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Carbon isotope discrimination as a means of evaluating drought resistance in barley, rice and cowpeas

by Roger B. AUSTIN [1], Peter Q. CRAUFURD [2], Michael A. HALL [3], Edmundo ACEVEDO [4], Beatriz da SILVEIRA PINHEIRO [5] and Eliud C.K. NGUGI [6]

[1] Institute of Plant Science Research (Cambridge Laboratory), Maris Lane, Trumpington, Cambridge CB2 2JB, UK.
[2] International Institute of Tropical Agriculture, Kano Sub-station, PMB 3112, Kano, Nigeria.
[3] Sub-Department of Quaternary Research, University of Cambridge CB2 3RS, UK.
[4] International Centre for Agricultural Research in the Dry Areas, P.O. Box 5466, Aleppo, Syria.
[5] Centro National de Pesquisa-Arroz, Feijão, Empresa Brasileira de Pesquisa Agropecuaria, CP 179, Goiania, Goias, Brasil.
[6] National Dryland Farming Research Centre, P.O. Box 340, Machakos, Kenya.

Summary.- In all the crops studied there was variation among genotypes in carbon isotope discrimination (Δ). In trials of barley genotypes there were strong positive correlations between grain yield and Δ, though in trials which were irrigated or received abundant rainfall, this correlation was non-significant or negative. Some of the variation in yield and Δ was related to date of heading, as early varieties in stressed trials had highest yields and had high Δ.

In an experiment with 8 early and 10 late flowering rice genotypes there were consistent positive correlations between yield and Δ in the early group, but in the late group the correlations tended to be negative. Among the early group the early genotypes were highest yielding, and may be presumed to have avoided the worst effects of drought and hence had higher Δ. In the late group, there was no correlation between yield and date of flowering.

In one experiment with cowpeas, a weak positive correlation between grain yield and Δ was observed, but in a second trial, in an abnormally wet season, no such correlation was observed.

In all three crops further work is needed before the usefulness of carbon isotope discrimination for screening for high yield in water-limited environments can be assessed.

Résumé.- Chez toutes les espèces cultivées étudiées, il a été observé des différences entre les génotypes en ce qui concerne la discrimination isotopique du carbone (Δ). Chez l'Orge, il existe une corrélation positive très étroite entre le rendement en grains et Δ, bien que chez les plantes bien irriguées ou ayant reçu une pluviosité abondante cette corrélation ne soit pas significative ou même devient négative. Certaines variations dans le rendement et Δ sont reliées à la date de montaison, les variétés précoces ayant des rendements plus élevés et une valeur plus grande du Δ, dans les lots stressés.

Chez le Riz (8 génotypes à floraison précoce et 10 à floraison tardive), on a observé une corrélation positive entre le rendement et Δ chez les génotypes précoces, mais chez les génotypes tardifs, les corrélations tendent à devenir négatives. Dans le groupe précoce, les génotypes précoces ont le rendement le plus élevé, et on suppose qu'ils ont évité les effets les plus néfastes de la sécheresse et donc on un Δ plus élevé. Dans le groupe tardif, il n'y a pas de corrélation entre le rendement et la date de floraison.

Chez le Niébé, dans une expérience, il a été observé une corrélation positive, mais faible entre la production en grains et Δ, mais dans une autre expérience qui a eu lieu au cours d'une saison anormalement humide, aucune corrélation n'a été mise en évidence.

Chez les 3 espèces cultivées, des études plus poussées sont donc nécessaires avant de conclure à la possiblilité d'utiliser la discrimination isotopique du carbone pour le screening de variétés à hauts rendements et résistants aux environnements limitants en eau.

Key words : barley - rice - cowpeas - carbon 13 - carbone isotope discrimination - drought - yield - water use efficiency.

