maturity, five spikes were collected per genotype. Four grains per spike were taken from mid-spike nodes to produce samples of 20 grains each (grains of six-rowed genotypes were collected from central rows of the spike). Dry weight of samples was obtained after drying to constant weight in a forced-air oven at 60°C, and used to calculate average grain weight at

sampling. Final individual grain weight (IGW), maximum grain filling rate (GFR) and grain filling duration (GFD) were calculated using the logistic regression equation suggested by Darroch and Baker (1990):

$$y = W/[1 + \exp(B - Cx)] \quad [1]$$

where: y = individual grain weight (mg)
 x = accumulated growing degree-days (GDD) from anthesis
 W = fitted final grain weight (mg)
 B and C = empirical values related to grain filling rate (GFR, mg GDD⁻¹) and grain filling duration (GFD, GDD)

Maximum GFR was obtained by finding the first derivative of equation [1]; GFD was calculated when 95% of the final grain weight was achieved by replacing y with $0.95W$ in equation [1]. Growing degree-days were calculated using a base temperature of 0°C (Bauer *et al.*, 1984). Curve fitting was performed using the NLIN procedure of SAS/STAT for nonlinear regression (SAS Inst., 1987).

2.3. Definition of environmental and genotypic variables

The environmental and genotypic variables selected in this study have been extensively described in a previous paper (Voltas *et al.*, 1998). Briefly, jointing (J), heading (H), and grain filling (GF) stages of plant development were characterized using average temperatures, total rainfall and water deficit experienced by the crop. The biomass accumulated by the crop at anthesis (Biomass), averaged over genotypes, was also included in an attempt to account for the differential contribution of soil fertility or cultural practices on G×E. Five genotypic variables, showing little or no G×E interaction, were used to characterize genotypes: average anthesis date of cultivars (AD), carbon isotope discrimination of mature grains (Δ_g), carbon isotope discrimination of peduncles sampled at anthesis (Δ_p), % decrease in individual grain weight (IGW_{decr}) and % increase in dry matter translocated to grains (DM_{trans}). The last two variables were obtained after application of a desiccation treatment with potassium iodide 0.5 % ten days after anthesis (Blum *et al.*, 1983). In addition to these five variables, the type of spike of each genotype (two- or six-rowed) was also included as qualitative genotypic variable.

2.4. Statistical analysis

A detailed description of the statistical procedures used for analysis of the data can be found in the companion paper to this paper (Voltas *et al.*, 1998). Here we will restrict ourselves to a brief summary.

Preliminary analyses

Fixed three-way ANOVA model were fitted, with the factorial treatment structure: Genotype by Sowing Date by Trial (G×S×T). Three-way G×S×T interaction mean squares were chosen as estimates for error. For further analyses, data were structured as a two-way genotype by environment tables, with the rows indexed by genotypes and the columns indexed by environments (Sowing Dates x Trials). The contribution of individual genotypes and environments to the interaction sum of squares (= ecovalence; Wricke, 1962) was calculated to quantify G×E interaction per genotype and environment.

AMMI models

AMMI and factorial regression models were fitted to the two-way G×E tables (Mandel, 1971; Kempton, 1984; Gauch, 1988; Denis and Gower, 1996). In AMMI models the analysis of variance interactions, ge_{ij} , are replaced by a sum of products of multiplicative genotypic and environmental interaction parameters (often called scores), and a residual term:

$$ge_{ij} = \sum_{k=1}^K c_{ik} d_{jk} + \delta_{ij},$$

where c_{ik} and d_{jk} are the genotypic and environmental scores, respectively, for the k -th multiplicative term, and δ_{ij} represents a random term giving the discrepancy between the original analysis of variance interaction and the approximative multiplicative formulation. The number of multiplicative terms necessary for adequate description of the interaction, K , can be assessed by first transforming the eigenvalues (=sum of squares) corresponding to the individual terms to mean squares (Gollob, 1968). The mean squares are obtained by dividing the eigenvalues by degrees of freedom: $I+J-3$ for the first term, $I+J-5$ for the second, etc. Subsequently, significance for these mean squares can be assessed by calculating variance ratios, with the mean squares to

be tested in the numerator and an estimate for error in the denominator. The variance ratios are assumed to follow F-distributions.

The genotypic scores c_{ik} can be interpreted as genotypic sensitivities to hypothetical environmental variables, d_{jk} . Genotypic scores can be related to genotypic covariables and environmental scores to environmental covariables in an attempt to relate the results of the AMMI analysis to explicit information on genotypes and environments. To that end, individual genotypic covariables can be regressed on the set of genotypic scores, i.e., on c_{i1} and c_{i2} for the case of only two multiplicative terms. Environmental covariables can be regressed on the set of environmental scores, d_{j1} and d_{j2} .

Biplots

Interaction as described by two multiplicative terms in an AMMI model can easily be visualized in biplots. Genotypes and environments are then depicted as points on a plane. The position of the points for the genotypes is given by the estimates for the genotypic scores, the environmental point coordinates come from the estimates for the environmental scores. For example, genotype i has position (c_{i1}, c_{i2}) and environment j is at (d_{j1}, d_{j2}) . Proximity of genotypes in a biplot indicates similar behaviour over environments, and little or no interaction between these genotypes. Distant genotypes are indicative of clear interaction. Distance from the origin is an indication of the amount of interaction that is exhibited by a genotype. The same rules apply to the environments.

In a vector interpretation of the biplot the genotypic and environmental points are the end points of vectors starting at the origin, where these vectors are constitutive for lines. The angle between a pair of genotypic vectors is indicative of the correlation: from 1 for fully coinciding directions to -1 for fully contrasting directions. From the biplot approximations to the original analysis of variance interactions, ge_{ij} , can be found by projecting environmental points on genotypic lines (or vice versa). The approximation follows from the length of the projection multiplied by the length of the direction vector of the line on which projection takes place. When projection and direction vector are at an obtuse angle a minus sign should be added to this product. AMMI biplots can be enriched with genotypic and environmental information. Regress genotypic/ environmental covariables on the set of genotypic/environmental scores and use the regression coefficients to define directions of greatest change for these covariables in the biplot. Say environmental covariable z_h is regressed on the scores d_{j1} and d_{j2} to give coefficients b_1 and

b_2 . Then the line with direction vector (b_1, b_2) , or slope b_2/b_1 , will give upon projection of the environmental points on this line an ordering of the environments with respect to that covariable. The angles between the direction vectors again give information about correlations.

Factorial regression models

In factorial regression models (Denis, 1988, 1991; van Eeuwijk *et al.*, 1996) the analysis of variance interaction, ge_{ij} , is replaced by a multiplicative formulation in which the interaction can be interpreted as differential genotypic sensitivity to explicit environmental covariables. As an example consider a situation with covariables $z_1 \dots z_H$:

$$ge_{ij} = \sum_{h=1}^H \beta_{ih} z_{jh} + \delta_{ij},$$

where β_{ih} stands for the genotypic sensitivity of genotype i to environmental variable z_h , and δ_{ij} is again a random discrepancy. To facilitate variable selection for the factorial regression model, enriched AMMI biplots are useful to pre-screen for possible redundancies (multi-collinearities) among the covariables. As another restriction it is sensible to first introduce covariables observed during grain filling, next those observed during heading, and finally those observed at the stage of jointing.

Factorial regression models were investigated for inclusion of environmental covariables. After having decided on a reasonable model containing only environmental covariables, the estimated genotypic coefficients were correlated to genotypic covariables to find out whether the sensitivities to particular environmental covariables were related to specific genotypic information.

For the analyses programs in SAS (SAS Inst., 1987) and GENSTAT (GENSTAT 5 Committee, 1993) were used.

3. RESULTS

3.1. Analyses of Variance

GFR and GFD were significantly affected by the environment (Table 1). GFR ranged from 0.0918 mg GDD⁻¹ (A_w93) to 0.1292 mg GDD⁻¹ (B_w92), and GFD varied between 449.1 GDD (A_w93) and 683.1 GDD (Z_w94). The effect of sowing time did not significantly affect GFR, but influenced GFD (Table 1). Trial×Sowing period interaction appeared significant for both traits. Significant genotypic effects were also detected for GFR and GFD. G×E was mostly present as Genotype by Trial (G×T) interaction, the G×T sum of squares (SS) for both parameters was even larger than the corresponding SS for the genotypic main effect. The magnitude of Genotype by Sowing period (G×S) interaction in the overall G×E for GFR was small, but G×S was at the border of significance ($p=0.059$) for GFD.

Table 1. Analyses of variance for grain filling rate (GFR) and grain filling duration (GFD) of ten barley genotypes grown at 12 environments (trial-sowing period combination).

Source of variation	df	GFR (mg GDD ⁻¹)			GFD (GDD)		
		Sum of squares (×10 ⁻⁵)	Variance ratio	P-value	Sum of squares (×10 ³)	Variance ratio	P-value
Trial (T)	5	1302.5	19.40	0.0001	2546.2	20.50	0.001
Sowing Date (S)	1	5.4	0.40	0.5286	173.1	6.97	0.011
T x S	5	270.3	4.03	0.0042	1557.3	12.54	0.001
Genotype (G)	9	513.8	4.25	0.0005	1112.5	4.98	0.001
G x T	45	1231.7	2.04	0.0093	1956.0	1.75	0.032
G x S	9	103.9	0.86	0.5669	450.4	2.01	0.059
Error	45	604.1			1117.6		

3.2. Model formulation of interaction for grain filling rate

For the AMMI model three axes (IPCA1 to IPCA3) were retained, together accounting for 76% of the G×E sum of squares. A biplot of the first two axes, which represents 62% of G×E, is shown in Fig. 1. Relevant genotypic and environmental information has also been included. Late flowering genotypes appear at the top, early ones at the bottom. Valladolid exhibited relatively low pre-anthesis temperatures and accumulated more biomass at anthesis, which in turn favoured GFR of six-rowed genotypes. On the whole, 'Sowing Date' was not important for describing G×E, perhaps with the exception of GIM92 and BEL92 (see their corresponding sowings lying quite apart in the biplot). Artesa exhibited low average GFRs, which did not seem to favour late flowering cultivars but increased the relative performance of early ones. IPCA3 was closely associated to just one specific environmental variable, R_H . For this reason, R_H was also tested for inclusion in the factorial regression model, together with T_{max_H}/T_{max_J} /Biomass (variables showing a lot of overlapping information, as suggested by their positions in the biplot) and GFR_{mean} .

A proposal for GFR is shown in Table 2. After R_H and T_{max_J} , the regression on the environmental mean (GFR_{mean}) was included in the model. This layout retained 53% of G×E with 27% of the df, and left a non-significant remainder. Genotypic sensitivities to R_H and T_{max_J} were partially explained by the ear type: six-rowed cultivars behaved relatively better under high rainfall conditions during heading and under low temperatures during jointing. Furthermore, environments with high overall GFR favoured late flowering genotypes (see the $AD \times GFR_{mean}$ indented cross-product in Table 2). Ecovalences (W_i) for GFR indicated that Monlon, Plaisant and Albacete were the most sensitive cultivars to environmental variation (Table 4). In particular, regression coefficients for the selected covariables revealed that Monlon, Albacete and also Orria were favoured by mild temperatures and/or high rainfall prior to anthesis (and also high biomass accumulation). On the contrary, Beka and Kym behaved opposite to the cultivars cited. The late flowering cultivars Plaisant and Tipper showed the highest sensitivities to high environmental GFRs. Alpha, which showed the lowest W_i , was the least sensitive cultivar to variable environmental conditions.

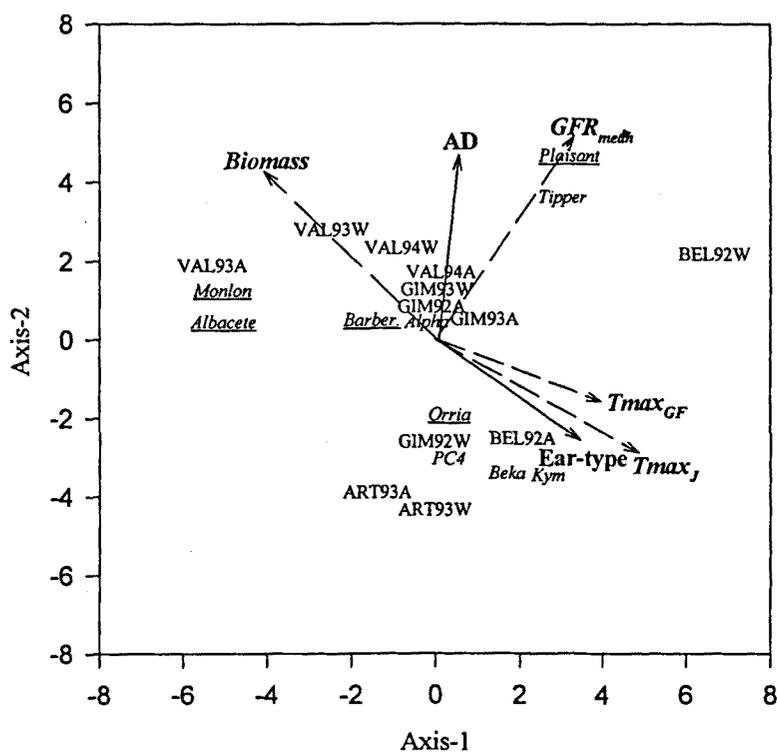


Figure 1. Biplot of the AMMI analysis for grain filling rate. Environments are represented in capitals, genotypes in italics. Six-rowed genotypes appear underlined. Relevant genotypic and environmental variables in the explanation of G×E have been included: AD (anthesis date), Ear-type (two- or six-rows), Biomass (biomass accumulated by the crop at anthesis), GFR_{mean} (mean environmental grain filling rate), $T_{max_{GF}}$ (average maximum temperature during grain filling), and T_{max_J} (average maximum temperature during jointing). The arrows indicate directions of greatest change for genotypic (solid lines) and environmental (dashed lines) variables.

Table 2. Factorial regression model proposal for the partitioning of G×E for grain filling rate

Source of variation	df	Sum of squares (SS) ($\times 10^{-5}$)	% SS G×E	Mean squares	Variance ratio	P-value
G×E	99	1940.1				
G×R _H	9	244.8	12.6	27.20	2.03	0.048
Ear-type×R _H	1	147.7	7.6	147.73	11.00	0.002
Deviations	8	97.1	5.0	12.51	0.93	0.501
G×Tmax _j	9	374.7	19.3	41.64	3.10	0.003
Ear-type×Tmax _j	1	137.6	7.1	137.62	10.25	0.003
Deviations	8	237.1	12.2	29.64	2.21	0.044
G×GFR _{mean}	9	398.9	20.6	44.32	3.30	0.002
AD×GFR _{mean}	1	211.2	10.9	211.23	15.73	0.000
Deviations	8	187.7	9.7	23.46	1.75	0.113
Remainder	72	921.4	47.4	12.80	0.95	0.584
Error (G×S×T)	45	604.2		13.43		

3.3. Model formulation of interaction for grain filling duration

The two IPCA axes of an AMMI2 model for interaction were significant and together explained about 55% of G×E. The biplot is shown in Fig. 2. Only a few genotypic and environmental covariables were related highly enough to the G×E interaction retained in the biplot to merit imposition: R/ETP_H and R_H, which provided similar information, and AD and Δ_g . In general, late flowering genotypes show positive IPCA1 values, early cultivars negative scores. Low Δ_g genotypes seemed to take advantage of environments with good water status by heading. It is noteworthy that the sowing effect, contrarily to its small influence on G×E for IGW and GFR, was important for determining differential genotypic responses for GFD. Hence, distances in the biplot between autumn and winter sowings within any specific Trial were substantial (Fig.

2). Autumn sowings tended to group to the right of the biplot, winter sowings to the left. Indeed, winter sowings experienced higher water availability immediately before flowering.

Given these considerations, a factorial regression model for GFD is proposed in Table 3. This outline accounted for 34% of $G \times E$ with 19% df, leaving a non-significant remainder. The differential genotypic behaviour to R/ETP_H was mostly related to contrasting Δ_g values. Also AD partially accounted (although considerably less than Δ_g) for the differential genotypic responses to water availability during heading. However, AD was included later in the model as $AD \times E$ because a large amount of $G \times E$ related to AD remained unexplained by the cross-product $AD \times R/ETP_H$. Contributions to $G \times E$ and sensitivities of individual genotypes to R/ETP_H are shown in Table 4. Only three genotypes exhibited significant responses to this variable. The largest interactive cultivar for GFD, PC4, showed the highest sensitivity to R/ETP_H . PC4, together with Beka, have constitutively low Δ_g values, but also early flowering dates. Plaisant performed the opposite, being a late cultivar also characterized by high Δ_g . The environmental potencies for AD (data not shown) indicated that, in general, winter sowings favoured early flowering cultivars and autumn sowings were more adequate for late genotypes. This is corroborated by the significance of the term $AD \times$ Sowing Date in the factorial regression model (Table 3).

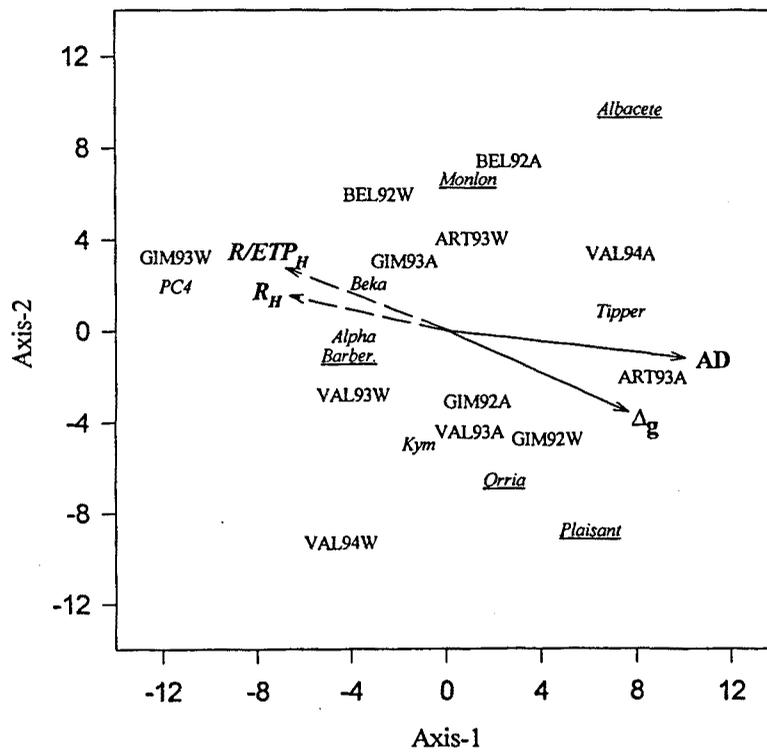


Figure 2. Biplot of the AMMI analysis for grain filling duration. Environments are represented in capitals, genotypes in italics. Six-rowed genotypes appear underlined. Relevant genotypic and environmental variables in the explanation of $G \times E$ have been included: AD (anthesis date), Δ_g (carbon isotope discrimination of mature grains), R_H (total rainfall during heading), and R/ETP_H (ratio of total rainfall to potential evapotranspiration during heading). The arrows indicate directions of greatest change for genotypic (solid lines) and environmental (dashed lines) variables.

Table 3. Factorial regression model proposal for the partitioning of G×E for grain filling duration

Source of variation	df	Sum of squares (SS) ($\times 10^3$)	% SS G×E	Mean squares	Variance ratio	P-value
G×E	99	3524.0				
G×R/ETP _H	9	533.3	15.1	59.26	2.39	0.019
$\Delta_g \times R/ETP_H$	1	392.7	11.1	392.74	15.81	0.000
Deviations	8	140.6	4.0	17.57	0.71	0.681
AD×E	10	675.7	19.2	67.57	2.72	0.006
AD×Sowing Date	1	162.2	4.6	162.17	6.53	0.014
Deviations	9	513.5	14.6	57.05	2.30	0.032
Remainder	80	2315.0	65.7	28.94	1.16	0.297
Error (G×S×T)	45	1117.6		24.84		

Table 4. Average genotypic values, ecovalences (W_i), contribution (%) of genotypes to $G \times E$ interaction sum of squares, and estimates of the regression coefficients of selected environmental covariates for individual genotypes according to factorial regression models for grain filling rate (GFR) and grain filling duration (GFD).

