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DETERMINATION OF ^{13}C DISCRIMINATION AS AN INDIRECT SELECTION CRITERION FOR BARLEY IN DRY ENVIRONMENTS

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1. EXECUTIVE SUMMARY

The main aim of the project was to establish whether the carbon isotope discrimination method would be a more efficient selection criteria for increased and more stable barley yields in dry Mediterranean environments.

Earlier studies had established a causal relationship between yield performance, water use efficiency and carbon isotope discrimination in many C3 plants (Farquhar, et al., 1982; Farquhar and Richards, 1984). In brief, ^{13}C discrimination might be used as a predictor of yield performance in specific environmental conditions and therefore could provide crop improvement scientists with a very useful screening method.

The project carried out over a period of six years, had three main research activities, which included both field and controlled environment experiments. Finally, the project developed more than 570 families from seven crosses, from six parents having high and low ^{13}C discrimination. Over 3500 samples were analyzed for ^{13}C discrimination at leading institutes in the USA and Australia.

Results indicate that ^{13}C discrimination (Δ), water use efficiency and yield are strongly correlated, as predicted by theory. Therefore, ^{13}C discrimination could be used as a predictor of yield performance and/or water use efficiency of barley under water limited conditions. However, the slopes of the relationships changed from negative to positive in response to changes in environmental conditions. In highly drought stressed environments, which are subject to extremes of temperature during crop growth cycle, a positive and significant relation consistently existed between yield and Δ. Therefore, it appears that selection for high Δ can be used effectively as a means to improve yield in barley in water limited Mediterranean environments. However the targeted environment in the Mediterranean is highly variable from season to season and under milder conditions the relationship changed to negative. Therefore this technique may not be appropriate for barley under these variable conditions. In the breeding program it was found that the efficiency of selection based on ^{13}C discrimination relative to that based on grain yield itself was on average 0.41 (in certain crosses it was substantially higher). Although this means that

selection based on yield of F_5 is more efficient, it still represents an important figure, since these estimations were based on selection for Δ at the F_2 generation, at a time when selection based on grain yield is not possible. In addition selection for grain yield requires several generations of selection and a large resource investment. Therefore, if the technique of ^{13}C discrimination measurements can be more efficient and cheaper then it could possibly still be used effectively as a substitutive technique to standard selection methods.

Finally, considerable experience has been gained by scientists involved in this project. Scientists from leading institutes in the USA, Europe and Australia may also benefit from the findings of the project. The project has undoubtedly contributed to our understanding of the physiology of carbon isotope discrimination and its association with yield and water use efficiency. It focused on new approaches to the way crop adaptation plays an important role in determining the relation between yield and Δ under various environmental conditions. In addition, Dr. Abdullah Dakheel, a Professor in Ecophysiology in Aleppo University, Syria, working on this project, has now become one of the more experienced and knowledgeable scientists on ^{13}C. He is highly regarded by his international colleagues in both the leading institutes in USA, UK and Australia.

2. RESEARCH OBJECTIVES

Progress in breeding for low and erratic rainfed environments has been extremely slow. Selection based on estimates of grain yield are often inefficient because of large variation in environmental conditions throughout the growth cycle. Therefore an important objective was to asses the usefulness of this new and novel ^{13}C discrimination method, as a selection tool under these harsh conditions.

Barley was chosen as the crop, as it is the predominant cereal grown in the dryland farming systems of the Mediterranean region. It was anticipated that the results of this project could be promptly and inexpensively utilized worldwide through the improvement of selection efficiency for varieties to be grown under less favorable, stressful environments.

2.1 Primary Objectives

The project had three main research objectives:

2.1.1 Assessment of the relation between ^{13}C discrimination, water use efficiency and yield under different moisture levels.

2.1.2 Assessment of the relation between ^{13}C discrimination, transpiration efficiency and dry matter production under controlled environmental conditions.

2.1.3 To develop a divergent selection experiment for ^{13}C discrimination and yield.

The specific objectives of 2.1.3 were to (i) determine the efficiency of selection for drought tolerant genotypes based on their ^{13}C discrimination when compared with selection based on yield performance under stress and non-stress conditions, and (ii) to study the genetic parameters and breeding implications for the ^{13}C discrimination technique. Seven crosses were made between four high and two low ^{13}C discrimination parents in 1987. All the families were analyzed for ^{13}C discrimination in the F_2 generation in 1994. At the F_5 stage there were 572 families from these crosses.

Two types of selection were planned:

a) selection based on high and low ^{13}C discrimination;
b) selection based on high and low yield under stress (Breda) and non-stress (Tel Hadya (ICARDA's headquarters), irrigated) moisture conditions.

Selection for High/Low ^{13}C discrimination and High/Low grain yield was done on the F_5 plants. This experiment was expected to provide valuable information on (i) the yield correlation between selections based on high or low ^{13}C discrimination, (ii) the appropriate environment for the selection, (iii) the efficiency of using ^{13}C discrimination as an indirect selection criterion compared with yield, and (iv) the heritability of ^{13}C discrimination.

2.2 Secondary Objectives Relating to the ^{13}C Discrimination Technique

Various experiments were conducted to address specific issues relating to the use of ^{13}C discrimination as a selection criterion for drought tolerance: viz.

- The effect of phenological stage on ^{13}C discrimination (time of sampling)
- The effect of type of material used in ^{13}C discrimination analysis (the correlation between ^{13}C discrimination in leaves vs. stems vs. peduncle vs. grain)
- Interseasonal variability in ^{13}C discrimination
- The relation between ^{13}C discrimination and crop traits which might be used as surrogates, such as:

  a) peduncle length
  b) specific leaf area (SLA)
  c) days to heading
  d) grain filling period

Similar work is being carried out in Australia and in the USA (Richards and Condon 1993; Condon and Richards 1993; Ehleringer et al. 1990). This is the only project where ^{13}C has been measured on successive breeding generations. Throughout this project, scientists from the University of Utah, USA and CSIRO Canberra, Australia have been involved.

3. METHODS AND RESULTS

3.1 Materials and Methods

3.1.1 Plant material and experimental site

Field experiments were conducted in northern Syria at Breda (35°56'N, 37°10'E), one of ICARDA's drier experimental sites. The site is characterized by 262 mm long-term average rainfall and a calcic xerosol soil type. The climate is Mediterranean with cold rainy winters and dry hot summers.

3.1.1.1 In both the field and plastic house (controlled environment) experiments (objectives 2.1.1 and 2.1.2) ten two-row barley genotypes were used. The genotypes were identified from among the best adapted to the harsh Mediterranean environments (Acevedo, 1993) and included 5 landraces (L) originating in the Syrian steppe and arid zone (Tadmor, SLB 39-99, SLB 62-35, SLB 62-99, and SLB 8-6) and 5 improved lines (I) developed at the Waite Institute in Australia (WI 2198 and WI 2198/WI 2291) and from ICARDA's barley breeding program (Harmal, Roho, and SBON 96). The genotypes have a similar phenology and posses some important traits for adaptation to Mediterranean environments (Acevedo, 1993).

3.1.1.2 In the divergent selection experiment six out of the ten barley genotypes with high and low ^{13}C discrimination were used to develop seven crosses in 1988. These are listed below:

Cross 1 SLB 8-6 x WI 2198

Cross 2 SLB 8-6 x Roho
Cross 3 Tadmor x SLB 62-99
Cross 4 Tadmor x Roho
Cross 5 Tadmor x SLB 62-99
Cross 6 SLB 8-6 x SLB 62-99
Cross 7 Tadmor x WI 2198

3.1.2 Experimental design and management

Field experiments relating to the first objective were conducted for three seasons from 1988/89 to 1990/91. They included 10 moisture levels ranging from natural rainfall (rainfed) to around 400 mm during the growing season (rainfall + irrigation) using a line-source sprinkler system (LSS). The LSS distributed water along both sides. Details on design, measurements and carbon isotope analysis are given in the attached publications (Dakheel et al., 1994; Acevedo, 1993).

In the controlled environment experiments the ten barley genotypes were grown in a plastic house for three seasons from 1988/89 until 1990/91. Four moisture regimes were used, a non-stressed treatment, fully stressed throughout the life cycle, a pre-heading and post-heading moisture stressed treatment. Details are given in the attached publications (Dakheel et al., (in prep); Acevedo, 1993).

In the divergent selection experiment ^{13}C discrimination (Δ) was determined in the F_2 progeny of each of the seven crosses for a total of 572 families. F_3 and F_4 bulks from each F_2 plant were grown during 1989/90 and 1990/91 seasons respectively. Yield data were available from unreplicated plots at the F_4 stage. Selection for high and low ^{13}C discrimination and high and low yield was delayed until the F_5 generation due to the delay in completing the carbon isotope analysis by the contracting laboratory (see previous annual and progress reports). At the F_5 generation a replicated experiment was planted at Breda and at Tel Hadya, which represented the minimum stress with respect to barley moisture requirements. At both locations, the 572 families from the seven crosses were included in 10 experimental units and each was designed as a 8 x 8 lattice. Three and two replications were used in Breda and Tel Hadya respectively. During the 1992/93 season (F_5 generation), 225 families from all crosses (plus 31 checks and parents) were selected for planting during 1992/93 season as the F_6 generation. Selections were based on seven different criteria at a 5% selection intensity, these are:

- selections based on high and low grain yield at the stressed site (Breda);
- selections based on high and low grain yield at the non-stressed site (Tel Hadya);
- selections based on high and low ^{13}C discrimination (Δ); and
- a random selection

Selection for high and low Δ was carried out according to the values obtained from the F_2 generation. These were not available until mid 1991. During the 1992/93 season the 256 selected F_6 families, parents and checks were planted in a 16 x 16 lattice design at both Breda and Tel Hadya. Later, during Dr. A. Dakheel's visit to the Department of Biology, University of Utah, more than 2400 samples were analyzed for ^{13}C discrimination representing the F_5 and F_6 generations, in addition to the field and plastic house experiments. In total, carbon isotope discrimination analysis was performed on more than 3500 samples.

3.2 Results

3.2.1. Assessment of the relation between ^{13}C discrimination, water use efficiency and yield in barley grown under field conditions at several moisture levels

Statistical analysis of the three years data from the Line Source Sprinkler (LSS) show significant genotypic variation in grain yield (GY), above ground dry matter production (AGDM), water use efficiency (*W*), and Δ measured in: leaves at 3, 5 and flag leaf stages; peduncles; and grains. All variables varied among moisture levels, with the exception of Δ measured during the early developmental stages (3 & 5 leaf stages) (Table 1), (Dakheel et al., 1994, p. 176).

3.2.1.1. Relation between yield and water use efficiency

Water use efficiency (*W*) correlated positively and significantly with both grain and biological yield in all seasons. Across all moisture levels correlation coefficients for the three seasons means were 0.47 for AGDM and 0.44 for GY ($P<0.01$). Within each moisture level the relation between W and GY or AGDM were very strong; correlations exceeded $r=0.90$ ($P<0.001$).

Variations in water use efficiency (*W*) within high and low moisture levels explained the high percentage (from 93 to 97%) of yield variability among genotypes. The high association between *W* and yield justified the efforts to search for variation in water use efficiency within crop species, and for traits which could be associated with variation in *W* such as ^{13}C discrimination (Δ).

3.2.1.2. Relation between carbon isotope discrimination and yield

Carbon isotope discrimination was highest in the leaves, the primary photosynthetic tissue; lower in peduncles and lowest in grains. Similarly, Δ values were higher at high moisture level.

The ^{13}C discrimination theory (Farquhar & Richards, 1984) predicts that water use efficiency (W) and Δ are negatively related at the leaf level; yield and *W* are positively related and consequently yield and Δ are negatively related (Richards & Condon, 1993).

In this study, the mean responses, over three seasons, for the relation between Δ and AGDM (or GY) were significantly negative at both the low ($r=-0.75$) and high moisture levels ($r=-0.85$). However, within each season the relations were not consistently negative. At the high moisture level the relation between Δ and yield (GY & AGDM) remained negative, but significant only in the 1991 season (for AGDM). At the low moisture level correlations were negative and significant in the 1991 season, while in 1989 relations between Δ and yield were positive, but significant only for GY, and in 1990 seasons relations were significantly positive for AGDM (Dakheel et al. (1994) Table 2, p. 178).

Across a gradient in moisture levels Δ measured in the grains and peduncles, correlated positively and highly significantly with both GY and AGDM ($r=0.86$ & 0.87 respectively) (Dakheel et al. (1994) Figure 1). While Δ measured in the leaves at 3-or 5-leaf stages correlated positively , but weakly or not significantly with both GY and AGDM.

Positive relation between Δ and yield were also reported in several studies (Condon et al., 1987), especially across a gradient in moisture levels, in several crops including wheat (Ehdaie et al., 1991), durum wheat (Kirda et al., 1992) and from barley in a similar environment in northern Syria (Craufurd et al., 1991; Acevedo, 1993). While a negative relationship was found in wheat in environments with a low yield potential (Condon & Richards, 1993), in peanut [Wright et al. (1988, 1993 & 1994)] and in range grasses (Johnson & Bassett, 1991).

3. Relation between ^{13}C discrimination and water use efficiency

The relation between Δ and *W* followed a similar pattern to the relation between Δ and yield. In the 1991 season within individual moisture levels, strong negative relationships between Δ, measured in grains, and yield were found in the 1991 season, at both high and low moisture levels (r ranged from -0.57 to -0.68). In 1989 and 1990 seasons relations were negative at high moisture levels, but significant only in 1990 between *W* and Δp, measured in peduncles. While at the low moisture level, slopes of the relations shifted to positive (Dakheel et al., 1994, Table 4, p. 180). The close resemblance of the relation between Δ and yield and between Δ and *W* suggested that the same factors influence both ^{13}C discrimination and water use efficiency as it was predicted by carbon isotope discrimination theory (Farquhar & Richards, 1984).

Δ is largely related to the ratio of intercellular to atmospheric carbon dioxide concentration (ci/ca). This ratio is in turn influenced by stomatal conductance and photosynthetic capacity of the plant. The relationship between yield and Δ is expected to be positive when the stomatal conductance increase and photosynthetic capacity remains constant, such that the ci/ca ratio increases. At the same time transpiration (or water use) efficiency is reduced (Condon et al., 1987). The reverse conditions would lead to a reduction in ci/ca ratio, an increase in *W*, and a negative relationship between yield and Δ (Wright et al., 1994). The two patterns, separately or in combination, were observed in several crop species (Ehleringer et al., 1990, Hubick et al., 1986, Condon et al., 1990).

The observed inconsistencies in the relationships between Δ and yield and Δ and *W* among the three seasons were mainly related to environmental conditions, in particular temperature and rainfall, during critical times of plant growth. In the 1989 and 1990 seasons, when positive relations were observed, an extended period of frost and below zero temperatures, exceeding 47 days, dominated from December until February. Rainfall was lower in those two seasons (183 mm in comparison with 241 mm in 1991) and were characterized by severe terminal moisture stress and high temperatures during grain filling. Such environmental stresses are expected to increase limitations on stomatal conductance and lead to a reduction in Δ and cause a positive or inconsistent relation between Δ and yield or water use efficiency. While less stressful conditions reduce effects on stomatal conductance they may lead to negative relations between Δ and yield or Δ and *W* (Condon et al., 1987) (more details are given in the attached publications by Acevedo and Dakheel).

It is concluded from this study that for barley, grown in a Mediterranean environment, the expected negative relation between Δ and yield and Δ and W were only maintained under high moisture availability, in association with mild temperatures during critical periods of plant growth. Under low moisture conditions and extremes of temperature, a positive relation between Δ and yield or *W* was dominant.

Therefore, selection for low Δ in barley in Mediterranean environments is only likely to lead to an improvement in yield and *W* in environments where moisture and temperature stresses are not dominant during critical periods of plant growth. In environments with low moisture availability and high frequency of low temperature and frost occurrence, as is most likely to be the case in Mediterranean environments, selection for **high ^{13}C discrimination (Δ)** will be associated with a higher yield potential.

3.2.2. Assessment of the relation between ^{13}C discrimination, transpiration efficiency and yield under controlled environmental conditions.

As in the field experiment, Δ, transpiration efficiency (*W*), grain yield (GY) and above ground dry matter production (AGDM) varied significantly among moisture levels and genotypes.

3.2.2.1. Relation between yield, transpiration efficiency and Δ

Across all moisture treatments yield (GY and AGDM) correlated negatively and significantly with *W* ($P<0.001$) (Dakheel et al., 1995, Figure 2). Yield and ^{13}C discrimination correlated highly and positively ($r> 0.85$, $P<0.001$). The correlation coefficient between Δ and GY and AGDM for the mean of two seasons and the three moisture treatments were 0.82 and 0.87 respectively (Dakheel et al. (in prep.), Figure 1 & 2).

When the relationships were examined within each moisture treatment, or between the two seasons, they appeared to be inconsistent with those observed across moisture levels. The relation between yield and Δ remained positive and strongly significant in the 1989 season within the non-stressed field capacity (FC) and fully stressed (1/3 FC) moisture treatments (Dakheel et al., (in prep.), Table 4). In the post and pre-heading moisture stress, the relations were also positive but weaker. In the 1991 season the relation between yield and Δ shifted to negative, but was not significant in the non-stressed treatment. In the other stressed treatments, relations were positive but only significant between AGDM and Δ in the fully stressed moisture treatment.

The relation between yield and *W* also remained negative, and significant in most cases, in the 1989 season. Similarly, in 1991 season the relation between yield and *W* changed to positive, but significant only in the non-stressed (field capacity, FC) and terminal moisture stressed (FC-1/3FC) treatments.

The stability of Δ, or other traits, measured during the two seasons were weak between the unstressed moisture treatments. Transpiration efficiency (*W*) was the only trait which was consistent during the two seasons. While among moisture stressed treatments Δ, GY and *W* all correlated significantly across the two seasons. Such correlations suggest that Δ in barley is more stable across moisture limited environments than unlimited.

3.2.2.2. Relation between ^{13}C discrimination and transpiration efficiency

As predicted by the ^{13}C discrimination theory, the relation between Δ and W was strongly negative across moisture treatments in both 1989 and 1991 seasons ($r=-0.76$ and -0.85

respectively, P<0.001) (see Dakheel et al., (in prep.), Figure 3 & 4). Within individual moisture treatments the relation between W and Δ remained negative. In 1989 correlations were highly significant in all moisture treatments. In 1991 relations were also negative, but significant only in the full and post-heading moisture treatments (Dakheel et al., (in prep.), Table 4).

Similar to the results from field experiments, the observed variation in the relationship between Δ and yield or yield and W between the two seasons were related to variations in environmental conditions. A positive relationship between yield and Δ dominated under conditions of high vapor pressure deficit (vpd) at critical times of spike development and grain filling during the first season, and in full moisture stressed treatments during both seasons. This implies that variation in stomatal conductance, rather than photosynthetic capacity, influenced ci/ca ratio, and consequently Δ, and led to the positive relation between yield and Δ.

We conclude from the plastic house experiments that: First, high Δ in barley is generally associated with high yield potential, especially in water limited environments. Second, higher transpiration efficiencies (*W*) were associated with lower Δ and consequently with lower yield potentials. This is inconsistent with the results obtained from the field study, where the relations between Δ and *W* were influenced by variations in temperature and moisture conditions. Third, the relation between Δ across moisture levels and seasons was consistent only under moisture stress. Therefore the results suggest that utilizing Δ in selection for dry areas is more appropriate when selection is carried out within the targeted environment. Finally, both the controlled environment and field studies (Dakheel et al., 1994), emphasize that the relation between Δ and yield across a moisture gradient does not necessarily reflect the relation observed within individual moisture regimes.

From the two studies (field and controlled environment) it is evident that the relationships between Δ and yield, and Δ and W in barley are influenced by both moisture and temperature regimes which prevail during critical periods of plant growth. However, under water limited environments, a positive and significant relation consistently existed between Δ and yield, regardless of other environmental conditions. Therefore, it appears that selection for high Δ can be used effectively as a means to improve yield in barley in water limited Mediterranean environments.

The relation between Δ and water use (or transpiration) efficiency *W*, was also influenced by variations in environmental conditions. In the plastic house the relation remained negative, as theoretically expected, despite changes in the relation between yield and Δ. In seasons with high requency of low temperatures it changed from negative at high moisture levels, to positive at low moisture, in seasons with high frequency of low temperatures. Therefore, selection for low Δ, as an indirect mean to increase *W* is dependent on whether yield and *W* are positively related under the targeted environment.

3.2.3. To develop a divergent selection experiment for ^{13}C discrimination and yield.

Prior to 1988 seven crosses were made between two parents with low ^{13}C discrimination and four parents with high ^{13}C discrimination. At the F_2 stage, 572 families were identified from the seven crosses. They were all analyzed for ^{13}C discrimination, and results were available by mid 1991.

In 1991 the F_5 families were planted in a replicated experiment in two locations, considered as highly and moderately moisture stressed.