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INTRODUCTION

About 99% of the carbon atoms in atmospheric carbon dioxide have a mass of 12, but the remaining 1% are of the heavier, non-radioactive isotope of mass 13. During photosynthesis, plants discriminate aginst the heavier isotope. Discrimination (4,4‰) occurs because $^{13}CO_2$ diffuses less rapidly than $^{12}CO_2$ from the atmosphere to the sites of carboxylation (Craig, 1954). Also, during the carboxylation of RuBP, there is a discrimination of about 27‰ (Park and Epstein, 1960 ; Wong, Benedict and Kohel, 1979). In C3 plants, the net discrimination (Δ) varies between 4,4 and 27‰ reflecting the balance between the stomatal control of photosynthesis and that exerted by carboxylation (Farquhar and Richards, 1984).These authors have pointed out that the measure of this balance, the ratio of the intercellular (pi) and atmospheric (pa) partial pressures of CO_2 is simply related to Δ by the following expression :

$$\Delta = (4,4 + 22,6\ pi/pa) \times 10^{-3}$$

At constant atmospheric vapour pressure, variations in either stomatal conductance or photosynthetic capacity affect the ratio of assimilation to transpiration, or water use efficiency (WUE). Thus genetic variation in WUE in a given environment should be correlated with variation in Δ, a low value of Δ being indicative of a high WUE. However, leaf transpiration (T) is affected directly by stomatal conductance (g_l) and the leaf-to-air difference in vapour pressure (e_i-e_a), T being equal to g_l (e_i-e_a), while assimilation (A) is not. Thus the actual relation between A/T (or leaf water use efficiency) and Δ is a function of g_l, carboxylation capacity and (e_i-e_a). In addition, crop water use efficiency is less than leaf water use efficiency because some water is lost by evaporation from the soil surface.

Condon, Richards and Farquhar (1987) have shown for rainfed wheat grown in South Australia that there is a positive phenotypic correlation between grain yield and Δ measured on grain (grain Δ). This result implies that high yield is associated with low WUE, which at first sight appears paradoxical. However, this result would be expected if there was a large diurnal variation in temperature and hence in leaf-to-air water vapour pressure difference and genotypes varied in the proportion of their daily photosynthesis that occurred at the beginning and end of the photoperiod, when vapour pressure difference was least and actual WUE greatest. It would also be expected in a cropping system in Mediterranean climates where drought became progressively more severe as the season advanced. In these conditions, grain filling of early flowering genotypes would occur when the vapour pressure difference was lower

than later in the season so that the actual WUE of the early flowering types would be greater, but Δ also greater.

In 1985, work at IPSR, Cambridge and ICARDA, Syria with spring barley was undertaken to investigate the relationships between Δ and grain yield and to ascertain the value of Δ for screening for high yield in early generations of a breeding programme. In 1988, the work was extended to rice and cowpeas, with the same objectives. This paper summarises the results of this work to date.

MATERIALS AND METHODS

Barley

Thirty-six genotypes, or subsets of them, were studied. They comprised landraces collected mostly from the drier barley-growing areas of Syria, as well as introduced material and derived breeding lines known to yield well in the moister areas of the country.

Field experiments comparing these genotypes of spring barley adapted for growing in Syria were grown at three locations in Syria, differing in expected rainfall. The moist site, Tel Hadya had a long term seasonal rainfall of 328 mm ; the intermediate site, Breda 281 mm, and the driest site, Bouider, 212 mm. All trials were of a randomised block design with three or four replicates. Plots were at least 1,5 x 5 m. Fertiliser was applied at modest rates and weeds and diseases controlled by application of proprietary agrochemicals. These trials were sown in 1985 and 1987. In 1988 and 1989 they were carried out at Breda only, at normal seeding rate and in plots with the plants spaced 30 x 10 cm.

At Cambridge, trials were sown with the same genotypes in 1986 and 1988. Each year one trial was subjected to a terminal drought, being sheltered from rain from the beginning of May to maturity, while the other one received natural rainfall supplemented by irrigation to maintain the soil moisture 15 mm of field capacity. In 1988, an additional experiment was sown at low density (plants spaced at 30 x 30 cm) and subjected to a terminal drought.