Genotype	Ear type	GFR					GFD				
		Average GFR (mg GDD ⁻¹)	W_i ($\times 10^{-5}$)	%SS G×E	R_H	T_{max_j}	GFR _{mean}	Average GFD (GDD)	W_i ($\times 10^3$)	%SS G×E	R/ETP _H
Albacete	Six-rowed	0.1070	261.4	13.5	-0.0683 [†]	-0.0854 [*]	-0.0112	614.6	530.1	15.1	-22.4
Alpha	Two-rowed	0.1106	67.4	3.5	-0.0187	-0.0014	638.4	415.9	11.8	9.6	
Barberousse	Six-rowed	0.1045	126.7	6.6	-0.0059	0.0023	577.2	112.3	3.2	3.5	
Beka	Two-rowed	0.1159	208.7	10.7	-0.0634 [†]	0.0525	566.4	293.7	8.4	28.4 [†]	
Kym	Two-rowed	0.1156	276.8	10.5	0.0018	0.1045 [*]	547.0	206.1	5.9	-15.3	
Monlon	Six-rowed	0.1292	171.2	14.3	0.0572	-0.0877 [*]	609.6	221.0	6.3	-12.1	
Oria	Six-rowed	0.1192	103.9	8.8	0.0668 [†]	0.0374	565.9	269.6	7.6	-12.8	
PC4	Two-rowed	0.1119	278.4	5.4	-0.0599	0.0127	578.3	656.2	18.6	45.1 [*]	
Plaisant	Six-rowed	0.1149	242.0	14.3	-0.0031	0.0099	588.6	440.1	12.5	-28.9 [*]	
Tipper	Two-rowed	0.1174	56.8	12.5	-0.0430	0.0170	532.4	379.1	10.8	4.9	

†, *, **: Regression coefficients with confidence intervals that do not include zero at $\alpha=0.1$, 0.05, and 0.01, respectively



4. DISCUSSION

4.1. Grain filling rate

Pre-anthesis climatic variables (R_H and T_{max}) explained a significant part of $G \times E$ for GFR. Thus, factors operating prior to the grain filling process seemed to account for differential genotypic responses to the environment in the rate of grain dry matter accumulation. The capacity of the grain to accumulate dry matter is established shortly after anthesis, being mainly dependent on the formation of endosperm cells (Brocklehurst, 1977). This initial phase of mitotic activity is stimulated largely by the capacity of the plant to supply assimilates to the growing grains (i.e., source activity) (Jenner *et al.*, 1991). Indeed, it is the balance between source strength and sink demand that determines the rate of dry matter accumulation in grains. Since the relative size of the photosynthetic tissue by anthesis (i.e., green leaf area) depends on pre-flowering environmental conditions, genotypic differences in source/sink balance, which have been reported in many studies on wheat (e.g. Ma *et al.*, 1990; Bruckner and Froberg, 1991), may have been responsible for $G \times E$ on GFR. For instance, larger grain weight responses to sink reduction treatments have been detected in genotypes bearing smaller grains (Blum *et al.*, 1988; Ma *et al.*, 1990). Preliminary work performed in a set of six- and two-rowed barleys, which partially differed from the genotypes used here, showed that the six-rowed group was clearly source-limited during grain filling (Voltas, unpublished data). It has been reported that two-rowed cultivars generally produce heavier grains than six-rowed types when grown in low-rainfall areas (Acevedo *et al.*, 1991). In this study, six-rowed cultivars exhibited overall larger sensitivities to pre-anthesis climatic constraints (low R_H and high T_{max}) than two-rowed types. Those environments characterized by high T_{max} , exhibited in turn poor biomass accumulation by anthesis. Actually, substituting T_{max} by Biomass in the factorial regression model would have been perfectly feasible, retaining a similar overall fit. It is then possible to speculate that those environments determining a limited plant photosynthetic system by anthesis (i.e., showing higher T_{max} , in this study) affected comparatively more the source/sink balance of six-rowed cultivars. Due to the substrate effect on endosperm cell division, GFR would decrease comparatively more in six- than in two-rowed genotypes. Nevertheless, this general behaviour may not be applicable to all genotypes and merits by itself further exploration.

In addition to R_H and T_{max} , the environmental mean for GFR was also included in the model. Probably, the uncertain factors determining high/low GFR_{mean} coincided with those ones responsive for IGW_{mean} (see the previous paper (Voltas *et al.*, 1998)), given the relationship found between IGW_{mean} and GFR_{mean} ($r=0.81, p<0.01$). Furthermore, reasons behind the observation that early-flowering genotypes accumulated dry matter at relatively higher rates in low GFR_{mean} environments are not completely clear, this being a very consistent response. Late cultivars might take advantage of the non-limiting grain filling conditions that also determined larger grains. Early ones, contrarily, might escape from the harmful conditions associated with an overall poor grain filling (but, on the other hand, AD could not be linked to IGW_{mean} in the factorial regression model for IGW).

4.2. Grain filling duration

The strong GFD dependence on environmental conditions reported elsewhere for wheat (e.g., Sofield *et al.*, 1977; Wiegand and Cuellar, 1981) has also been detected in this study. In fact, mean environmental GFD values and average mean temperatures during grain filling were negatively related ($r=-0.481; p<0.09$). Presence of thermic stress along the grain filling period was not associated, however, to differential genotypic responses in GFD. As for the explanation of $G \times E$ for GFR, factors operating prior to flowering accounted for such responses. Particularly, water availability during heading (R/ETP_H) was the one and only environmental variable entering the factorial regression model. The AMMI2 biplot revealed that this climatic variable was associated with sowing date, the winter sowings having higher R/ETP_H values than the autumn ones.

Plaisant, a cultivar with high Δ_g (and low WUE), showed an increased GFD in those environments characterized by high water deficits (low R/ETP_H) during heading, i.e., most of the autumn sowings. At first, such response may seem inconsistent. However, while winter sowings experienced considerable pre-flowering rainfall, autumn ones tended to receive the same rain during the early phase of grain filling due to their approximately 10-15 days shift in phenology (there were some exceptions, however: GIM92 sowings showed both low R/ETP_H , while GIM93 sowings exhibited relatively high R/ETP_H). Rainfall during grain filling would avoid premature leaf senescence, permitting the maintenance of a constitutively high stomatal conductance (associated with a high Δ_g) during a long grain filling period. On the contrary, the more water-

conservative low Δ_g genotypes (PC4 and Beka) would benefit, in relative terms, of the harsher grain filling conditions associated in our study with favourable water status at heading. Such behaviour has also been pointed out by Blum (1988), in the sense that lower yielding varieties, i.e. with lower Δ_g (Craufurd *et al.*, 1991; Sayre *et al.*, 1995), would use comparatively less water after anthesis because they are able to maintain a more favourable water potential under drought.

Beyond genotypic differences in Δ_g , flowering earliness also accounted for cultivar responses to the environment in terms of GFD. As mentioned above, AD partially accounted for differences in genotypic behaviour to changes in R/ETP_H , changes that in turn were mainly driven by a sowing time effect. As suggested by the factorial regression model, one possible interpretation to such genotypic differences would be to associate early flowering cultivars with late sowings, and late flowering cultivars with early sowings. Longer GFDs have often been linked to early flowering cultivars (Metzger *et al.*, 1984; Van Sanford, 1985). These cultivars, by using an escape strategy, may suffer to a lesser extent the higher temperatures and lack of water that usually experience late sowings at the end of the cycle. It should be recognized, however, that in this context it is rather difficult to discern between the confounding effects of R/ETP_H and Sowing Date on cultivars varying in Δ_g and AD. Both the pair of genotypic variables and the pair of environmental variables, although not being perfectly coincident in explaining $G \times E$ for GFD, contained much overlapping information.

4.3. Grain filling process: a general discussion

Individual grain weight (IGW) can be thought of as the result of two growth parameters: GFR and GFD. From a physiological point of view, however, these parameters express very different phenomena (Jenner *et al.*, 1991): GFR reflects the rate of biochemical reactions involved in the accumulation of starch and protein, while GFD indicates the grain developmental program. Under near-optimal pre-anthesis conditions, the influence of moderate post-anthesis water and thermic stresses decreases GFD of wheat to a larger extent than the concomitant increase experienced by GFR (Wardlaw and Moncur, 1995; Savin *et al.*, 1997). In our study, however, the relationship between mean environmental values for GFR and GFD was far from negative ($r=+0.566$, $p=0.06$), suggesting that pre-anthesis conditions partially hid the effects of post-anthesis climatic constraints on grain growth. In a previous paper, Voltas *et al.* (1997) found contrasting estimates of potential grain weight, by changing the source/sink ratio, at some of the

environments used in this study. This result was attributed to large differences in pre-anthesis conditions among environments. Some authors also indicate that the genotypic responses to high temperatures during grain filling can be altered by changes in source-sink balance (Wardlaw *et al.*, 1980; Wardlaw, 1994). Particularly, the interaction between photosynthate supply and post-anthesis constraints probably determined differential genotypic responses to the environments in terms of individual grain weight and grain filling parameters.

The factorial regression models indicated that pre-anthesis climatic conditions were important for the explanation of G×E for both GFR and GFD, while the water availability and temperature regime during post-anthesis accounted for most of G×E on IGW (Voltas *et al.*, 1998). This finding seems somehow contradictory: does it make sense that the covariables selected for GFR and GFD differ so markedly from those for IGW, the resulting outcome of the two grain filling components? However, if we introduce the selected variables from GFR and GFD we obtain as good a description of G×E for IGW as it was found in the previous paper. Thus a new factorial regression model was built using T_{max} and R_H (covariables previously selected for GFR), and R/ETP_H (covariable selected for GFD). This model explained 42.9% of G×E for IGW, an amount that did not differ from that of the model initially proposed for IGW (43.1% of G×E) (Voltas *et al.*, 1998). This result implies that the application of statistical tools for modelling G×E on grain growth, without a previous knowledge of the phenomenon at the ecophysiological level, may be hazardous and lead to rigorousless (erroneous) conclusions. Should we have used another selection strategy of covariables, we would have arrived at an equally good model, just due to chance correlations within the set of environmental variables. It seems, therefore, entirely recommendable to conduct a preselection of variables as a first step prior to develop any particular factorial regression model. AMMI fulfilled this goal, emerging as a valid methodology to be applied in studies aimed to explore the unknown nature of G×E. Once AMMI was used to propose candidate external descriptors to fit factorial regression models, it seemed sensible to attempt including first those environmental variables taking place during grain filling, and to continue later on with those ones related to previous stages of crop development. The suitability of this selection strategy is supported by feasibility of results, as well as by their consistency with previous findings reported in related studies. The extent to which grain weight of barley genotypes depends specifically on pre-flowering conditions in contrast to other conditions, however, remains uncertain, although such conditions undoubtedly exerted a strong influence on grain growth by modifying genotypic behaviour of both GFR and GFD.

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REFERENCES

- Acevedo, E., Craufurd, P.Q., Austin, R.B., and Pérez-Marco, P., 1991. Traits associated with high yield in barley in low-rainfall environments. *J. Agric. Sci., Camb.* 116, 23-36.
- Bauer, A.C., Fanning, C., Enz, J.W., Eberlein, C.V., 1984. Use of growing degree-days to determine spring wheat growth stages. *North Dakota Agricultural Experimental Station Bulletin* 37.
- Blum, A., Mayer, J., Golan, G., 1983. Chemical desiccation of wheat plants as a simulator of post-anthesis stress. II. Relations to drought stress. *Field Crops Res.* 6, 149-155.
- Blum, A., 1988. *Plant breeding for stress environments*. CRC Press Inc. Boca Raton, Florida.
- Brocklehurst, P.A., 1977. Factors controlling grain weight in wheat. *Nature* 266, 348-349.
- Bruckner, P.L., Frohberg, R.C., 1991. Source-sink manipulation as a postanthesis stress tolerance screening technique in wheat. *Crop Sci.* 31, 326-328.
- Craufurd, P.Q., Austin, R.B., Acevedo E., Hall, M.A., 1991. Carbon isotope discrimination and grain yield in barley. *Field Crops Res.* 27, 301-313.
- Darroch, B.A., Baker R.J., 1990. Grain filling in three spring wheat genotypes: statistical analysis. *Crop Sci.* 30, 525-529.
- Denis, J.B., 1988. Two-way analysis using covariates. *Statistics* 19, 123-132.
- Denis, J.B., 1991. Ajustement de modèles linéaires et bilinéaires sous contraintes linéaires avec données manquantes. *Revue de Statistique Appliquée* 39, 5-24.
- Denis, J.B., Gower, J.C., 1996. Asymptotic confidence regions for biadditive models, interpreting genotype-environment interaction. *Applied Statistics* 45, 479-493.

- Gauch, H.G., 1988. Model selection and validation for yield trials with interaction. *Biometrics* 44, 705-715.
- Gauch, H.G., 1992. *Statistical analysis of regional yield trials: AMMI analysis of factorial designs*. Elsevier, Amsterdam.
- Gollob, H.F., 1968. A statistical model that combines features of factor analysis and analysis of variance techniques. *Psychometrika* 33, 73-115.
- Ho, K.M., Jui, P.Y., 1989. Duration and rate of kernel filling in barley. *Cereal Research Communications* 17, 69-76.
- Hunt, L.A., van der Poorten, G., Pararajasingham, S., 1991. Postanthesis temperature effects on duration and rate of grain filling in some winter and spring wheats. *Can. J. Plant Sci.* 71, 609-617.
- Jenner, C.F., Ugalde, T.D., Aspinall, D., 1991. The physiology of starch and protein deposition in the endosperm of wheat. *Aust. J. Plant Physiol.* 18, 211-226.
- Jenner, C.F., 1994. Starch synthesis in the kernel of wheat under high temperature conditions. *Aust. J. Plant. Physiol.* 21, 791-806.
- Kempton, R.A., 1984. The use of biplots in interpreting variety by environment interactions. *Journal of Agricultural Science, Cambridge* 103, 123-135.
- Kobata, T., Palta, J.A., Turner N.C., 1992. Rate of development of postanthesis water deficits and grain filling of spring wheat. *Crop Sci.* 32, 1238-1242.
- Ma, Y.Z., MacKown, C.T., Van Sanford, D.A., 1990. Sink manipulation in wheat: compensatory changes in kernel size. *Crop Sci.* 30, 1099-1105.
- Mandel, J., 1971. A new analysis of variance model for non-additive data. *Technometrics* 13, 1-18.
- Metzger, D.D., Czaplowski, S.J., Rasmusson, D.C., 1984. Grain filling duration and yield in spring barley. *Crop Sci.* 19, 5-9.
- Nass, H.G., Reiser, B., 1975. Grain filling period and grain yield relationships in spring wheat. *Can. J. Plant Sci.* 55, 673-678.

- Richards, R.A., 1996. Increasing the yield potential of wheat: manipulating sources and sinks. In: Reynolds, M.P., Rajaram, S., McNab, A. (Eds.), *Increasing Yield Potential in Wheat: Breaking the Barriers*. CIMMYT, Mexico D.F., pp. 134-149.
- Savin, R., Stone, P.J., Nicolas, M.E., Wardlaw, I.F., 1997. Grain growth and malting quality of barley. 1. Effects of heat stress and moderately high temperature. *Aust. J. Agric. Res.* 48, 615-624.
- SAS Institute Inc. 1987. *SAS/STAT guide for personal computers*. Version 6 Edition. Cary, NC.
- Sayre, K.D., Acevedo, E., Austin, R.B., 1995. Carbon isotope discrimination and grain yield of three bread wheat germplasm groups grown at different levels of water stress. *Field Crops Res.* 41, 45-54.
- Slafer, G.A., Savin, R., 1994. Source-sink relationships and grain mass at different positions within the spike in wheat. *Field Crops Res.* 37, 39-49.
- Sofield, I., Evans, L.T., Cook, M.G., Wardlaw, I.F., 1977. Factors influencing the rate and duration of grain filling in wheat. *Aust. J. Plant Physiol.* 4, 785-797.
- van Eeuwijk, F.A., Denis, J.B., Kang, M.S., 1996. Incorporating additional information on genotypes and environments in models for two-way genotype by environment tables. In: Kang, M.S., Gauch, H.G. (Eds.), *Genotype by environment interaction: New perspectives*. CRC Press. Boca Raton, pp. 15-49.
- Van Sanford, D.A., 1985. Variation in kernel growth characters among soft red winter wheats. *Crop Sci.* 25, 626-630.
- Volta, J., Romagosa, I., Araus, J.L., 1997. Grain size and nitrogen accumulation in sink-reduced barley under Mediterranean conditions. *Field Crops Res.* 52, 117-126.
- Volta, J., van Eeuwijk, F.A., Sombrero, A., Lafarga, A., Igartua, E., Romagosa, I., 1998. Integrating statistical and ecophysiological analyses of genotype by environment interaction for grain filling of barley in Mediterranean areas. I. Individual grain weight. *Field Crops Res.* (submitted).
- Wardlaw, I.F., Dawson, I.A., Munibi, P., 1980. Factors limiting the rate of dry matter accumulation in the grain of wheat grown at high temperature. *Aust. J. Plant Physiol.* 21, 731-739.
- Wardlaw, I.F., Dawson, I.A., Munibi, P., Fewster, R., 1989. The tolerance of wheat to high temperatures during reproductive growth. I. Survey procedures and general response patterns. *Aust. J. Agr. Res.* 40, 1-13.

Wardlaw, I.F., 1994. The effect of high temperature on kernel development in wheat: variability related to pre-heading and post-anthesis conditions. *Aust. J. Plant Physiol.* 21, 731-739.

Wardlaw, I.F., Moncur, L., 1995. The response of wheat to high temperature following anthesis. I. The rate and duration of kernel filling. *Aust. J. Plant Physiol.* 22, 391-397.

Wiegand, C.L., Cuellar, J.A., 1981. Duration of grain filling and kernel weight of wheat as affected by temperature. *Crop Sci.* 21, 95-101.

Wricke, G., 1962. Über eine Methode zur Erfassung der ökologischen Streubreite in Feldversuchen. *Z. Pflanzenzüchtg.* 47, 92-93.

Wych, R.D., McGraw, R.L., Stuthman, D.D., 1982. Genotype×year interaction for length and rate of grain filling in oats. *Crop Sci.* 22, 1025-1028.

CHAPTER V



Genotype by environment interaction for grain yield and carbon isotope discrimination of barley in Mediterranean Spain

ABSTRACT

Carbon isotope discrimination (Δ) has been found to be diversely related to grain yield of small grain cereals when grown in contrasting environments. In order to clarify a possible association between grain yield of barley (*Hordeum vulgare* L.) and Δ of mature kernels under semiarid conditions, five six-rowed and five two-rowed barley genotypes were evaluated in 22 rainfed environments of northern Mediterranean Spain. Analyses of variance revealed that the genotypic expression for grain yield was considerably more affected by the growing environment than that for Δ . Genotype by environment (G×E) interaction for grain yield was further explored by fitting an AMMI (Additive Main Effects and Multiplicative Interaction) model. The first two multiplicative axes were found significant. The AMMI2 model provided more reliable estimates of genotypic yields within environments than the conventional unadjusted means across replicates. AMMI2 estimates were used for input into cluster analysis, grouping environments that ranked genotypic yields similarly. Three major groups were obtained, with average yields of 2.42 t ha⁻¹ (cluster I), 3.06 t ha⁻¹ (cluster II) and 5.16 t ha⁻¹ (cluster III). Genotypic ranking for Δ was nearly constant across clusters, but it changed substantially for grain yield. The low-yielding cluster I ranked average genotypic yields rather oppositely to cluster III, suggesting the existence of a crossover point in an intermediate yield level. The association between grain yield and Δ for genotypic means within clusters was variable. In cluster I, yield and Δ tended to be negatively related, being positively related in clusters II and III. Genotypes with lower Δ , i.e., with higher transpiration efficiency, performed better in low-yielding environments (mostly those grouped in cluster I). On the contrary, a high genotypic Δ was of advantage in medium (cluster II) and high-yielding environments (cluster III). This observation supports the assumption that drought resistance and high yield potential under non limiting growing conditions are antagonistic concepts in barley. Genotypic means for kernel number m⁻² and Δ were consistently and positively related within clusters, suggesting that a constitutively high Δ may be driven by a large genotypic reproductive sink. The convenience of using Δ as a selection criterion in areas exhibiting a considerable G×E interaction is discussed.