3.2.3.1. Relation between yield and ^{13}C discrimination in the F_5 generation (1991/92 season):

In 1994, analysis for ^{13}C discrimination in all families at the F_5 generation (572 families plus families which were separated into black and white seeded entries), was completed. Results show that Δ and grain yield were positively and significantly related in six out of the seven crosses and in the parents. The only exception was the first cross which showed a significantly negative relationship. This cross was between a landrace from Syria (SLB 8-6) with low ^{13}C discrimination and an improved variety from Australia (WI 2198), which was identified as having a high ^{13}C discrimination (Acevedo, 1993) (Figure 1).

3.2.3.2. Relation between yield and ^{13}C discrimination in the F6 generation (1992/93 season):

Contrary to the results of the F_5 generation, carbon isotope discrimination analysis which was carried out on 225 selected families in the F_6 generation show that the relationship between above ground dry matter production (AGDM) and Δ, and grain yield and Δ were negatively and significantly related in all seven crosses (Figures 2 & 3) Correlations were higher for AGDM than GY.

The shift in the relation between yield and Δ in the F_5 and F_6 generations is likely to be associated with variation in environmental conditions between the two seasons (Figure 4).
Rainfall average for 1991/92 and 92/93 seasons were 263 and 283 mm respectively. However, the two seasons were different in rainfall distribution and, in particular, in their temperature regimes. During the critical period of grain filling in March and April rainfall was substantially higher in the 1992/93 season in comparison with 1991/92 (45 vs. 20 mm). Moreover, mean temperature during the winter months was higher in 1992/93 in comparison with 1991/1992 season (7.2 vs. 4.6 °C). Frost occurrence and severity were much higher in 1991/92 season (Figure 4), especially during the rapid growth period in March and April (37 days in comparison with 12).

In the field experiment a negative relationship existed between yield and Δ in environments with a high amount and favorable distribution of rainfall in combination with mild temperatures during critical periods of plant growth. Such conditions reduce environmental effects on stomatal conductance and imply that variations in ci/ca ratio, and consequently in Δ, are less affected by stomatal conductance and more controlled by variation in the photosynthetic capacity of the genotypes (Condon et al., 1987). Therefore, the observed negative relation between yield and Δ in the F_6 generation, grown in 1992/93 season, is most likely related to the milder temperature and more favorable distribution of rainfall during that season. In environments where low temperature occur during early growth and high temperature and low moisture availability occur during grain growth stage, such as the 1991/92 season, it is expected that variations in Δ among genotypes are more controlled by variations in ci/ca caused by variations in stomatal conductance, than by variation in photosynthetic capacity, or by combinations of the two factors. The positive relation

between yield and Δ which is expected in such situations, has been observed in the F_5 generation during 1991/92 season (Figure 1). The consistent negative relation between yield and Δ observed in cross 1 (between SLB 8-6 and WI 2198) indicates that stomatal conductance in genotypes of this cross is less affected by variations in environmental conditions. Therefore, Δ (or ci/ca) is influenced by variation in photosynthetic capacity of the genotypes and a negative relation dominated. The stability of the relationship in this cross need to be studied further in different years and environments (or locations). If the relation between yield and Δ prove to be consistent in response to a changing environment, then crosses might provide valuable material for utilizing Δ in selection for dry Mediterranean environments.

3.2.3.3. Relations between ^{13}C discrimination measured at different generations

Regressions between Δ measured at the F_2, F_5 and F_6 generations showed that significant correlations existed between the F_5 and F_6 in four crosses and in the mean of all crosses (Table 1).

Correlation between Δ measured at F_2 and F_5 were not significant in five crosses. There was a significant positive relation in cross 1, and a significant negative correlation in cross 4. Similarly, correlations between F_2 and F_6 generations were not significant in five crosses but there was a positive and significant in cross 4 and a negative and significant correlation in cross 5 (Table 1).

An important measure of the suitability of Δ as a selection criteria for barley yield improvement in water limited environments is the stability of the trait across environments and generations. The response across generations showed that early (F_2) and late generations (F_5 and F_6) correlated poorly, while later generations (F_5 and F_6) had better correlations despite the fact that the relation between yield and Δ was mostly reversed between the two generations (Figures 1 & 2). Heritability of Δ was estimated as realized heritability measured between F_2 and F_6 generations according to the following:

Realised Heritability = Direct response to selection / selection differential (in standard units)

$$h^2 = \frac{(^{13}C^{+} - R) - (^{13}C^{-} - R)}{(^{13}C^{+} \text{ at } F_2 - {}^{13}C^{-} \text{ at } F_2)}$$

where:

$^{13}C^{+}$, mean Δ in F6 entries selected for high ^{13}C discrimination in F_2

$^{13}C^{-}$, mean Δ in F6 entries selected for low ^{13}C discrimination in F2

R , mean Δ of randomly selected entries

$^{13}C^{+}$ at F_2 , mean F_2 Δ for entries selected for high ^{13}C discrimination

$^{13}C^{-}$ at F_2 , mean F_2 Δ for entries selected for low ^{13}C discrimination

Heritability values for mean response of all crosses or for individual crosses were very weak ranging from 0.016 (for crosses mean) to a maximum of 0.13 in cross 7. Low heritability values in wheat grown in stressful environments have been recently reported by Ehdaie and Waines (1994).

An important objective of the divergent selection experiment was to estimate the efficiency of selection based on ^{13}C discrimination (correlated response) relative to selection based on grain yield itself (direct response to selection). The following formula was used for such estimation:

$$\frac{CR}{R} = \frac{(GYS\ of\ ^{13}C^{+} - GYS\ of\ R) - (GYS\ of\ ^{13}C^{-} - GYS\ of\ R)}{(GYS\ of\ GYS^{+} - GYS\ of\ R) - (GYS\ of\ GYS^{-} - GYS\ of\ R)}$$

Where:
GYS of $^{13}C^{+}$, grain yield under stress for entries selected for high Δ
GYS of $^{13}C^{-}$, grain yield under stress for entries selected for low Δ
GYS of R, grain yield under stress for randomly selected entries
GYS^{+}, grain yield for entries selected for high grain yield under stress
GYS^{-}, grain yield for entries selected for low grin yield under stress.

The advantages of using Δ in barley selection relative to yield itself (CR/R) ranged from 0.41 for the mean of all crosses to 0.62, 0.48 and 1.03 in crosses 1, 7 and 3 respectively. In the other crosses values were low. Only in one cross values exceeded 1, i.e. Δ was higher than selection for grain yield. However the mean value of 0.41 represents a reasonable figure given the fact that selection for Δ was based on measurements done at an early generation (F_2), whereas selection based on grain yield requires several generations of selection and a large amount of resource investment (Acevedo, 1993). Measurements were made on peduncles at the F_2 stage and results from the field work showed that measurements made on grains are better correlated with grain yield (Dakheel et al., 1994). Therefore, if the technique of ^{13}C discrimination measurements can be tuned to become more efficient and cheaper (this already has been achieved in New Zealand) then it might be used effectively as an early assessment of yield potential of F_2 plants, thus reducing the amount of lines for yield testing which is an expensive, resource demanding and time consuming process.

The crossing experiment gave results similar to the line source and plastic house experiment, showing that the relation between ^{13}C discrimination and yield (and consequently with water use efficiency) is variable and influenced by variation in both rainfall amount and distribution and the temperature regime. However, within a season the relation between yield and Δ were highly significant more than any other trait measured in these experiments (data not shown) such as days to heading, harvest index, peduncle length all of which usually have high association with yield. Therefore, the main limitation to using this trait effectively (which has a strong physiological link with yield and transpiration efficiency, as an effective selection criteria to improve or achieve higher yield stability in barley in rainfed Mediterranean environments) is the shift in the relationship with environmental change. To select for a high or low Δ (or low and high water use efficiency respectively), a detailed analysis of the long term pattern of environmental conditions that prevail in the targeted environment where selection is planned should assist in making the appropriate selection decisions.

A pattern from the three components of the project emerged which showed that in highly moisture stressed conditions, in particular in association with temperature stress at early growth stages and high temperature stress at grain filling period, a positive relation between yield and Δ is dominant,

and therefore selection for high ^{13}C discrimination is an effective tool to improve yield in barley in such environments However, if the targeted environment is highly variable from one season to the other, then the use of the technique has to be made in relation to the environmental conditions of the season in which selection is made.

A most interesting finding of the selection experiment is the clear shift in the relation between yield and Δ in such a large number of genotypes which were examined in those consecutive seasons where temperature and rainfall pattern differ. We are unaware of any other work which involve this number of genotypes and which clearly demonstrate such a shift in relationships among seasons. Therefore, these results may stimulate discussions and new research initiatives focusing on physiological mechanisms which control the relation between yield and ^{13}C discrimination in relation to temperature and vapour pressure deficit (VPD) extremes. Genotypes, such as those of the first cross which show stability in response to a changing environment are of particular importance from both a scientific and breeding point of view.

The conclusions on barley are enhanced by results from other work which was also conducted at ICARDA on the relation between yield and ^{13}C discrimination in durum wheat. The results of this work showed that a positive relation between grain yield and Δ consistently existed in five different environments, overlapping with those when the barley experiments were conducted. The consistent positive relationships indicate that for durum wheat in a Mediterranean water limited environment, the controlling factor over ci/ca ratio, and consequently Δ, is variation in stomatal conductance rather than variation in photosynthetic capacity. While in barley, which is better adapted to harsh environmental conditions, variation in stomatal conductance or photosynthesis capacity dominate according to the level of environment stress, and consequently could give either a positive or a negative relation between yield and ^{13}C discrimination (Condon et al., 1987, Wright et al., 1994). These findings are worth further investigation.

4. IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER

As stated above the project findings should have a major impact on the way the relation between ^{13}C discrimination and yield is evaluated in the future, especially for crops grown in marginal environments which are subject to multiple stresses such as the drought prone areas in the world where there are also significant changes in temperature during the growing season. Major institutes in the USA and Australia working on the technique could be interested in the results of this work. With regard to the secondary objectives of the project, several measurements were made to address the specified objectives, in particular those which are related to traits which can be used as surrogates for Δ. Results show that none of the traits considered; such as peduncle length, specific leaf area, days to heading and grain filling period; gave a stable relation with Δ. This subject should be further examined in moisture stressed and non-stressed environments.

The project undoubtedly will contribute significantly to our understanding of the physiology of carbon isotope discrimination and its association with yield and water use efficiency. It will focus on new approaches in looking at the way crop adaptation may play an important role in determining the relation between yield and Δ under various environmental conditions. In terms of technology transfer there is a long way to go but only after a few seasons of working on the technique, considerable experience has been accumulated and both international and local

scientists have benefited from the project. In addition, Dr A. Dakheel a Syrian national, who has been actively involved in the project during the last 4 years, is now one of the more experienced and knowledgeable scientists in this area of research. He is regarded by his international associates in the same field, in leading institutes in USA and Australia. As a Professor in Ecophysiology in Syria and now lecturing in UAE, his experience and research findings have been included in his lecture series to many students throughout the Mediterranean region.

5. PROJECT ACTIVITIES/OUTPUTS

Drs. Acevedo, Dakheel and Peacock attended the International ^{13}C meeting held in University of California, Riverside, California in January 1992. A paper summarizing some of the early work was presented by Acevedo (1993). The paper "Carbon isotope discrimination and water use efficiency in barley under field conditions" (Dakheel et al., 1994) was presented at the Association of Applied Biologist meeting held at the University of Reading, UK, in July 1994. A paper on the plastic house experiments has been recently prepared (attached) and will be submitted to the Australian Journal of Plant Physiology. Other papers relating to the line source sprinkler work and two papers related to the selection experiments are planned for completion during the coming year. A synthesis paper relating to the technique of ^{13}C discrimination to barley and durum wheat yield response in Mediterranean environments is also planned.

Dr. Dakheel spent 6 months at the University of Utah, from October 1993 to April 1994, where he assisted in the analysis of 2400 samples and gained a close working knowledge of the technique and instrumentation involved. He presented a number of seminars in the USA and in Syria on the research topic.

6. PROJECT PRODUCTIVITY

At the beginning Dr. Edmundo Acevedo, then the principal physiologist at ICARDA, was nominated as the Principal Investigator and Professor Jim Ehleringer, from the Department of Biology, The University of Utah, Utah, USA, agreed to do the ^{13}C analysis in their Stable Isotope Ratio Facility for Environmental Research. This was in accordance with the financial protocol of the USAID grant. In late 1990, Dr. Abdullah Dakheel was appointed as a part-time researcher to carry out the project. The line source sprinkler experiment and the transpiration efficiency (TE) plastic house experiments were both completed after three seasons of field work. However, completion of the research required analysis of the plant material for ^{13}C discrimination.

In July 1991, one year before the project was due to end, Dr. John M. Peacock was appointed Principal Physiologist, following Dr. Acevedo's departure to CIMMYT, Mexico, and thus inherited the position of Principal Investigator of the project. Dr. Dakheel remained as the principal research scientist in the project.

In January 1992, 6 months before the project was to end, the first set of results from Utah was received. The results were extremely encouraging and some of the analysis was presented by Dr. E. Acevedo at a the Symposium on ^{13}C held in Riverside California (Acevedo, 1993). During the Symposium Drs. Peacock and Dakheel had detailed discussions with the two leading groups on

^{13}C from USA and Australia and it was agreed that, although the project was well behind schedule, the preliminary results warranted the need to continue, as the work was pertinent to the current research of these two groups, (Richards and Condon, 1993, Condon and Richards, 1993). More samples were sent to Utah, but because of custom problems they did not arrive in USA until May 1992 and were eventually analysed in September 1992, after the project had officially ended.

In August 1992, based on the problems mentioned above, and the need to complete the third objective, ICARDA applied to the USAID R & D office for a "no cost" extension. This was subsequently granted, for two further growing seasons, until June 1994. It was also proposed at the same time that Dr. Dakheel go to Utah to carry out the analysis of the samples in order to speed up the completion of the project. He was scheduled to leave at the end of 1992, once the May samples had been analysed and the results examined. This was necessary for the selection of the remaining samples from the later generations of crosses. Again due to problems in getting samples analysed in Utah, Dr. Dakheel finally left in October 1993 and completed the analysis in April 1994.

In December 1994, when Dr. Dakheel was still analyzing data, he was requested by Aleppo University to conduct the Plant Ecology course at the University of UAE. Dr. Dakheel left on February 1st, 1995, and returned at the end of June, 1995. He has now taken up a full time teaching position at the same university.

Up to this stage the project accomplished the proposed objectives. The initial design of the project was overly complicated including field, plastic house and a largely crossing program, conducted over several seasons requiring large amounts of resource investment. In certain seasons experimental plots exceeded 3500 entries. The project was too ambitious for the time allocated. Despite the delays in sample analysis, the field work for all components of the project was completed within the extension period. The number of samples required for the analysis of ^{13}C discrimination were extremely large and the fact that the sampling analysis and field work was completed by Dr. Dakheel in 1994 is notable. Only the main results are presented in this report. Further work on the vast amount of data will hopefully be presented in further publications during the coming two years.

7. FUTURE WORK

Clearly this project should lead to future work. Both the lead institutes in the USA and Australia are intrested in the findings. The scale of this project and the harsh conditions under which the research was done make it unique. We believe that the findings have marked implications for the future applicability of the technique in harsh environments such as these. Already some of the findings have generated considerable interest, especially the shift in the relation between yield and Δ with changing environmental conditions. This will stimulate research directed toward better understanding the physiological mechanisms that affect Δ, and the way they are influenced by environmental factors.

The fact that the parental material which was used in the crossing program had a lower variability in Δ than was anticipated, induces us to believe that an excellent opportunity now exists to select genotypes with larger differences in Δ and use them in a new crossing program to develop new

barley material suitable for stressed Mediterranean environments. Further, the crosses that were consistent in the relation between yield and ^{13}C discrimination need to be studied further and tested in a wider range of environmental conditions and more seasons. These two points could be addressed in a future proposal which could be submitted to the USAID for support. Finally, the work on durum wheat needs to be considered together more general with the work on barley needs to be considered together so as to reach conclusions about the suitability of Δ as a selection criteria in cereals in stressed environments.

We had hoped that ICARDA would have had the funds to continue with this initiative, but at present, given the current budgetary constraints within the CGIAR system, and staff reductions within the physiology group and cuts in other resources, ICARDA will be unable to continue this initiative without a major injection of funds and resources.

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Table 1. Correlation coefficients between Δ measured in seven bariey crosses, grown in dry environments, at F_2, F_5 and F_6 generations.

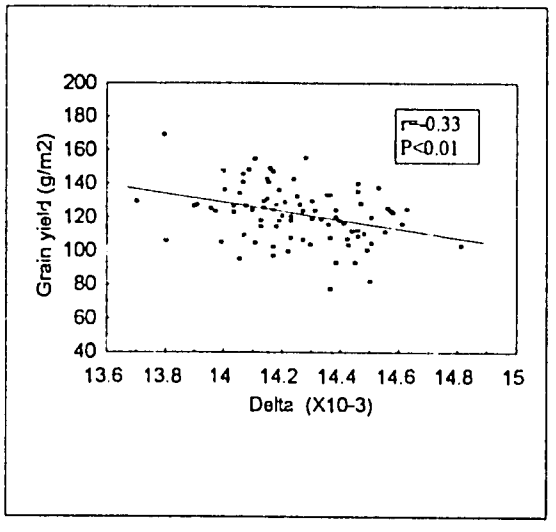
	ΔF_2	ΔF_5
Cross 1 (n=27)		
ΔF_5	0.35*	
ΔF_6	-0.08ns	0.21ns
Cross 2 (n=34)		
ΔF_5	0.22ns	
ΔF_6	0.05ns	0.49**
Cross 3 (n=12)		
ΔF_5	0.36ns	
ΔF_6	0.22ns	0.41ns
Cross 4 (n=23)		
ΔF_5	-0.38*	
ΔF_6	0.40*	-0.22ns
Cross 5 (n=8)		
ΔF_5	-0.51ns	
ΔF_6	-0.74**	0.82**
Cross 6 (n=32)		
ΔF_5	0.053	
ΔF_6	0.024ns	0.58***
Cross 7 (n=21)		
ΔF_5	0.31ns	
ΔF_6	0.12ns	0.63***
All crosses (n=163)		
ΔF_5	0.15*	
ΔF_6	0.07ns	0.22**

*,**,*** denote a significant correlation at P<0.1, 0.05, and 0.01 respectively.

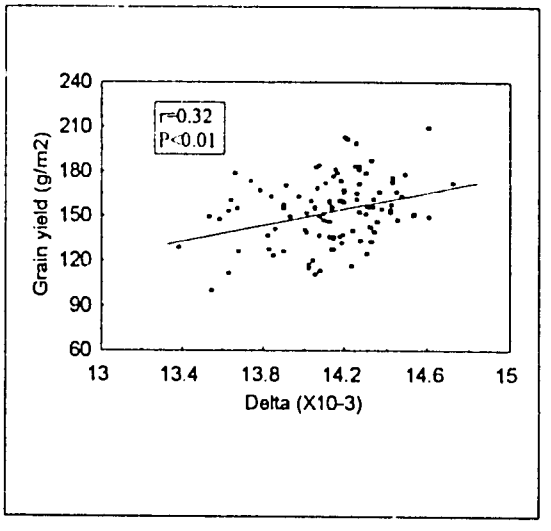
Fig. 1

Relationship between 13C discrimination and grain yield in seven barley crosses in F5 generation planted in dry environmnet (Breda) during 1991/199 season.