In all experiments, dates of anthesis were recorded and at maturity grain and sometimes straw yields were recorded and samples of grain and straw analyzed for Δ. In 1988, from both the spaced- and dense-planted experiments, plants were also sampled during vegetative growth, and at anthesis and the plant material analysed for Δ.

All values of Δ were expressed relative to the international Pee Dee Belemnite (PDB) standard. Relative to this standard, the apparent isotopic discrimination of air was -8,07‰. As air contains trace amounts of nitrous oxide, a correction for this was made by adding 0,22‰ to the apparent air value. To obtain values of plant Δ relative to air, the apparent discrimination of air (-8,07 + 0,22 = 7,85‰) was subtracted from the plant Δ values relative to PDB.

Rice

In 1988, a trial was carried out to compare 18 rainfed rice genotypes, of which ten were late flowering and eight early flowering. There were four sowing dates, at weekly intervals, from 14 January (late genotypes) and 28 January (early genotypes). The trial was of a split plot design with four replicates, the sub-plots being 1,5 x 5,0 m. The entire experiment was irrigated by sprinklers throughout growth except during a stress period from 24 April to 20 May. Dates of appearance of first flowers and grain yield were measured. Subsequently, grain samples were analyzed for Δ, corrected as described for barley.

Cowpeas

In April 1988 and in October 1988 trials were sown at the Kiboko substation of the NDFRC, Kenya. Both trials compared the same 72 genotypes of cowpea in plots 1,2 x 4,0 m in a randomised block experiment with two replications. The date of appearance of the first flowers, and date of maturity were recorded for each plot, and grain yield (and in the experiment sown in October, 1988, straw yield) taken. Grain samples were analyzed for Δ, and corrected as described for barley.

RESULTS AND DISCUSSION

Barley

Table 1 gives trial mean grain yields and grain Δ for all 12 trials harvested in 1986 and 1988 and the phenotypic correlation coefficients between

grain yield and Δ. Considering only the drilled trials, the greater the yield, the greater the Δ. As grain Δ is a crude measure of the severity of drought during grain filling, the relationship between grain yield and Δ (Fig. 1 A) shows that water availability was the main factor causing variation in trial mean yields.

Table 1.- Trial mean, grain yield, grain Δ and phenotypic correlation between yield and Δ.

Experiment		N[1]	Grain yield g m^{-2}	Grain Δ %	r[2]
(a) Drilled trials					
Tel Hadya	1986	21	346	17,37	0,216
	1988	21	285	17.58	-0,019
Breda	1986	21	139	14,41	0,836
	1988	21	291	17,44	0,262
Bouider	1986	21	135	14,80	0,730
	1988	21	277	17,75	0,257
Cambridge irrigated	1986	23	374	18,84	-0,534
	1988	21	362	19,29	-0,385
Cambridge droughted	1986	23	221	16,54	0,490
	1988	21	294	17,56	0,569
(b) Spaced-plant trials					
Breda	1988	24	348	16,74	0,433
Cambridge droughted	1988	21	105	17,42	0,763

[1] number of genotypes in trial
[2] correlation coefficient, degrees of freedom N-2

In general, for those trials which experienced drought, there was a positive correlation between genotype mean grain Δ and grain yield (Table 1, Fig. 1 B). Thus, in the droughted trials, high yielding genotypes had high Δ and so, apparently, were less efficient in water use. There are two possible explanations for this paradoxical result, as noted in the Introduction. When stomata are well open Δ will be higher than when they are less open. Stomata will tend to be more open earlier in the season than at its end. Thus genotypes which are early flowering and maturing will have a higher grain Δ than later flowering ones. As previous studies on these genotypes have shown, the early ones are also high yielding (Acevedo *et al.*, in press) because, it is presumed, they escape the detrimental effects of late drought. It follows from this reasoning that a positive correlation between grain yield and grain Δ may be expected in this set of genotypes. Although a high Δ is indicate of low water use efficiency, this is only true at a fixed atmospheric water vapour pressure deficit (see Introduction). Assuming a fixed stomatal conductance, transpiration will be lower in humid than in drier air, but photosynthesis, and Δ will remain constant. Early in the season, when the air is humid, stomata will be open, but water use