1. INTRODUCTION

Understanding of the association between grain yield of cereals and carbon isotope discrimination in rainfed Mediterranean environments is still limited. Numerous pot studies corroborate existence of a negative relationship between Δ and transpiration efficiency (TE) in C_3 species (e.g., Farquhar and Richards, 1984; Hubick and Farquhar, 1989; Condon *et al.*, 1993). High-TE genotypes accumulate more ^{13}C in their tissues, thus increasing the ratio of ^{13}C to ^{12}C and decreasing the plant discrimination against ^{13}C (Δ), compared with less efficient genotypes. However, field experiments have proved that a high TE, i.e., a low Δ , may not always be a beneficial genotypic attribute for maximizing total biomass or economic yield under drought. Several studies report positive relationships between Δ and productivity in wheat (Ehdaie *et al.*, 1991; Araus *et al.*, 1993) and barley (Craufurd *et al.*, 1991; Voltas *et al.*, 1998). Among the reasons cited for the explanation of such an apparent contradiction follows (Richards and Condon, 1993): (i) the plant part used in the determination of Δ , and (ii) the existence of physiological or genetic associations that override the expected negative relation between Δ and biomass.

Recently, Richards (1996a) points out, in order to improve yield under drought, the need to understand the above-mentioned trade-offs between Δ and growth in specific environments. In Mediterranean climates, an accurate definition of the target environment represents an additional obstacle in the development of cereal breeding programs. Outyielding genotypes in non limiting locations or years often perform deficiently under harsher conditions (Ceccarelli, 1989; van Oosterom *et al.*, 1993). Therefore, G×E is qualitative, or crossover, i.e., the ranking of genotypes changes from one environment to another. In such conditions, selection efficiency for yield itself may be meagre, as may also be the correlated response from indirect selection for Δ .

It has been reported that the magnitude of G×E for Δ is relatively low compared with that for grain yield (Romagosa and Araus, 1991; Condon and Richards, 1992). In this regard, the potential of Δ to account for G×E of grain yield under complex interaction patterns remains uncertain (Romagosa and Araus, 1991). If the expression of Δ was relatively constant in a range of environments, it is clear that such a trait would be advantageous in some conditions and detrimental in others. Therefore, an inconsistent correlation between grain yield and Δ would be expected across environments. Craufurd *et al.* (1991) reported a positive relation between Δ of kernels and grain yield of barley genotypes measured under severe drought, and a negative association when assessed in less droughted trials. In that study, the adaptation pattern to drought

was mainly caused by an escape strategy of the earliest flowering lines, thus having higher Δ and also greater yields (Acevedo *et al.*, 1993). Conversely, Condon and Richards (1993) found a negative relationship between Δ measured in vegetative tissues and grain yield only in the driest environment, with positive correlations at sites where drought was not severe.

Differential genotypic performance across environments is commonly identified by standard analysis of variance (ANOVA). A common extension of ANOVA for studying G×E is the additive main effects and multiplicative interaction (AMMI) model (Gauch, 1992). Applications of AMMI have led to more insight in the complicated patterns of genotypic responses to changing environmental conditions (Royo *et al.*, 1993; Romagosa *et al.*, 1996). Together with the parallel use of multivariate techniques like cluster analysis, it permits to describe the maximum variation from G×E data sets in a few dimensions (Williams, 1976). Environments can be considered in a multidimensional space with each dimension a genotype and, thus, they can be grouped based on the relative similarities of the yields of the different genotypes (Fox and Roseille, 1982). The clustering classification minimizes G×E for grain yield within groups, i.e., it assembles environments that rank genotypic classes similarly. For each group of homogeneous environments, a better assessment of the relationship between grain yield and Δ can be presumably achieved.

This study was aimed at examining the relationship between grain yield and carbon isotope discrimination (Δ) in a large set of rainfed environments. Ordination (AMMI) and classification (cluster analysis) techniques were used first to interpret and provide more insight into G×E for grain yield. Besides, they provided the analytical basis for a proper evaluation of the possible links between grain yield, yield components and Δ .

2. MATERIALS AND METHODS

2.1. Experimental design and plant material

Field experiments were conducted for three consecutive years (1992, 1993 and 1994) in various locations situated in two northern provinces of Spain (Lleida and Navarra) (Table 1). Gimenez and Artesa de Segre were selected as target locations of the moderate and harsher semiarid Mediterranean environments of northeastern Spain (Lleida province), respectively. They will be referred to in the text as L1 (Gimenez) and L2 (Artesa de Segre). Locations in Navarra were attempted to represent both the transition to the Atlantic-type climate of the province northern part (denoted as N1), and the severe, continental climate of its southern extreme (N2). Although not the same locations were used in Navarra throughout the three-year study, those situated within a distance range inferior to 15 km were assumed to belong to the same homogeneous climatic area (N1 or N2).

The term 'trial' will be used herein to define the combination of a particular location/area in a given year. In all trials, Fall and Winter sowings were carried out giving a total of 22 'year-location-sowing period' combinations (hereafter referred to as environments) (Table 1). The experimental layout in each environment was a completely randomised block design with four replicates. Plots consisted of six 12-m rows, spaced 20 cm, and seeded at rates of 350 seeds m⁻², and were fertilized according to standard cultural practices. Ten genetically diverse barley genotypes differing in adaptation to rainfed environments were used: Alpha, Beka, Kym, PC4 and Tipper (two-rowed types), and Albacete, Barberousse, Monlon, Orria and Plaisant (six-rowed types).

2.2. Plant sampling and grain yield

At each environment, mid-anthesis (ZDC 65) and maturity (ZDC 91) stages of development were recorded according to the Zadocks decimal code (ZDC) (Zadocks *et al.*, 1974). Prior to harvest, plant samples composed of two 1-m row segments were taken from each plot of one replicate per environment, and kernel number m⁻² and thousand kernel weight (TKW) were obtained. Samples of kernels of the same genotype were collected from one 1-m row segment at each of the four replicates of a particular environment. They were subsequently bulked to provide

Table 1. Characterization of the 22 environments of this study

Year	Province	Location	Sowing period	Environmental code	R/ETo ₀ ¹	R/ETo ₀ ²	Grain yield (t ha ⁻¹)	Δ (%)
1992	Lleida (L)	Gimenells (L1)	Autumn	L1A2	0.584	0.481	5.49	16.98
			Winter	L1W2	0.570	0.722	3.64	17.03
1993		Gimenells (L1)	Autumn	L1A3	0.443	0.912	4.23	17.24
			Winter	L1W3	0.568	0.371	4.16	17.31
1994		Artesa de Segre (L2)	Autumn	L2A3	0.519	0.695	2.61	16.12
			Winter	L2W3	0.582	0.250	1.63	16.13
		Gimenells (L1)	Autumn	L1A4	0.292	0.326	2.74	16.55
			Winter	L1W4	0.267	0.325	1.63	16.82
1992	Navarra (N)	Artesa de Segre (L2)	Autumn	L2A4	0.457	0.224	2.66	15.00
			Winter	L2W4	0.455	0.220	1.40	14.48
		Solchaga (N1)	Autumn	N1A2	1.012	0.820	5.65	16.05
			Winter	N1W2	0.809	0.324	4.01	16.11
1993		Falces (N2)	Autumn	N2A2	0.690	0	1.44	12.82
			Winter	N2W2	0.526	0.598	1.50	14.63
		Pueyo (N1)	Autumn	N1A3	0.893	0.822	5.46	17.05
			Winter	N1W3	0.874	0.822	3.69	17.12
1994		La Venta (N2)	Autumn	N2A3	0.828	0	4.53	16.10
			Winter	N2W3	0.546	0.389	1.27	16.20
		Garinoain (N1)	Autumn	N1A4	0.938	0.791	6.43	17.61
			Winter	N1W4	0.913	0.533	6.06	17.74
1994		Peralta (N2)	Autumn	N2A4	0.490	0.644	1.53	15.43
			Winter	N2W4	0.267	0.325	1.15	15.61

¹Pre-anthesis period; ²Post-anthesis period

a balanced mixture of plant material (kernels). Grain yield was recorded at maturity in each environment.

2.3. Carbon isotope analysis

Kernel samples were oven-dried and ground to a fine powder. $^{13}\text{C}/^{12}\text{C}$ ratios were determined by mass spectrometry (Isotope Services, Inc. Los Alamos, NM 87544, USA). Results were expressed as $\delta^{13}\text{C}$ values (Farquhar *et al.*, 1989), where:

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

and R is the $^{13}\text{C}/^{12}\text{C}$ ratio. The standard for comparison was a secondary standard calibration against Pee Dee Belemnite (PBD) carbonate. Sample sizes of five to ten mg were used, and precision of analysis was less than 0.10 ‰. Carbon isotope discrimination (Δ) was then calculated in kernels from δ_a and δ_p , where a and p refer to air and plant, respectively (Farquhar *et al.*, 1989):

$$\Delta = (\delta_a - \delta_p) / (1 + \delta_p)$$

The value of $\delta^{13}\text{C}$ for the air was assumed to be -8.00 ‰ (Farquhar *et al.*, 1989).

2.4. Environmental characterization

The water deficit experienced by the crop (by means of the ratio of rainfall to total evapotranspirative demand obtained according to the Hargreaves method, R/ET_0), calculated at two growth periods, was used as climatic measurement to characterize environments (Table 1). The two growth periods were defined as follows:

- pre-anthesis period: from sowing to mid-anthesis (ZDC 65) ($R/ET_{0(1)}$).
- post-anthesis period: from mid-anthesis to maturity (ZDC 91) ($R/ET_{0(2)}$).

2.5. Statistical analyses

Analyses of variance

Fixed three-way ANOVA models were fitted to analyze grain yield, yield components and carbon isotope discrimination (Δ) data. The factorial structure was: Genotype by Sowing Date by Trial (G×S×T). For Δ determinations and yield components, in which only one sample per genotype and environment was available, the three-way GxSxT interaction mean square was chosen as an estimate for error in the ANOVAs. For further analyses involving grain yield, data were structured as a two-way genotype (G) by environment (E) table, with I rows (genotypes) and J columns (environments). Each environment referred to a particular Sowing Date by Trial combination.

AMMI analysis of grain yield

AMMI was fitted to the two-way GxE table for grain yield. AMMI models (Gauch, 1992) are special cases of biadditive models, models that are characterized by terms that consist of products of row and column parameters (Denis and Gower, 1994; van Eeuwijk, 1995). The general formulation for the AMMI model for the expectation of genotype i in environment j is

$$E(Y_{ij}) = \mu + g_i + e_j + \sum_{k=1}^K c_{ik} d_{jk}$$

where μ is the overall mean, g_i and e_j are genotypic and environmental main effects, and c_{ik} and d_{jk} are the genotypic and environmental scores, respectively, for the k -th multiplicative term. In AMMI the usual GxE interaction term, ge_{ij} , of the standard two-way ANOVA model, $E(Y_{ij}) = \mu + g_i + e_j + ge_{ij}$, is replaced by a sum of products $c_{ik} d_{jk}$ and a random remainder, ϵ_{ij} . AMMI generates a family of models based on the number of multiplicative terms, K , necessary for adequate description of the interaction, number that can be obtained by two different processes (Gauch and Zobel, 1988). They used the terms postdictive and predictive accuracy.

The best postdictive model is assessed by first transforming the eigenvalues (=sum of squares) corresponding to the individual terms to mean squares (Gollob, 1968). The mean squares are obtained by dividing the eigenvalues by degrees of freedom: I+J-3 for the first term, I+J-5 for the second, etc. Subsequently, significance for these mean squares can be assessed by calculating variance ratios. In prediction, individual replicates are randomly allocated for each genotype-environment combination to two subgroups: modelling and validation data. The sum

of squared differences between validation data and predicted values for the different AMMI models, across the data matrix, is then divided by the number of validation observations. The square root of this quantity is the root mean square predictive difference (RMS PD), for which smaller values indicate more accurate prediction. Predictive modelling is preferred to postdiction since it considers usually a fewer number of multiplicative terms, associated with pattern, with subsequent terms, retained by a postdictive assessment, being often associated with noise (Gauch, 1992). The best AMMI model for our grain yield data was then chosen based on predictive accuracy after 50 validation runs. Estimates for all genotype-environment combinations were generated based on this model and used for subsequent analyses. This approach stems from Stein (1955), who first demonstrated that estimators from a multivariate model could be more precise and efficient than the original data averaged across replications. It has proved useful to unmask adaptation patterns of specific genotypes to particular environments, otherwise secluded by noise in the data set (Gauch and Zobel, 1989; Crossa *et al.*, 1991).

Interaction as described by two multiplicative terms in an AMMI model can easily be visualized in biplots. Genotypes and environments are then depicted as points on a plane. The position of the points for the genotypes is given by the estimates for the genotypic scores, the environmental point coordinates come from the estimates for the environmental scores. Proximity of genotypes in a biplot denotes similar behaviour over environments while distant genotypes are indicative of different performance. Distance from the origin is an indication of the amount of interaction that is exhibited by a genotype. The same rules apply to the environments. In a vector interpretation of the biplot, the genotypic and environmental points are the end points of vectors starting at the origin, where these vectors are constitutive for lines. From the biplot, inference about specific interactions of individual genotypes and environments can be found by projecting environmental points on genotypic lines (or vice versa). The approximation follows from the length of the projection multiplied by the length of the direction vector of the line on which projection takes place. When projection and direction vectors are at an obtuse angle, a minus sign should be added to this product.

Classification of genotypes and environments and genotypic ranking

Cluster analysis of the standardized AMMI estimates for grain yield was carried out as suggested by Fox and Rosielle (1982). For each environment, standardization to a mean of zero and a unit phenotypic standard deviation is performed to adjust for yield differences between

environments. The Ward or incremental sum of squares method is used as a clustering method to group environments that rank genotypic classes similarly, and vice versa. At each fusion point of clusters, the new group formed arises from the minimization of the increment in the within-groups sum of squares. Changes in genotypic ranking across clusters for grain yield and Δ were quantified using Spearman's rank correlations.

Relationships between grain yield, Δ and yield components

Partial correlation analyses were used to assess the degree of statistical independence between grain yield, yield components, and carbon isotope discrimination (Δ). Partial correlation is a measure of the strength of the linear relationship between two variables, after controlling for the effect of other variables. In this study, first order partial correlations were determined after controlling for the effect of ear type, i.e. number of rows. Overall, two-rowed barleys bear heavier grains but produce fewer grains per square meter than six-rowed types. It seemed sensible to account for the influence of this particular genotypic feature before characterizing any possible relationship involving yield components of barley.

For the analyses, programs in SAS (SAS Inst., 1987) were used.

3. RESULTS

3.1. Environmental characterization

There were large differences among environments in R/ETo ratios during both pre- and post-anthesis periods (Table 1). Usually, those locations classified *a priori* as having a harsher climate (L2 and N2) exhibited lower R/ETo_(t) ratios, i.e., higher water deficits from sowing to flowering. There were exceptions, however: L1 environments during the 1994 crop season exhibited, due to the scarce winter rainfall, very low R/ETo values. ETo was especially low for N1 environments (data not shown), and their R/ETo_(t) ratios were close to unity. During the grain filling period, variation in R/ETo was rather unpredictable across locations, years and sowings, ranging from 0 to 0.912 among environments. Differences in rainfall rather than in ETo were important in accounting for such variation (data not shown).

3.2. Analyses of variance

Most of the total variation for both grain yield and Δ was due to differences among environments (87.7% and 88.6%, respectively) (Table 2). Mean grain yields ranged from 1.15 t ha⁻¹ (N2W4) to 6.43 t ha⁻¹ (N1A4). Mean Δ values ranged from 12.82‰ (N2A2) to 17.74‰ (N1W4). The ANOVAs showed that differences among trials rather than between sowing dates were important in accounting for environmental variation in grain yield and Δ . Though significant in both cases, the proportion of the total sum of squares due to differences among genotypes was much lower for grain yield (0.3%) than for Δ (5.9%), whereas it was analogous for G×E (8.2% in both cases) (Table 2). This suggests that the environmental influence on genotypic expression was considerably larger for grain yield than for Δ . Significant differences among genotypes and environments were also detected for yield components (kernel number m⁻² and TKW) (data not shown).

3.3. AMMI analysis of grain yield

Based on postdiction, the AMMI3 model for grain yield was statistically significant. The first multiplicative term accounted for 41% of the interaction sum of squares, the second term explained 30% and the third 11% of the G×E sum of squares. However, in the predictive sense the AMMI2 model was superior, showing the least deviation from validation data of all possible AMMI models based on 50 randomizations (RMS PD of 643 kg ha⁻¹). AMMI2 accounted for 71% of the G×E sum of squares and captured 90.6% of the total variation. The poorer fit of models with a higher number of multiplicative terms indicated that such terms could be rather associated with noise in the original data set than with pattern, i.e., systematic variation, in the interaction between genotypes and environments. Estimates of the AMMI2 model based on four replicates were obtained and compared to the unadjusted means of the two-way matrix of genotypes and environments. AMMI2 estimates changed the top-yielding genotype in 13 out of 22 environments. Spearman's rank correlations between AMMI2 estimates and unadjusted means were not significant anymore in 6 out of 22 environments. Next, AMMI2 estimates were used for input into cluster analysis.

Table 2. Analysis of variance for grain yield and carbon isotope discrimination (Δ) of ten barley genotypes grown at 22 environments

Source of variation	Yield (t ha ⁻¹)				Δ (%)					
	df	Sum of squares	Mean squares	Variance ratio	R ² (%) ¹	df	Sum of squares	Mean squares	Variance ratio	R ² (%) ¹
Total	879	3086.1	3.51			219	341.5	1.56		
Environment (E)	21	2608.7	124.21	653.7**	84.5	21	293.1	13.95	116.3**	85.8
Trial (T)	10	2130.4	213.04	132.2**	69.0	10	274.7	27.47	233.3**	80.4
Sowing Date (S)	1	290.0	289.84	179.8**	9.4	1	2.2	2.2	18.8**	0.6
T x S	10	188.3	18.83	11.7**	6.1	10	16.2	1.62	13.8**	4.8
Error 1 (Reps/E)	66	106.3	1.61		3.4		(²)			
Genotype (G)	9	8.2	0.91	4.9**	0.3	9	20.3	2.26	19.2**	5.9
G x E	189	252.1	1.33	7.0**	8.2	189	28.1	0.15	<i>nt</i>	8.2
G x T	90	184.1	2.05	11.0**	60	90	16.0	0.18	1.5*	4.7
G x S	9	5.9	0.63	3.5**	0.2	9	1.5	0.17	1.4	0.4
G x T x S	90	62.1	0.69	3.7**	2.0	90	10.6	0.12	<i>nt</i> ⁽²⁾	3.1
Error 2	594	110.8	0.19		3.6		(²)			

*, ** Significant at the 0.05 and 0.01 probability levels, respectively; *nt*, not testable hypothesis (¹) Fraction of sum of squares associated to each term or interaction (²) No error terms are listed for Δ since one sample was available per genotype and environment. Instead, 3-way interaction acts as experimental error

3.4. Classification of Genotypes and Environments

Cluster analysis classified environments in three major groups (Table 3), which accounted for 74% of the environments and G×E sum of squares. Cluster I, the largest group, had an average yield of 2.42 t ha⁻¹ and it was composed by 11, mostly low-yielding, environments. Cluster II showed a moderate average yield (3.06 t ha⁻¹) and was integrated by five environments. Cluster III included six highly productive environments (average of 5.16 t ha⁻¹). Genotypes were also clustered in three groups accounting for 76% of the genotypes and G×E sum of squares. Cluster classification using raw yields, rather than AMMI2 adjusted means, modified partially grouping of environments and genotypes: three environments (N1W2, N2A2 and N1A3) moved from cluster I to cluster III, one environment (L1A2) from cluster III to cluster II; cultivar Monlon was also grouped in a different cluster of genotypes.