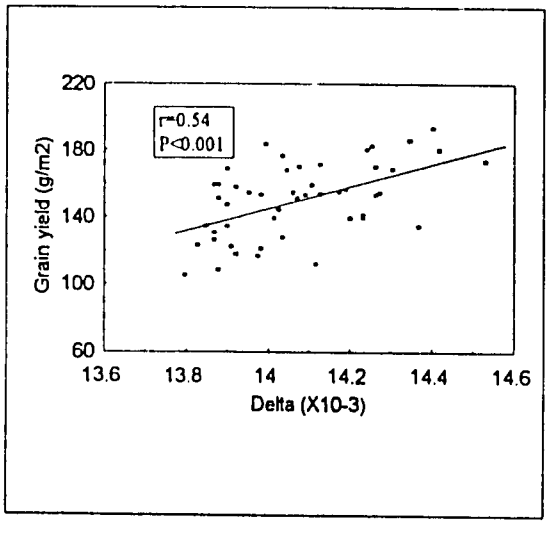
Cross 1: SLB 8-6 (L) X WI 2198 (I)



Cross 2: SLB 8-6 (L) X ROHO (I)



Cross 3: Tadmor (L) X SLB 62-99 (L)



Cross 4: Tadmor (L)X Roho (I)

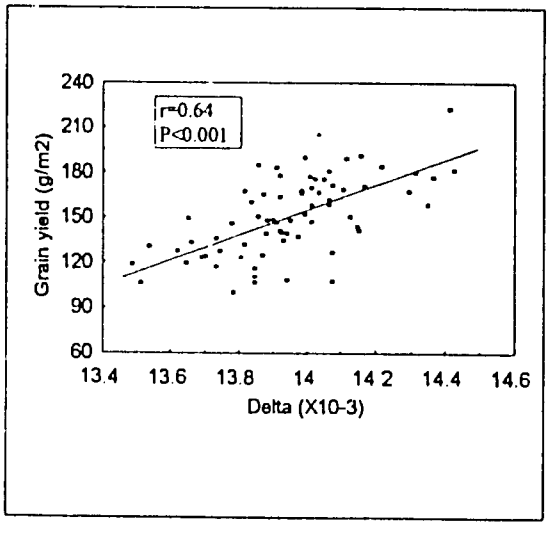
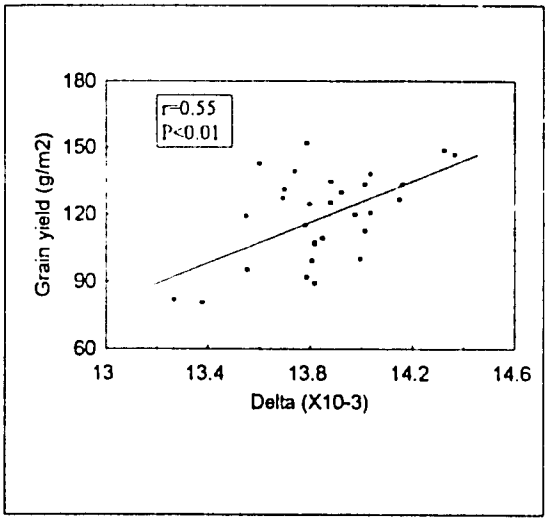
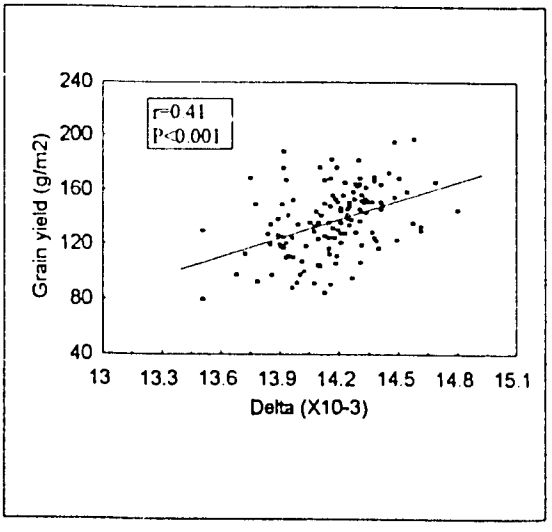


Fig. 1. (Cont'd..)

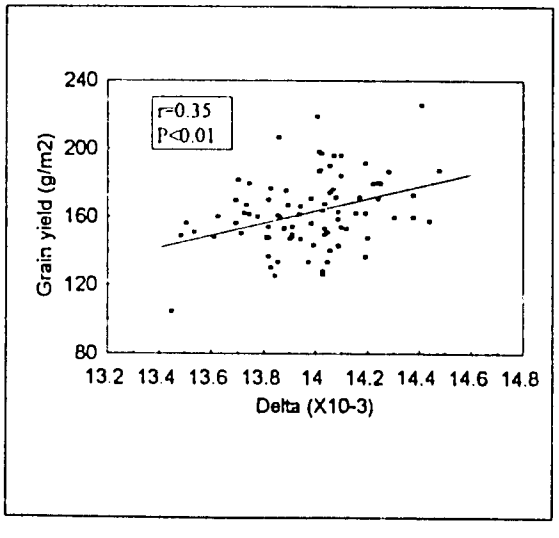
Cross 5: SLB 62-35 (L) X Tadmor (L)



Cross 6: SLB 8-6 (L) X SLB 62-99 (L)



Cross 7: Tadmor (L) X WI 2198 (I)



Parents (P) plus checks (C)

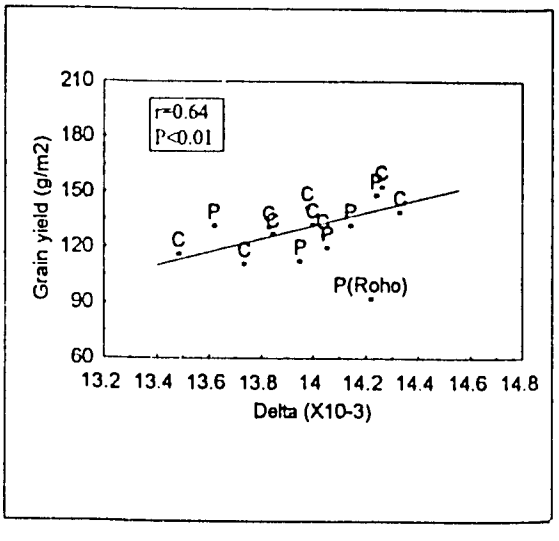
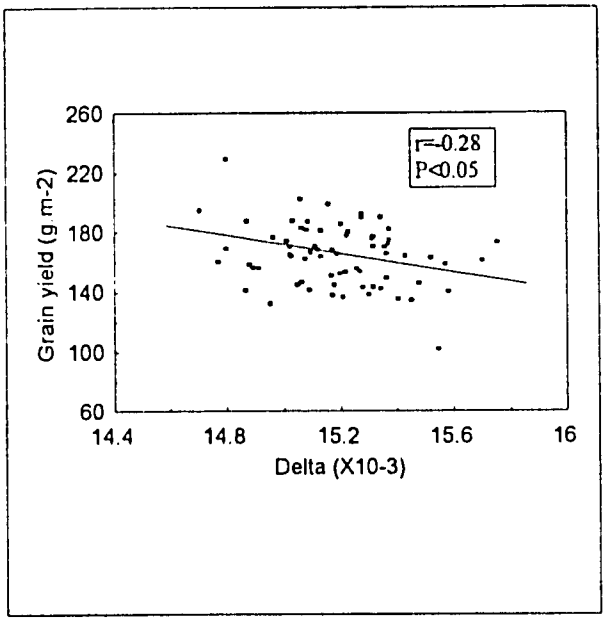


Fig. 2

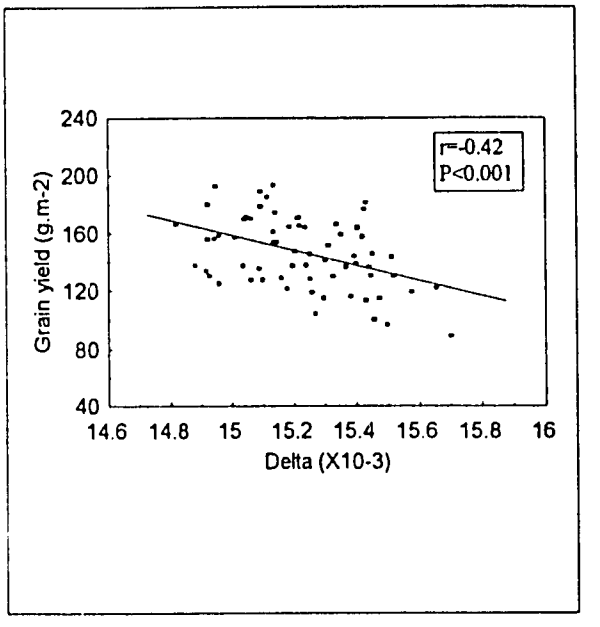
Relationship between 13C discrimination and grain yield in seven barley crosses in F6 generation planted in dry environment : 1992/93 season

Crosses 1 & 2



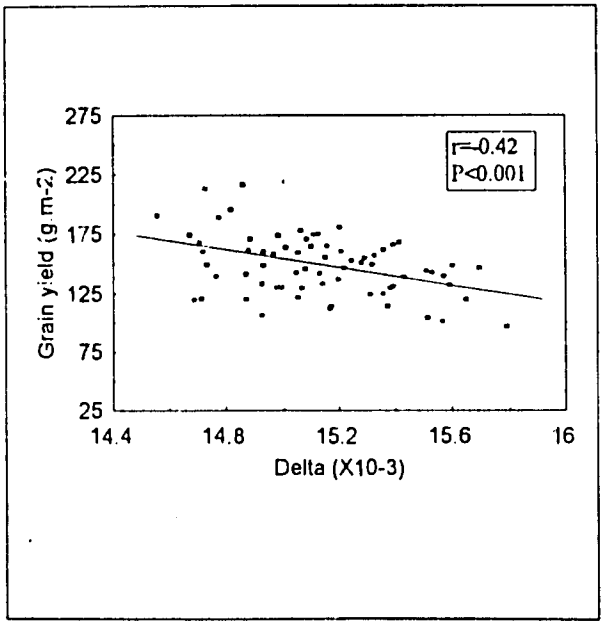
n=69

Crosses 3, 4 & 5



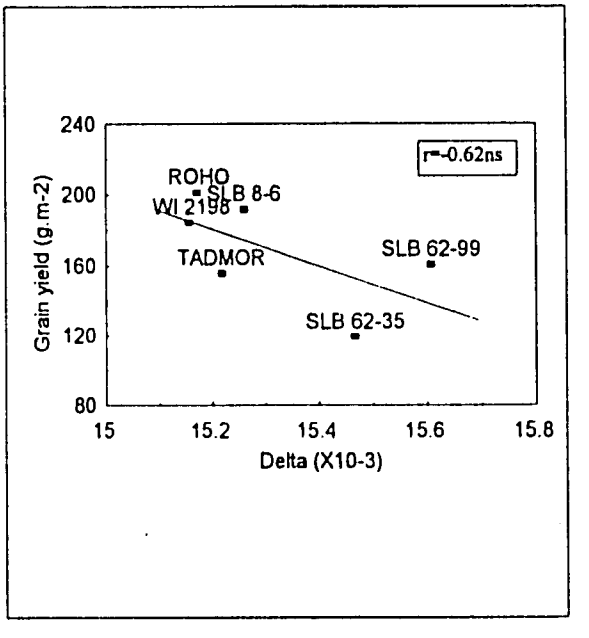
n=67

Crosses 6 & 7



n=69

Parents

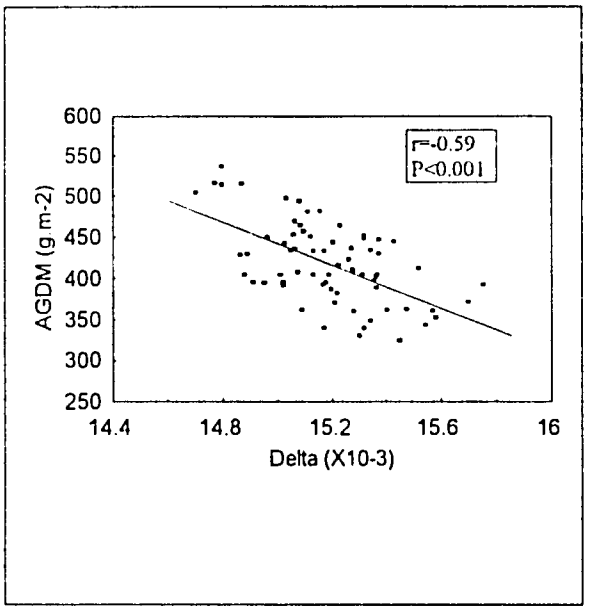


n=6

Fig. 3

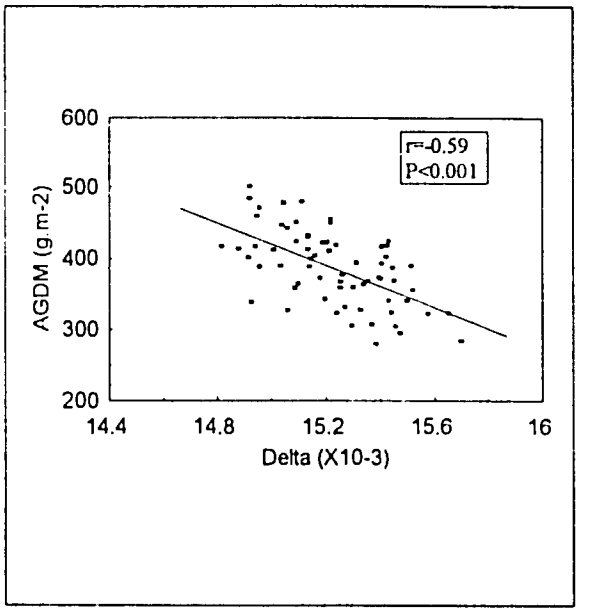
Relationship between 13C discrimination and above ground dry matter production (AGDM) in seven barley crosses in F6 generation planted in dry environment : 1992/93 season

Crosses 1 &2



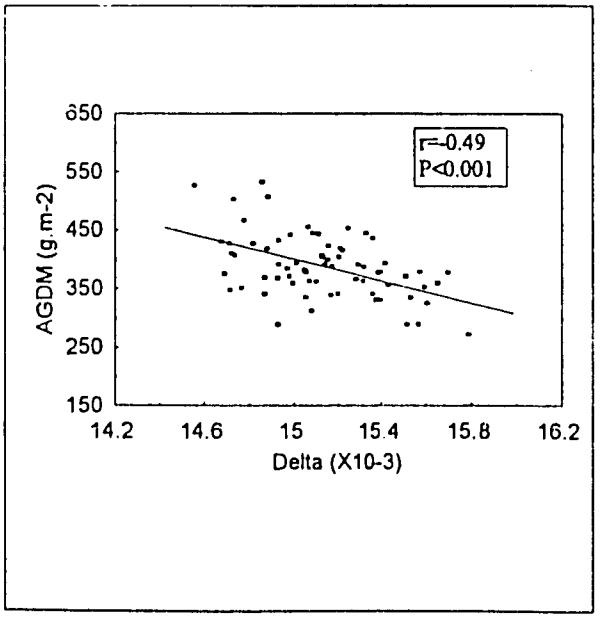
n=69

Crosses 3, 4 & 5



n=67

Crosses 6 & 7



Parents

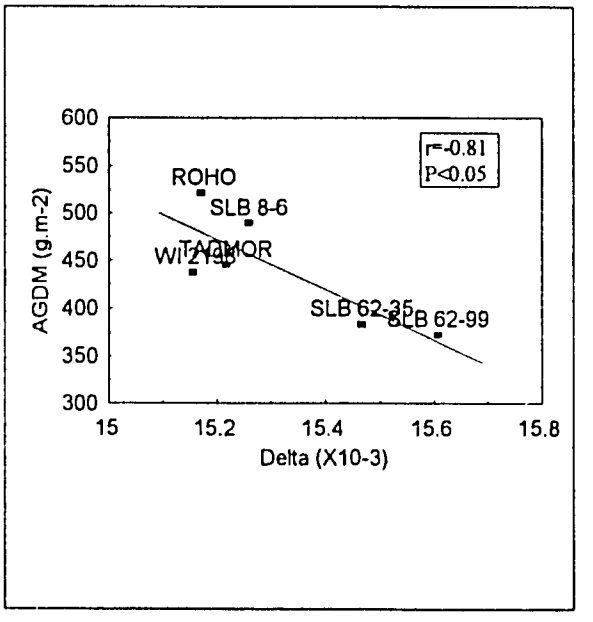
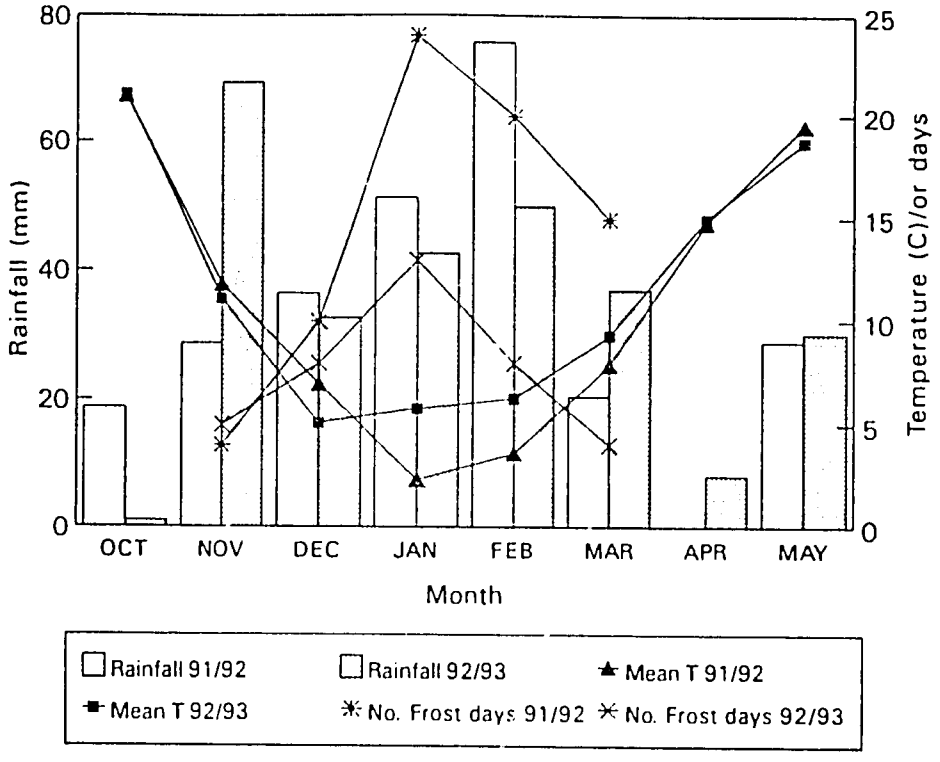


Figure 4. Weather data at the dry experimental site (Breda) during 1991/92 & 92/93 cropping seasons



26

Potential of Carbon Isotope Discrimination as a Selection Criterion in Barley Breeding

E. Acevedo

I. Introduction

The effectiveness of selection for yield per se is relatively low because of the large number of genes involved and the additional attributes required by new cultivars. Hence, it is difficult to find desired gene combinations in the progenies. Many economic traits like grain yield have low heritability values since they are controlled by many genes and their expression is greatly influenced by the environment (Kronstad and Moss, 1991). Therefore, the use of associated traits, such as physiological attributes, in selecting for yield is becoming more important. In determining the possible role of a physiological trait, it is useful to keep in mind the various steps in a breeding program. These include (1) identifying factors that limit yield; (2) determining if genetic variability exists and the nature of this variation for specific attributes; (3) identifying the most promising parents to hybridize; (4) applying appropriate selection pressures to isolate the desired progeny; and (5) evaluating, multiplying, and disseminating new cultivars. Physiological traits may play a role in the first four steps.

The associations between physiological mechanisms and yield potential as well as adaptation of winter cereal genotypes to abiotic stresses are being intensively studied with the aim to define breeding goals and increase efficiency (Evans, 1987; Acevedo *et al.*, 1991b; Quisenberry, 1982; Srivastava *et al.*, 1987). Plant breeders have only slowly adopted traits related to these mechanisms for use in selection because of (1) the difficulty in measuring the trait or mechanism in a wide range of genetic materials, (2) the lack of convincing evidence to support a relationship with increased yield or increased stability of production, possibly due to the small number of appropriate selection experiments (Ceccarelli *et al.*, 1991. Acevedo *et al.*,

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1991c), (3) insufficient knowledge about inheritance of the character, and (4) inadequate cross-disciplinary (physiology/breeding) communications.

There now appears to be widespread acceptance that if physiological traits are to be useful in breeding, they must comply with some minimum criteria. These include (1) a greater heritability than yield, (2) a significant correlation with yield and/or stability of yield, (3) a causal relationship with yield, (4) physiological assays that are easy to use, and (5) the possibility of screening early generations when yield per se cannot be assessed (Acevedo and Fereres, 1992). Ideally physiological techniques that are nondestructive, repeatable, and adaptable to evaluating large numbers of plants at specific stages in the growth cycle would be most useful to breeders in providing a greater degree of selection efficiency.

In this chapter, the potential of carbon isotope discrimination (Δ) as an indirect selection criterion in barley breeding for dry environments is analyzed. The analysis refers to information obtained at the International Center for Agricultural Research in the Dry Areas (ICARDA) during 1985–1990. Part of the work was done in collaboration with the Institute of Plant Science Research (IPSR), Cambridge, United Kingdom.

II. Carbon Isotope Discrimination

Carbon isotope discrimination has been proposed as an indirect selection trait to assist in genotype selection of C_3 species for drought environments (Farquhar and Richards, 1984; Austin *et al.*, 1990; Richards, 1991). Both stomatal control of assimilation and the assimilative capacity of photosynthetic organs affect the discrimination against atmospheric CO_2 and contribute to determining the Δ value. From the values of inherent discrimination of $^{13}CO_2$ by diffusion and carboxylation, it can be inferred that as the stomata open wider, thus increasing the internal partial pressure of CO_2 (p_i), the discrimination by RuBisCO will be greater as compared to discrimination by diffusion, increasing the overall Δ value. The overall balance between photosynthesis and transpiration is such that increased p_i, due to the wider stomata openings and hence a greater Δ value, is associated with decreased transpiration efficiency (W). Our work shows that the relation between Δ and W is indeed negative in barley, which agrees with results reported by Hubick and Farquhar (1989) (Fig. 1).

