Pak.J.Bot., 30(1): 87-93, 1998.

CARBON-ISOTOPE DISCRIMINATION IN WHEAT UNDER SALINE CONDITIONS

R. ANSARI, S.S.M. NAQVI, A.N. KHANZADA AND K.T. HUBICK*

Atomic Energy Agricultural Research Centre,
Tandojam, Pakistan.

Abstract

A study using 21 cultivars of wheat (*Triticum aestivum* L.) and a salt tolerant barley (*Hordeum vulgare* L.) showed significnat variation in Δ (discrimination against ^{13}C during photosynthetic CO_2-uptake) among cultivars and salinity levels. Salinity reduced straw, grain weight and the average value of Δ in all the cultivars. The reduction caused by salinity varied among cultivars. Barley had larger values of Δ than did wheat and there was a positive correlation between Δ and production of straw (r=0.36), grain (r=0.44) and total biomass (r=0.47).

Introduction

Plants fix CO_2 containing the lighter of the naturally-occuring stable isotopes of carbon, ^{12}C, faster than the heavier isotope, ^{13}C, resulting in a smaller ratio of $^{13}C/^{12}C$ in plant tissue than that of the atmosphere. This discrimination against ^{13}C, expressed as Δ varies with photosynthetic pathway. Species with the C_4 photosynthetic pathway discriminate much less than those with the C_3 pathway because of the different isotope effects of PEPCase and Rubisco (Farquhar *et al.*, 1989). Smaller, but significant variation occurs in Δ because of the genetic and the environmental effects (Hubick *et al.*, 1988).

Theoretically Δ and W (molar ratio of carbon gain by a plant to water loss) should be correlated because of independent links to p_i/p_a, the ratio of partial pressures of internal CO_2 (p_i) and ambient CO_2 (P_a). Experimentation has established that there is a negative correlation between Δ and W in pot grown and field grown C_3 plants (Wright *et al.*, 1988). A number of plants including field crops e.g., cotton (Lu *et al.*, 1996), wheat (Condon *et al.*, 1990), barley (Walker & Lance, 1991), coffee (Meinzer *et al.*, 1992), groundnut (Nageswara Rao *et al.*, 1995) and trees e.g., mangroves (Lin & Sternberg, 1992), *Acacia, Eucalyptus, Melaleuca* sp., (Stewart *et al.*, 1995) have been tested and it has been concluded that in general, plants with greater values of Δ will have smaller W in a particular environment.

Availability of water is a basic requirement for all plant growth and most of the above studies deal with Δ in foliage in relation to substrate moisture status. The constraints of present day agriculture include increasing salinity in many parts of the world affecting crop yields on vast areas. This emphasizes the need to find a suitable marker imparting salt tolerance in plants. A suitable criterion may be Δ among others like accumulation or exclusion of ions, production of compatible osmotic solutes

*Department of Industry, Technology and Commerce, GPO Box 9839, Canberra 2601, Australia.

Table 1. Isotope Discrimination (Δ) and yield in wheat as affected by salinity.

	$\Delta \times 10^3$	Yield/plant Grain	Straw	Biomass
		Normal plants		
Mean	18.98	17.51	15.61	33.12
Maximum	20.12	29.19	25.75	54.94
Minimum	17.55	5.38	6.90	13.03
		Stressed plants		
Mean	16.97	5.87	6.23	12.10
Maximum	20.16	16.14	14.57	30.71
Minimum	14.57	2.08	3.00	5.53
S.E.	0.54	0.77	0.67	1.15

in the tissue under salinity stress (Flowers *et al.*, 1977, Greenway & Munns, 1980). There are some reports of the effect of the relationship between Δ and salt tolerance (Flanagan & Jeffries, 1989; Guy *et al.*, 1986), but detailed investigations have yet to be undertaken. This study may help to emphasize the requirement for a systematic study of this relationship, particularly among different cultivars within a species like *Triticum*, where the genetic control of Δ has been reported to be fairly strong (Farquhar *et al.*, 1989).

Materials and Methods

Twenty one cultivars of wheat (*Triticum aestivum* L.) and a barley (*Hordeum vulgare* L. cv. California mariout) were grown in cemented tanks (9x 1.2x0.3 m) filled with acid-washed river sand which was supplemented with 0.4% (w/v) NaCl and irrigated periodically with full strength Hoagland solution. Care was taken to irrigate well before any symptoms of drought stress (leaf wilting) were visible. Seeds were sown 20 cm apart in rows at a distance of 3 cm between them. The experiment was laid out according to a randomized complete block design with three replicates. Plants were harvested at maturity and the weights of the straw, grain and whole plants were recorded.

A subsample of approximately 10 mg of finely ground ($<100\ \mu$ particle size) material was analysed for stable carbon isotopes content at the Australian National University, Canberra and the value of Δ was calculated according to Hubick *et al.*, (1986), using a value of 7.6 per mil on the PeeDee Belemnite scale for the isotopic composition of the atmosphere. The precision of duplicate analyses of any one sample was approximately 0.1 per mil.