Specific adaptation patterns could be inferred more easily by means of biplots. The biplot for the AMMI2 model, together with the classification of genotypes and environments according to the cluster analysis of AMMI2 estimates, is visualized in Fig. 1. Axis-1 separated highly productive environments (cluster III, positive scores) from the rest (clusters I and II, negative scores). Axis-1 was, in turn, strongly associated with the water status of the crop from sowing to anthesis. This observation was brought about by the positive correlation of Axis-1 environmental scores with R/ET_{o(1)} ratios ($r=0.65^{**}$, $n=22$). Though included in different groups, modern, highly productive genotypes like Orria or Plaisant, with positive Axis-1 scores, exhibited an overall outstanding performance under non limiting environmental conditions. Old, low-yielding genotypes like Albacete and Beka performed relatively better in poor environments. Axis-2 discriminated basically among environments belonging to cluster I or II. Particularly, N2A3 dominated this second axis (it showed the most negative Axis-2 score, -49.2), together with N1A3 (it showed the most positive score, 20.4). While both N2A3 and N1A3 showed a favourable crop water status prior to anthesis (R/ET_{o(1)}), they differed markedly with regard to the incidence of terminal water stress (R/ET_{o(2)}) (Table 1). This fact explains the differential behaviour, according to the biplot, of Orria and Plaisant: Orria seemed to support adequately the terminal drought of N2A3, and Plaisant behaved relatively better under the optimum water availability of N1A3 during grain filling.

Table 3. Classification of environments and characterization of groups according to the cluster analysis. Values are environment means within clusters. Standard errors of the means are shown between brackets.

Groups	Environments	R/ET _{0(n)}	R/ET ₀₍₂₎	GY	Gr m ²	TKW	Δ_t
Cluster I	L1A3, L2W3, L1W4,	0.558	0.402	2.42	10171	32.9	15.57
	L2A4, L2W4, N1W2,	(0.0516)	(0.0874)	(0.071)	(348.3)	(0.82)	(0.093)
	N2A2, N2W2, N1A3,						
	N2A4, N2W4						
Cluster II	L1W3, L2A3, N2A3,	0.551	0.356	3.06	11366	35.3	16.45
	N2W3, L1A4	(0.0770)	(0.0998)	(0.202)	(835.7)	(1.36)	(0.212)
Cluster III	L1A2, L1W2, N1A2,	0.815	0.572	5.16	16381	38.7	17.09
	N1W3, N1A4, N1W4	(0.0714)	(0.1039)	(0.201)	(958.6)	(1.18)	(0.113)

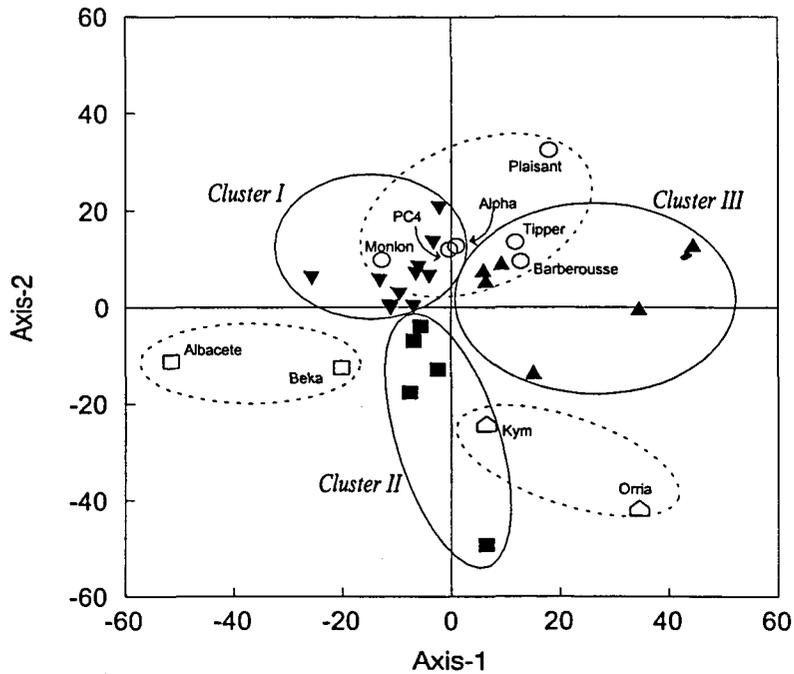


Figure 1. Biplot of the AMMI analysis for grain yield. Groups of environments from cluster analysis (clusters I, II and III) are shown by solid lines, and groups of genotypes by dashed lines. Genotypes/environments represented by different open/filled symbols according to the cluster classification.

3.5. Changes in genotypic ranking across clusters

Rank correlations for grain yield involving different groups of environments indicated a large differential genotypic performance across clusters (Table 4). This is especially observed after comparing genotypic means for grain yield of clusters I (the least productive on average) and III (the most productive on average): the rank correlation coefficient obtained was negative and close to significant ($p=0.06$). Overall, the winner genotype in cluster I (Albacete) was the poorest yielding one in cluster III, and showed an intermediate position in cluster II (data not shown). On the contrary, Orria and Plaisant, outstanding genotypes in cluster III, displayed the lowest yields in cluster I. Rank correlations for Δ indicated consistency of genotypic ranking

across clusters. For instance, Orria always exhibited the highest Δ values across clusters, whereas Beka had the lowest Δ values for clusters I and III.

Table 4. Spearman's rank correlation matrix between groups of environments classified according to the cluster analysis for grain yield. Calculations are based on genotypic means across environments for grain yield (above diagonal) and Δ (below diagonal). Values below the correlation coefficients represent $\text{Prob}>|R|$ under $H_0: \rho=0$, $n=10$.

	Cluster I	Cluster II	Cluster III
Cluster I	-	+0.152 (0.68)	-0.612 (0.06)
Cluster II	+0.806 (0.00)	-	-0.079 (0.83)
Cluster III	+0.927 (0.00)	+0.661 (0.04)	-

3.6. Relationships between grain yield, yield components and Δ

Relationships between grain yield, yield components and Δ were studied for genotypic means within groups of homogeneous environments according to the cluster analysis for grain yield. It was noticed, however, that simple correlations could be blurred by huge differences among genotypes varying in number of rows for most of these traits. Thus, the statistical contrast two *versus* six rows was found highly significant ($p < 0.01$) for Δ , kernel number m^{-2} and TKW (data not shown). Six-rowed genotypes showed consistently higher Δ values and kernel number m^{-2} , but lower TKW than two-rowed types. Partial correlations were then performed as a method for controlling for the ear-type effect. For completeness, such correlations have been obtained for both the AMMI2 estimates and the unadjusted means (Table 5).

Table 5. Partial correlation matrix, adjusted for number of rows, between grain yield, kernel number per square meter (KN m²), thousand kernel weight (TKW) and carbon isotope discrimination (Δ). Calculations are based on genotypic means across environments grouped according to the cluster analysis of AMMI2 estimates (above diagonal) or unadjusted means (below diagonal). Values below the correlation coefficients represent Prob>|R| under Ho:p=0, n=10.

	Cluster I				Cluster II				Cluster III			
	GY	KN m ²	TKW	Δ	GY	KN m ²	TKW	Δ	GY	KN m ²	TKW	Δ
GY	-	-0.325 (0.39)	+0.514 (0.16)	-0.594 (0.09)	-	+0.705 (0.03)	+0.561 (0.11)	+0.867 (0.00)	-	+0.495 (0.17)	-0.103 (0.80)	+0.611 (0.08)
KN m ²	-0.306 (0.42)	-	-0.811 (0.01)	+0.525 (0.15)	+0.814 (0.00)	-	-0.032 (0.94)	+0.691 (0.04)	+0.584 (0.10)	-	-0.853 (0.00)	+0.742 (0.02)
TKW	+0.616 (0.08)	-0.635 (0.07)	-	-0.278 (0.47)	+0.382 (0.31)	-0.061 (0.88)	-	+0.406 (0.28)	+0.003 (0.99)	-0.711 (0.03)	-	-0.630 (0.07)
Δ	-0.428 (0.25)	+0.634 (0.07)	-0.210 (0.59)	-	+0.891 (0.00)	+0.827 (0.01)	+0.301 (0.43)	-	+0.368 (0.33)	+0.419 (0.26)	-0.549 (0.13)	-

We will focus first on correlations based on the AMMI2 estimates. Grain yield correlated positively with Δ in cluster II and, to a lesser extent, in cluster III, and was clearly related to kernel number m^{-2} in cluster II. On the contrary, grain yield and Δ tended to be negatively related in cluster I. Kernel number m^{-2} and Δ were positively associated across clusters, whereas no consistent relation was observed between TKW and Δ . Overall, correlation coefficients based on unadjusted means corroborated the tendencies obtained for the AMMI2 estimates, but the latter detected more efficiently the association pattern of grain yield with Δ within clusters. This is especially seen for groups I and III, in which the correlations calculated for AMMI2 estimates were at the border of significance, being clearly not significant for unadjusted means.

4. DISCUSSION

Differences in water availability among locations, seasons and sowing dates originated an extreme range of responses for grain yield and Δ . In Mediterranean areas, precipitation and temperatures are associated with environmental mean grain yield (Yau *et al.*, 1991; Nachit *et al.*, 1992). In our study, $R/ETO_{(1)}$ and grain yield were strongly related across environments ($r=0.724^{**}$, $n=22$), which suggests that the productive potential for a given location and year depends on the plant water status before flowering. This observation coincides with the assumption that cereal yields are better related to kernel number m^{-2} than to individual kernel weight (Slafer *et al.*, 1996). On the other hand, variation in environmental mean Δ was better related to water availability from anthesis to maturity ($R/ETO_{(2)}$) ($r=0.584^{**}$, $n=22$). Several studies report that the water regime during grain filling strongly affects the Δ values of mature kernels (Condon *et al.*, 1992; Araus *et al.*, 1997).

$G \times E$ for grain yield and Δ were comparatively similar. However, the magnitude of the G term in the ANOVAs suggested that the genotypic Δ values were more consistent across environments than the genotypic yields (Table 2). This assumption agreed with the observation that different clusters ranked genotypic Δ values similarly (Table 4). In this context, Δ can be defined as a constitutive trait (Ceccarelli, 1989), its expression being more environment-independent than that of yield. This is in accordance with previous studies in wheat (Condon and Richards, 1992) and cowpea (Hall *et al.*, 1994), among others.

AMMI and cluster analysis provided more insight into G×E for grain yield. For instance, this and also other studies have shown that increasingly higher Axis-1 scores are related to higher environmental mean yields and, in turn, to better crop water availability during growth in Mediterranean environments (Nachit *et al.*, 1992; van Oosterom *et al.*, 1993). Environments with positive Axis-1 scores were grouped mainly in cluster III, which included only N1 and L1 locations, i.e., *a priori* non limiting environments. Poor yielding environments were classified in cluster II and, especially, in cluster I. Clusters I and III ranked genotypes for grain yield in a rather opposite way. Cluster II, showing an intermediate average yield, was unrelated to clusters I and III regarding genotypic ranking (Table 4). Old genotypes (Beka) and landraces (Albacete) dominated genotypic performance under drought (cluster I). Modern, widely adapted lines (Orria and Plaisant) led genotypic ranking under favourable, non limiting conditions (cluster III). This behaviour indicates existence of a crossover point in an intermediate productivity level, in agreement with other studies (Ceccarelli, 1989; Blum, 1996). Blum (1996) concludes that, below certain point, high yield potential and drought resistance become incompatible. Morphophysiological traits associated with high yield potential are no longer valid to ensure high yields in low-rainfall areas (Acevedo *et al.*, 1991). Thus, it has been claimed that breeding for low-yielding environments should be performed preferentially under stress, i.e., in trials below the crossover point (Ceccarelli and Grando, 1991).

Clustering of environments in such contrasting groups permitted to clarify the relationship between grain yield and Δ of mature kernels. Overall, a high Δ seemed detrimental for genotypes growing in poor yielding environments. However, it was of advantage in those environments of medium and high productivity. Similar results have been reported by Condon and Richards (1993) using vegetative tissues for Δ determinations in environments differing in water stress. On the contrary, our results are in opposition to those reported by Acevedo (1993), in which the most droughted trials showed the largest positive relationships between Δ and grain yield. This author suggests that variations in earliness brought about differences in productivity in the driest environments. In our study, however, anthesis date of genotypes and grain yield were mostly unrelated within environments (data not shown).

Several straightforward implications can be derived from the results obtained in this study: (i) in non limiting environments (cluster III), high Δ genotypes possess some yield advantage compared to low Δ ones, despite their lowered transpiration efficiency. Those locations included in cluster III (N1 and L1) were thought to be representative of stress-free, rainfed

Mediterranean areas; (ii) in medium-yielding environments (cluster II), high Δ genotypes are still superior. These genotypes may continue expressing their larger yield potential under not extremely severe environmental conditions; and (iii) in low-yielding environments (cluster I), and according to the expectation, a low Δ , i.e., a high TE seems beneficial to achieve higher yields. These results suggest indirectly the existence of a crossover point at below the average yield of cluster II (3 t ha^{-1}) which might be taken into account for definition of particular breeding strategies according to the target environment. In this study, however, unpredictable seasonal variation often originated that a given location (especially L2 or N2, but also L1) was classified either in cluster I or in cluster II (Table 3). In such conditions, the use of Δ as an indicator of grain yield may not be warranted due to year-to-year fluctuations in rainfall and temperatures, which may cause both traits to be either positively or negatively related, or just unrelated. If spatial variation dominates seasonal fluctuations, then homogeneous growing regions can be defined and appropriate genotypes can be targeted for each of these regions (Gauch and Zobel, 1997). Unfortunately, this does not seem to be the case in the Mediterranean region (Ceccarelli *et al.*, 1991).

What can cause grain yield and Δ to be related in such different ways? A possible explanation arises from the positive relationship found between Δ and kernel number m^{-2} within clusters (Table 5). It has been pointed out that a greater sink strength, as indicated by higher kernel number m^{-2} , may force the plant to increase the stomatal conductance to account for the increased demand for assimilates (Blum *et al.*, 1988; Richards, 1996b). Slafer *et al.* (1996) indicate that kernel number m^{-2} has been the yield component most largely modified by wheat breeding in the last decades. In addition, Muñoz *et al.* (1998) report a positive association between year of release and Δ of barley cultivars. It may be feasible that breeding for high yield potential could have resulted in an indirect downward genetic shift in TE (Muñoz *et al.*, 1998) through an increase in sink strength of modern genotypes. Blum (1996) points out that a large reproductive sink is antagonistic to drought resistance if water is limiting grain filling. Under these circumstances, grain yield can be often limited by source supply during grain filling, especially for those genotypes having a higher yield potential. For instance, a previous study (Voltas *et al.*, 1997) carried out with modern six-rowed genotypes (Barberousse, Orria and Plaisant), confirmed that some of the environments used here were strongly source-limited during grain filling (e.g., L2W3, cluster I). High-yielding genotypes, by having a large sink and, therefore, high constitutive Δ values, may be penalized under severe stress, i.e., where the yield level is well

below that of the crossover point, cluster I (Blum, 1996). Provided that all cultivars extract the same amount of available soil water, a high TE (i.e., a low Δ) seems a desirable trait for maximizing grain yield under such conditions. On the contrary, it has been suggested that a greater sink strength of the plant when water is not limiting crop growth critically is compensated by a higher photosynthetic capacity, i.e., an increased source strength, sustained by a concomitant increase in stomatal conductance (Richards, 1996b). Those plant keeping their stomata more open would also augment their transpiration flux, being able, in turn, to capture more water from the soil and to increase their total water use. Accordingly, Δ would augment and plants would be able to fill a larger number of grains without reduction in kernel weight. Therefore, when genotypes differ in their capacity to capture water from the soil and water is not restricting growth drastically, variation in total water use probably overrides that in transpiration efficiency. Such interpretation may apply to the environments of the present study classified in clusters II and III.

If the dependence of Δ on sink strength is confirmed, it may be preferable to breed for genotypes having a high yield potential through an increase in kernel number m^{-2} rather than an increase in Δ , its indirect correlated response (Richards, 1996b). On the other hand, this study has shown that a high TE may be a beneficial genotypic trait in low-rainfall areas. In breeding for such zones, Δ appears as a promising trait for maximizing TE in barley. It should be noted, however, that an extensive use of Δ for screening barley lines resistant to drought requires a proper definition of the target environment in which perform the selection. Delimitation of homogeneous agroclimatic regions seems a difficult challenge for breeders in Mediterranean areas. In this study, seasonal variation caused a given location to be classified in different groups of uniform environments. Accordingly, selection for Δ may have different consequences, with regard to grain yield, due to unpredictable environmental fluctuation from year to year. In order to warrant the suitability of Δ as indirect selection criterion for semiarid areas, high-TE genotypes must be selected in and targeted for locations usually experiencing average yields well below that of the crossover point, which in Mediterranean Spain seems to appear at a level of about $3 t ha^{-1}$.

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REFERENCES

Acevedo, E., Craufurd, P.Q., Austin, R.B., and Pérez-Marco, P., 1991. Traits associated with high yield in low-rainfall environments. *J. Agric. Sci. (Cambridge)* 116, 23-36.

Acevedo, E., 1993. Potential of carbon isotope discrimination as a selection criterion in barley breeding. In: Ehleringer, J.R., Hall, A.E., and Farquhar, G.D. (Eds.), *Stable Isotopes and Plant Carbon - Water Relations*. Academic Press, Inc., San Diego, CA, pp. 399-417.

Araus, J.L., Reynolds, M.P., and Acevedo, E., 1993. Leaf posture, grain yield, leaf structure, and carbon isotope discrimination in wheat. *Crop Sci.* 33, 1273-1279.

Araus, J.L., Febrero, A., Buxó, R., Rodríguez-Ariza, M.O., Molina, F., Camalich, M.D., Martín, D., and Voltas, J., 1997. Identification of ancient irrigation practices based on the carbon isotope discrimination of plant seeds: a case study from the south-east Iberian peninsula. *Journal of Archaeological Science* 24, 729-740.

Blum, A., Mayer, J., and Golan, G., 1988. The effect of grain number (sink size) on source activity and its water relations in wheat. *J. Exp. Bot.* 39, 106-114.

Blum, A., 1996. Yield potential and drought resistance: are they mutually exclusive. In: Reynolds, M.P., Rajaram, S., and McNab, A. (Eds.), *Increasing Yield Potential in Wheat: Breaking the Barriers*. CIMMYT, Mexico D.F., pp. 90-100.

Ceccarelli, S., 1989. Wide adaptation: How wide? *Euphytica* 40, 197-205.

Ceccarelli, S., Grando, S., 1991. Environment of selection and type of germplasm in barley for low-yielding conditions. *Euphytica* 57, 207-219.

Ceccarelli, S., Acevedo, E., and Grando, S., 1991. Breeding for yield stability in unpredictable environments: single traits, interaction between traits, and architecture of genotypes. *Euphytica* 56, 169-186.