The Δ value is an interesting parameter to look at as an indirect selection criterion from a plant-breeding perspective, since Δ is a measure of gas exchange integrated over time and hence provides a seasonal average of W. This is an important advantage for a physiological trait to be used in breeding since the instantaneous and/or short-term processes of photosynthesis (A) and transpiration (E) are too variable with weather fluctuations to be of much value as selection criteria. It can be argued that the Δ value is high in the hierarchy of processes determining biomass production in C_3 plants, a requirement for a trait to be useful in breeding at present (Passioura, 1986;

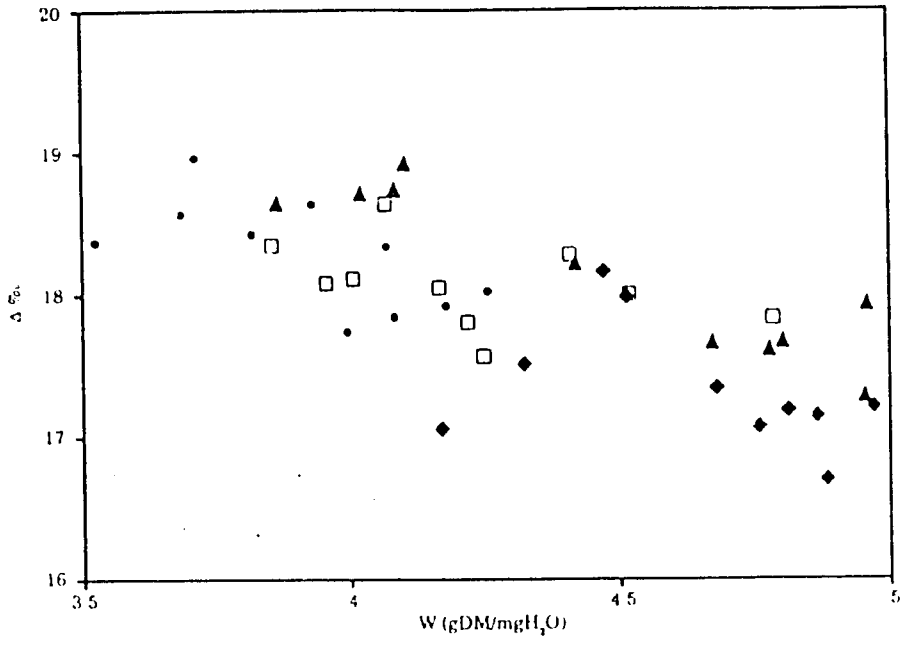

Figure 1. Relation between Δ measured in the peduncle and transpiration efficiency (*W*) calculated based upon the mean growing period vapor pressure deficit (vpd = 0.53 kPa). Ten barley genotypes were grown in a greenhouse at temperatures of 25°C maximum and 5°C minimum through heading, the temperature regime was changed to 25/10 from heading to maturity. Four watering treatments in 5-kg soil (3 sand : 1 clay) pots were given immediately after planting: watering to field capacity when the pots reached one-half of the available water (FC, dots), watering to one-half available water when the pots reached the wilting point (½ FC, diamonds), ½ FC through heading and FC from heading through physiological maturity (½ FC—FC, triangles), and FC through heading followed by ½ FC to maturity (FC—½FC, squares). The surface of the pots was sealed with aluminum foil perforated to allow two plants per pot to pass through (Acevedo, unpublished).

Acevedo and Ceccarelli, 1989). At the plant level, yield under drought stress can be expressed as the product of three terms that are part of an identity: transpiration (*E*), transpiration efficiency (*W*), and harvest index (HI) (Passioura, 1977, Fischer and Turner, 1978). If these terms are largely independent, increasing any of them would increase yield. It follows that, in dry environments, genotypes with low Δ would perform better (higher *W*)—other factors being similar and the interactions between the three terms being nonsignificant, but the latter is highly unlikely.

A. Experimental Work Being Conducted at ICARDA on Carbon Isotope Discrimination by Barley

The experimental approach that ICARDA used to evaluate the potential of carbon isotope discrimination as a selection criterion in barley breeding for abiotically stressed environments involved studying the relationship between genotypes for Δ values and barley grain yield under stress. After a relationship between Δ and yield was shown to exist for the most stressed

environments, experiments were designed to test if the relationship would hold in spaced plants, typical of F_2 generation plantings. The correlation between dense and spaced plantings indicated that spacings used in breeding nurseries would not affect genotype ranking significantly and therefore the study should be pursued further.

A set of two-row barley genotypes differing in grain Δ was planted in a line source sprinkler system (LSS) to determine if there was a genotype × drought-stress interaction. The plantings were done during the 1989 season at Breda in northern Syria, an experimental site with a Mediterranean-type climate. Although the site has a long-term average rainfall of 281 mm, only 180 mm fell during the 1989 season.

The same set of genotypes studied in the LSS was grown in a greenhouse at Tel Hadya, ICARDA's headquarters, under four moisture regimes in order to reassess the correlations between Δ and transpiration efficiency (total biomass production/transpiration). Positive correlations between Δ and grain yield under drought stress had been found in previous studies, implying a negative relationship between *W* and yield (e.g., Condon *et al.* 1987; Craufurd *et al.*, 1991).

Crosses were made between high and low Δ two-row barley genotypes and Δ was determined in the F_2 progeny of each of seven crosses. F_3- and F_4-derived bulks from each F_2 plant were grown to study genetic parameters and the breeding implications of early-generation (F_2) selection for Δ.

B. Carbon Isotope Discrimination and Grain Yield

A strong positive correlation ($r = 0.952$) between mean trial Δ in grain and mean grain yield was found for barley trials grown in dry environments (years and locations) as different as the dry Mediterranean types of northern Syria and trials in which drought was imposed by rain-out shelters in Cambridge in the United Kingdom (Craufurd *et al.*, 1991). High-yielding trials had higher Δ values and possibly lower *W*s (Fig. 2). The genotypes were the same in all trials, hence this is essentially an environmental effect on Δ values. As drought increased in severity (lower rainfall and higher vapor pressure deficit, vpd), Δ values decreased presumably mainly as a result of increased stomatal control of transpiration. Associated with the decrease in Δ values was a corresponding decrease in grain yield. Similar drought effects on Δ and yield have been found for wheat grown in an LSS in the Yaqui Valley of Mexico by CIMMYT (Acevedo, Sayre, and Austin, unpublished). The within-trial relationship between variety Δ values and grain yield was found to vary with environment. Where barley crops were droughted, the relationship was positive, as reported by Condon *et al.* (1987) for wheat in well-watered conditions. But for wheat the positive correlation was stronger as the water-stress level increased. In mild water-stress environments, the phenotypic correlation dropped to zero and it became negative when water was nonlimiting in apparent contrast to the results of Condon *et al.* (1987). We have found a significant correlation ($r = -0.853$) between trial mean grain yield and the correlation coefficient be-

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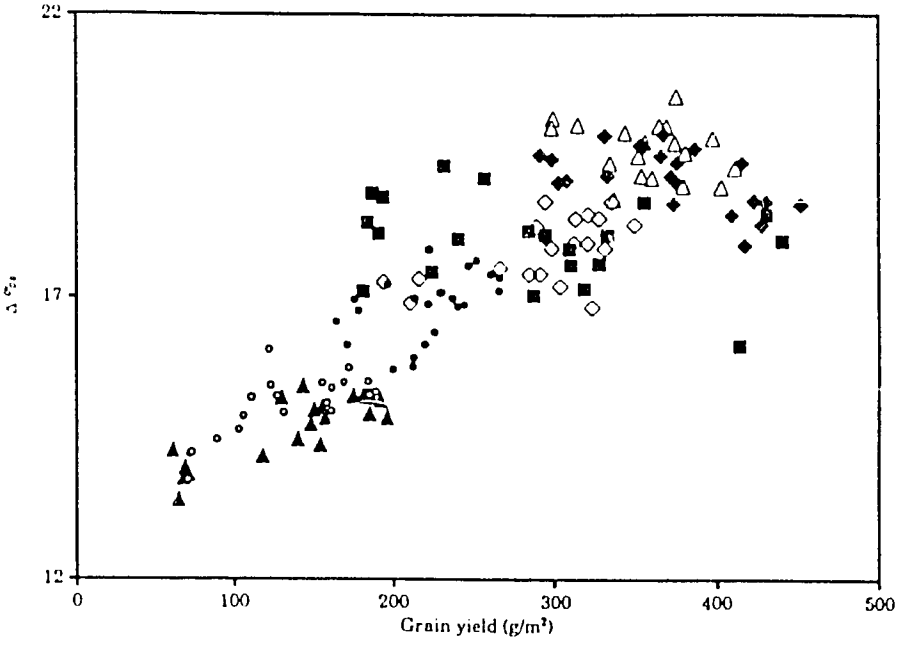

Figure 2. Grain yield has been found to be positively related to grain Δ. The data in the figure show the values of grain Δ and grain yield of 21 or 23 two-row barley genotypes grown under field conditions either in Cambridge (●, ◇, △) or in northern Syria (▲, ○, ■). The grain yield is indicative of the stress intensity.

tween variety grain yield and Δ as shown in Fig. 3 from Austin *et al.* (1990). Varieties with a high Δ value had higher yields in stressed environments with the reverse occurring in nonstressed environments. Furthermore, barley landraces from the Fertile Crescent tended to have a lower *W* than improved genotypes (Table II). We also noted a strong negative correlation between Δ and days to ear emergence (Craufurd *et al.*, 1991). It is possible that variations in earliness may have been responsible for differences in grain yield in the dry environments.

As expected from the previous discussion, significant correlations between Δ value and grain yield were not always found between genotypes. This correlation was weak but significant ($r = 0.316$, $P < 0.05$, $n = 45$) in a breeder's advanced barley yield trial at ICARDA in northern Syria (Acevedo and Ceccarelli, unpublished) despite significant variations in grain Δ values (ranging from 12.86 to 14.86‰) and very low seasonal rainfall (186 mm). The correlation in days to ear emergence and grain yield was nonsignificant in this trial. A barley yield trial showed no significant association between grain Δ value and grain yield nor grain Δ value and days to ear emergence despite a highly significant association ($r = -0.559$, $P < 0.001$, $n = 45$) between days to ear emergence and grain yield. The variation in Δ values across genotypes in the preliminary yield trial was highly significant, ranging from 12.88 to 14.42‰.

Many reasons have been suggested for the contrasting relationships

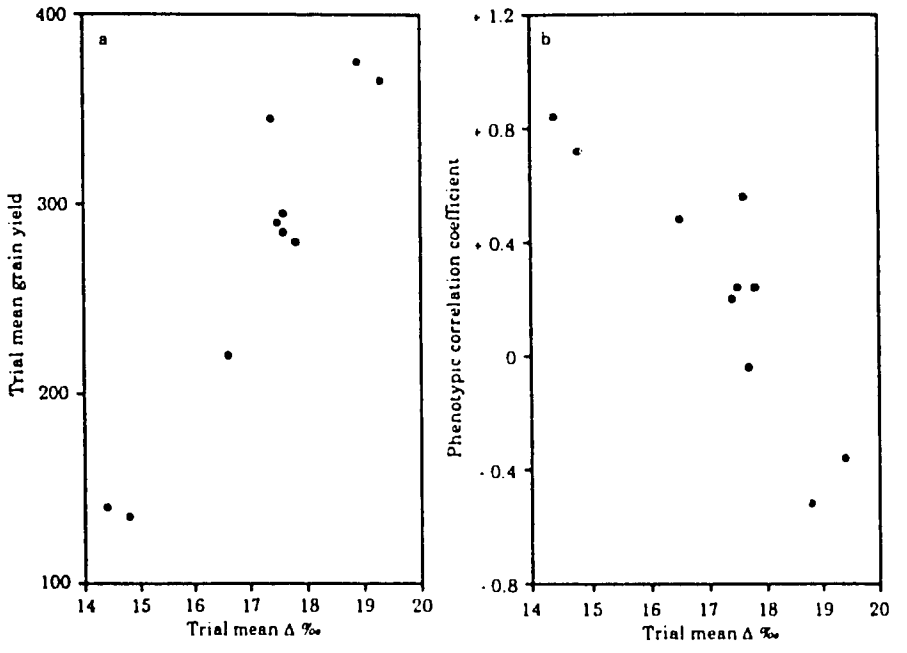

Figure 3. As the water stress decreased (a), mean trial grain yield and mean trial grain Δ increased. The within-trial phenotype correlation coefficient between grain yield and grain Δ was negatively related to trial mean Δ (b). Of the 10 trials in the figures, 6 were conducted in northern Syria and 4 in Cambridge. After Austin *et al.* (1990).

found between grain yield and genotype in dry and wet environments. Barley genotypes with early ear emergence, which are higher yielding in Mediterranean environments (Acevedo *et al.*, 1991a), can fix much of their grain carbon in a relatively drought-stress-free period and hence the value of Δ would be dominated by a relatively high p_i/p_a. The relationship between Δ and grain yield would be positive. Second, earliness is generally associated with early vigor and better ground cover and hence higher water-use efficiency at the crop level (by reducing soil surface evaporative losses) but not necessarily at the plant level (*W*) (Acevedo and Ceccarelli, 1989; López-Castañeda, 1992), resulting in higher yield under terminal drought stress. Low *W* genotypes that cover the soil quickly may have high crop water-use efficiency and high Δ values due possibly to a higher specific leaf area (SLA, m^2/g) (López-Castañeda, 1992). It is also possible that the tissue sampled may have fixed most of its carbon at a time when water stress was low, e.g., tissue produced before anthesis in terminal drought environments. At the crop level, the relationship between Δ and *W* may be affected because of an uncoupling of stomatal openings and transpiration caused by the leaf and crop boundary layer (Farquhar *et al.*, 1988; see chapters in this volume). High *W* may be associated with high Δ values in this case. The relocation of pre-anthesis assimilates to the grain may affect grain Δ values. This relocation increases with drought stress (Richards and Townley-Smith, 1987) and there seems to be variation among genotypes in

relocation of pre-anthesis assimilates (Blum *et al.*, 1983; Turner and Nicholas, 1987). Grain Δ would reflect p_i/p_a of the leaves that produced the stored assimilates. If relocation of assimilates substantially contributes to grain yield, grain Δ and grain yield would be positively associated. Under non-stress conditions, grain Δ will reflect carbon gain by leaves and other photosynthetic organs during grain filling because the proportion of relocated assimilates to the grain is low. A relatively low Δ due to partial stomatal closure may be observed in long-duration varieties that have high grain yields. This would show a negative relationship between grain Δ and grain yield. If stomatal conductance is equally high in all genotypes throughout the season, variations in p_i/p_a and hence Δ values would be a reflection of variation in photosynthetic capacity. Genotypes having a lower p_i/p_a and low Δ would have a higher yield.

The relationship between genotype Δ and grain yield is complex. It is dependent on stress level and stresses are usually present in combination. Other stresses may also influence yield in dry rain-fed Mediterranean environments (Ceccarelli *et al.*, 1991) where low winter temperatures and terminal heat stress are nearly always present. This fact would also complicate relationships between Δ values and yield.

C. Carbon Isotope Discrimination and Transpiration Efficiency

Carbon isotope discrimination has been shown, in theory and experimentally, to be related to transpiration efficiency (Farquhar *et al.*, 1982; Farquhar and Richards, 1984), and variation in Δ and W across genotypes has been reported in several C_3 species by various authors (e.g., Farquhar and Richards, 1984; Hubick *et al.*, 1986; Farquhar *et al.*, 1989). It has therefore been suggested that crop physiologists should search for exploitable variation in W by using Δ. Figure 1 shows that W and Δ are negatively related in barley, a finding that was also reported by Hubick and Farquhar (1989). Many authors have reported a linear variation of dry matter (DM) with water transpired, which implies a constant W (e.g., Arkley, 1963; Tanner and Sinclair, 1983), but as much as 35% of the variation among barley genotypes has been found to be unexplained by transpiration by Hubick and Farquhar (1989). Furthermore, the conclusion of a constant W is valid only if the regression of DM on E passes through the origin. Tables I and II show the significant variation in W and Δ among barley genotypes. Stressed plants had a larger W value than did well-watered ones. The rankings of stressed and mildly stressed genotypes were similar. The carbon isotope discrimination values for barley in Hubick and Farquhar (1989) were highly and negatively correlated to W for both whole plants and plant parts (leaves, heads, stems, and roots). Our greenhouse studies are in agreement with this finding, but they also show a negative relation between W and total biomass (Fig. 4) and a highly significant positive relationship between Δ measured in the peduncle or grain at harvest and total biomass and grain yield (ranging from 0.46 to 0.60, $P < 0.001$), probably indicating that in barley the greater leaf area is more than enough to counteract the effect of

Table I Δ at Various Irrigation Levels in a Line Source Sprinkler System[a]

Variety	Irrigation (mm)			
	82	181	325	Mean
Harmal	16.42	17.32	18.58	17.43
WI2198	16.18	17.78	18.96	17.67
WI2198/WI2291	16.49	17.43	18.79	17.61
Tadmor	16.21	17.29	18.96	17.49
Roho	16.46	18.02	18.89	17.77
SLB 39-99	16.53	17.95	19.00	17.76
SLB 62-35	16.32	18.19	19.28	17.97
SLB 62-99	17.39	18.09	19.80	18.36
SLB 8-6	16.70	18.54	19.66	18.24
SBON 96	16.45	17.26	18.79	17.47
Mean	16.51	17.78	19.07	

Note. LSD0.05 for genotypes at each irrigation level, 0.58; LSD0.05 for genotype means at each irrigation level, 0.18; LSD0.05 for irrigation levels at each genotype level, 0.55; LSD0.05 for irrigation means for each genotype, 0.26. The variety × irrigation interaction is insignificant.

[a]The Δ measurements were done in peduncles collected at harvest. The experiment was conducted at Breda, northern Syria, in the 88/89 season. Total seasonal rainfall was 180 mm.

Table II Transpiration Efficiency (*W*, mg DM/g H_2O kPa)[a] of Selected Two-Row Barley Genotypes[b]

Type	Genotype	FC		½ FC	
		W	Δ	*W*	Δ
Improved					
	Harmal	4.1	18.68	4.7	19.09
	WI2198	4.0	19.14	4.8	18.61
	WI2198/WI2269	3.9	18.93	4.7	18.68
	ROHO	3.7	19.45	4.6	18.54
	SBON 96	3.8	18.96	4.0	18.51
Landraces					
	Tadmor	3.9	20.08	4.6	19.20
	SLB 62-99	3.6	20.71	4.3	19.03
	SLB 39-99	3.5	20.18	4.3	19.48
	SLB 62-35	3.4	20.18	4.2	19.42
	SLB 8-6	3.8	20.76	4.5	18.61

Note. LSD0.05 for genotype *W* at the same level of watering, 0.51; LSD0.05 for watering treatment at the same level of cultivar, 0.56; LSD0.05 for Δ at the same level of watering, 0.67. The genotype × watering interaction is insignificant for *W* and Δ.

[a]Dry mass includes roots. Mean day time vpd: 0.53 kPa.

[b]Plants grown in pots in a greenhouse and irrigated up to field capacity (FC) or ½ FC. In the FC treatment the pots were allowed to dry until they reached one-half of the available water. In the ½ FC treatment the pots reached the wilting point before irrigation.

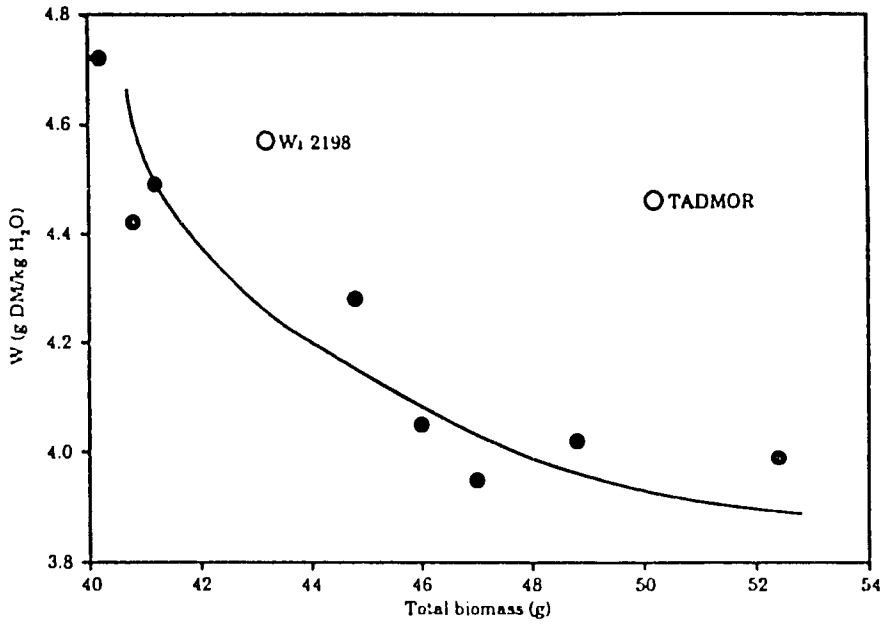

Figure 4. Mean transpiration efficiency and mean biomass production of 10 barley genotypes grown in a greenhouse under four irrigation treatments (described under Fig. 1). Each point in the figure represents the mean transpiration efficiency for a genotype.

a decreased W due to higher SLA (López-Castañeda, 1992). The significant difference in Δ values found for various plant parts by Hubick and Farquhar (1989) was attributed to chemical composition, to different contribution to carbon fixation by PEP carboxylation, or to temporal variations in p_i/p_a while carbon was being laid down in the various plant parts. Our barley work also shows such differences in Δ values between plant parts at a given time of measurement (Fig. 5). The grain tends to have lower Δ values when compared with leaf lamina and peduncle. This may be related to the time at which assimilates are laid down in the various organs. Of interest, however, is that for given sampling dates, anthesis, and maturity, the values of various organs were found to be highly correlated (Table III). Strong correlations among Δ values of different plant parts at various growth stages were observed by Craufurd *et al.* (1991). In other cases, weak correlations have occurred for genotypes sampled at the five-leaf stage, anthesis, and maturity in a Mediterranean environment (Table IV). This result may be explained by the temporal variation of plant-water status at the various growth stages.