efficiency high, and Δ will be high because it is dominated by the discrimination arising from carboxylation. These predicted relationships are illustrated in Fig. 2 for typical values of atmosphere and plant parameters.

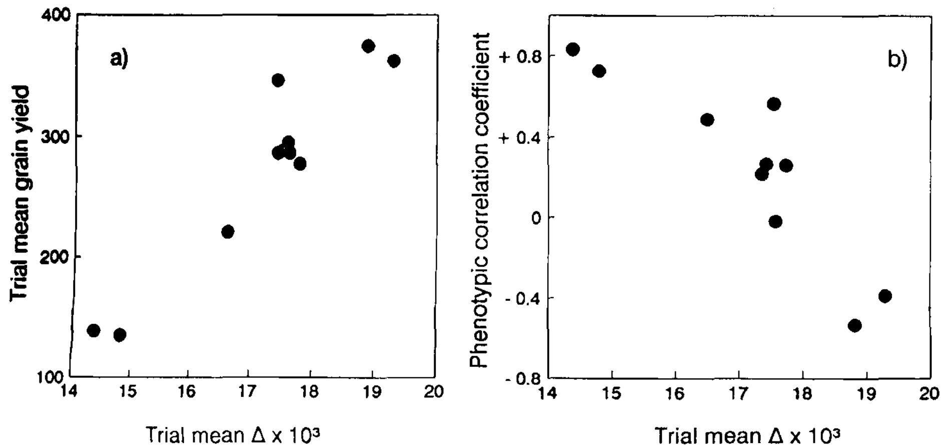

Fig. 1.- Trials with barley

A.- Relationship between trial mean grain yield and trial mean grainΔ.

B.- Relationship between trial mean Δ and the phenotypic correlation coefficient between grain yield and grain Δ.

The second possible reason for the positive correlation between grain yield and grain Δ involves the same general reasoning, but for diurnal changes in atmospheric water vapour pressure deficit. In this case it is assumed that in the higher yielding genotypes, more photosynthesis occurs at the beginning of the day when the air is more humid, water use efficiency is greater and Δ higher, than is so for lower yielding genotypes. Fig. 3 shows two simulations of this effect for typical values of plant parameters and diurnal changes in atmospheric humidity.

It should be emphasised that although these scenarios are consistent with the observed results, we have as yet no direct evidence that they are correct, or if so, of the relative importance of the seasonal and diurnal effects. It seems unlikely that the seasonal effects on Δ can fully account for the observed relationship between Δ and grain yield. Table 2 shows that Δ measured on plants at the tillering stage and at heading is correlated with grain yield. Furthermore, Δ at tillering was consistently positively, though weakly, correlated ($p = 0{,}10 - 0{,}05$) with plant weight at this stage for spaced and drilled trials at Breda and Cambridge.

To be useful in screening for high yield in stressed environments, any character apart from yield itself must be highly heritable and strongly correlated with yield. In barley, as for other small grain cereals, no selection for yield is possible until the F4 or later generations. In earlier generations, where selection for yield would be desirable, progenies are still segregating and it is normal