Condon, A.G., and Richards, R.A., 1992. Broad sense heritability and genotype x environment interaction for carbon isotope discrimination in field-grown wheat. *Aust. J. Agric. Res.* 43, 921-934.

Condon, A.G., Richards, R.A., and Farquhar, G.D., 1992. The effect of variation in soil water availability, vapor pressure deficit and nitrogen nutrition on carbon isotope discrimination in wheat. *Aust. J. Agric. Res.* 43, 935-947.

- Condon, A.G., and Richards, R.A., 1993. Exploiting genetic variation in transpiration efficiency in wheat: an agronomic view. In: Ehleringer, J.R., Hall, A.E., and Farquhar, G.D. (Eds.), *Stable Isotopes and Plant Carbon - Water Relations*. Academic Press, Inc., San Diego, CA, pp. 435-450.
- Condon, A.G., Richards, R.A., and Farquhar, G.D., 1993. Relationships between carbon isotope discrimination, water use efficiency and transpiration efficiency for dryland wheat. *Aust. J. Agric. Res.* 44, 1693-1711.
- Craufurd, P.Q., Austin, R.B., Acevedo, E., and Hall, M.A., 1991. Carbon isotope discrimination and grain yield in barley. *Field Crops Res.* 27, 301-313.
- Crossa, J., Fox, P.N., Pfeiffer, W.H., Rajaram, S., and Gauch, H.G., 1991. AMMI adjustment for statistical analysis of an international wheat yield trial. *Theor. Appl. Genet.* 81, 27-37.
- Denis, J.B., and Gower, J.C., 1994. Biadditive models. Letter to the editor. *Biometrics* 50, 310-311.
- Ehdaie, B., Hall, A.E., Farquhar, G.D., Nguyen, H.T., and Waines, J.G., 1991. Water-use efficiency and carbon isotope discrimination in wheat. *Crop Sci.* 31, 1282-1288.
- Farquhar, G.D., and Richards, R.A., 1984. Isotopic composition of plant carbon correlates with water use efficiency of wheat genotypes. *Aust. J. Plant Physiol.* 11, 539-552.
- Farquhar, G.D., Ehleringer, J.R., and Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40, 503-537.
- Fox, P.N., and Roseille, A.A., 1982. Reducing the influence of environmental main effects in pattern analysis of plant breeding environments. *Euphytica* 31, 645-656.
- Gauch, H.G., and Zobel, R.W., 1988. Predictive and postdictive success of statistical analyses of yield trials. *Theor. Appl. Genet.* 76, 1-10.
- Gauch, H.G., and Zobel, R.W., 1989. Accuracy and selection success in yield trial analyses. *Theor. Appl. Genet.* 77, 473-481.
- Gauch, H.G., 1992. *Statistical analysis of regional yield trials*. Elsevier, Amsterdam.
- Gauch, H.G., and Zobel, R.W., 1997. Identifying mega-environments and targeting genotypes. *Crop Sci.* 37, 311-326.

- Gollob, H.F., 1968. A statistical model that combines features of factor analysis and analysis of variance techniques. *Psychometrika* 33, 73-115.
- Hall, A.E., Thiaw, S., and Krieg, D.R., 1994. Consistency of genotypic ranking for carbon isotope discrimination by cowpea grown in tropical and subtropical zones. *Field Crops Res.* 36, 125-131.
- Hubick, K.T., and Farquhar, G.D., 1989. Carbon isotope discrimination and the ratio of carbon gains to water lost in barley cultivars. *Plant, Cell and Environment* 12, 795-804.
- Muñoz, P., Voltas, J., Araus, J.L., Igartua, E., and Romagosa, I., 1998. Changes in adaptation of barley releases over time in northeastern Spain. *Plant Breeding* (in press).
- Nachit, M.M., Sorrells, S.E., Zobel, R.W., Gauch, H.G., Fischer, R.A., and Coffman, W.R., 1992. Association of environmental variables with sites' mean grain yield and components of genotype-environment interaction in durum wheat. II. *J. Genet. Breed.* 46, 50-55.
- Richards, R.A., and Condon, A.G., 1993. Challenges ahead in using carbon isotope discrimination in plant-breeding programs. In: Ehleringer, J.R., Hall, A.E., and Farquhar, G.D. (Eds.), *Stable Isotopes and Plant Carbon - Water Relations*. Academic Press, Inc., San Diego, CA, pp. 451-462.
- Richards, R.A., 1996a. Defining selection criteria to improve yield under drought. *Plant Growth Regulation* 20, 157-166.
- Richards, R.A. 1996b. Increasing the yield potential of wheat: manipulating sources and sinks. In: Reynolds, M.P., Rajaram, S., and McNab, A. (Eds.), *Increasing Yield Potential in Wheat: Breaking the Barriers*. CIMMYT. Mexico D.F., pp. 134-148.
- Romagosa, I., and Araus, J.L., 1991. Genotype-environment interaction for grain yield and ^{13}C discrimination in barley. *Barley Genetics* VI, 563-567.
- Romagosa, I., Ullrich, S.E., Han, F., and Hayes, P.M., 1996. Use of the additive main effects and multiplicative interaction model in QTL mapping for adaptation in barley. *Theor. Appl. Genet.* 93, 30-37.
- Royo, C. Rodríguez, A., and Romagosa, I., 1993. Adaptation of complete and substituted triticale to acid soils. *Plant Breeding* 111, 113-119.
- SAS Institute, 1987. *SAS/STAT Guide for Personal Computers, Version 6*. SAS Institute, Inc., Cary, NC.

Slafer, G.A., Calderini, D.F., and Miralles, D.J., 1996. Yield components and compensation in wheat: opportunities for further increasing yield potential. In: Reynolds, M.P., Rajaram, S., McNab, A. (Eds.), *Increasing Yield Potential in Wheat: Breaking the Barriers*. CIMMYT, Mexico D.F., pp. 101-133.

Stein, C., 1955. Inadmissibility of the usual estimator for the mean of a multivariate normal distribution. In: Proc. 3rd Berkeley Symp. Math. Stat. Prob., Vol. 1. Berkeley, CA. December 1954 and July and August 1955. Univ. of California Press, Berkeley, pp. 197-206.

van Eeuwijk, F.A., 1995. Multiplicative interaction in generalized linear models. *Biometrics* 51, 1017-1032.

van Oosterom, E.J., Kleijin, D., Ceccarelli, S. and Nachit, M.M., 1993. Genotype-by-environment interaction of barley in the Mediterranean region. *Crop Sci.* 33, 669-674.

Voltas, J., Romagosa, I. And Araus J.L., 1997. Grain size and nitrogen accumulation in sink-reduced barley under Mediterranean condition. *Field Crops Res.* 52, 117-126.

Voltas, J., Romagosa, I., Muñoz, P., and Araus, J.L., 1998. Mineral accumulation, carbon isotope discrimination and indirect selection for grain yield in two-rowed barley grown under semiarid conditions. *Eur. J. Agron.* (in press).

Williams, P.T., 1976. *Pattern analysis in agricultural science*. Elsevier, Amsterdam.

Yau, S.K., Ortiz-Ferrara, G., and Srivastava, J.P., 1991. Classification of bread wheat-growing environments based on differential yield responses. *Crop Sci.* 31, 571-576.

Zadocks, J.C., Chang, T.T., and Konzak, C.F., 1974. A decimal code for the growth stages of cereals. *Weed Res.* 14, 415-421.

CHAPTER VI



Mineral accumulation, carbon isotope discrimination and indirect selection for grain yield in two-rowed barley grown under semiarid conditions

ABSTRACT

Mineral accumulation in vegetative plant parts and in mature kernels has been proposed as an indirect selection criterion, either in addition to or in substitution of carbon isotope discrimination (Δ), to assess grain yield of temperate cereals in Mediterranean areas. However, the association between mineral concentration, in different plant parts, and grain yield is not yet fully understood. In order to study these relationships, four rainfed trials were established in northeastern Spain involving ten two-rowed barley (*Hordeum vulgare* L.) cultivars. Carbon isotope discrimination and total ash concentration were measured at maturity in kernels and straw. As expected, Δ of kernels (Δ -K) and straw (Δ -S) were positively correlated within environments. By contrast, Δ -K and ash concentration in kernels were often negatively related within environments, which suggests that mineral accumulation in kernels is not associated with the transpiration efficiency of the plants during grain filling. The lack of a positive relationship between ash concentration in the straw and either Δ -K or Δ -S indicates that ash concentration in vegetative tissues sampled at maturity may be of limited value as a surrogate of Δ . Grain yield correlated positively with either Δ -K or Δ -S, and negatively with ash concentration in kernels, especially in the poorest rainfed environments. However, ash concentration in the straw was not consistently associated with grain yield. While our results confirm that Δ traits are valid indicators of grain yield under Mediterranean conditions, the use of ash concentration in kernels for screening purposes is not warranted at this time due to the lack of a more accurate understanding of the physiological mechanisms underlying mineral accumulation in kernels. Prediction of grain yield through multiple linear regression has shown, however, that ash concentration in kernels could be used as a complementary criterion to Δ in poor rainfed environments.

1. INTRODUCTION

Carbon isotope discrimination against ^{13}C (Δ) measured in plant tissues has been proposed as a useful integrated indicator of transpiration efficiency (TE, ratio of net assimilation to water transpired) in C_3 plants (Farquhar and Richards, 1984; Hubick and Farquhar, 1989). Several studies corroborate existence of a negative relationship between Δ and TE (Ehdaie *et al.*, 1991; Condon *et al.*, 1993). High-TE genotypes accumulate more ^{13}C in their tissues, thus increasing the ratio of ^{13}C to ^{12}C and decreasing the plant discrimination against ^{13}C (Δ), compared with less efficient genotypes. Some interesting features of Δ when considering its application to screen germplasm in cereal breeding programs are: (1) a consistent relationship with TE across treatments (Condon *et al.*, 1990; Ehdaie *et al.*, 1991); (2) its high broad-sense heritability (Richards and Condon, 1993); (3) a relatively low genotype by environment interaction (Romagosa and Araus, 1991; Condon and Richards, 1992); and (4) the existence of a phenotypic correlation with grain yield (Condon *et al.*, 1987; Craufurd *et al.*, 1991). Theoretically, high-TE (i.e., low Δ) genotypes should have an advantage for higher yields under semiarid conditions, but several studies have reported positive relationships between Δ and grain yield (Condon *et al.*, 1987; Sayre *et al.*, 1995). This is feasible because in many cases, the increased TE may have been accompanied by a substantial reduction in total water use. Other possible explanations to such phenomenon are also discussed by Richards (1996).

Alternatively, Masle *et al.* (1992) have reported the existence of a positive linear relationship between total mineral concentration in vegetative tissues and either the transpiration ratio ($1/\text{TE}$) or Δ . Therefore, mineral accumulation in plant parts could be used instead of Δ because of the high cost involved in isotope analysis. Although the physiological basis of the relationship between Δ and TE is well established, the mechanisms involved in the relationship found between mineral accumulation in vegetative parts, Δ and TE remain to be fully elucidated. The passive mineral transport driven by transpiration would be an important factor responsible for such association (Masle *et al.*, 1992; Mayland *et al.*, 1993). Low-TE (i.e., high Δ) genotypes may maintain a higher stomatal conductance and sustain, in turn, a higher transpiration (Richards, 1996). Such genotypes may, therefore, accumulate more minerals in their tissues than high-TE genotypes. Nevertheless, other factors different from a passive mineral transport might also be involved, since the relation between Δ and mineral concentration has been found to differ under varying environmental conditions, even disappearing in severe droughted trials (Masle *et al.*,

1992; Mayland *et al.*, 1993). The relationship between mineral accumulation and TE improves when using total ash concentration instead of any mineral alone like silicon or potassium (Masle *et al.*, 1992; Mayland *et al.*, 1993).

Furthermore, Febrero *et al.* (1994) proposed the total ash concentration, not in green tissues but in mature kernels, as a complementary criterion (in addition to Δ) to assess genotypic differences in grain yield of barley grown in rainfed Mediterranean environments. These authors reported a negative association between yield and ash concentration in kernels only under drought conditions. A first possible explanation to such negative association might be a pure dilution effect: in water-stressed plants, ash concentration in mature kernels would be negatively related with kernel weight on a dry-weight basis. However, other factors could also be involved: for instance, ash concentration in mature kernels could indicate the importance of retranslocation processes during grain filling (Febrero *et al.*, 1994).

The first objective of this study was to clarify the relationship between Δ and ash concentration measured in vegetative tissues (hereafter referred to as straw, a mixture of stems, leaf blades and sheaths) and kernels, both taken at maturity, in a set of 10 two-rowed barley cultivars grown in four rainfed Mediterranean environments. Further, the association between these traits and grain yield, as well as with kernel weight and anthesis date of cultivars, were evaluated to elucidate their usefulness in breeding programs.

2. MATERIALS AND METHODS

2.1. Plant material and experimental design

Ten genetically diverse two-rowed barley genotypes, differing in sensibility to photoperiod and vernalization, and also in adaptation to poor rainfed environments, were used in this study: Albada, Alexis, Alpha, Beka, Cameo, Iranis, Kym, Mogador, Tipper, and Zaida. All genotypes were tested in four rainfed trials carried out in northeastern Spain involving two contrasting locations: Artesa de Segre and Gimènells (Table 1). The experimental design in each trial was a randomized complete block with four replications. Plots consisted of six 6-m rows, spaced 20 cm, and seeded at rates of approximately 350 viable seeds m^{-2} . Plots were provided with 50 kg N ha^{-1} , 20 kg P ha^{-1} , and 35 kg K ha^{-1} at sowing, and 60 kg N ha^{-1} at the jointing stage.

2.2. Grain yield and sampling of plant tissue

The grain yield and thousand kernel weight (TKW) of each trial were recorded at maturity. Before harvest, separate samples of kernel and straw (including leaf blades, sheaths and stems) were collected from one 1-m row segment at each plot. Samples of the same genotype obtained from each of the four blocks of a particular trial were subsequently bulked to provide a balanced mixture of plant material (kernel or straw). The anthesis date (AD) was also recorded for each of the 10 genotypes in each trial.

2.3. Carbon isotope analysis

Straw and kernel samples were oven-dried and ground to a fine powder. $^{13}\text{C}/^{12}\text{C}$ ratios were determined by mass spectrometry (Isotope Services, Inc. Los Alamos, NM 87544, USA). Results were expressed as $\delta^{13}\text{C}$ values (Farquhar *et al.*, 1989), where:

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

and R is the $^{13}\text{C}/^{12}\text{C}$ ratio. The standard for comparison was a secondary standard calibration against Pee Dee Belemnite (PBD) carbonate. Sample sizes of 5-10 mg were used, and precision of analysis was less than 0.10 ‰. Carbon isotope discrimination (Δ) was then calculated in straw ($\Delta\text{-S}$) and kernels ($\Delta\text{-K}$) from δ_a and δ_p , where *a* and *p* refer to air and plant, respectively (Farquhar *et al.*, 1989):

$$\Delta = (\delta_a - \delta_p) / (1 + \delta_p)$$

The value of $\delta^{13}\text{C}$ for the air was assumed to be -8.00 ‰ (Farquhar and Richards, 1984).

2.4. Ash concentration

The ash concentration was determined in ground samples of straw and mature kernels, as made for carbon isotope analyses. Approximately 1.5 g of dry matter (either straw or kernels) was burnt in a furnace at 450°C for 10 h. At least two replicates were obtained per sample. The ash concentration was expressed as milligrams per gram of sample dry weight.

Table 1. Trial characterization and climatic data.

Location	Coordinates	Soil type	Trial code	Sowing date	Anthesis date ¹	Rainfall (vegetative period) (mm)	T _{max}	T _{mean}	T _{min}	R (mm)
Artesa de Segre	42°11'N, 0°32'E	Fluventic Xerochrept	A93	19/11/92	3/5/93	182.4	23.1	16.6	10.1	89.0
Gimenells	41°36'N, 0°13'E	Xerollic Paleorthid	G90	10/1/90	18/4/90	89.6	25.6	18.7	11.9	70.8
			G92	8/11/91	21/4/92	145.4	24.8	18.4	12.1	122.3
			G93	20/11/92	19/4/93	138.2	23.9	17.2	10.5	102.2

¹Earliest flowering genotype

2.5. Statistical analyses

Two-way analysis of variance was used to determine genotypic (G) and environmental (E) effects in the parameters measured, as well as the presence of GxE interaction for grain yield and TKW. In those parameters related to Δ and ash concentration, in which only one bulked sample per environment was determined, GxE was used as experimental error to test for significance of the main effects. Broad-sense heritabilities and their standard errors were determined by estimation of variance components (Hallauer and Miranda, 1981) using the VARCOMP procedure (SAS Institute, 1987).

3. RESULTS

Grain yield varied significantly among environments between 3.18 t ha⁻¹ (A93) and 6.24 t ha⁻¹ (G92) (Table 2). There were also highly significant differences in thousand kernel weight (TKW), which varied between 35.80 g (A93) and 50.99 g (G92). The mean ash concentration and carbon isotope discrimination (Δ) values differed significantly according to the growing environment (Table 2). The largest differences were found in ash concentration in the straw, which increased by 52% from A93 to G90. The ash concentration in the kernels was 12% higher in G93 than in A93, whereas the mean Δ of straw and kernels increased in absolute terms by 0.85 ‰ and 2.09 ‰ from G90 to G92. Significant genotypic differences ($p < 0.01$) were detected in grain yield and TKW, as well as in Δ and ash concentration in both kernels and straw (ANOVAs not shown). GxE interaction was also present for grain yield and TKW, but it could not be evaluated for Δ and ash concentration since, as mentioned above, only one sample per genotype and environment was analysed.

Table 2. Mean values of grain yield (t ha^{-1}), thousand kernel weight (TKW, g), ash concentration (mg g^{-1} dry weight), and carbon isotope discrimination (Δ , ‰) of straw and kernels sampled at maturity for each of four environments involving 10 barley cultivars.

	Environments				SE ¹
	A93	G90	G92	G93	
Grain yield	3.18	4.24	6.24	3.30	0.099
TKW	35.80	41.46	50.99	45.21	0.883
Straw ash	59.33	90.19	80.18	74.24	0.393
Kernel ash	20.62	20.57	21.30	23.03	0.101
Δ straw	18.03	17.67	18.52	18.07	0.136
Δ kernel	17.00	15.68	17.77	16.91	0.117

¹Standard error of the means

To check whether the variation in Δ and ash concentration among genotypes was due to differences in phenology, leading to different growing conditions, the relationship between anthesis date (AD) of each genotype and these parameters was initially studied (Table 3). Since the correlation coefficients obtained were not significant, it could be concluded that the genotypic differences in Δ and ash concentration were not driven mainly by differences in phenology. The relationship between ash concentration in kernels and TKW was also inferred to assess whether mineral accumulation was independent of kernel size. Although negative, correlations were not significant (Table 3). In fact, after correcting for the covariant effect of TKW on ash concentration in kernels, there were still significant differences among genotypes in this trait (data not shown).

In general, the relationship between ash concentration in kernels and in the straw tended to be positive within environments, but it was only significant in one case (A93) (Table 3). The correlation coefficients obtained between Δ measured in kernels (Δ -K) and straw (Δ -S) were also positive and often significant within environments (Table 3). In fact, the correlation found for genotypic means was highly significant (Table 3). Δ -K tended to be negatively related with both ash concentration in kernels and in the straw, and such a relationship was often significant (Fig. 1). A similar trend was observed between Δ -S and either ash concentration in the straw or in kernels, with negative and sometimes significant correlation coefficients (Fig. 1).