D. Carbon Isotope Discrimination, Drought Resistance, and Stability of Yield

Breeders measure drought resistance as the ability of a genotype to yield under stress compared with standard cultivars. In low-rainfall environments, there is a high year-to-year variability of rainfall that results in a

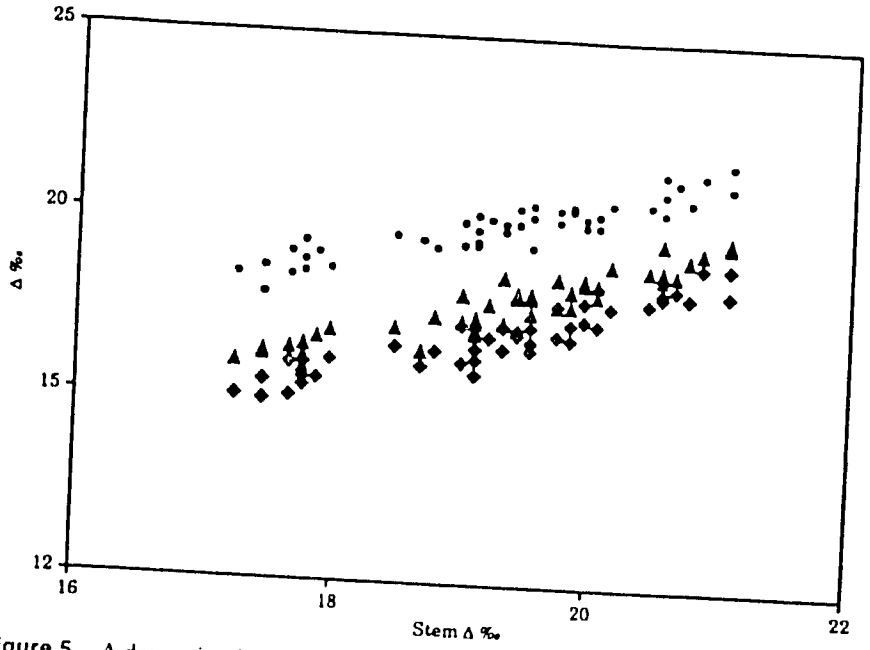

Figure 5. Δ determination at harvest of various plant parts of barley at various irrigation levels: leaf lamina (dots), peduncle (triangles), and grain (diamonds). Data from Breda, northern Syria, in the 1989 season.

high genotype × environment interaction for crops sown without irrigation. The joint regression analysis (Finlay and Wilkinson, 1963; Blum, 1988) has been used to assess both stress resistance and stability of yield across environments. In the joint regression analysis, a nursery of genotypes is grown in various environments and the yield of each genotype is regressed against the nursery mean yield of each environment. The nursery mean yield is used as an environmental index. At least two parameters

Table III Simple Correlation Coefficients for Δ of Various Plant Parts within Each of Two Sampling Times (n = 45; Line Source Sprinkler, Breda, Northern Syria)

		Stem	Peduncle	Leaf lamina
Anthesis				
	Stem	1.000	—	—
	Peduncle	0.903***	1.000	—
	Leaf lamina	0.945***	0.878***	1.000
	Heads	0.899***	0.887***	0.920***
Maturity				
	Stem	1.000	—	—
	Peduncle	0.946***	1.000	—
	Leaf lamina	0.931***	0.910***	1.000
	Grain	0.933***	0.959***	0.927***

***Significance at 0.001% level.

Table IV Correlations between Δ Measured in Various Plant Tissues at Various Growth Stages (n = 45; Line Source Sprinkler, Breda, Northern Syria)

		Anthesis			
	Five-leaf stage	Stem	Leaf lamina	Peduncle	Heads
Five leaf (whole plant)	1.000	0.195	0.154	0.210	0.172
Maturity					
Stem	0.004	0.226	0.223	0.320*	0.186
Leaf lamina	0.133	0.232	0.226	0.310*	0.188
Penducle	0.168	0.296*	0.284	0.383**	0.275
Grain	0.130	0.283	0.274	0.371*	0.234

*,**Significance at 0.05 and 0.01%, respectively.

characterize each genotype, the intercept of extrapolated genotype yield for a mean nursery yield of zero and the slope of the regression that represents the stability (or responsiveness) of the yield of the genotype as the environment changes. If the slope of the regression has a value of 1, the genotype has average (of the genotypes in the nursery) stability; a slope above 1 indicates below-average stability; a slope below 1 indicates above-average stability. In the joint regression analysis, the intercept may be

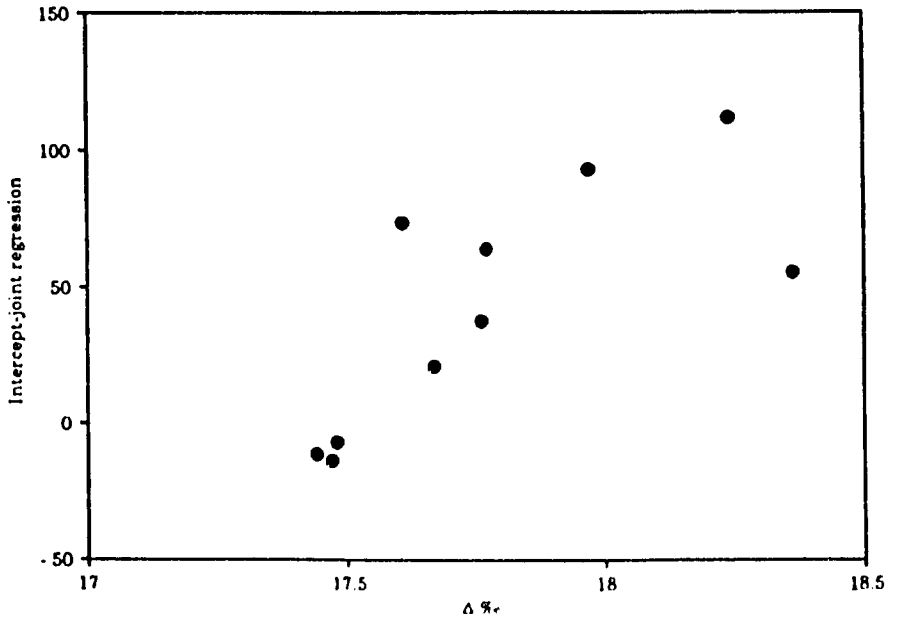

Figure 6. Intercept of a joint regression analysis and Δ. The values of Δ are the mean across irrigation levels in a line source sprinkler (Breda, northern Syria, 1988/1989). The genotypes are the same as those in Table II, grown across nine environments in northern Syria. The joint regression analysis was done using the nine Syrian environments.

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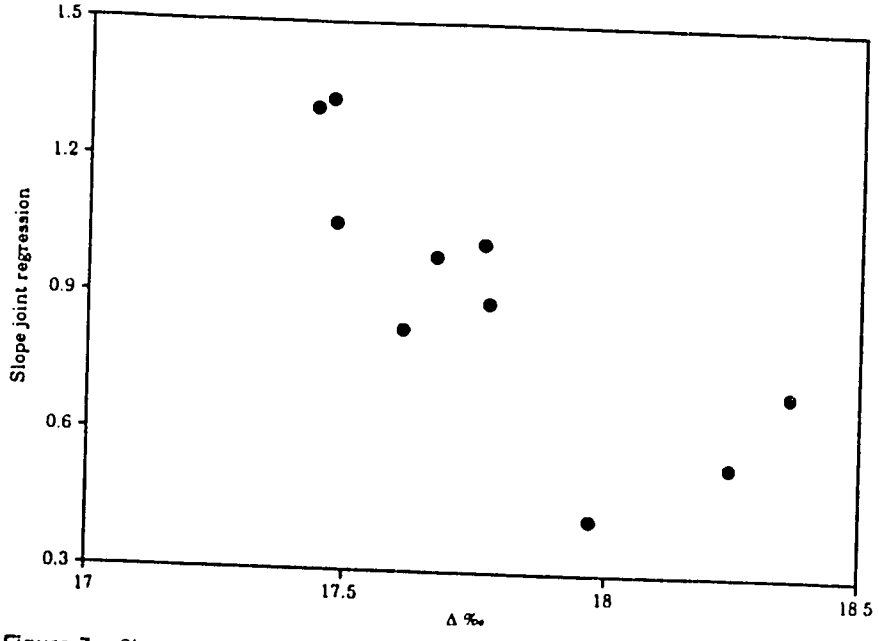

Figure 7. Slope of the joint regression analysis versus Δ. Data obtained as for Fig. 6

interpreted as a measure of the genotype yield under severe stress (stress resistance in breeder terminology) and the slope as a measure of how stable the yield will be with changing environments. Ideally, breeders are searching for genotypes with high stress resistance as well as responsiveness to an improving environment, or stable across years if the year-to-year variability is high.

The 10 barley genotypes in Table II were grown in nine environments in northern Syria (years and locations) and the joint regression analysis was performed for each genotype. Figure 6 shows the intercept of the joint regression plotted against the nursery mean grain Δ. There is a clear tendency in Mediterranean environments for a higher grain Δ to be associated with a higher yield under drought. In addition, the barley genotypes with a high Δ had high stability of production (Fig. 7).

III. Screening for Carbon Isotope Discrimination in Early Generations

The number of crosses and segregating populations handled in a breeding program can be massive. In a typical cycle for CIMMYT's spring bread wheat-breeding program, which operates two cycles per year, from 500,000 to 750,000 segregating genotypes at the F_2 level are reduced to 10,000 to 15,000 bulks at the F_3 level (S. Rajaram, personal communication). This large reduction in the number of F_2 plants is based essentially on screening for biotic stress resistance (mainly diseases) and visually assessed

morphological traits that may bear a relation to yield potential and/or abiotic stresses. These include plant height, spike size, maturity types, head number, and leaf posture. There is no objective assessment of yield potential or yield under stress until at least the F_5 generation when the grain yield can be measured. An objective method is needed to determine the potential for grain and/or yield under stress, such as drought, in earlier generations. There is a high probability that among the voluminous material discarded between the F_2 and F_5 stages of selection (98%), many high-yielding and/or stress-resistant materials are discarded as well.

Several questions need to be answered before a trait such as Δ can be recommended for use in early-generation screening. Does the Δ measured in spaced plants reflect the value of a dense plant situation? The what extent do the measurements made in early generations (F_2) reflect the correlated responses that the breeder is looking for in later generations? What fraction of the trait's variance is heritable?

A. Carbon Isotope Discrimination in Spaced versus Dense Plantings

To allow selection, F_2 plants are grown as spaced (30 × 10 cm) plants. The Δ value would increase its potential for screening early-generation material if the values determined in a spaced-plant situation would show similar rankings as those measured in dense plantings. To answer this question, we undertook an experiment in which we grew 24 genotypes in spaced and dense plots. The trials were grown at Breda in northern Syria and at Cambridge, United Kingdom, under drought. Table V shows that grain Δ as well as Δ values of other plant parts measured on dense plantings strongly correlated with that measured on spaced plants. These results supported the proposed use of Δ to screen in early generations.

Table V Correlation between Δ Values Measured in Various Plant Parts on Spaced and Drilled Plots[a]

	Breda[b] 1988	Breda[b] 1989	Cambridge[c] droughted 1988
Grain	0.714**	0.635**	0.661**
Peduncle at maturity	0.943**	0.810**	0.894**
Straw at maturity	0.854**	NA	0.542**
Whole plant at heading	0.511*	NA	0.668**
Peduncle at anthesis	NA[d]	0.678**	NA
Whole plant at tillering	0.740**	0.758**	0.665**

[a]The seasonal rainfall at Breda was 415 mm in 1988 and 195 mm in 1989. Total evapotranspiration at Cambridge for plants grown in a rain-out shelter was 109 mm.

[b]N = 24.

[c]N = 21.

[d]NA, not available.

*,**Significance at 0.05 and 0.01% respectively.

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B. Genetic Parameters and Correlated Yield Response to Carbon Isotope Discrimination

Knowledge of genetic correlations and the heritability of direct and indirect characters is required to obtain the expected response of a character (e.g., grain yield or *W*) when selection is applied to another character (e.g., Δ). Table VI gives estimated broad-sense heritabilities for grain yield and *W* and the genetic correlations between grain yield, *W*, and Δ values. The values used in the calculation were replicated plots of the 10 genotypes grown in the LSS and greenhouse experiments. The calculated heritability for *W* and Δ values is in the order of 81%. These values compared well with those reported by Condon *et al.* (1987) for wheat Δ (60 to 90%) (see other chapters in this volume). The broad-sense heritability of Δ values calculated by Hubick *et al.* (1988) for 16 peanut genotypes across 10 sites was 81% and Hall *et al.* (1990) reported a value of 76% for leaf Δ in cowpeas.

The merit of indirect selection relative to that of direct selection may be expressed as the ratio of the expected responses, CRx/Rx, where CRx is the correlated response to selection in character X when selection is done for character Y and Rx is the direct response when selection is done for character X (Falconer, 1983). Using a 10% selection intensity for the two characters and the value of the square root of the heritabilities as well as the genetic correlations given in Table VI, selecting indirectly for grain yield and *W* using Δ would have an expected merit of 0.82 for grain yield and 0.98 for transpiration efficiency.

With the above results in mind, we proceeded to cross two-row barley genotypes with similar phenology having low and high Δ's measured in previous trials. Seven crosses were made, the F_2's were grown in the field at Breda in the 1988/1989 season (rainfall, 180 mm), Δ was determined in the straw at harvest, and yield was measured in F_4 families (1990/1991, 290 mm rainfall).

Table VI Genetic Correlations (rg) between Δ, Grain Yield, and Transpiration Efficiency (*W*), and Broad-Sense Heritabilities (*H*) Obtained from 10 Barley Genotypes (pure lines)[a]

	Genetic correlation (rg)	Broad-sense heritability *H*
Grain yield	0.775 (0.760)	0.71
W	−0.910	0.82
Δ	—	0.81

[a]Values of rg were estimated in a greenhouse experiment. Those in parentheses were estimated from a line source sprinkler experiment. All heritability estimates are from the line source experiment (Breda, northern Syria, 1989).

Table VII ^{13}C Discrimination of Parents and Mean F_2 Progenies of Seven Barley Crosses with Parents Diverging in Δ[a]

Cross	^{13}C discrimination (‰) P_1	P_2	$\bar{x}F_2$ (range)	Shoot biomass (g/m²) $\bar{x}F_4$ (range)	Grain yield (g/m²) $\bar{x}F_4$ (range)
SLB8-6/WI2198	18.32c[b]	18.88a	18.64a (16.39–20.15)	1290ab (736–1739)	404a (177–604)
SLB8-6/Roho	18.32c	18.66b	18.26c (16.28–19.31)	1306a (892–1733)	405a (146–579)
Tadmor/SLB62-99	18.59b	18.93a	18.61a (17.2–20.4)	1007d (550–1428)	256e
Tadmor/Roho	18.59b	18.66b	18.07d (16.28–19.52)	1257b (819–1642)	363b (126–513)
Tadmor/SLB62-35	18.59b	19.00a	18.36a (17.32–19.10)	1010d (656–1317)	251e (83–441)
SLB8-6/SLB62-99	18.32c	18.93a	18.66a (17.12–19.84)	1117c (653–1564)	274d (78–498)
Tadmor/WI2198	18.59b	18.88a	18.22c (16.80–20.15)	1274ab (589–1756)	315c (129–587)

[a]The mean shoot biomass and grain yield of F_4 progenies are also given

[b]Cross means followed by different letters differ at 0.05%. Values of Δ for parents of a cross followed by a different letter differ at 0.05%.

Despite significant variation in F_2 Δ, F_4 grain yield, and aboveground biomass (Table VII), no subsequent correlation was found between F_2 Δ values and F_4 family yields either within or between crosses (Table VIII) (Acevedo, Ceccarelli, and Dakeel, unpublished). The relation between F_2 Δ values and days to ear emergence in the F_4 was nonsignificant. *W* and Δ values have not been measured in the F_4 progenies. This experiment is in progress; the results obtained so far with indirect selection, using Δ as a surrogate for grain yield, indicated however that the correlated responses

Table VIII Phenotypic Correlation Coefficient between Straw Δ in F_2 and Grain Yield (g/m²) or Shoot Biomass (g/m²) in F_4 Families

Cross	No. (g/m²)	Grain yield (g/m²)	Shoot biomass
SLB8-6/WI2198	90	0.109	0.070
SLB8-6/ROHO	120	0.126	0.120
TADMOR/SLB62-99	51	−0.161	−0.267[a]
TADMOR/ROHO	70	−0.047	−0.122
TADMOR/SLB62-35	35	0.007	0.166
SLB8-6/SLB62-99	129	−0.155	−0.107
TADMOR/WI2198	86	−0.081	0.044

[a]Significance at 0.1% level

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were limited. The distributions of the Δ values were normal for the seven F_2 progenies indicating that Δ is a quantitative character. Out of seven crosses of genotypes differing in Δ value, the F_2 distributions showed a significant skewness to lower values only in three, indicating that some degree of dominance of low Δ may not be a general phenomenon in barley as suggested by Hubick *et al.* (1988) for peanuts.

C. Genotype × Environment Interaction of Δ

The genotype × environment (G × E) interaction of Δ in our experiments has been low and usually nonsignificant (Craufurd *et al.*, 1991). Additional evidence of low G × E comes from our greenhouse study on *W* of barley (Table II) and from the LSS work conducted at Breda in the 1988/1989 season (Table I). Both experiments had the same genotypes. The varieties in these experiments were chosen because they had similar flowering dates when grown in the field in northern Syria and they represented a range of Δ values measured in the previous experiments.

The interaction between irrigation levels and variety Δ in the LSS and in the pot-grown varieties was nonsignificant. Varieties at each irrigation level and varietal means across irrigation levels differed significantly in Δ values (Tables I and II). The LSS and the greenhouse created different environments. As a result, varieties differed significantly ($P < 0.001$) in their flowering date in these experiments.

A strong G × E in Δ would be expected for locally adapted barley genotypes, which are usually responsive to temperature and photoperiod. The similar genotype rankings for Δ values in the greenhouse experiment and the LSS (Tables I and II, rank correlations for means $r = 0.70$, $P < 0.05$) indicate, however, that G × E in this parameter is low despite the large dissimilarity between the two environments. This subject warrants further analysis.

IV. Summary

Carbon isotope discrimination may have the potential to assist yield-based selections in barley breeding for drought-stressed environments. Experimental evidence showed that, for barley, there is genetic variability for Δ and that the G × E is low, and the broad-sense heritability is high. In Mediterranean environments, the trait can be measured at anthesis or at the end of the growing cycle in any plant tissue as long as consistency is maintained in the sampling procedure. The Δ values for dense and spaced plantings were highly correlated, which suggests that measurements could be done in early generations, a time when yield and/or transpiration efficiency cannot be assessed. However, the efficiency of this trait for selection in early barley generations remains to be shown. There were no correlations between Δ values measured in F_2 plants of seven barley crosses and the grain yield of the F_4 families derived from each F_2 plant. Work is in

progress through a divergent selection experiment to study this point further.

The experiments conducted showed relations between Δ, transpiration efficiency, and grain yield. The relation of Δ with grain yield was found to be generally strong and positive under water stress, but it was also generally found that Δ was related to earliness in flowering. Transpiration efficiency was negatively related to Δ across barley genotypes.

Carbon isotope discrimination was positively related to genotype grain yield at the intercept of a joint regression analysis, which included nine Mediterranean environments, i.e., the larger the Δ values, the higher the yield under drought stress. The Δ values were negatively related to the slope of genotype yield over nursery mean yield. The higher the discrimination, the higher the genotypic stability as well as yield under stress. The apparent paradox between higher yield under water-limited conditions and lower transpiration efficiency needs to be resolved. Factors involved may be, among others, variations in earliness of flowering and/or in specific leaf area.