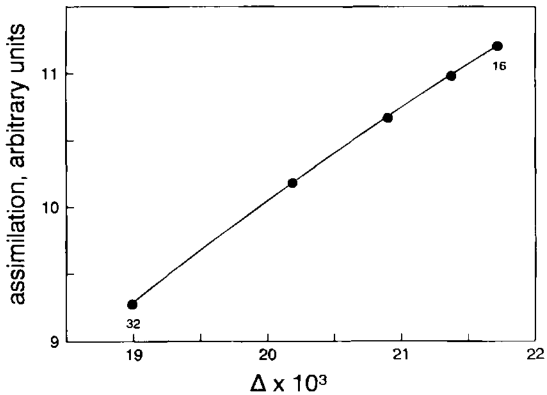

Fig. 2.- Simulated relationship between Δ (‰) and assimilation during grain filling (arbitary units) as a function of date of anthesis (16 = earliest anthesis, 32 = latest anthesis). The model simulated assimilation, transpiration and Δ during grain filling. The main features of the model were : there was a seasonal increase in air temperature and leaf to air vapour pressure deficit (VPD) ; transpiration was computed between an arbitary starting date and the date of anthesis was a function of stomatal conductance and the prevailing VPD. Green area started to decline 10 days after anthesis and photosynthetic capacity from the date of anthesis. Stomatal conductance during grain filling was a function of the available soil moisture and of VPD. Duration of grain filling was 30 day ; computation were done on a daily basis and Δ is the mean for the total assimilation during the grain filling period. Temperatures and VPD's and dates of anthesis are appropriate for barley grown in Tel Hadya, Syria.

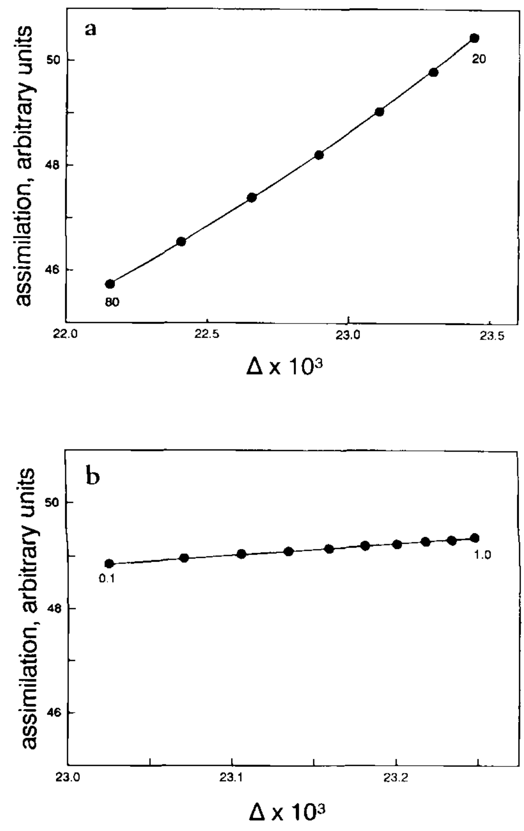

Fig. 3.- Simulated relationships between Δ (‰) and assimilation during a day (arbitary units not comparable with those in Fig. 2). A diurnal change in air temperature and VPD typical of that encountered at Tel Hadya, Syria, during the grain filling period of a barley crop are assumed, VPD and air temperature rising to a maximum at midday (down = 0°, midday = 90°, dusk = 180°). Photosynthetic capacity was assumed to be maximum at 10° and 170°, rising from and falling to zero at 0° and 180° and declining sinusoidally to a minimum at midday. In (a) times of maximum stomatal conductance (g_s = 1,0) vary from 20° and 160° to 80 and 100°, conductance decreasing sinusoidally before and after these maxima to g_s = 1,0. In (b) the time of maximum g_s (= 1,0) was fixed at 40° and 140°, but the midday minimum g_s ranged from 0,1 to 1,0. Other conditions were as for (a). Note that the relation between assimilation and Δ is much more sensitive to the times when g_s are maximum than it is to the extent of midday stomatal closure in these simulations.

to select individual plants for characters other than yield (ear type, stature, disease reaction, etc.), and for this to be practicable, they have to be relatively widely spaced. It may be expected that the expression of some characters, including those which affect water use efficiency will vary with plant spacing. To test the usefulness of grain Δ measured on speced plants as a predictor of yield at normal seeding rates in droughted trials, we grew two trials at wide spacing and measured grain Δ and yield. Table 1 shows that yield and grain Δ in these trials were significantly positively correlated. The correlations between Δ measured on various tissues and stages of growth on spaced plants and grain yield measured in adjacent trials at normal density is given in Table 2. Evidently, grain Δ measured on spaced plants is very strongly correlated with that measured in drilled trials at the same site and year.