As expected, the broad-sense heritability ($H^2 \pm SE$) for grain yield showed the lowest value of the parameters evaluated (0.601 ± 0.0909), probably due to the presence of GxE, which partially concealed genotypic effects. The H^2 estimate for TKW was slightly higher (0.653 ± 0.0910). The highest H^2 of the four traits studied based on Δ and mineral accumulation was for ash concentration in kernels (0.785 ± 0.0908), and the lowest H^2 estimate for Δ -K (0.693 ± 0.0907). Other H^2 values obtained were 0.740 ± 0.0908 (ash concentration in the straw), and 0.742 ± 0.0917 (Δ -S).

For each environment, correlations between grain yield and the traits evaluated based on Δ and ash concentration are shown in Table 4. Δ -K and Δ -S correlated positively with grain yield, such relationships always being significant or almost significant. The correlations calculated based on genotypic means were also highly significant for Δ traits. The ash concentration in kernels was negatively related with grain yield in all environments, and the relationship was significant at A93 and G90, and also for genotypic means across environments. Conversely, the relationship between ash concentration in the straw and grain yield did not show a consistent trend among environments, being negative and significant only in the poorest yielding environment (A93). In the best yielding environments (G90 and G92), correlations with yield changed based on the trait evaluated: whereas the ash concentration in kernels showed negative relationships with yield, the ash concentration in the straw tended to be positively, although not significantly, correlated with yield. Correlations of TKW and AD date with yield were also calculated to ascertain to which degree these parameters could predict yield compared with traits based on Δ and ash concentration (Table 4). TKW was found to be unrelated to grain yield within environments. Similarly, differences in AD among cultivars were not related to grain yield at any of the four environments studied.

Multiple linear regressions were used to assess the combined effect of Δ (either straw or kernel) and ash concentration in kernels to predict grain yield (Table 5). Together, they explained 35.8-78.3% (by using Δ -K) and 30.2-75.3% (by using Δ -S) of the total variability for grain yield. In the least favoured environments (i.e., the poorest yielding environment, A93, and the most water stressed environment, G90), the ash concentration in kernels contributed the most to the accounted variation in grain yield, whereas in others (G92 and G93), Δ was more important in explaining grain yield. Overall, regressions based on genotypic means across environments showed that ash concentration in kernels added little information after Δ in accounting for grain yield variation.

Table 3. Correlation coefficients of different relationships between ash concentration in straw (Ash-S), ash concentration in kernels (Ash-K), carbon isotope discrimination in straw (Δ -S), carbon isotope discrimination in kernels (Δ -K), thousand kernel weight (TKW), and anthesis date of each genotype referred to the earliest flowering genotype (AD). Correlations were calculated for 10 two-rowed barley cultivars at each of four environments and for genotypic means over environments (*df*=8).

Environment	AD/Ash-K	AD/Ash-S	AD/ Δ -K	AD/ Δ -S	TKW/Ash-K	Ash-K/Ash-S	Δ -K/ Δ -S
A93	0.284	0.535	-0.497	-0.435	-0.391	0.670*	0.746*
G90	-0.134	0.206	-0.388	0.206	-0.618	-0.361	0.485
G92	-0.016	0.261	0.241	-0.271	-0.006	0.215	0.396
G93	-0.107	0.087	-0.195	-0.015	-0.387	0.423	0.802**
Genotype means	0.014	0.358	-0.267	-0.134	-0.205	0.215	0.783**

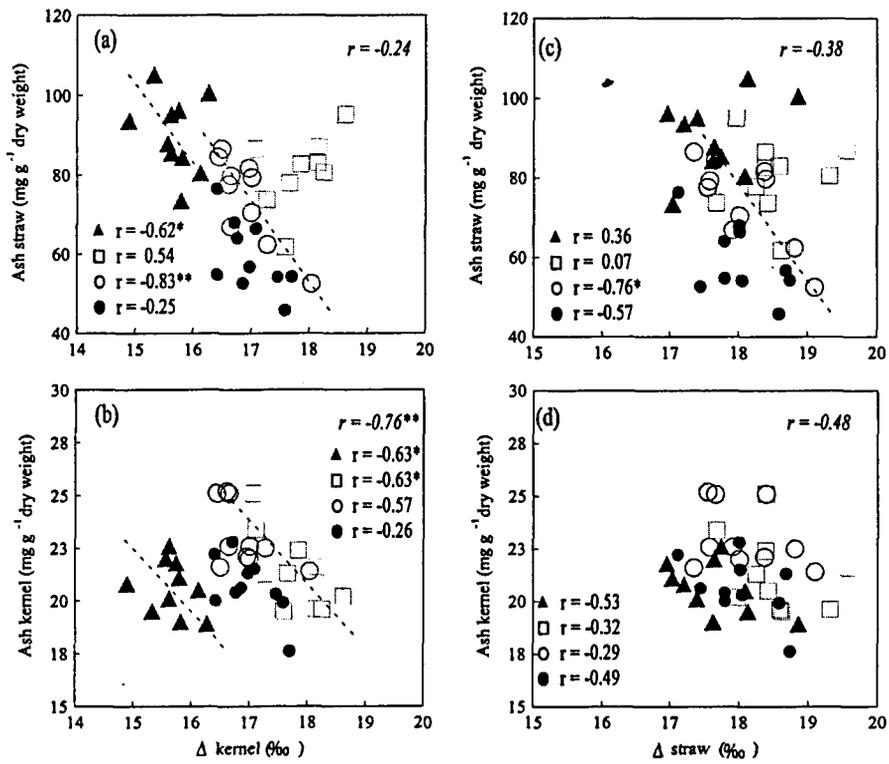


Figure 1. Relationship between Δ measured either in kernels (a, b, left figures) or in the straw (c, d, right figures), and the ash concentration in the straw (a, c, upper figures), or in kernels (b, d, lower figures), for 10 barley cultivars. Genotypes were grown in four different environments: A93 (\bullet), G90 (\blacktriangle), G92 (\square), and G93 (\circ). For a given environment, each point represents an individual genotype. Correlation coefficients based on genotypic means shown in italics.

Table 4. Correlation coefficients of the relationship between grain yield and the following traits: ash concentration in straw (Ash-S), ash concentration in kernels (Ash-K), carbon isotope discrimination in straw (Δ -S), carbon isotope discrimination in kernels (Δ -K), thousand kernel weight (TKW), and anthesis date of each genotype referred to the earliest flowering genotype (AD). Correlations calculated for 10 two-rowed barley cultivars at each of four environments and for genotypic means over environments ($df=8$).

Parameter	A93	G90	G92	G93	Genotype means
Ash-S	-0.670*	0.529	0.231	-0.244	0.030
Ash-K	-0.861**	-0.708*	-0.349	-0.387	-0.629*
Δ -S	0.460	0.759**	0.776**	0.486	0.763**
Δ -K	0.692*	0.473	0.587	0.678*	0.840**
TKW	0.118	0.494	-0.295	0.340	-0.233
AD	-0.116	0.278	0.104	-0.290	0.025

Table 5. Percentage of grain yield variation (r^2) among genotypes explained by Δ traits (Δ -K/ Δ -S), and in combination with ash concentration in kernels (Ash-K). Calculations were performed independently for each environment and for genotypic means over environments by fitting grain yield to simple linear (for one independent variable, $df=8$) and multiple linear (for two independent variables, $z = ax + by + c$, $df=7$) equations.

	A93	G90	G92	G93	Genotype means
Δ -K	47.9	22.4	34.5	46	71.6
Δ -K + Ash-K	78.3	59.3	35.8	47.4	72.1
Δ -S	21.1	57.6	60.2	23.6	58.2
Δ -S + Ash-K	75.3	71.2	61.5	30.2	66.3

4. DISCUSSION

A positive relationship between Δ traits and ash concentration in vegetative tissues, which has been reported in the literature (Walker and Lance, 1991; Masle *et al.*, 1992), has not been observed in this study for barley. On the contrary, ash concentration in the straw was often negatively related with Δ -K and Δ -S within environments. Differences in ontogeny of the vegetative tissue examined in this compared with other works could be a relevant factor influencing such a relationship. Sampling the whole straw instead of just a given plant part (e.g. flag leaf) could be an additional factor also involved. Thus, most studies used plant parts taken before jointing (e.g. Masle *et al.*, 1992) or prior to anthesis (e.g. Walker and Lance, 1991; Masle *et al.*, 1992), but never after physiological maturity. Sampling at maturity a priori includes interesting features for screening purposes, especially ease of collection and processing, and homogeneity in the developmental stage among genotypes. However, a possible limitation concerning the sampling of vegetative tissue at the end of the growing cycle could be the previous reallocation of part of leaf constituents to the reproductive sink (Loehwing, 1951; Hill, 1980; Noodén, 1980). The existence of a positive correlation between ash concentration in the straw and Δ traits would indicate that some cultivars maintain higher stomatal conductances and transpiration rates either during the vegetative period of development (as would be suggested by higher Δ -S) or during grain filling (by higher Δ -K), thus accumulating more minerals in vegetative tissues. Conversely, the negative relationship often observed in this study between ash concentration in the straw and Δ -K within environments suggests that a passive transport of nutrients through the transpirative stream was not the only factor that determined mineral concentration in vegetative tissues at the end of the crop cycle. As suggested above, a fraction of accumulated minerals is downloaded to the phloem during the later stages of kernel development (i.e., after the onset of senescence) to feed the reproductive organs (Wardlaw, 1990).

Although, in this study, the ash concentration in kernels and the ash concentration in the straw tended to be positively correlated within environments, minerals accumulate in kernels and vegetative tissues in different ways. Minerals are primarily transported and deposited in vegetative parts through the transpirative stream (via xylem), but they enter the growing kernels through phloem transport (Duffus and Cochrane, 1992). Reallocation of nutrients to the reproductive organs after the onset of senescence may have determined the relationship between both traits. For instance, those genotypes exhibiting a higher mineral concentration in vegetative

parts would be able to reallocate more minerals to growing kernels. The lack of any positive relationship between ash concentration in kernels and Δ traits suggests that the former may be not related to the integrated TE of the plant during grain filling. In fact, ash concentration in kernels and Δ -K (or Δ -S) were often negatively (and significantly) related within environments. Since photosynthesis is more drought-restricted than translocation (Loss and Siddique, 1994), those genotypes unable to maintain high rates of stomatal conductance and photosynthesis during grain filling (i.e., with a lowered Δ -K) would fill their kernels through retranslocation of photoassimilates from pre-anthesis reserves, and of minerals from early-senescent vegetative tissues. The proportion of minerals to carbohydrates in kernels would rise in those cultivars showing a constitutively lower Δ because of their lower photosynthetic rates during grain filling. This explanation may apply to G90, the driest and hottest of the four environments during grain filling (as also suggested by its lowest mean Δ -K value, Table 2), in which the ash concentration in kernels and Δ -K were found to be negatively correlated ($r=-0.63^*$).

The variation in Δ and/or ash concentration found among environments was unrelated to the corresponding fluctuation in overall productivity (Table 2). This could be due to the limited set of environments used in this study. The accumulated rainfalls during the whole growing period were, respectively, 161 mm, 268 mm, and 240 mm in G90, G92 and G93, and 271 mm in A93. Such a variation closely follows that found in Δ among environments (the more water available, the higher the Δ). Whereas a variation in mean Δ among environments probably reflects differences in water availability, mean grain yield and accumulated rainfall were not positively related among environments since the low yields achieved in A93 could be caused by a shallow soil rather than by a deficient crop water status. The variation found in ash concentration in kernels among environments was not explained either based upon water availability. For instance, the low ash concentration of the straw found in A93 could also reflect a limited soil fertility or nutrient availability (Table 2).

The identification of traits most likely to improve yield in water-limited environments is currently conducted under the framework proposed by Passioura (1977). Thus, grain yield is explained as the product of three components: water use by the crop, water use efficiency (WUE), and harvest index. Transpiration efficiency (TE), a physiological measure of WUE at the plant level, is one of the possible traits associated with an increase in yield under drought. The positive relationship between grain yield and either Δ -K or Δ -S observed in this study within environments is well documented elsewhere for barley (Romagosa and Araus, 1991) and wheat



(Condon *et al.*, 1987; Araus *et al.*, 1993), under both irrigation and drought conditions. At first, such a positive relationship may seem contradictory. It is generally accepted, however, that plant production in water-limited environments depends not only on high TE (as would be indicated by a low Δ), but mostly on the genotypic capacity to sustain transpiration (Blum, 1993). Hence, genotypes exhibiting a low stomatal conductance may have limited their final yield because of the negative effect on photosynthetic activity and the inability to use the available water (Morgan *et al.*, 1993). The decrease in mean Δ values from vegetative tissue (Δ -S) to mature kernels (Δ -K) in all environments suggests that genotypes suffered water limitation during grain filling. Nevertheless, both Δ -S and Δ -K predicted grain yield similarly within environments. Under the influence of severe terminal drought, not present in this study, Δ -K rather than Δ -S could provide better information for assessing genotypic behaviour to drought during grain filling.

Ash concentration in kernels was negatively correlated with grain yield within environments. Such a relationship was significant in the poorest yielding (A93) and the most water-stressed (G90) environments, but it disappeared under the higher yielding conditions (far from drought stress) found in G92. This is in agreement with a previous study in barley (Febrero *et al.*, 1994), in which only the rainfed environment showed a negative correlation with grain yield. As mentioned above for the relationship between mineral accumulation in kernels and Δ traits, the differential genotypic sensibility of photosynthesis and translocation to drought may lead to a negative relation between ash concentration in kernels and grain yield. On the other hand, the ash concentration in the straw was not consistently related with grain yield within environments. The reallocation of nutrients to growing kernels probably reduces the value of mineral accumulation in vegetative tissues, taken at the end of the crop period, for screening purposes.

Globally, our results confirm that Δ traits are valid indicators of grain yield under Mediterranean conditions. Hence, Δ can be used for defining desirable genotypic attributes determining TE in water-limited environments. The adoption of Δ in breeding programs may be limited by the high cost involved in isotope analysis, but it can be easily applied as a complementary criterion for parental selection. Richards (1996), however, points out that the cost of Δ is not excessive if compared with other techniques such as the use of DNA markers or even the establishment of field plots. Therefore, indirect selection for Δ may be effective also for screening purposes aimed at reducing a large number of entries during early generations of breeding programs, prior to an extensive multilocation testing for grain yield. On the contrary,

the use of ash concentration in kernels for screening purposes, either alone or in addition to Δ , is not warranted at this time due to the lack of a more accurate understanding of the physiological mechanisms underlying the accumulation of minerals in kernels. The addition of explanatory traits predicting grain yield through multiple linear regression has shown, however, that the ash concentration in kernels could be used as a complementary criterion to Δ in poor rainfed environments. Its high broad-sense heritability estimate suggests that the genotypic expression of this trait is equal or even less affected by the environmental conditions than that of Δ . Extended studies are currently underway to ascertain the physiological basis of the association between mineral accumulation in kernels and productivity.

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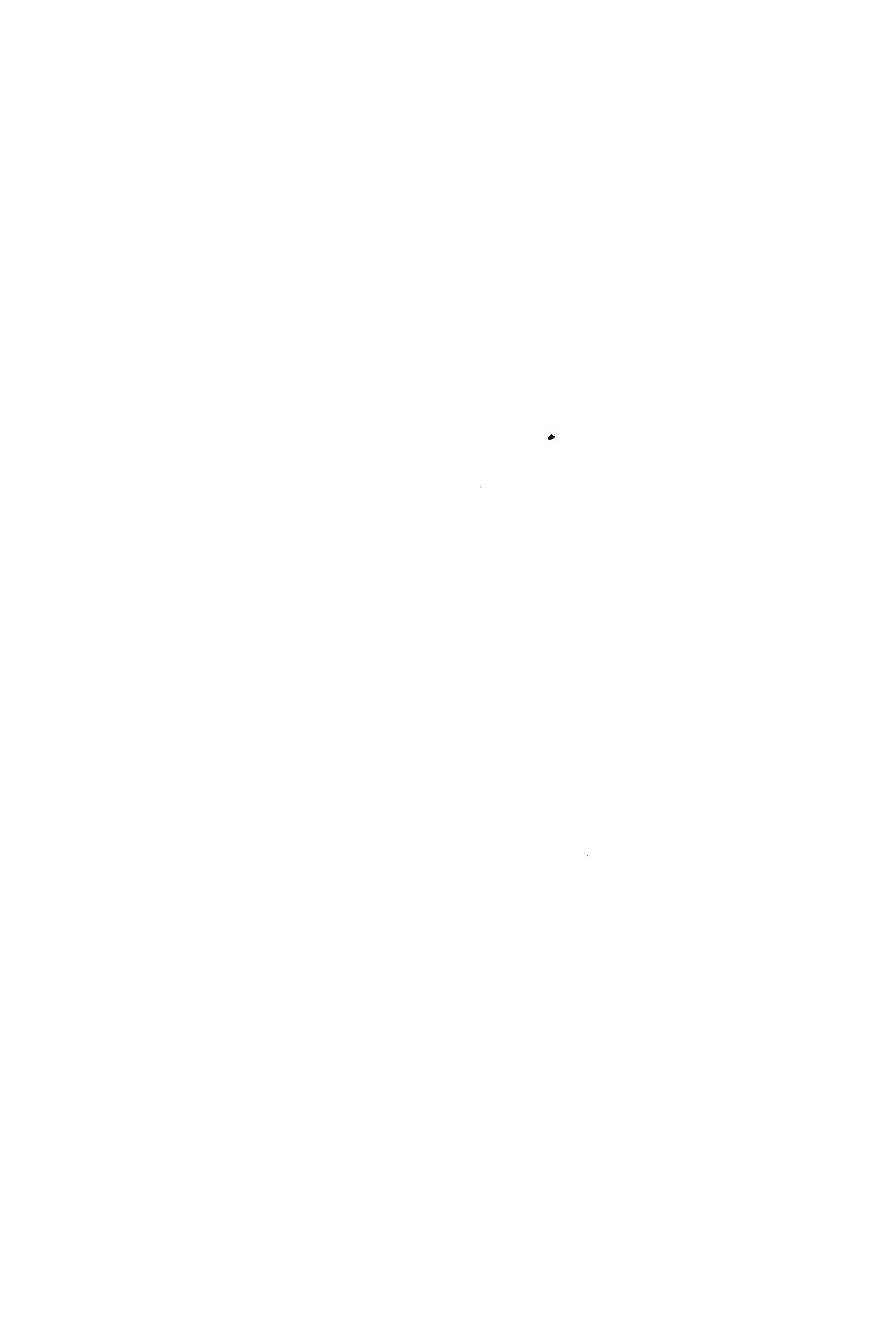
REFERENCES

- Araus, J.L., Reynolds, M.P., and Acevedo, E., 1993. Leaf posture, grain yield, leaf structure, and carbon isotope discrimination in wheat. *Crop Sci.* 33, 1273-1279.
- Blum, A., 1993. Yield potential and drought resistance: are they mutually exclusive? In Reynolds, M.P., Rajaram, S., McNab, A. (Eds), *Increasing yield potential in wheat: breaking the barriers*. CSSA, Madison, pp. 343-347.
- Condon, A.G., Richards, R.A., and Farquhar, G.D., 1987. Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat. *Crop Sci.* 27, 996-1001.
- Condon, A.G., Farquhar, G.D., and Richards, R.A., 1990. Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat. Leaf gas exchange and whole plant studies. *Aust. J. Plant Physiol.* 17, 9-22.
- Condon, A.G., and Richards, R.A., 1992. Broad sense heritability and genotype x environment interaction for carbon isotope discrimination in field-grown wheat. *Aust. J. Agric. Res.* 43, 921-934.
- Condon, A.G., Richards, R.A., and Farquhar, G.D., 1993. Relationships between carbon isotope discrimination,