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Carbon isotope discrimination and water use efficiency in barley under field conditions

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Summary

The relationships among yield, carbon isotope discrimination (Δ) and water use efficiency (*W*) were investigated in a set of ten barley genotypes under different moisture levels for three seasons in northern Syria. A line-source sprinkler irrigation system was used to create ten moisture levels ranging from natural rainfall to around 400 mm/growing season. Amount and distribution of rainfall and temperature differed substantially between seasons. Water use was estimated by the neutron probe method. Δ was measured in separate plant parts at several developmental stages and moisture levels. Results show significant genotypic variations in yield, *W* and Δ measured in peduncles, grains and in flag leaves. Across the moisture gradient, Δ and *W* were highly and positively correlated with above ground dry matter (AGDM) and grain yield (GY). Environments with a higher yield potential always had higher Δ values. Within individual moisture regimes and for mean genotypes response over all moisture levels, yield and *W* were highly positively related (r=0.93 to 0.99). The relation between AGDM and Δ, and between W and Δ at high moisture level were negative in all seasons but significant only in 1991. At low moisture level the relation was also negative in 1991, but significantly positive in the other two seasons. It is concluded that, first for barley in Mediterranean environments selection for low Δ would lead to improvement in water use efficiency and consequently in yield in environments provide that temperature and moisture stress are not dominant during critical crop growth periods. Second, frost and low temperatures alone or in combination with low moisture availability influence the expected negative relationship and lead to a positive relation between yield and Δ and/or *W* and Δ.

Key words: ^{13}C discrimination, Δ, water use efficiency *W*, line source sprinkler irrigation system, *Hordeum vulgare*

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Introduction

Improving crop water use efficiency (or transpiration efficiency) as an integrated means to improve yield, has received increasing attention with the development of the ^{13}C discrimination theory in C3 plants. The theory predicted that ^{13}C discrimination (Δ), water use efficiency and growth are linked together at the leaf level (Farquhar,O'Leary & Berry,1982; Farquhar & Richards,1984). In brief, during photosynthesis processes C3 plants discriminate against the heavier naturally occurring ^{13}C carbon isotope (Farquhar *et al.*, 1982). Such process is dependent on the ratio of intercellular to atmospheric carbon dioxide concentration (ci/ca). Similarly, but independently, transpiration rate is linked to ci/ca at the leaf level (Farquhar *et al.*, 1982). ^{13}C discrimination is higher when stomata are widely open and ci is high. Under such conditions transpiration rates are high due to higher conductances, while transpiration efficiency (ratio of photosynthesis/transpiration) is low due to high water loss in transpiration in comparison with carbon gain in photosynthesis (Ehleringer *et al.*, 1990). The net result is that ^{13}C carbon isotope discrimination should be negatively related to water use efficiency. Consequently, Δ was proposed as a means to find variations in transpiration efficiency among genotypes of crop species for use in breeding programs (Farquhar & Richards, 1984; Condon, Richards & Farquhar, 1987). The uniqueness of ^{13}C discrimination is that it is not only an indirect measure of W (which has been shown in many studies), but it also represents an integrated measure of the efficiency of photosynthesis processes (the long term ci/ca ratio) in response to the environmental conditions that prevail during the plant growth cycle prior to its estimation (Condon *et al.*, 1990; Meinzer *et al.*, 1993). Therefore, it also can be used as a predictor of yield performance of plants in specific environmental conditions.

Among winter cereal crops, barley (*Hordeum vulgare* L.) occupies the drier marginal region of cereal cultivation. In such environments, increasing yield potential and/or stability is a prime objective in barley breeding programs (Ceccarelli, Acevedo & Grando, 1991).

At the International Center for Agricultural Research in Dry Areas (ICARDA), a research project was initiated in 1988/89 season to examine the potential of the ^{13}C discrimination technique in barley improvement under water limited conditions and extremes of temperature. Several questions related to the use of ^{13}C discrimination in breeding programs are addressed.

In this paper we report the results from a field study conducted over three growing seasons, from 1988/89 until 1990/91, with the objectives of determining: (1) the relation between Δ, water use efficiency, and yield performance of barley under different moisture conditions. (2) the potential of ^{13}C discrimination as an indirect selection criterion for water use efficiency in crop plants.

Materials and Methods

Plant material and experiment site

The experiments were conducted in northern Syria at Breda (BR, 35°56'N, 37°10'E), one of ICARDA's drier experimental sites. The site characterised by 262 mm long term average rainfall and calcic xerosol soil type. The climate is Mediterranean with cold rainy winters and dry hot summers. During the three growing seasons rainfall was 180mm in 1989, 183mm in 1990, and 241 mm in 1991.

Ten related, two-row barleys were used in this study. The genotypes were identified from among the best adapted to the harsh Mediterranean environments (Acevedo, 1993) and

included 5 landraces (L) originating in the Syrian steppe and arid zone (Tadmor, SLB 39-99, SLB 62-35, SLB 62-99, and SLB 8-6) and 5 improved lines (I) developed at the Waite Institute in Australia and from ICARDA's barley breeding program (WI 2198, WI2198/WI2291, Harmal, Roho, and SBON 96).

The genotypes have similar phenology and posses the important traits for adaptation to Mediterranean environments. They differ in grain Δ, as determined previously (Acevedo, 1993).

Experimental design and management

The experiments were conducted under 10 moisture levels ranging from natural rainfall to around 400 mm during the growing season (rainfall + irrigation). A line-source sprinkler system (LSS) created the required gradient in soil moisture levels. The LSS distributed water along both sides. The experimental area was divided into four blocks, two on each side of the LSS, and were used as four replications. Each replication comprised 10 main plots (perpendicular to the LSS) one for each genotype. Each main plot was 2.4m X 18m , which was split to 10 sub-plots alcng the 10 moisture levels created by the LSS. Each subplot (2.4m X 1.8m) had 12 rows of crop plants, 20 cm apart. Fertilisers were applied as a top dressing at a rate of 40 kg ha^{-1} superphosphate (40%) in addition to 20 kg ha^{-1} of ammonium phosphate (21%). Sowing at a seeding rate of 100 kg ha-1 was done between 11 and 27 November. Harvest was between mid to late May each season. At maturity a 1.2 m^2 sample was harvested from each plot. Height, peduncle length, total above ground biomass, grain yield, harvest index and other yield related traits were estimated. The remainder of each plot was mechanically harvested.

Water use and water use efficiency (W)

The amount of water received by each plot through natural rainfall and LSS water was measured from tin cans placed at crop height. Crop water use was monitored using the neutron probe. A total of 160 access tubes in four replications were installed in plots receiving high moisture treatment (level 9) and low moisture treatment (level 4). Water use at the other moisture levels was estimated by extrapolation from water used at levels 4 and 9. Neutron probe measurements were taken at least 10 times during the growth period.

Carbon isotope discrimination analysis

^{13}C discrimination analysis was performed on separate plant parts at several developmental stages and moisture levels. Each year peduncles and grains were analysed at maturity. In the 1989 and 1991 seasons leaves were also analysed at the 5-leaf stage. In addition, in 1991 leaves at the 3-leaf stage and green flag leaves were analysed. Plant materials were oven dried at 70°C for 48hr, finely ground and thoroughly mixed. A 2 mg subsample from each sample were combusted in an elemental analyser (Heraeus CHN-O RAPID Elemental Analyzer, Germany). Carbon isotopic composition in the combustion gas was determined by a ratio mass spectrometer (Finnigan MAT, Gennany) according to the method described by Wright *et al.* (1988). Analysese were done on three replications from each treatment. Δ values were obtained according to Hubick *et al.* (1986) assuming that the isotopic composition of the air relative to the international standard (Pee Dee Belemnite) is -8‰.

Statistical analysis

Analysis of variance for split-plot design was used to assess variations among genotypes and

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moisture treatments for all the measured variables (Steele & Torrie, 1980). Moisture levels were the main plots and genotypes the subplots. Each year was analysed separatly and regressions and correlation analysis among several variables were also performed. The statistical package MSTAT were used in performing all statistical analysis.

Results

Relation between yield and water use efficiency

Analysis of variance showed that grain yield (GY), above ground dry matter production (AGDM), and water use efficiency (W) varied significantly among genotypes and moisture levels in all seasons (table is not shown). There were no significant interactions between genotype response and irrigation level for GY, *W* or AGDM in any year.

Grain and biological yields differed between years (Table 1). Average grain and biomass yields were highest in 1991 (GY=265 and AGDM=646 g m^{-2}) and lowest in 1990 (GY=144 and AGDM=396 g m^{-2}). Differences between years were largest at the lower moisture levels. Amounts of water used at high and low moisture levels were significantly different among moisture levels and genotypes (Table 1). At low moisture (level 3) genotypes were different by 9.4% of the top value, while at high moisture (level 8) by 5.1%. Differences in total water use between levels 1 and 10 reached 229 mm (151 mm between levels 3 and 8), which corresponded to a 370% increase in grain yield and 300% in biomass (190% and 160% between levels 3 and 8).

Table 1. *Mean values of AGDM, GY, total water used by plants(W U), water use efficiency (W) and ^{13}C discrimination (Δ measured in peduncles (P), grains (G) and leaves at the 5 leaf stage(L5) during three cropping seasons at low and high moisture levels*

	Low moisture (level 3)				High moisture (level 8)			
	1989	1990	1991	Mean	1989	1990	1991	Mean
AGDM(g.m-²)	364	275	546	395	540	579	774	631
SEM	16	9	12	9	13	18	19	11
GY(g.m-²)	148	68	209	142	248	238	345	275
SEM	5	4	7	5	9	10	10	6
W U(mm)	174	187	242	201	332	387	336	352
SEM	3.1	1.1	1.1	1.4	1	4.4	1.6	2
W_u (g.mm)	2.1	1.47	2.26	1.94	1.63	1.5	2.3	1.8
SEM	0.08	0.05	0.04	0.04	0.04	0.05	0.06	0.04
Δ-L5	20.6		21.3	20.96	20.7		21.1	20.9
SEM	.09		.10	.09	.10		.08	.09
ΔP	16.7	16.8	18.7	17.4	18.4	8	19.5	18.7
SEM	.08	.29	.07	.1	.14	.17	.13	.10
ΔG	15.4	13.3	16.2	14.7	17.4	16.6	17.7	17.1
SEM	.25	.11	.07	07	.25	.11	.08	.07

(n=10)

· Water use efficiency (W) increased slightly at low moisture levels in comparison with higher levels (Table 1). This indicates that water use and yield declined proportionally along the gradient in moisture levels. Genotypic variations in W were statistically significant. At low moisture genotypes varied by a maximum of range 18%, and by 17.2% at high moisture level.

Across moisture levels, above ground biomass and grain yield correlated highly with total amount of water used during the three-seasons (r=0.91 to 0.95, $P < 0.001$). Water use efficiency correlated positively with biological yield. Correlation coefficients for three seasons means were 0.47 for AGDM and 0.44 for GY ($P < 0.01$). The relation between mean genotypes response over all moisture levels and yield was highly significant and positive ($r > 0.95$, $P < 0.001$) for both grain and biological yield (Table 3).

Within each moisture level the relation between water use efficiency and yield were stronger than across moisture levels, all correlations exceeded r=0.90 ($P < 0.001$).

Relation between Δ and yield

Carbon isotope discrimination (Δ) was measured in separate plant parts at several moisture levels and developmental stages. Genotypic variations were not significant in Δ measured in leaves at 3-& 5-leaf stages (analysis not shown). Δ measured in flag leaves, peduncles and grains varied significantly between genotypes and moisture levels. As expected Δ values were highest in leaves (ΔL5) the primary photosynthetic tissue, lower in peduncles (ΔP) and lowest in grains (ΔG) (Table 1). Similarly, Δ values were higher at high moisture level. Within moisture levels the range in Δ was higher at high moisture levels so that the difference was highest in grains (2.4 X 10^{-3}) and least in leaves (Table 1). The range in ΔP between genotypes was 1.6 X 10^{-3} at high moisture, and 1.4 at low moisture. Variations in ΔG were in the range of 1.0 and in ΔSL 0.90 X 10^{-3}.

Across moisture levels (1,3,5,8 &10) Δ measured in peduncles and grains correlated positively and highly significantly with both grain yield and above ground biomass in all seasons (Figure 1). Environments with high yield potential always had high Δ values in all plant parts (Table 1). Δ measured in leaves at 3-or 5-leaf stages correlated weakly or not significantly with both GY and AGDM.

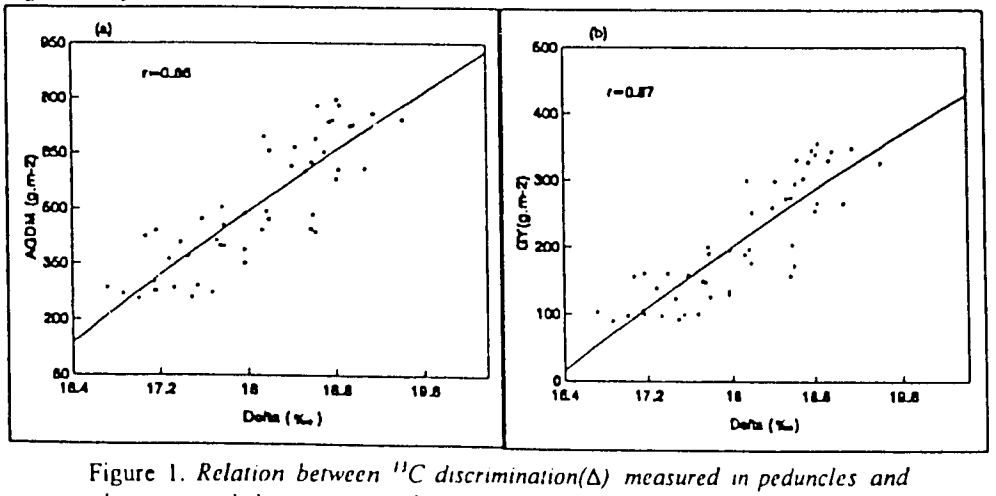

Figure 1. *Relation between ^{13}C discrimination(Δ) measured in peduncles and above ground dry matter production (AGDM) (a) and grain yield (GY) (b) in 10 barley genotypes across five moisture levels Values are means of three seasons*

When individual moisture levels were considered, or mean genotypes response over all moisture levels, the relation between Δ and yield became different. For mean response over three seasons the relation between Δ and both grain and biomass were significantly negative at either low or high moisture (levels 3 & 8). Figure 2 shows the relation for above ground dry matter. Similar correlations were observed for grain yield (data are not shown).

Within each season the relations were not consistently negative. In 1989 seasons ΔP correlated positively and significantly with GY at low moisture level (Table 2). While at high moisture level a weak negative relation was observed. Similarly in 1990 Δ measured in grains correlated significantly and positively with AGDM at low moisture level. At high moisture levels correlations were negative but non-significant (Table 2). During 1991 seasons Δ measured in all plant parts correlated negatively with both grain and biomass yield within individual moisture levels. In 1991 correlations were significant between AGDM, GY and Δ measured in leaves at different stages (data not shown) and in grains at maturity at both high and low moisture levels (Table 2).

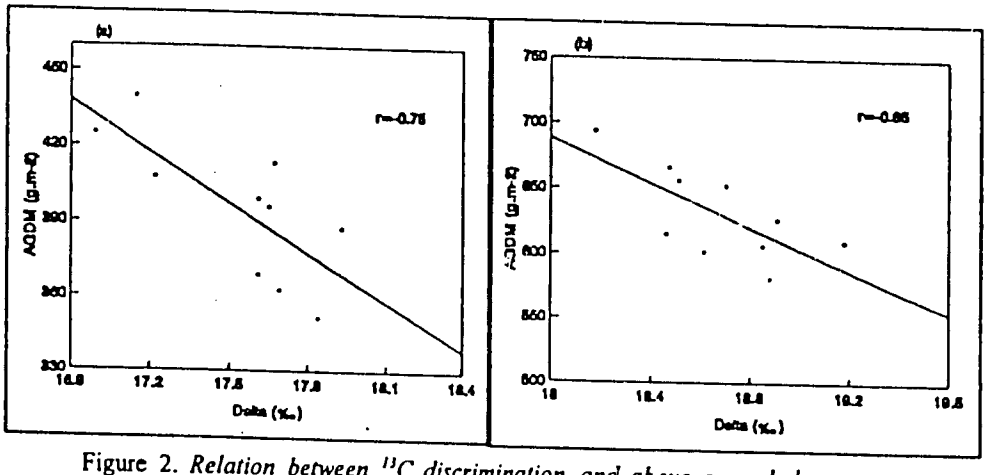

Figure 2. *Relation between ^{13}C discrimination and above ground dry matter production (AGDM) at low moisture (level 3) (a) and high moisture (level 8) (b) in 10 barley genotypes. Values are means of three seasons.*

Table 2. *Correlation coefficients between AGDM, GY and ^{13}C discrimination (Δ) measured in peduncles (P) and grains (G)during three seasons at low and high moisture levels*

Season	Trait	ΔP	ΔG	ΔP	ΔG
		Low Moisture (level 3)		High Moisture (level 8)	
1989	AGDM	0.51ns		-0.24ns	
	GY	0.69*		-0.02ns	
1990	AGDM	-0.05ns	0.62*	-0.42ns	-0.47
	GY	-0.15ns	0.32ns	-0.23ns	-0.09
1991	AGDM	-0.46ns	-0.62*	-0.59*	-0.74**
	GY	-0.46ns	-0.71**	-0.32ns	-0.40ns

n=10(df=8), *,**,*** significant correlating at P<0.05,0.01, and 0.001 respectively, ns not significant.

For genotypes mean response over all moisture levels, the relations between yield and Δ measured in peduncles or grians were highly negative as predicted by ^{13}C theory (Table 3).

Table 3. *Correlation coefficients between AGDM, GY and W and ^{13}C discrimination (Δ)measured in different plant parts for mean genotypes response over all moisture levels. Values are means of three seasons*

	AGDM	GY	W_b	W_c
$W_{b\ or\ c}$	0.97***	0.96***		
ΔP	-0.83***	-0.75**	-0.70*	-0.72**
ΔG	-0.69*	-0.73**	-0.67*	-0.80**

n=10(df=8), significance levels as in previous tables.

Relation between ^{13}C discrimination and water use efficiency

Across moisture levels, Δ and *W* (for above ground biomass or grain yield) were weakly related, either positively or negatively depending on the season. This was mainly due to the low variability in *W* across moisture levels (Table 1). Such response is contrary to the expected negative relationship between Δ and *W*, which has been found in many studies (Farquhar & Richards, 1984), and for the same set of barley genotypes when grown under different moisture regimes under plastic house conditions (Dakheel *et al.*, unpublished).

However, for mean genotypes response over all moisture levels the relation between Δ and *W* was significantly negative for grain and biomass production (Table 3).

Within individual moisture levels, mean values over three seasons showed significant negative relation between ^{13}C discrimination and water use efficiency for both low and high moisture levels (Figure 3). Within each season the relation was not consistent, at low moisture level the relation between *W* and Δ was positive in 1989 and 1990, and negative in 1991. While at high moisture level the relation between Δ and *W* was consistently negative in all seasons, although significantly so only in 1990 and 1991 (Table 4).

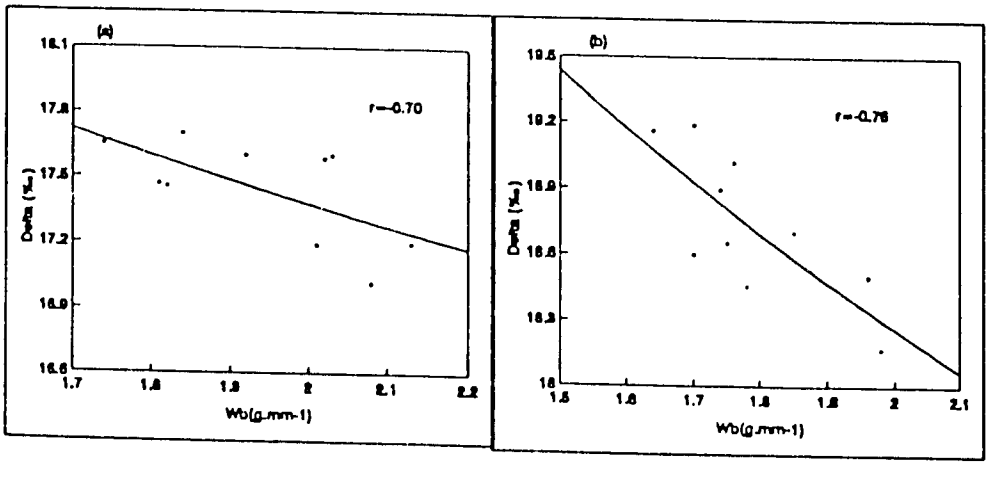

Figure 3. *Relation between ^{13}C discrimination(Δ) and water use efficiency (W) at low moisture (level 3)(a) and high moisture (level 8) (b) in 10 barley genotypes. Values are means of three seasons.*

Δ measured in leaves at 3-& 5-leaf stages, and in flag leaves in 1991, also correlated significantly with *W* (r= -0.59 to -0.88).