Results and Discussion

Carbon-isotope discrimination measured in the grain, straw and total plant material was significantly reduced in all salinity stressed plants compared to control (Table 1). Differences in the response to salt treatment occured among cultivars, indicated by significant interaction for salinity x cultivar treatment effects (Table 2). The mean values of Δ , for the cultivars (Table 3), were in the range (16.7 to 15.6 x 10^3) previously reported for wheat and barley (Condon *et al.*, 1987; Hubick & Farquhar, 1989).

It is also evident that the grain or biomass yield, in the cultivars under test, generally followed the pattern observed in changes in Δ irrespective of salinity (Fig.1). Earlier data on salt tolerance (Khanzada *et al.*, 1986) compared with Δ values of the same cultivars, tested in the present study (Table 3), indicate a general association of improved salt tolerance with greater values of Δ . Comparison between Δ and grain, straw and total biomass, however, showed a positive correlation only under salt stress (Table 4).

The reduction in Δ indicates a reduction in the ratio of intercellular to ambient partial pressures of CO_2, $_{pi/pa}$ and therefore, an increase in W at the level of the leaves. There are two main sources of change which could cause reduction in $_{pi/pa}$, which is a measure of the relative rates of leaf conductance and photosynthetic CO_2 uptake. On the one hand, stomata may have closed and reduced leaf conductance more relative to any change in photosynthetic rate. On the other hand, photosynthetic rate may have increased relatively more than any reduction in leaf conductance. Since leaf conductance was not measured so it is not known whether the reduction in $_{pi/pa}$ could be attributed to an increased photosynthetic capacity or to the stomatal closure. If Δ decreased because of stomatal closure, then it is less likely that whole-plant W increased with the change in Δ than if the main change was in photosynthetic capacity. Other changes may have occurred, such as increased losses of carbon due to respiration which would affect plant biomass independent of any changes in $_{pi/pa}$ and W.

Plants have adaptations to a certain extent, to survive with elevated root zone salinity. Some adaptations also allow survival during periods of water shortage which have given rise to the concept of physiological drought. One physiological drought

Table 2. Mean squares for various characters in wheat under normal and saline conditions.

Source of variation	Degrees of Freedom	Discri-mination	Grain per plant	Straw per plant	Biomass per plant
Salinity	1	86.487**	2980.655**	1936.032**	9721.119**
Cultivars	21	2.114**	80.84**	228.475**	281.706**
S x C	21	1.762*	37.05**	82.525**	109.009**
Error	44	0.580	1.174	0.911	0.632

*,**indicate significance at 0.05, 0.01 level of significance.

Table 3. Effect of salinity on carbon discrimination ($\Delta \times 10^3$) in wheat and barley

Sr.No.	Cultivars	0	0.4	Cultivars mean
1.	M-154	18.42	14.96	16.69
2.	Punjab-81	18.83	14.57	16.70
3.	M-7/81	18.86	14.95	16.90
4.	Sarsabz	19.31	15.47	17.39
5.	Pavon	18.16	16.75	17.46
6.	Sind-83	18.19	16.89	17.54
7.	Jauhar-78	18.79	16.32	17.56
8.	PKV-1610	18.37	16.77	17.57
9.	C-591	17.55	17.88	17.72
10.	H-68	18.54	17.42	17.98
11.	Mehran	18.92	17.03	17.98
12.	PKV-1800	19.08	16.88	17.98
13.	Lu-26	18.55	17.74	18.15
14.	PKV-1600	19.18	17.13	18.15
15.	Shorawaki	18.64	17.82	18.23
16.	Sonalika	19.21	17.46	18.33
17.	Sind-81	19.80	16.93	18.36
18.	PKV-7050	19.67	17.23	18.45
19.	Pak-81	20.05	17.06	18.55
20.	Kharchia	20.13	17.64	18.88
21.	Karto	19.72	18.30	19.01
22.	Barley	19.01	20.16	19.59
	LSD (0.05)	1.51	1.51	1.07

adaptation is that of osmotic adjustment which occurs when the osmotic potential of the soil solution is reduced either by the addition of salts or by water limitation. A morphological adaptation to both stresses is a reduction of leaf area. This may help in reducing transpiration and, if achieved at minimum cost to growth, it amounts to increased water use efficiency and an indicator of better tolerance. Leaf conductance and p_i/p_a, and consequently Δ have been reported to be positively correlated in well-watered and water-stressed wheat plants (Condon *et al.*, 1990). However, salt stress and drought are different, in part, because the physiological drought caused by increased salt concentration coincides with the potential for increased effects from specific ions.