- water use efficiency and transpiration efficiency for dryland wheat. *Aust. J. Agric. Res.* 44, 1693-1711.
- Craufurd, P.Q., Austin, R.B., Acevedo, E., and Hall, M.A., 1991. Carbon isotope discrimination and grain yield in barley. *Field Crops Res.* 27, 301-313.
- Duffus, C.M., and Cochrane, M.P., 1992. Grain structure and composition. In: Shewry, P.R. (Ed), *Barley: Genetics, biochemistry, molecular biology and biotechnology*. CAB International, Wallingford, UK, pp. 291-317.
- Ehdaie, B., Hall, A.E., Farquhar, G.D., Nguyen, H.T., and Waines, J.G., 1991. Water-use efficiency and carbon isotope discrimination in wheat. *Crop Sci.* 31, 1282-1288.
- Farquhar, G.D., and Richards, R.A., 1984. Isotopic composition of plant carbon correlates with water use efficiency of wheat genotypes. *Aust. J. Plant Physiol.* 11, 539-552.
- Farquhar, G.D., Ehleringer, J.R., and Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40, 503-537.
- Febrero, A., Bort, J., Català, J., Marzabal, P., Voltas, J., and Araus, J.L., 1994. Grain yield, carbon isotope discrimination and mineral content in mature kernels of barley under irrigated and rainfed conditions. *Agronomie* 2, 127-132.
- Hallauer, A.R., and Miranda, J.B., 1981. *Quantitative genetics in maize breeding*. Iowa State Univ. Press. Ames.
- Hill, J., 1980. The remobilization of nutrients from leaves. *Journal of Plant Nutrition* 2, 407-444.
- Hubick, K.T., and Farquhar, G.D., 1989. Carbon isotope discrimination and the ratio of carbon gains to water lost in barley cultivars. *Plant, Cell and Environment* 12, 795-804.
- Loehwing, F.W., 1951. Mineral nutrition in relation to the ontogeny of plants. In: Troug, E. (Ed), *Mineral nutrition of plants*. University of Wisconsin Press, Madison, pp. 343-358.
- Loss, S.P., and Siddique, K.H.M., 1994. Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. *Advances in Agronomy* 52, 229-277.
- Masle, J., Farquhar, G.D., and Wong, S.C., 1992. Transpiration ratio and plant mineral content are related among genotypes of a range of species. *Aust. J. Plant. Physiol* 19, 709-721.
- Mayland, H.F., Johnson, D.A., Asay, K.H., and Read, J.J., 1993. Ash, carbon isotope discrimination and silicon as

- estimators of transpiration efficiency in crested wheatgrass. *Aust. J. Plant. Physiol* 20, 361-369.
- Morgan, J.A., Lecain, D.R., McCaig, T.N., and Quick, J.S., 1993. Gas exchange, carbon isotope discrimination, and productivity in winter wheat. *Crop Sci.* 33, 178-186.
- Noodén, L.D., 1980. Senescence in the whole plant. In: Thimann, K.V. (Ed), *Senescence in plants*. CRC Pres, Inc., Boca Raton, FL., pp. 219-58.
- Passioura, J.B., 1977. Grain yield, harvest index and water use of wheat. *J. Aust. Inst. Agric. Sci.* 43, 117-120.
- Richards, R.A. and Condon, A.G., 1993. Challengers ahead in using carbon isotope discrimination in plant breeding programs. In: Ehleringer, J.R., Hall A.E., Farquhar, G.D. (Eds.), *Stable Isotopes and Plant Carbon-Water Relations*. Academic Press, New York, pp. 451-462.
- Richards, R.A., 1996. Defining selection criteria to improve yield under drought. *Plant Growth Regulation* 20: 157-166.
- Romagosa, I., and Araus, J.L., 1991. Genotype-environment interaction for grain yield and ¹³C discrimination in barley. *Barley Genetics VI*: 563-567.
- SAS Institute, 1987. *SAS/STAT Guide for Personal Computers, Version 6*. SAS Institute, Inc., Cary, NC.
- Sayre, K.D., Acevedo, E., and Austin, R.B., 1995. Carbon isotope discrimination and grain yield of three bread wheat germplasm groups grown at different levels of water stress. *Field Crops Res.* 41: 45-54.
- Walker, C.D., and Lance, R.C.M., 1991. Silicon accumulation and ¹³C composition as indices of water-use efficiency in barley cultivars. *Aust. J. Plant. Physiol.* 18, 427-434.
- Wardlaw, I.F., 1990. *The control of carbon partitioning in plants*. *New Phytol.* 116, 341-381.

GENERAL DISCUSSION



1. DROUGHT AND PRODUCTIVITY

Barley growth is usually limited in Mediterranean environments by the effect of various abiotic constraints, basically inadequate rainfall and solar radiation, and high temperature (Loss and Siddique, 1994). Rainfall uses to be abundant during winter, but becomes less frequent or even disappears during spring. Solar radiation may limit growth in spring when the lower canopy of the crop becomes shaded by the upper leaves. High temperature at the end of the growing cycle increases respiration losses while maintaining assimilation rates below a certain level. In all cases crop development is sub-optimal. Water availability for growth is in close association with the amount of rainfall from sowing to maturity, but the temperature and solar radiation regimes have a large influence on crop evapotranspiration. The joint effect of inadequate rainfall, high temperatures and intense solar radiation generates drought, a critical water limitation to growth and the most serious factor curtailing grain yield in rainfed Mediterranean environments.

Along this thesis the incidence of drought on plant growth has habitually been characterized by the proportion of rainfall to total evapotranspirative demand during particular stages of crop development. This ratio summarizes the simultaneous effect of the above-mentioned constraints to growth and can be used to determine the magnitude and timing of water limitation to crop productivity in Mediterranean environments. To that end, grain yield can be divided into two major numerical components, grain number m^{-2} and individual grain weight. Both components are determined with minimum overlapping in time and, therefore, can be considered as being largely independent (Slafer *et al.*, 1996). We can attempt to relate these components, as well as the overall productivity, with the water deficit experienced by the crop in specific periods of the crop cycle. An application to the set of 22 environments used in Chapter V is presented (Fig. 1). The most striking result is that differences in overall productivity among environments can be attributed, to a very large extent (52.4%), to concomitant differences in water availability for growth from sowing to anthesis (Fig. 1a). If we consider that contrasting sites were used in this study not only with regard to the incidence of abiotic stresses, but also concerning soil type and management, it can be concluded that year-to-year variations in productivity for a particular location must be essentially driven by changes in water availability from sowing to anthesis (Austin *et al.*, 1998). A convenient water availability in such a period translates, in turn, into a high number of grains m^{-2} (Fig. 1c). It has often been reported that grain yield relates better to grain number m^{-2} than to individual grain weight (e.g. Slafer and Andrade,

1989), and this finding is also confirmed here: the relationship between grain number m^{-2} and productivity is considerably higher ($r=0.918^{**}$, $n=22$) than that between thousand kernel weight (TKW) and productivity ($r=0.586^{**}$, $n=22$) across environments.

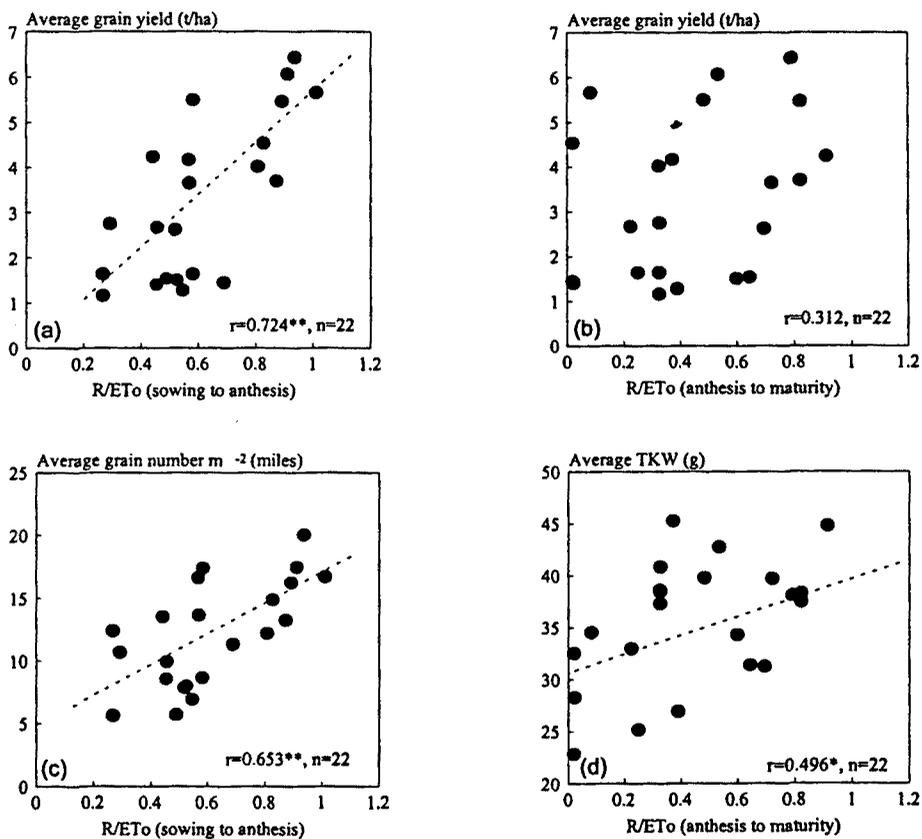


Figure 1. Relationships between either average grain yield (a) or average grain number m^{-2} (c) and the ratio of rainfall to total evapotranspirative demand from sowing to anthesis, and between either average grain yield (b) or average thousand kernel weight (TKW) (d) and the ratio of rainfall to total evapotranspirative demand from anthesis to maturity. Each point is the average of ten cultivars at each of 22 environments.

This study also confirms that post-anthesis drought is often detrimental for achieving large grains (Fig. 1d). However, it seems that a severe water limitation does not always result *per se* in poorly-filled grains. For instance, R/ETo ratios close to zero may still produce considerably

high TKW (Fig. 1d). Pre-anthesis conditions play an important role not only in the determination of the number of grains m^{-2} (i.e., sink size), but also in the attainment of a good plant photosynthetic capacity (source strength) and in the availability of photoassimilates stored in vegetative tissues for grain filling (Bidinger *et al.*, 1977). Due to this last reason, grain growth may be supported to a greater or lesser extent by remobilisation of pre-anthesis reserves under post-anthesis drought, and this results in relatively less variation in TKW as compared with grain number m^{-2} (Gallagher *et al.*, 1975). Accordingly, grain yield is rather independent of post-anthesis water deficits (Fig. 1b), although the attainment of an adequate TKW is crucial to guarantee either good nutritional properties for feed barley or low nitrogen concentration to meet the quality standards for malting. Chapters I and II of this thesis were partially devoted to address this issue.

2. COMPETITION FOR ASSIMILATES BETWEEN GRAINS: AN EXCESSIVE LOAD ON THE SOURCE?

Source availability rather than sink size arises as the main factor limiting grain weight of modern six-rowed barley cultivars in Mediterranean environments (Chapter I). The modification of source-sink ratios during post-anthesis has shown contradictory responses in the literature (see review in Slafer and Savin, 1994). Such an inconsistency may be ascribed to either differences in genotypic behaviour to altered source-sink ratios, different pre- and post-anthesis environmental conditions influencing source strength, or both factors acting together. However, studies on wheat demonstrate that, under near optimal growing conditions, grain weight appears as largely limited by sink size, i.e., the plant photosynthetic capacity largely exceeds the requirements for assimilates by the growing grains (Slafer *et al.*, 1996). For instance, wheat plants with their original leaf area halved shortly after anthesis are able to maintain similar grain weights despite the reduction of green tissue (Rawson *et al.*, 1976). Accordingly, Richards (1996) suggests that wheat cultivars currently cultivated possess a photosynthetic system that is operating at a level substantially below its potential, and concludes that grain yield for irrigated spring wheat may not be limited by source supply.

Chapter I attempts to quantify grain weight responses to sink halving in rainfed conditions (see Fig. 3, Chapter I). Results obtained suggest a post-anthesis sink limitation to grain yield only

in those environments favouring large grains (>45 mg). Below this point, individual grain weight is mostly source-limited, more as grains of control spikes decrease in size. This source limitation to grain development can be attributed to two possible and not mutually excluding explanations:

i) Pre-anthesis drought reduces source strength to a greater extent than sink size (i.e., number of grains). It has been shown that estimates of potential grain weight, as obtained by doubling the source/sink ratio, are diverse in environments varying in water availability, increasing as grains of control spikes increase in size (see Y-axis in Fig. 1, Chapter I). This finding may be related to a limited availability of assimilates at the initial phase of grain filling, i.e., when the potential grain weight is determined (Brocklehurst, 1977), caused by sub-optimal pre-anthesis conditions. For instance, if all estimates of potential grain weight have reached a similar plateau across environments, further reductions in grain weight under control conditions in each particular trial would be imputable solely to the occurrence of post-anthesis abiotic stresses, namely drought and high temperature. In contrast, our results suggest that pre-anthesis drought may have limited the plant photosynthetic capacity at anthesis to a greater extent than the plant sink size, and this should translate into a higher number of grains per unit of biomass compared with near-optimal conditions. Therefore, unless pre-anthesis conditions do not limit crop growth, sink size may become often an excessive load on the source, and grain filling is prone to be sub-optimal in relation to its maximum potential growth, with independence of the post-anthesis growing conditions. Once the maximum potential grain weight is determined shortly after anthesis, the incidence of post-anthesis drought usually induces leaf senescence and, therefore, grain growth may be further limited by the supply of carbon. Remobilisation of pre-anthesis assimilates, however, may attenuate the effect of post-anthesis drought on grain growth.

ii) Drought tolerance is in opposition to a large genotypic sink. In the last century, cereal breeding has successfully increased yield potential of the released cultivars mainly through a concomitant increase in grain number m^{-2} (i.e., sink strength) (Slafer *et al.*, 1994). Thus, a large sink can be considered as an inherent feature of the high yielding genotype. Comparisons between old and modern wheat cultivars show that breeders continue increasing grain number without changes in the availability of assimilates per grain (Koshkin and Tararina, 1989; Slafer *et al.*, 1996). In non-limiting conditions, the capacity of the source to fill the potential sink seems still sufficient (Richards, 1996) but, once water availability becomes inadequate, sink size may represent an

excessive load on the source, as mentioned above, and comparatively more in modern than in old cultivars. It has been documented that a high grain number m^{-2} may have driven a corresponding increase in stomatal conductance of modern cultivars (Richards, 1996), and this idea has been pointed out indirectly in this thesis through carbon isotope discrimination (Δ) analysis (Chapters V and VI). But a large sink also accelerates drought-induced leaf senescence during grain filling (Blum, 1996a), suggesting that this genotypic feature may be in opposition to drought tolerance in modern, high-yielding genotypes. Two observations support this hypothesis. First, it is shown in Chapter II how a 50% reduction in sink strength lengthened grain filling duration of six-rowed Barberousse, Orria and Plaisant in poor rainfed environments, and it is suggested that such a response might be mediated by a delay in the plant senescence pattern in strongly source-limited conditions. Second, as reported in Chapter V, Barberousse, Orria and Plaisant exhibited an outstanding behaviour in favourable environments and displayed in turn high constitutive Δ values. However, this superiority disappeared completely under harsher growing conditions. Although it was not evaluated, it would be reasonable to expect that old, low-yielding genotypes such as Albacete exhibited lower responses to sink halving under those conditions determining poorly-filled grains.

Based upon these considerations, evidence has been presented suggesting the existence of a frequent source limitation to grain yield in Mediterranean climates. The degree of such a limitation may be genotype-dependent, and indirect arguments have also been listed that support this expectation. Improving the performance of barley cultivars through an increase in sink strength may not be a reasonable breeding strategy in poor rainfed areas. The adoption of this approach does not necessarily imply *per se* a deficient genotypic behaviour in terms of overall productivity but rather a poor grain filling, in contradiction with the plump, well-filled grains that are required to accomplish the feed and malting barley quality standards.

3. DROUGHT, HIGH TEMPERATURE AND GRAIN FILLING

In Chapters III and IV differential genotypic sensitivities to drought and high temperature during grain filling were reported under field conditions. This result agrees with previous observations in that both stresses do not affect genotypic performance identically (e.g. Kobata *et*

al., 1992). In this thesis, the interaction between photosynthate supply and post-anthesis constraints probably brought about, to a greater or lesser extent, such genotypic responses to the environment in terms of individual grain weight and grain filling rate. For instance, some studies conclude that a reduction in the source-sink ratio (i.e., a possible source limitation to growth during grain filling) enhances sensitivity of wheat to high temperature (Wardlaw *et al.*, 1989). Six-rowed Barberousse and Orria were *a priori* more prone to present a source limitation to grain growth than old cultivars like six-rowed Albacete or two-rowed Beka, as discussed above, and indeed they showed a higher sensitivity to high temperatures (see Table 6, Chapter III). Thus, the magnitude of the response might be modified in some way by a change in the source-sink balance, with a greater response under source limiting conditions. This point is merely speculative and deserves by itself further investigation. Genotypic responses to drought are also likely to be mediated by the photosynthate supply during grain filling relative to sink size. Barberousse and Plaisant showed high sensitivities to drought and a considerable source limitation in poor rainfed environments. However, Orria performed relatively better in environments suffering drought at the end of the growing cycle, an unexpected response provided its source limitation to grain growth. Differences in remobilisation of pre-anthesis assimilates may have accounted for this differential behaviour, and it is noticeable that Orria showed the lowest average response to sink halving (14.6%) in comparison with the other cultivars evaluated (25.1 % for Barberousse, 21.3% for Plaisant).

Overall, six-rowed genotypes displayed larger sensitivities to drought and high temperatures than two-rowed types for individual grain weight. This result might be a direct consequence of the limited number of genotypes used in the study, but it may also be related to differences in source-sink balance among genotypes. For instance, preliminary work performed in a partially different set of six- and two-rowed showed that the six-rowed group was clearly source-limited, whereas two-rowed cultivars exhibited a comparatively less important response to sink halving (Fig. 2).

Two-rowed barleys usually bear heavier grains than six-rowed types in low rainfall areas (Acevedo *et al.*, 1991). This represents an important feature for optimizing grain quality in sub-optimal growing conditions. According to our results, two-rowed genotypes seem to fill their grains comparatively better under the harmful incidence of abiotic stresses. Such an advantage could be partially driven by a more favourable source-sink balance of two-rowed genotypes during the post-anthesis period. Further detailed research, however, would be necessary to really

uncover to which extent tolerance to terminal abiotic stresses is dependent on the plant source-sink balance. The observation that differential genotypic responses exist with respect to drought and thermic stress suggests that mechanisms other than the source-sink balance may also influence the process of photosynthate incorporation into growing grains.

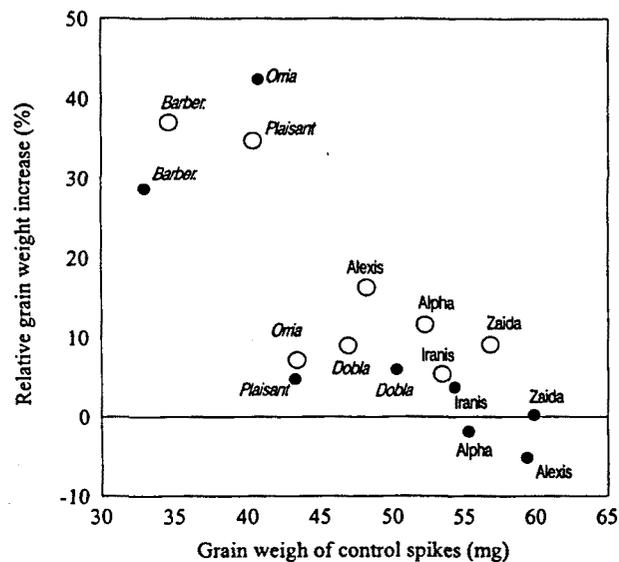


Figure 2. Relative grain weight increase (%) in response to sink halving in relation to grain weight of control spikes for five six-rowed (in italics) and five two-rowed barley genotypes grown in two different environments (*,○) in 1991 (Volta, unpublished data).