Table 4. *Correlation coefficients between W (B and G) and ^{13}C discrimination (Δ) measured in different plant parts during three seasons at low and high moisture levels*

Season	Trait	ΔP	ΔG	ΔP	ΔG
		Low Moisture (level 3)		High moisture (level 8)	
1989	W_B	0.57*		-0.12ns	
	W_G	0.29ns		-0.12ns	-0.10ns
1990	W_B	0.03ns	0.69*	-0.32ns	
	W_G	-0.10ns	0.05ns	-0.61*	
1991	W_B	-0.36ns	-0.59*	-0.57*	-0.68**
	W_G	-0.46ns	-0.61*	-0.13ns	-0.60*

n=10(df=8), significance levels as in previous tables.

Discussion

The results of this study demonstrated the large effect of water availability on barley yield in stressed Mediterranean environments. Regressions fitted to the relation between mean yield at each moisture level and amount of water used by genotypes (y=-3.89+1.79x, r=0.98 for AGDM and y=-62.03+0.94x, r=0.99 for GY) showed that grain yield with potential less than 1000 kg ha^{-1} in environments receiving between 160-200 mm/season could double (from 90 to 180 $g.m^{-2}$) when amount of water used by plants increased by 70 mm/season. Yield potential of 1.5-1.6 t ha^{-1} corresponded to 230-250 mm of water use. This yield was also doubled when amount of water use increased by 165-175 mm to exceed 400 mm/season.

Across all moisture levels the amount of water used (WU) by plants explained on the average 86% of the variations in total biomass production and 88% in grain yield. Such relation implies that other factors beside absolute amount of water used influenced genotypes yield performance.

On the other hand, variations in water use efficiency (*W*) within high and low moisture levels accounted for a high percentage of yield variability among genotypes which ranged from 93 to 97%. Such high association between W and yield justifies the efforts to search for variations in water use efficiency and for traits which could be associated with such variations.

^{13}C discrimination (Δ) was proposed as an effective mean for searching for variations in water use efficiency (Farquhar & Richards, 1984) and as a predictor of yield performance (Acevedo, 1993; Meinzer *et al.*, 1993).

In this study a positive relation between Δ measured in various plant parts and yield was observed across moisture levels. Similar studies have been reported for a range of crops including wheat (Condon *et al.*, 1987; Ehdaie *et al.*, 1991), durum wheat (Kirda *et al.*, 1992), and barley in a similar environment in northern Syria (Craufurd *et al.*, 1991; Acevedo, 1993).

It is expected that Δ is highest due to increased stomatal conductance and photosynthesis and *W* is lowest at higher moisture availability so that that water use efficiency *W* and Δ are negatively related (Farquhar & Richards,1984). Yield and *W* are positively related, and

consequently yield and Δ are negatively related (Richards & Condon, 1993). Our results showed that within individual moisture levels yield and ^{13}C discrimination were consistently negatively related in 1991 season and in mean response of three seasons. At the same time water use efficiency and Δ were negatively related (Figures 2 & 3) as predicted by ^{13}C theory. In the other two seasons relation were either positive or weakly negative. Negative relation between yield and Δ was reported in wheat in environments with low yield potential and positive in high yield environments (Condon & Richards,1993). Also negative relation were observed in peanut (Wright *et al.*, 1988) and in range grasses (Johnson & Bassett, 1991).

Under field conditions the relation between yield and Δ is not as straight forward as expected from theory. There are several possible explanations for the positive relation observed in many studies, detailed by Richards & Condon (1993) and by Acevedo (1993). Differences between genotypes in phenological development, the proportion of carbon fixed early in plant development, degree of early plant growth at time when stress is minimal, and the capacity for retranslocation of assimilates to reproductive parts from reserve laid down in plant tissues before anthesis, are some of the important factors which may cause differences in the relation between yield and Δ.

Δ is related to the ratio of intercellular to atmospheric CO_2 concentration (ci/ca), which is influenced by both stomatal conductance and photosynthetic capacity (Condon & Richards, 1993). According to Condon *et al.* (1987) the relation between Δ and yield is expected to be positive when stomatal conductance increases and photosynthetic capacity remains constant. This increases the ci/ca ratio and at the same time reduces water use efficiency. In contrast changes in photosynthetic capacity at constant stomatal conductance leads to a negative relation between Δ and yield. Variations in the relation between yield, W and Δ among the three seasons could be related to variations in environmental conditions. Amount and distribution of rainfall varied substantially among seasons. In 1991 the season rainfall was higher (241 mm) and large proportion fell during the critical period of head formation and grain filling between March and May. The other two seasons had less rainfall (183 mm) and most rainfall came before February. More importantly, during 1989 and 1990 seasons an extended period of frost and below zero temperatures, exceeded 47 days, dominated from December until February. While in 1991 frost was minimal and there were only 17 days of below zero temperature. Moreover, during grain filling in May mean daily temperature was 17.9°C in 1991 in comparison with 22.2°C in the other two seasons. These factors caused grain and biological yields to vary among seasons even at high moisture levels where total amount of water received were similar or higher in seasons with lower yields. The mild environmental conditions in 1991 season and the amount and favorable distribution of rainfall are expected to reduce environmental limitations on stomatal conductance. This would imply that variations in ci/ca (and consequently in Δ) are less affected by stomatal conductance and more controlled by variations in a genotype photosynthetic capacity. In such situations it is expected that Δ would be negatively related to biomass production (Condon *et al.*, 1987) which was observed in this study. In 1989 and 1990 seasons when low temperatures during early growth and high temperature and low atmospheric humidity during grain growth stages dominated. It is expected that variations in Δ among genotypes are more controlled by variations in ci/ca caused by variations in stomatal conductance than by variations in photosynthetic capacity or by a combination of the two factors. This situation is more likely to cause the inconsistency in relation between yield and Δ which was observed to be weakly negative or positive at low moisture level. While at high moisture levels the relation between yield and Δ or between W and Δ were negative in all seasons, but significant only in 1991 (Tables 2 & 4).

Similarly, the expected negative relation between Δ and water use efficiency, which has been

demonstrated in several studies under plastic house conditions (Richards & Condon, 1993) including the same set of barley genotypes, might not hold consistent under field conditions. Difficulties in estimating the transpirational component out of total water used by a crop canopy and changes in canopy characteristics in scaling from single plant to canopy level where aerodynamics and diffusion processes are different, are some of the major reasons for inconsistent relation between Δ and *W* under field conditions (Richards & Condon, 1993). In this study the relation between Δ and *W* were identical to the observed relation between Δ and yield within individual moisture levels. Strong negative relationships were found in 1991 season, while in 1989 and 1990 the relation were either positive or weakly negative (Table 4). The close resemblance of the relation between Δ and yield and between Δ and *W* suggested that the same controlling factors influence both ^{13}C discrimination and water use efficiency as it was predicted by carbon isotope discrimination theory (Farquhar & Richards, 1984).

We conclude from the observed relation, under field conditions, between ^{13}C discrimination, water use efficiency and yield that: First, a strong positive relation exists between water use efficiency and yield regardless of environmental conditions and moisture level. Second, significant negative relation exists between Δ and both biological and grain yield and between Δ and water use efficiency in environments where temperature and moisture stresses are not dominant at critical stages of crop growth. In such environments selection for low Δ (either at high or at lower moisture levels) would be associated with higher water use efficiency, and consequently with higher yield potentials. Third, frost and low temperatures alone or in combination with low moisture availability may influence the expected negative relation and lead to a positive relation between yield and Δ and/or *W* and Δ. Fourth, when the relation among yield, *W* and Δ evaluated across moisture level gradient the relation among all three components are more likely to be positive as observed in this study. However, such response over moisture gradient may not be relevant to the actual relations observed within individual moisture environment.

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Carbon isotope discrimination, yield and transpiration efficiency in barley grown in a plastic house

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Abstract

The relationships among carbon isotope discrimination (Δ), transpiration efficiency (W) and yield were investigated in ten barley genotypes grown in a plastic house in northern Syria. Four moisture treatments were used. A non-stressed treatment with soil moisture content near to field capacity (FC), a fully stressed treatment kept at 1/3 FC throughout the life cycle, a pre-heading treatment kept at 1/3 FC before heading and a post-heading treatment kept at 1/3 FC after heading. Soil moisture contents were adjusted daily by weighing the pots and adding water. Δ was measured in the peduncles at physiological maturity. Results show that Δ, W, grain yield (GY) and above ground dry matter production (AGDM) varied significantly with moisture level and genotype. Across moisture levels the relations between Δ and yield (GY & AGDM) were highly significant and positive ($r>0.85$, $P<0.001$). Yield and W, and Δ and W were negatively and significantly correlated ($r>-0.70$ and -0.78 respectively, $P<0.001$). However, within individual moisture treatments the strength and slopes of the relationships were inconsistent. The relation between Δ and W remained consistently negative, although not significant in all cases. Δ and yield were positively related in the fully stressed treatments during both seasons, while in the variable moisture stress treatments (pre- and post-heading) relations were positive but significant only in the first season. In the second season Δ and yield were negatively, but insignificantly, related. The relation between W and yield changed from negative to positive between the two seasons.

Stability of genotype ranking in Δ between moisture stressed and unstressed treatments was significant only in the first season. Interseasonal stability of Δ was significant only between the means of the stressed treatments, while Δ measured in the unstressed treatments was poorly correlated. Generally, landraces had higher yields, higher Δ, and lower transpiration efficiencies than improved varieties under all moisture levels. It is concluded from this study that, first, selection for higher Δ in barley is associated with a high yield potential, under both low and high moisture levels. Second, selection for lower Δ will probably lead to an improvement in transpiration efficiency (W), however yield potential might be negatively affected. Third, genotype ranking in Δ is more stable across seasons in moisture stressed rather than unstressed treatments, therefore selection for this trait for moisture stressed areas is more appropriate within the targeted environment.

Introduction

Yield in a water limited environment is viewed as the product of three factors (Passioura, 1977; Condon & Richards 1993) namely: amount of water transpired by the plant (E), water use efficiency (or transpiration efficiency) (the ratio of biomass produced /water consumed by plants, *W*), and the efficiency of allocation of dry matter to harvestable crop products, approximated by the harvest index (HI). Maximizing any of the three factors is expected to improve yield potential given that interactions between the three components are minimum.

Improving crop water use efficiency (or transpiration efficiency) to improve yield, has received increasing attention with the development of the ^{13}C discrimination theory in C3 plants. The theory predicted that ^{13}C discrimination(Δ), water use efficiency and yield are linked at the leaf level (Farquhar et al.,1982; Farquhar & Richards,1984). During photosynthesis, C3 plants discriminate against the heavier naturally occurring ^{13}C carbon isotope (Farquhar et al.,1982). The process is dependent on the ratio of intercellular (ci) to atmospheric carbon dioxide (ca) concentration (ci/ca). Discrimination is higher, when stomata are widely open and ci is high. Similarly, but independently, transpiration rate is linked to ci/ca at the leaf level(Farquhar et al. 1982). Higher transpiration efficiency (*W*) (ratio of photosynthesis/ conductance) is associated either with a reduced stomatal conductance or an increase in photosynthesis capacity (Condon & Richards 1992). Therefore, theory predicts that an increase in *W* would be associated with a reduced discrimination

against ^{13}C (Δ) (Farquhar & Richards 1984; Ehleringer et al.1990). Consequently, Δ was proposed to predicts variation in transpiration efficiency among genotypes of crop species and subsequently to utilize it in breeding programs (Farquhar & Richards 1984; Condon, Richards & Farquhar, 1987). The uniqueness of ^{13}C discrimination, is that it is not only an indirect measure of *W* (which has been shown in many studies), but it also represents an integrated measure of the efficiency of photosynthesis, in response to the prevailing environmental conditions during growth cycle prior to its estimation (Condon et al.1990; Meinzer et al.1993).

At the International Center for Agricultural Research in Dry Areas (ICARDA), a research project, related to the potential of ^{13}C discrimination technique as an in indicator of water use efficiency and as a predictor of yield performance under stress in barley, was initiated in the 1988/89 season.

The objective of this study was to determine the relationship among Δ, transpiration efficiency and yield performance in barley under different moisture regimes.

Materials and Methods

Location

The experiment was carried out during three seasons from 1988/89 until 1990/91 in a plastic house at Tel Hadya, ICARDA's headquarters and main research site in northern Syria. However, the 1989/90 experiment suffered from an unidentified toxicity which affected yield, therefore the final results are not considered here. In the first season planting was done in October 1988, and plants were harvested in May 1989. In the 1990/91 season planting was commenced in December 1990 and were harvested in June 1991.

Plant material

Ten barley genotypes with similar phenology were selected, these included 5 landraces (L) which originated in the Syrian steppe and arid zone (Tadmor, SLB 39-99, SLB 62-35, SLB 62-99, and SLB 8-6), and 5 improved lines (I) developed at the Waite Institute in Australia (WI 2198, WI 2198/WI 2291) and from ICARDA's barley breeding program (Harmal, Roho, and SBON 96).

The ten genotypes differ in grain Δ, as determined previously (Acevedo,1993) and possess the majority of traits identified as important in adaptation to harsh Mediterranean environments.

Growth conditions

Seeds of each genotype were germinated in petri dishes in an incubator at 20°C. Two seedlings of equal size were transplanted into a clay soil and sand mix (2:1 ratio) in a six kg plastic

pots. A base fertilizer consisting of 0.42 g of superphosphate, 1.0 g ammonium sulphate, and 0.016 g urea was applied to each pot. The soil was fully irrigated to field capacity and surface was sealed with aluminum foil leaving two holes for seedlings and a third for watering. A plastic tube (15 cm length and 1 cm diameter) was inserted in the hole and used for water addition. When not in use the tube was closed with a cork to prevent direct evaporation. A 1/10 Hoagland nutrient solution was used for irrigation.

In both seasons the plastic house temperature was set at a maximum day time /minimum night time regime of 22/5°C during the first period of plant growth, and 25/10°C from ear emergence to maturity. Temperature and relative humidity were monitored with a hygrothermograph and vapor pressure with a ventilated psychrometer in order to normalize amount of water transpired by plants according to vapor pressure deficit (vpd).

Moisture treatments

There were four moisture treatments. The first a non-stressed treatment with soil moisture content near to field capacity (FC) throughout the growth cycle. The second was a fully stressed treatment kept at 1/3 field capacity (1/3 FC). The third treatment was subject to pre-heading moisture stress receiving 1/3 FC, then kept near to FC (1/3 FC-FC). The fourth was a post-heading (terminal) moisture stressed treatment which was kept at 1/3 FC moisture regime from heading afterward (FC-1/3FC).

Moisture content of the soil mix at field capacity and permanent wilting point was 30.8% and 16.7% respectively. Soil moisture

contents were adjusted daily by weighing of the pots and adding water according to the predesigned moisture treatment. Total amount of water used in each pot were estimated. Watering was terminated at physiological maturity. Direct soil water evaporation was also calculated using twelve pots without plants, which were randomly distributed throughout the treatments. Treatments were arranged in a randomized complete block, in a split-plot design, where soil moisture level were the main plots and genotypes were the subplots. Each treatment was replicated three times giving a total of 120 pots for each experiment.

Plant harvest

At maturity, plants were harvested and separated into heads, leaves, stems, and peduncles. The roots were carefully washed and extracted from the soil through a fine steel mesh sieve, all the plant parts were oven dried at 70°C for 48h. Total and above ground biomass were calculated. Seeds were threshed and harvest index estimated as the ratio of seed weight to above ground dry matter. Transpiration efficiency was calculated by dividing total or above ground dry matter over amount of water transpired by plants (g/kg).

Isotope discrimination analysis

The peduncles at final harvest in all treatments was used for determination of carbon isotopic composition, they were finely ground, mixed and analyzed. A 2 mg subsample from each sample was combusted separately in an elemental analyzer (Heraeus CHN-O RAPID Elemental Analyzer, Germany). Carbon isotopic composition

in the combustion gas was determined by a ratio mass spectrometer (Finnigan MAT, Germany) according to the method described by Wright et al.(1988). Δ values were obtained according to Hubick et al.(1986) with the assumption that the isotopic composition of the air relative to the international standard (PDB) is -8.0‰.

Statistical analysis

Variations among genotypes and moisture treatments and the interactions between the two factors for all variables measured were assessed by analysis of variance for the split-plot design (Steel & Torrie 1980). Each season was analyzed separately, in addition to the mean of the two seasons. Variations within each moisture level were assessed by a one-way analysis of variance. Correlation analysis and regressions between various variables were also performed. The statistical package MSTAT was used in performing all the statistical analysis.

Results

Yield response and ^{13}C discrimination

Grain yield (GY), above ground dry matter production (AGDM) and ^{13}C discrimination (Δ) varied significantly among barley genotypes and moisture treatments in both seasons (Table 1). Moisture stress reduced yield by 48% in the fully stressed treatment, and 43% in the terminal (post-heading) moisture stress treatment (Table 2). In the pre-heading stressed treatment grain yield was only reduced by 24% and biological yield was equal or higher than other moisture stress treatments (Table 2), emphasizing the importance of terminal moisture stress during grain filling period in determining final yield potential. Harvest index (HI) did not vary significantly among either moisture treatments or genotypes, with the exception of the terminal stress treatment (Table 2). Therefore, it might be expected that according to the equation given by Passioura (1977) other factors, namely E (amount of water transpired by the plant) and *W* (transpiration Efficiency), affected variations in yield potential among genotypes.

Δ was as expected (Richards & Condon, 1993) lowest at high moisture stress levels (fully moisture stressed treatments, 1/3FC).

Across all moisture treatments above ground dry matter production (AGDM) and grain yield (GY) appear to be correlated positively and highly significant with Δ in both 1989 and 1991 seasons (Table 3). Correlation coefficients were substantially higher

when only the high and low moisture treatments considered, or when the pre-heading stress treatment was eliminated (Table 3). In the later moisture treatment there was little variation among genotypes in Δ with the relief of stress during the critical period of grain filling. Mean correlation coefficients between GY and Δ, and AGDM and Δ across the three moisture treatment (FC, 1/3 FC and terminal stress treatments) for the two seasons was 0.87 and 0.82 respectively ($P<0.001$)(Figures 1 & 2).

Relationship between yield, transpiration efficiency, and Δ

Across moisture treatments yield and transpiration efficiency (*W*) were negatively related (Table 3). The mean correlation coefficient of the two seasons was higher ($r=-0.76$) when pre-heading moisture stress treatment was not included (Figure 3). As expected from the theory (Farquhar & Richards,1984) transpiration efficiency and ^{13}C discrimination (Δ) were negatively and significantly related during both seasons (Table 3). The mean correlation of the two seasons across the three moisture treatments was -0.85 (Figure 4).

Relationship between yield, W and Δ within moisture treatments and seasons

The relationships among yield (GY and AGDM), *W* and Δ appeared to be inconsistent when they were examined within individual moisture treatments or seasons. The relationship between yield and Δ showed a strong positive relation across moisture levels and was positive in 1989 experiment in the nonstressed (FC) and fully stressed treatments (1/3FC) (Table 4). In the split

moisture stress regimes (post and pre-heading stress) the relation were also positive but weaker (Table 4). Similarly, transpiration efficiency (*W*) was negatively and significantly related to yield in the non-stress treatment. In the other treatments relationships were also negative but not significant. The relation between yield and Δ in the 1991 experiment was negative but not significant in the unstressed treatment. In the fully stressed treatment (1/3 FC) the relation was positive as in the relation across moisture levels. Contrary to the 1989 season, the slopes of the relation between *W* and yield in the 1991 season were positive in all moisture treatments, but significant in the non-stressed and in terminal moisture stress treatments (Table 4).

Despite these differences in response between the two experiments the slope of the relation between Δ and transpiration efficiency *W* remained negative, although not significant in all cases in the two experiments (Table 4).

Correlations between genotypes response at different moisture levels and seasons

The relation between yield, *W* and Δ in the non-stressed treatment and the mean response over the other three moisture stressed treatments in both seasons are summarized in table (5). In the first experiment correlations were high between ^{13}C discrimination at high and low moisture levels, and in all other traits (Table 5). While in the second experiment, grain yield was the only trait which correlated significantly between high and low moisture treatments.

Interseasonal correlations between various traits (*W*, yield and Δ) were high and significant between treatments which were subject to moisture stress. The unstressed treatments did not correlate significantly between the two seasons with the exception of *W* (Table 6).

Response of landrace and improved varieties

In both experiments correlations among Δ, *W* and yield (GY and AGDM) were higher in the improved genotypes than in landraces, with the exception of the relation between *W* and Δ, which was similar (Table 7). Grain yield, above ground dry matter production and Δ were significantly higher in landraces than improved genotypes under all moisture treatments (Table 8), while transpiration efficiency (*W*) was lower in landraces, as previously reported (Acevedo,1993; Mahalakshmi et al.,1994).