Table 2.- Correlations between Δ measured on grain in drilled (dense) trials and that measured on various organs of plants from drilled and spaced-plant (spaced) trials.

	Breda[1] 1988		Cambridge[2] (droughted) 1988	
	dense	spaced	dense	spaced
grain	1,000	0,714	1,000	0,825
peduncle at maturity	0,877	0,850	0,708	0,711
straw at maturity[3]	0,868	0,752	0,403	0,748
whole plant at heading	0,443	0,513	0,725	0,617
whole plant at tillering	0,428	0,369	0,788	0,414

[1] N = 24
[2] N = 21
[3] leaves, stems but not chaff

Rice

A summary of the mean yields, grain Δ and days from sowing to flowering by sowing and variety group is given in Table 3. From this table it can be seen that yield decreased with late sowing, particularly in the late flowering genotypes. Also, Δ decreased with late sowing and was lower for the late than for the early varieties. As low grain Δ is an integrated measure of stress experienced during grain filling, it follows that late sowings experienced more stress and late genotypes were more stressed than early ones. All these results are consistent with theoretical expectations.

The next step in the statistical analysis was to seek correlations, within variety groups and sowings, between yield, time to flowering and Δ. The correlation coefficients are given in Table 4.

Taken individually, it can be seen that only four coefficients were significant (as indicated by an asterisk in the table). This is because each coefficient was based on only 6 or 8 degrees of freedom. inspection of the data suggests that the early varieties behaved differently from the late ones. The coefficients for each group were combined using Fisher's z transformation. The three combined coefficients for the early genotypes were all significant at $p < 0,05$, indicating a positive phenotypic correlation between yield and Δ and negative ones between flowering and Δ and yield and flowering. In other words,

Table 3.- Yields, Δ and days from sowing to flowering of eight early and ten late flowering rice varieties in the trial at CNPAF-EMBRAPA in 1988, for four sowings.

	Sowing 1	Sowing 2	Sowing 3	Sowing 4
(a) yield g m^{-2}				
Early	253	253	216	154
Late	265	224	161	96
(b) Δ (grain), parts per thousand				
Early	18,67	18,16	17,73	17,07
Late	18,24	17,39	17,17	16,71
(c) days from sowing to appearance of first flowers				
Early	72,2	70,7	68,6	67,3
Late	94,0	91,7	92,0	91,9

Table 4.- Correlations among yield, days to flowering and Δ for early and late rice varieties sown on four occasions at CNPAF-EMBRAPA, Brazil.

		Yield & Δ	Yield & flowering	Δ & flowering
(a) early genotypes (6degrees of freedom)				
Sowing	1	0,681	0,083	-0,447
	2	0,095	-0,689	-0,260
	3	0,746*	-0,505	-0,579
	4	0,768*	-0,530	-0,802*
	Average	0,572	-0,542	-0,472
(b) late genotypes (8 degrees of freedom)				
Sowing	1	-0,394	0,185	-0,502
	2	-0,743*	0,359	-0,375
	3	-0,339	0,046	-0,183
	4	+0,017	-0,447	-0,121
	Average	-0,364	-0,036	-0,295

among early genotypes, late flowering ones gave low yield and had lower Δ (were more stressed). This result is similar to that found by Condon, Richards and Farquhar (1987) for wheat and in the present study for barley.