4. ADAPTATION TO DROUGHT AND YIELD POTENTIAL: TWO CONFLICTING MECHANISMS?

In an agronomic context, a genotype can be considered adapted to a given set of conditions (e.g., under drought stress) when it yields well relative to the productive potential of test environments in such conditions (Romagosa and Fox, 1993). Whether a drought-adapted genotype is able to perform satisfactorily in a wide range of contrasting environments, including those where water availability is plentiful, is still a subject of strong debate. The theoretical framework of this issue is essentially the identification of a crossover, or qualitative, Genotype

× Environment (G×E) interaction, and contradictory positions arise so far. CIMMYT's wheat breeding methodology is centred around the development of widely adapted germplasm with high and stable yields across a wide range of environments (Rajaram *et al.*, 1997). It assumes that G×E is unimportant or, if existing, has a non-crossover nature. In other words, yield potential *per se* is beneficial also under drought. By contrast, ICARDA's barley breeding strategy is based in the conviction that the most efficient way to improve yield in low-rainfall areas is by direct selection in water-limited environments (Ceccarelli, 1997), i.e., it assumes the presence of a crossover-type G×E.

Would it be then impossible to combine drought tolerance with high yield potential or, on the contrary, both traits could perfectly merge in a particular genotype? At the physiological level, it appears that changes occurring as a response to drought may be divided into two different categories: those conferring the plants the ability to tolerate events of extreme stress, and those maximising yield under a relatively large range of water limitation. Whereas the expression of the former produces yield losses under less-severe drought, the latter expresses with independence of the degree of stress, being still essential in sustaining relatively high yields under moderate drought (Blum, 1996b). CIMMYT's breeders have been unable to identify such *drought tolerance* traits in wheat (Rajaram *et al.*, 1997). Actually, below a certain yield level, barley is the natural substitute of wheat since it possesses an intrinsically higher tolerance to drought (López-Castañeda and Richards, 1994). Barley breeding programs at ICARDA aim to utilise traits conferring drought tolerance by selecting for yield *per se* under extremely harsh conditions, and results from this thesis support this approach. Unfortunately, such traits are nowadays far from clear (Passioura, 1996).

Chapter V provided evidence on the existence of such a crossover-type G×E interaction in a set of environments covering most productive situations (from one to six t ha⁻¹) found in the Mediterranean region, and for cultivars with contrasting genetic backgrounds (including landraces and modern, high-yielding lines). Old cultivars such as Albacete tended to outyield modern lines such as Orria or Plaisant in environments with average yields below 3 t ha⁻¹, and vice versa in environments with average yields surpassing 3 t ha⁻¹. The exact crossover point is difficult to determine since it depends on many variables, namely timing and intensity of stress/stresses at each stage of development, and parameters such as soil type and depth whose levels define the responses to varying climatic factors. A crossover-type G×E has also been documented in studies performed in barley in the Mediterranean region (Ceccarelli and Grando, 1991; van Oosterom *et*

al., 1993); in some others, however, modern wheat cultivars have consistently outyielded older cultivars even in the lowest-yielding conditions of each particular study (Perry and d'Antuono, 1989; Koshkin and Tararina, 1994; Sayre *et al.*, 1997). This contradiction, which is in turn source of controversy, has to do mainly with two different species, barley and wheat, with different growth strategies under drought. Barley is more vigorous than wheat already from the time of emergence, captures more water for transpiration, and produces a greater biomass (Siddique *et al.*, 1989, López-Castañeda and Richards, 1994). This feature translates into significantly higher yields for barley in water-limited environments (López-Castañeda and Richards, 1994). Early vigour is recognized as a trait of interest for wheat and it is being incorporated in recently-released cultivars (Richards, 1996). In combination with early flowering it may be of advantage for genotypes grown in poor rainfed areas, conferring a yield benefit over high-yielding, widely adapted wheat lines.

Presence of a qualitative (i.e., crossover) G×E interaction for barley grown in the Mediterranean region has important breeding implications. Since timing and intensity of water stress vary from year to year, a drought-tolerant line may exhibit a manifest superiority in a given year that can be counterbalanced by a deficient behaviour in the following years. A considerable genotype×year interaction hinders the definition of homogeneous areas for cultivar recommendation (i.e., megaenvironments) (Gauch and Zobel, 1997) and, if such areas are defined, limits considerably their use and function. The results in Chapter V suggest the existence of such an important yearly variation, but more extended studies are needed to quantify to which extent G×E for a previously defined 'homogeneous' agroclimatic region is dominated by either seasonal or geographical fluctuation.

The assumption that drought tolerance and yield potential are rather antagonistic concepts, as stated by Blum (1996a), is also supported in this thesis by data on carbon isotope discrimination (Δ). The measurement of genotypic Δ appears rather environment-independent, in opposition with the genotypic yield (Chapter V). At first, a lower Δ (i.e., a higher transpiration efficiency, TE) may be a beneficial trait for the attainment of greater yields under drought. This feature seems constitutive in those genotypes with an outstanding performance in environments below the crossover point (i.e., in severely water-limited areas) provided that there are not significant differences in phenology among cultivars. On the contrary, the association between Δ and grain yield changes to positive in environments with little or moderate water-stress (Chapters V and VI), while maintaining a nearly constant genotypic ranking for Δ . It has been

suggested that a high TE is not, *per se*, a negative trait for achieving higher yields under non-limiting conditions, but rather the decrease in water use that may be associated with a high TE (Richards and Condon, 1993; Turner, 1993). High-yielding cultivars are characterized by a large sink (i.e., a high grain number m^{-2}) that requires, in turn, a large source activity. Their constitutively high Δ and low TE may be governed by a strong sink demand, which is responsible for keeping plant stomata more open, thus increasing transpiration losses. By virtue of their high stomatal conductance, high-yielding cultivars are capable of capturing more water, if available, for a larger growth. The increase in total water use overrides the expected negative relation between Δ and biomass or yield as result of a higher TE.

5. CONCLUDING REMARKS

Physiological understanding of factors limiting grain yield under drought is augmenting rapidly. Presumably, it will permit in a near future to face more efficiently the increase in barley yields in Mediterranean environments. However, some gaps still exist that physiologists need to overcome conveniently before particular traits could be used for indirect selection by which to complement the conventional empirical approach to plant breeding, or selection for yield *per se*. This thesis has shown that a profounder comprehension of topics such as the source-sink balance and the functioning of carbon partitioning in plants are key steps for targeting such important traits. Undoubtedly, an excessive sink strength and, consequently, an important source-sink unbalance, causes a poor grain filling and depreciates grain quality under severe drought. Accordingly, it is partially responsible of the non-optimal performance of modern cultivars in such conditions. In this context, optimization of the plant carbon partitioning to developing sinks seems one of the two reasonable means of increasing yields when water is limiting. The other should be to increase the efficiency by which the plant uses all the water available to produce photoassimilates. Matching the phenology of the crop to the environment has been the most straightforward procedure adopted by breeders to maximize carbon partitioning (i.e., harvest index) and water use efficiency. However, if we intend to continue increasing yields under drought, other traits conferring these genotypic attributes must be targeted. We are in the correct way to identify and utilise them for increasing yield-based selection.

REFERENCES

- Acevedo, E., Craufurd, P.Q., Austin, R.B., and Pérez-Marco, P., 1991. Traits associated with high yield in barley in low-rainfall environments. *J. Agric. Sci. (Camb.)* 116, 23-36.
- Austin, R.B., Cantero-Martínez, C., Arrúe, J.L., Playán, E., and Cano-Marcellán, P., 1998. Yield-rainfall relationships in cereal cropping systems in the Ebro river valley of Spain. *Eur. J. Agron.* 8, 239-248.
- Bidinger, F., Musgrave, R.B. and Fischer, R.A., 1977. Contribution of stored pre-anthesis assimilate to grain yield in wheat and barley. *Nature* 270, 431-433.
- Blum, A., 1996a. Yield potential and drought resistance: are they mutually exclusive? In Reynolds, M.P., Rajaram, S., and McNab, A. (Eds.), *Increasing Yield Potential in Wheat: Breaking the Barriers*. CIMMYT. Mexico D.F., pp. 90-100.
- Blum, A., 1996b. Constitutive traits affecting plant performance under stress. In Edmeades, G.O., Bänziger, M., Mickelson, H.R., and Peña-Valdivia, C.B. (Eds.), *Developing Drought- and Low Nitrogen -Tolerant Maize*. CIMMYT. Mexico D.F., pp. 131-135.
- Brocklehurst, P.A., 1977. Factors controlling grain weight in wheat. *Nature* 266, 348-349.
- Ceccarelli, S., and Grando, S. 1991. Environment of selection and type of germplasm in barley for low-yielding conditions. *Euphytica* 57, 207-219.
- Ceccarelli, S., 1997. Adaptation to low/high input cultivation. In Tigerstedt, P.M.A. (Ed.), *Adaptation in Plant Breeding*. Kluwer Academic Publ., pp. 225-236.
- Gallagher, J.N., Biscoe, P.V. and Scott, R.K., 1975. Barley and its environment. V. Stability of grain weight. *J. Appl. Ecol.* 12, 319-336.
- Gauch, H.G., and R.W. Zobel, 1997. Identifying mega-environments and targeting genotypes. *Crop Sci.* 37: 311-326.
- Kobata, T., Palta, J.A. and Turner, N.C., 1992. Rate of development of postanthesis water deficits and grain filling of spring wheat. *Crop Sci.* 32, 1238-1242.

Koshkin, E.I., and Tararina, V.V., 1989. Yield and source/sink relations of spring wheat cultivars. *Field Crops Res.* 22, 297-306.

López-Castañeda, C. and Richards, R.A., 1994. Variation in temperate cereals in rainfed environments. I. Grain yield, biomass and agronomic characteristics. *Field Crops Res.* 37, 51-62.

Loss, S.P., and Siddique, K.H.M., 1994. Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. *Advances in Agronomy* 52, 229-276.

Passioura, J.B., 1996. Drought and drought tolerance. *Plant Growth Regulation* 20, 79-83.

Perry, M.W., and D'Antuono, M.F., 1989. Yield improvement and associated characteristics of some Australian spring wheat cultivars introduced between 1860 and 1982. *Aust. J. Agric. Res.* 40, 457-472.

Rajaram, S., Braun, H.J., and van Ginkel, M., 1997. CIMMYT's approach to breed for drought tolerance. In Tigerstedt, P.M.A. (Ed.), *Adaptation in Plant Breeding*. Kluwer Academic Publ., pp.161-168.

Rawson, H.M., Gifford, R.M., and Bremner, P.M., 1976. Carbon dioxide exchange in relation to sink demand in wheat. *Planta* 132, 19-23.

Richards, R.A., and Condon, A.G., 1993. Challenges ahead in using carbon isotope discrimination in plant-breeding programs. In Ehleringer, J.R., Hall, A.E., and Farquhar, G.D. (Eds.), *Stable Isotopes and Plant Carbon - Water Relations*. Academic Press, Inc., pp. 451-462.

Richards, R.A. 1996. Increasing the yield potential of wheat: manipulating sources and sinks. In Reynolds, M.P., Rajaram, S., and McNab, A. (Eds.), *Increasing Yield Potential in Wheat: Breaking the Barriers*. CIMMYT. Mexico D.F., pp. 134-149.

Romagosa, I., and Fox, P.N., 1993. Genotype×environment interaction and adaptation. In Hayward, M.D., Bosemark, N.O., and Romagosa, I. (Eds.), *Plant Breeding. Principles and Prospects*. Chapman & Hall, London, pp. 373-390.

Sayre, K.D., Rajaram, S., and Fischer, R.A., 1997. Yield potential progress in short bread wheats in northwest Mexico. *Crop Sci.* 37, 36-42.

Siddique, K.H.M., Belford, R.K., Perry, M.W., and Tennant, D. 1989. Growth development and light interception of old and modern wheat cultivars in a Mediterranean-type environment. *Aust. J. Agric. Res.* 40, 473-487.

Slafer, G.A., and Andrade, F.H., 1989. Genetic improvement in bread wheat (*Triticum aestivum*) yield in Argentina. *Field Crops Research* 21, 289-296.

Slafer, G.A., and Savin, R., 1994. Source-sink relationship and grain mass at different positions within the spike in wheat. *Field Crops Res.* 37, 39-49.

Slafer, G.A., Satorre, H., and Andrade, F., 1994. Increases in grain yield in bread wheat from breeding and associated physiological changes. In Slafer, G.A. (Ed.), *Genetic Improvement of Field Crops*. Marcel Dekker Inc., pp. 1-68.

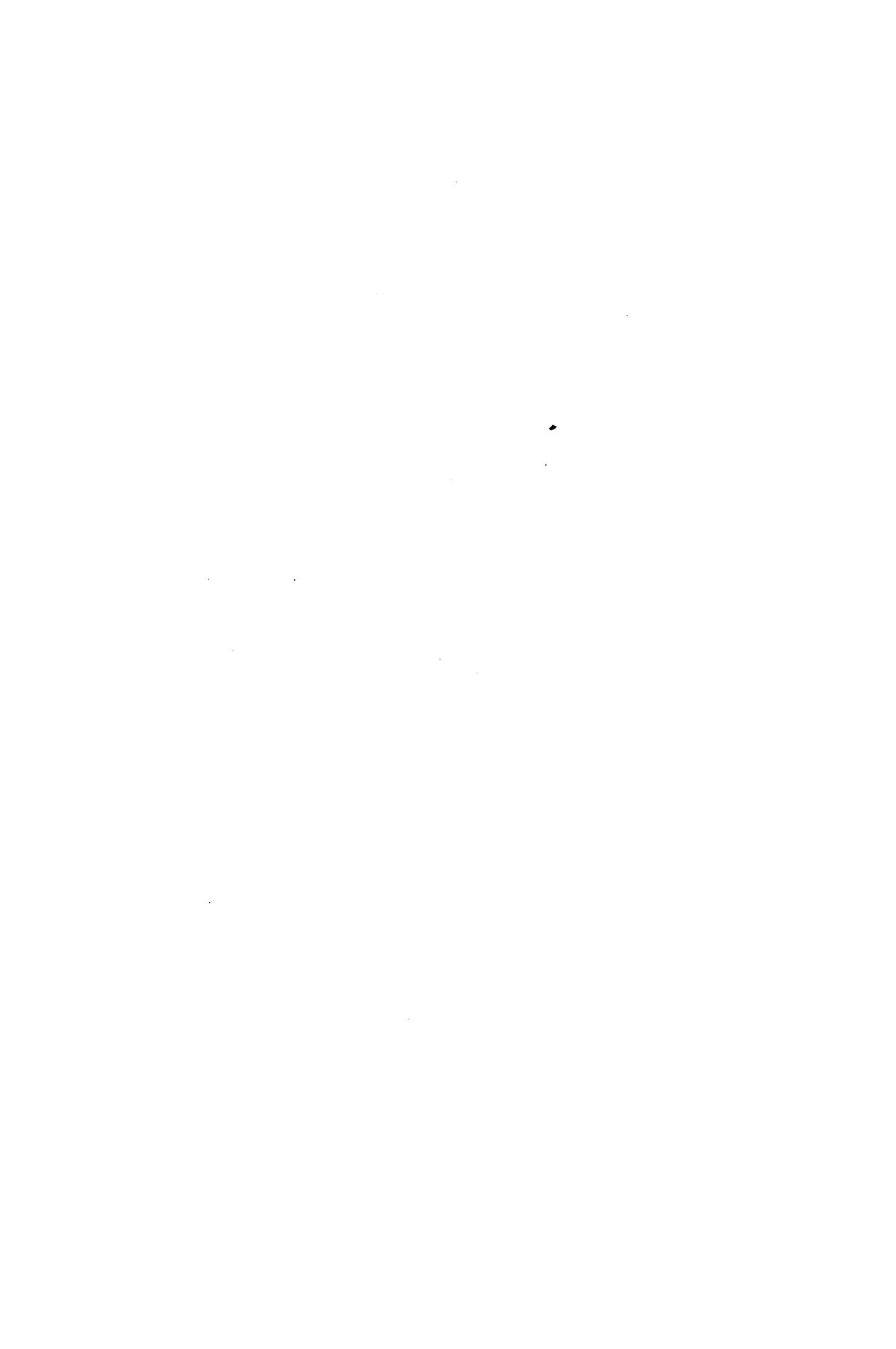
Slafer, G.A., Calderini, D.F., and Miralles, D.J., 1996. Yield components and compensation in wheat: opportunities for further increasing yield potential. In: Reynolds, M.P., Rajaram, S., McNab, A. (Eds.), *Increasing Yield Potential in Wheat: Breaking the Barriers*. CIMMYT. Mexico D.F., pp. 101-133.

Turner, N.C., 1993. Water use efficiency of crop plants: potential for improvement. In Buxton, D.R., Shibles, R., Frosberg, R.A., Blad, B.L., Asay, K.H., Paulsen, G.M., and Wilson, R.F. (Eds.), *International Crop Science*. CSSA, Madison, pp. 75-82.

van Oosterom, E.J., Kleijin, D., Ceccarelli, S. and Nachit, M.M. 1993. Genotype-by-environment interaction of barley in the Mediterranean region. *Crop Sci.* 33, 669-674.

Wardlaw, I.F., Dawson, I.A., Munibi, P., Fewster, R., 1989. The tolerance of wheat to high temperatures during reproductive growth. I. Survey procedures and general response patterns. *Aust. J. Agr. Res.* 40, 1-13.

CONCLUSIONS



1. Drought is the most limiting factor to barley growth and productivity in rainfed Mediterranean environments. It has an important detrimental effect on grain yield especially if it develops before the crop reaches anthesis.
2. A source limitation to grain growth in six-rowed, highly productive barleys, is common to most rainfed Mediterranean environments, determining an inadequate grain filling and producing, to a greater or lesser extent, an important proportion of poorly-filled or shrivelled grains.
3. In opposition to carbohydrate deposition, post-anthesis drought and/or high temperatures do not seem to affect adversely protein synthesis in grains.
4. Central grains of six-rowed genotypes have a greater intrinsic potential weight. Differences in weight between central and lateral grains do not decrease if plenty of assimilates are available for grain filling. The advantage of central grains is mostly caused by a higher dry matter accumulation rate rather than by a more prolonged grain filling period.
5. Barley genotypes do not react identically, in terms of individual grain weight and grain filling parameters, to varying environmental conditions. Genotype by environment interaction ($G \times E$) for individual grain weight can be explained by differential genotypic sensitivities to post-anthesis drought and thermic stress.
6. The balance between source strength and sink demand determines the rate of dry matter accumulation in grains. Accordingly, the existence of $G \times E$ for grain filling rate seems to be related largely to differences in source/sink balance between two- and six-rowed barleys at anthesis.

7. Differential genotypic responses to the environment ($G \times E$) for grain filling duration are brought about mainly by differences in phenology (i.e., anthesis date) among genotypes. Some cultivars, by using an escape strategy, may suffer to a lesser extent the effect of high temperatures and drought in particular environments at the end of the growing cycle.
8. A low carbon isotope discrimination (Δ), i.e., a high transpiration efficiency, is a beneficial genotypic attribute for maximizing grain yield of barley in severely water-limited environments.
9. By virtue of their large reproductive sink, an increase in total water use of high-yielding genotypes under moderate drought probably overrides the expected negative relation between Δ and biomass or yield. Indeed a positive genotypic association between Δ and grain yield is here reported.
10. Ash concentration in vegetative tissues sampled at maturity is of limited value as a surrogate of Δ . Ash concentration in mature grains could be used as a complementary criterion to Δ in semiarid environments, though a more accurate physiological understanding of the mechanisms underlying mineral accumulation in grains is still needed.
11. Drought tolerance and yield potential appear as rather antagonistic concepts in barley. The presence of a crossover $G \times E$ interaction for grain yield, as well as changing relationships between productivity and Δ depending on the intensity of water stress, support this proposition.

EXCLÒS DE PRÉSTEC