Discussion

The relationship between carbon isotope discrimination (Δ) and yield in crop species has been reported in the literature to vary from strongly negative (Hubick et al.,1986; Wright et al.,1988 & 1994), positive, or to no relation according to species and growth conditions (Craufurd et al.,1991; Condon et al., 1987; Condon & Richards,1993; Ehdaie et al.,1991; Johnson & Bassett,1991).

Δ is largely related to the ratio of intercellular to atmospheric carbon dioxide concentration (ci/ca). This ratio is in turn influenced by stomatal conductance and photosynthesis capacity of the plant. The relationship between yield and Δ is expected to be positive when stomatal conductance increases and photosynthetic capacity remains constant. Such that ci/ca ratio increases and at the same time transpiration (or water use) efficiency is reduced (Condon et al.,1987). The reverse conditions would lead to a reduction in ci/ca ratio, an increase in *W*, and a negative relationship between yield and Δ (Wright et al., 1994). The two patterns, separately or in combination, were observed in several crop species (Ehleringer et al., 1990; Hubick et al.,1988; Condon et al.,1990).

In this study under the plastic house conditions, the relation between Δ and either grain or dry matter production in barley was strongly positive across all moisture levels. In other studies similar responses were observed in durum wheat (Kirda et al., 1992) and in barley (Austin et al., 1990). Such a positive relationship over an increased moisture gradient is expected,

since stomatal conductance will increase and so will ci/ca and Δ. Similarly, transpiration efficiency will decrease and a negative relationship between yield (GY and AGDM) and *W* might also be expected (Table 3; Figures 1,2 & 3). However, it should be noted that the relationships were inconsistent when they are examined within individual moisture levels. In the first experiment the relationship within each moisture treatment was similar across all moisture levels. Yield correlated highly and positively with Δ and negatively with transpiration efficiency, in the nonstressed and in the fully stressed treatments. While the negative relationships between yield and *W* were weaker in the split moisture treatments (Table 4). In the second experiment the relationship between yield and Δ was significantly positive only in the fully stressed treatment, in the nonstressed moisture regime the relation was weakly negative. Similarly, the relationship between biomass production and *W* was reversed in the nonstressed treatment and became positive (Table 4).

These differences could be partially related to differences in environmental conditions inside the plastic house, and by the minimum differences in yield among moisture treatments in the first experiment. Temperatures were milder in the second experiment, mean maximum temperature was 18.02°C in comparison with 21.8°C in the first experiment. Similarly, relative humidity was 80% and 71% respectively. Consequently, mean daily seasonal vpd was 0.34 kpa in 1991 and 0.53 in 1989, and growing season was longer in 1991. Similar responses in wheat, grown under plastic house conditions, were observed by Ehdaie et al (1991).

A strong positive relation between Δ and yield during the first

season and the higher vpd, especially during the period between heading and maturity (0.71 kpa), suggest that variation in stomatal conductance, rather than photosynthetic capacity, greatly influenced ci/ca ratio and led to the positive relationship. While in the second experiment at a high moisture level, vpd was low, water used in transpiration was high and the negative relation between yield and Δ, indicate that variations in photosynthesis capacity was the controlling factor over the ci/ca variations and caused the negative relationship. Moreover, in the fully stressed moisture treatment, water used in transpiration was similar between the two seasons and regardless of ambient environmental conditions the relation between yield and Δ was positive in both seasons, although weak in 1991 (Table 4). Positive relations between yield and Δ were observed in a close set of barley genotypes grown under field conditions in similar Mediterranean environments (Craufurd et al.,1990), also in durum wheat (Kirda et al.,1992) and in bread wheat (Condon et al.,1987).

Contrary to the relation between Δ and yield the relation between Δ and transpiration efficiency (*W*) were mostly significant and consistently negative as theory predicts (Farquhar & Richards,1984) under all moisture treatments and during both seasons.

An important measure of the suitability of Δ as an indirect selection criterion is the stability of Δ across moisture levels and the relation between Δ and other plant traits under various environmental conditions. In this study, under controlled environmental conditions, the relation between Δ measured at

maturity under unstressed and stressed moisture conditions varied. Correlations were high in one season and low during the other. Similarly the relations between Δ measured at high moisture level and yield or *W* at low moisture levels were significant in the first season and weak in the second (Table 5). Although grain yield rankings were similar between high and low moisture levels during both seasons.

The stability of Δ, or other traits, measured during the two seasons were also weak between the unstressed moisture treatments. Transpiration efficiency (*W*) was the only trait which was consistent during the two seasons. While among moisture stressed treatments Δ, GY and *W* all correlated significantly across the two seasons (Table 6). Therefore, it is concluded that Δ is more stable across moisture limited environments than unlimited ones.

Finally barley landraces from the fertile crescent are known to be among the best adapted genotypes to the harsh Mediterranean environments (Ceccarelli et al., 1991). In this study they had higher yield potentials and ^{13}C discrimination than improved varieties even under unlimited water conditions. However, their transpiration efficiency (*W*) were lower than improved varieties under all moisture levels. Lower *W* at the plant level is not necessarily associated with lower *W* at the crop level (Acevedo, 1993). Canopy growth and soil cover occurs quickly in landraces and therefore reduces direct water evaporation from the soil, and improving crop water use efficiency (*W*). Field studies on the same barley genotypes showed that landraces had a similar or higher *W* than improved varieties at low moisture levels, while

at high moisture levels improved genotypes were higher in *W*. Yet Δ were consistently higher in landraces regardless of moisture levels (Dakheel et al., unpublished).

In conclusion the relationships among yield, transpiration efficiency, and ^{13}C discrimination (Δ) in barley under controlled environmental conditions showed that: First, selection for high Δ is generally associated with higher yield potentials under either high or low moisture levels. Second, higher transpiration efficiencies (*W*) were associated with lower Δ and consequently with lower yield potentials. This is inconsistent with the results obtained from field studies on the same genotypes where the relations between Δ and W were influenced by variation in temperature and moisture levels (Dakheel et al.,1994). Third, the relation between Δ across moisture levels and seasons was consistent only under moisture stress. Suggesting therefore, that utilizing Δ in selection for dry areas is more appropriate when selection is carried out within the targeted environment. Finally, it is emphasized from the controlled environment, as will as field studies (Dakheel et al.,1994), that the relation between Δ and yield across a moisture gradient does not necessarily reflect the relation observed within individual moisture regimes.

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Table 1. ANOVA summary for above ground dry matter production AGDM), grain yield (GY), seasonal water transpired (SWTR), transpiration efficiency (*W*), and ^{13}C discrimination (Δ) measured in peduncles at harvest in 10 barley genotypes grown in plastic house under four moisture treatments during 1989/90 & 1990/91.

		Source of variation		
Variable	Season	moisture	genotype	M X G interactions
	1989	***	***	ns
AGDM	1991	***	*	ns
GY	89	*	***	ns
	91	**	ns	ns
W	89	***	***	ns
	91	*	***	*
Δ	89	***	*	ns
	91	**	**	ns
SWTR	89	***	***	**
	91	***	***	ns

*,**,*** denote significant differences at P<0.05, 0.01 and 0.001 respectively, **ns** not significant .

Table 2. Mean values of different plant traits (symbols as in table 1) in 10 barley genotypes grown under four moisture treatments in the plastic house during 1989/90 and 1990/91.

Moisture treatment	SWTR	GY g/pot	AGDM g/pot	Δ ($X10^{-3}$)	W g.kg	HI
Non-stressed	6615	21.2	47.8	19.44	3.41	0.45
Stressed	3405	12.96	28.4	18.3	3.91	0.46
Pre-heading stress	4433	17.03	36.7	19.8	3.75	0.46
Post-heading stress	5028	14.03	37.2	19.34	3.3	0.37
LSD	453	2.35	3.3	0.310	0.38	0.03

*,**,*** significant differences at P<0.08, 0.05, and 0.01 respectively.

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Table 3. Correlations between grain yield (GY), above ground dry matter production (AGDM), transpiration efficiency (*W*) and ^{13}C discrimination (Δ) in 10 barley genotypes across different moisture treatments, unstressed (FC), and fully stressed (1/3 FC) during two seasons.

	Across four moisture treatments(n=40)				Moisture treatments FC and 1/3 FC (n=20)			
Trait	Δ		*W*		Δ		*W*	
Year	1989	1991	1989	1991	1989	1991	1989	1991
GY (g/pot)	0.75***	0.59***	-0.64***	-0.39***	0.91***	0.85***	-0.70***	0.79***
AGDM (g/pot)	0.65***	0.63***	-0.54***	-0.61***	0.85***	0.90***	-0.74***	-0.84***
W ($g.kg^{-1}$)	-0.70***	-0.20ns			-0.78***	-0.85***		

*,**,*** significant correlation at P<0.05, 0.01,0.001 respectively, **ns** not significant.

Table 4. Correlations between GY, AGDM, *W* and Δ within each moisture treatment for each season in 10 barley genotypes grown under plastic house conditions.

	Moisture treatment							
	FC		1/3 FC		1/3FC-FC		FC-1/3FC	
Trait	Δ	*W*	Δ	*W*	Δ	*W*	Δ	*W*
				1989 season				
GY	0.88***	-0.69*	0.82***	-0.46ns	0.69*	-0.67*	0.59*	-0.42ns
AGDM	0.75**	-0.76**	0.77**	-0.44ns	0.50ns	-0.17ns	0.25ns	-0.46ns
W	-0.83***		-0.81***		-0.84***		-0.74**	
				1991 season				
GY	-0.36ns	0.22ns	0.35ns	0.36ns	0.14ns	0.41ns	0.45ns	0.57*
AGDM	-0.20ns	0.72**	0.66*	0.20ns	0.40ns	0.41ns	0.45ns	0.72**
W	-0.10ns		-0.69*		-0.45ns		-0.66*	

n=10. *,**,*** significant correlations at P<0.05, 0.01 and 0.001 respectively, ns not significant.

Table 5. Correlations of Δ *W*, GY and AGDM between moisture levels in 10 barley genotypes grown under plastic house conditions within each growing season.

Unstressed treatment

1989 season

Mean of all stressed treatments	Δ	*W*	GY	AGDM
Δ	0.94***			
W	-0.66*	0.84***		
GY	0.71***	-0.67*	0.91***	
AGDM	0.45ns	-0.35ns	0.73***	0.85***

1990 season

Δ	-0.41ns			
W	0.14ns	0.44ns		
GY	-0.50ns	-0.26ns	0.83***	
AGDM	-0.50ns	0.01ns	0.10	0.10ns

n=10. *,**,*** significant correlations at P<0.05, 0.01 and 0.001 respectively, ns not significant.

Table 6. Correlations between the two seasons among moisture stressed and unstressed treatments for Δ, W ,GY and AGDM in 10 barley genotypes grown under plastic house conditions.

	1989 season			
	Δ	GY	AGDM	W
	Unstressed			
1991 season				
Unstressed	-0.43ns	0.32ns	-0.30ns	0.57*
	Stressed			
Stressed	0.58*	0.72*	0.54ns	0.85***

n=10. *,**,*** significant correlations at P<0.05, 0.01 and 0.001 respectively, ns not significant.

Table 7. Correlation coefficients among various traits in landrace (L) and improved (I) barley genotypes grown under plastic house conditions. (Values are means of two seasons).

	Δ		W	
	I	L	I	L
GY	0.67***	0.46*	0.58**	-0.44*
AGDM	0.63**	0.43	0.66**	-0.42
W	-0.46*	-0.50*		

n=10. *,**,*** significant correlations at P<0.05, 0.01 and 0.001 respectively, ns not significant.

Table 8. Mean values of different plant traits for improved (I) and landrace (L) varieties grown under plastic house conditions during two seasons (1989 & 1991).

Moisture treatment	Type	GY g/pot	AGDM g/pot	Δ ($X10^{-3}$)	W g.kg
Non-stressed	I	19.3	46.1	19.20	3.23**
	L	23.2**	49.5*	19.7**	2.99
	LSD	2.00	1.76	0.216	0.17
Fully stressed	I	11.6	26.0	18.05	3.98
	L	14.3***	30.8***	18.6**	3.84
	LSD	0.894	2.16	0.365	0.29
Pre-heading	I	16.7	36.6	19.53	3.84
stress	L	17.3*	36.8	20.0***	3.66
	LSD	0.96	1.91	0.216	0.24
Post-heading	I	13.0	36.8	19.21	3.49**
stress	L	15.0**	37.6	19.5*	3.11
	LSD	1.05	2.08	0.19	0.225
Mean	I	15.15	36.4	18.99	3.64**
	L	17.45	38.7	19.45***	3.40
	LSD	2.2	3.61	0.197	0.168

*,**,*** significant differences at P<0.08, 0.05, and 0.01 respectively.

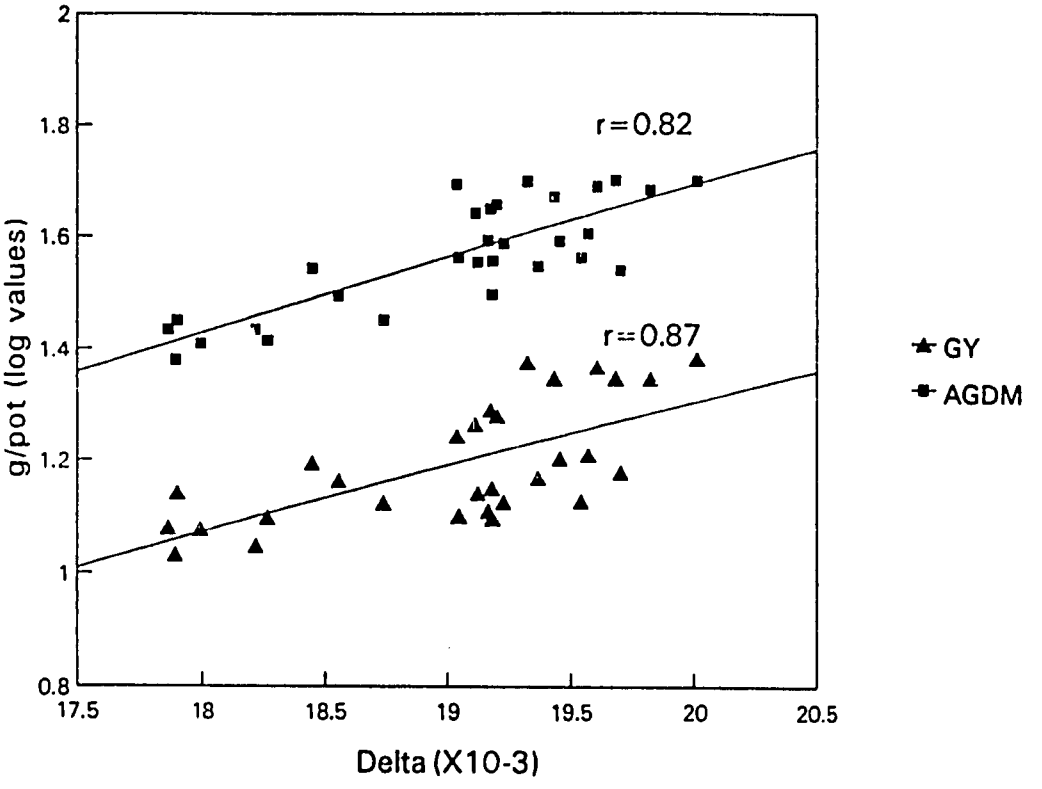

Fig. 1 & 2. Relation between ^{13}C discrimination (Δ) and yield (GY and AGDM) across the three moisture treatments (FC, 1/3 FC and terminal stress) for the two seasons.

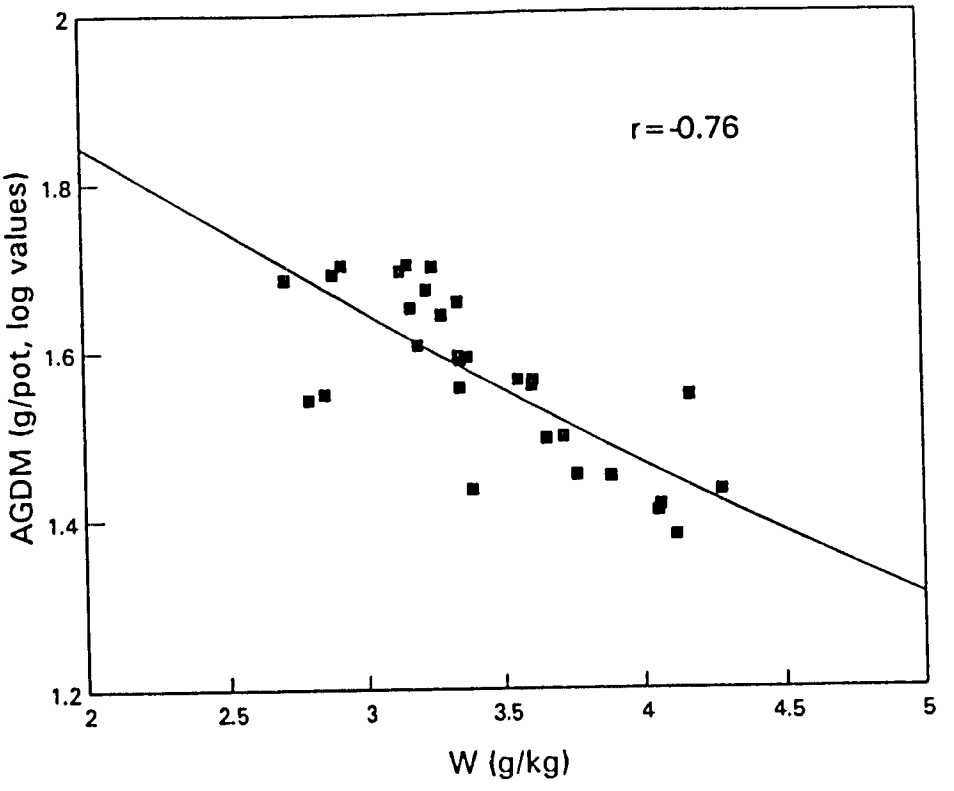

Fig. 3. Relation between yield (AGDM) and transpiration efficiency (W) across all but the pre-heading moisture stress treatment for the two seasons.

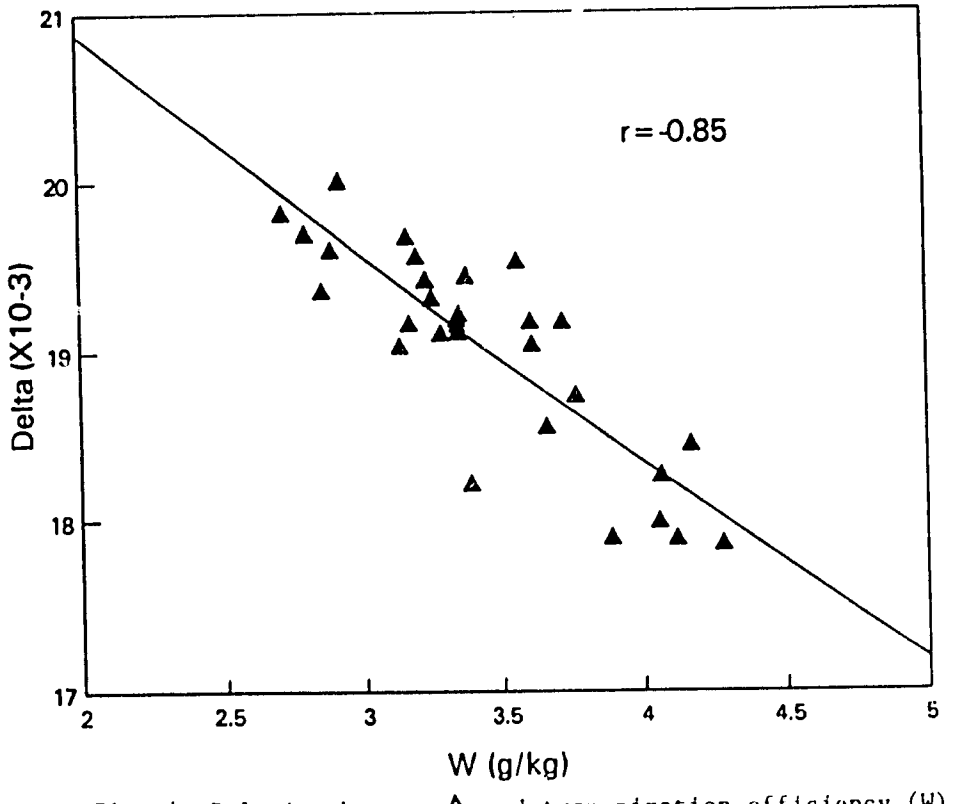

Fig. 4. Relation between Δ and transpiration efficiency (W) across the moisture treatments (FC, 1/3 FC and terminal stress) for the two seasons.