In contrast, among the late flowering genotypes, there were generally no significant relations among yield, days to flowering and Δ, even though the late group were more stressed (as judged by Δ, Table 3) than the early group. A possible reason for this result is that there was no association between yield and days to flowering among the late group and so late ones were not more stressed than early ones. In consequence, the weak negative association between yield and Δ reflected the influence of factors other than earliness on water use efficiency. From theoretical considerations, where there is no variation in date of flowering among a set of genotypes, a negative association between yield and Δ may be expected, i.e. high yielding varieties are most water use efficient.

Cowpeas

In both experiments there was significant variation among the 72 genotypes in both grain yield and grain Δ In the first season the plants experienced some drought and the mean Δ was 14,29‰. The second season was extremely wet and the mean Δ was 18,16‰. Mean grain yields in the two seasons were 150 and 288 g m^{-2} respectively.

In the first season there was a weak positive correlation between grain yield and grain Δ ($r = +\ 0,192$), but in the second season the correlation was close to zero. There was only a moderate correlation between grain yield in the two seasons ($r = 0,334$) but no correlation over seasons for Δ. However, Δ in the second season was significantly correlated with grain yield in the first season ($r = +\ 0,285$). One interpretation of these results is as follows : in the first season when the plants were moderately stressed, the higher yielding genotypes were those which avoided drought (as was the case in the experiments with barley), partly because they were early flowering. In the second season, the varieties that produced the largest plants (heaviest straw), which tended to be the later flowering ones ($r = 0,484$), gave the greatest yields (the correlation between grain and straw yield was + 0,360). There was some evidence that high Δ in the wet year was a reflection of physiological properties which were beneficial in the dry year. These may include midday stomatal closure which would increase both Δ and water use efficiency.

Clearly date from variety trials in more seasons is needed to establish these associations more securely, and such trials are in progress. Genetic studies to investigate the inheritance of Δ, and to examine genetic associations with yield have also been undertaken.

CONCLUSION

In barley, rice and cowpeas there was significant variation among varieties in carbon isotope discrimination (Δ). In experiments with these species where there was a drought during grain filling, there was usually a positive correlation between grain yield and Δ measured on grain samples. Especially in barley, this occured in part because early flowering varieties escaped the worst effects of drought, had on average greater stomatal conductance and so a higher Δ. It is also possible that the extent of diurnal variations in stomatal conductance and photosynthesis may differ among genotypes and this could be an additional factor making for a positive association between yield and Δ.

A reliable screening method for high yield in water limited environ-

ments would be of great value to plant breeders. More research is needed to establish whether carbon isotope discrimination meets the criteria needed for a practicable test.

BIBLIOGRAPHY

ACEVEDO E., P.Q. CRAUFURD, R.B. AUSTIN et P. PEREZ-MARCO.- Traits associated with high yield in barley with low-rainfall environments. J. agric. Sci., Camb. (in press).

CONDON A.G., R.A. RICHARDS et G.D. FARQUHAR, 1987.- Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field grown wheat. *Crop Sci.*, **27**, 996 - 1001.

CRAIG H., 1954.- Carbon 13 in plants and the relationships between carbon 13 and carbon 14 variations in nature. *J. geology*, **62**, 115 - 149.

FARQUHAR G.D. et R.A. RICHARDS, 1984.- Isotope composition of plant carbon correlates with use efficiency of wheat genotypes. Aust. *J. Plant Physiol.*, **11**, 539 - 552.

O'LEARY M.H., 1981.- Carbon isotope fractionation in plants. *Phytochem.*, **20**, 553 - 567.

PARK R. et S. EPSTEIN, 1960.- Carbon isotope fractionation during photosynthesis. Geochim. Cosmochim. *Acta*, **21** 110 - 126.

WONG W.W., C.R. BENEDICT et R.J. KOHEL, 1979.- Enzymic fractionation of the stable isotopes of carbon dioxide by ribulose-1,5-bisphosphate carboxylase. *Plant Physiol.*, **63**, 852 - 856.