In order for Δ to be useful as a screening method for genetic variation in salt tolerance, the basis of differences in Δ must be understood. Further studies will be undertaken to understand the physiological effects of salt on leaf conductance and on assimilation rate in order to identify the nature of the effects of salt on p_i/p_a and the parameters that cause it to vary. There is need to carry out studies to confirm that

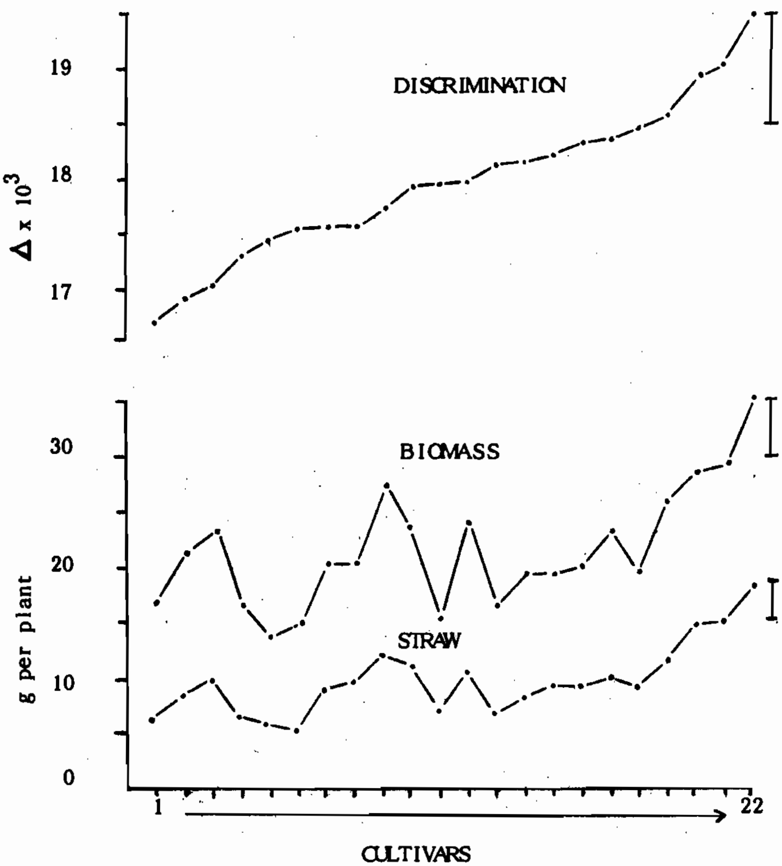

Fig.1. Changes in grain, biomass and ^{13}C discrimination in wheat and barley irrespective of salinity. For details of cultivars (1--->22) see Table 3. Vertical bars show L.S.D.

Table 4. Correlation (r) and slope of regression line (b) values for comparison of Δ with yield components in wheat.

	'r' values		'b' values	
	Normal	Saline	Normal	Saline
Grain	0.121	0.360*	0.014	0.139
Straw	-0.048	0.443*	-0.003	0.162
Biomass	0.001	0.472*	0.001	0.103

*Significant at $p = 0.05$

variation in Δ coincides with the expected negative correlation with W when salinity is the environmental variable.

The utility of using Δ as a measure of salt tolerance will depend on the ease with which selections may be made for further analysis or breeding purposes. This analysis was conducted at crop maturity and allowed the observation of some broad trends. The most useful criterion would be one at which selection could be made early in the life cycle. Future studies should include sequential harvests from seedling stages to maturity so that any cultivar differences in salt stress effects might be exploited at the earliest age but also which translate from a simple measure such as Δ in biomass of seedlings to salt tolerance in mature plants with increased yield.

References

Condon, A.G., G.D. Farquhar and R.A. Richards. 1990. Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat. Leaf gas exchange and whole plant studies. *Aust. J. Plant Physiol.*, 17: 9-22.

Condon, A.G., R.A. Richards and 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.

Farquhar, G.D., J.R. Ehleringer and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 40: 503-537.

Flanagan, L.B. and R.L. Jeffries. 1989. Effect of increased salinity on CO_2 assimilation, O_2 evolution and the $d^{13}C$ values of leaves of *Plantago maritima* L., developed under low and high NaCl levels. *Planta*, 178: 377-384.

Flowers, T.J., P.F.Troke and A.R. Yeo. 1977. The mechanism of salt tolerance in halophytes. *Ann. Rev. Plant Physiol.*, 28: 89-121.

Greenway, H. and R. Munns. 1980. Mechanisms of salt tolerance in non-halophytes. *Ann. Rev. Plant Physiol.*, 31: 149-190.

Guy, R.D., D.M. Reid and H.R. Krouse. 1986. Factors affecting $^{13}C/^{12}C$ ratios of inland halophytes. I. Controlled studies on growth and isotopic composition of *Puccinellia nuttalliana* L. *Can. J. Bot.*, 64: 2693-2699.

Hubick, K.T., and G.D. Farquhar. 1989. Carbon isotope discrimination and the ratio of carbon gained to water lost in barley cultivars. *Plant Cell Env.*, 12: 795-809.

Hubick, K.T., G.. Farquhar and R. Shorter. 1986. Correlation between water-use-efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. *Aust. J. Plant Physiol.*, 13: 803-816.

Hubick, K.T., R. Shorter and G.D. Farquhar. 1988. Heritability and genotype x environment interactions of carbon isotope discrimination and transpiration efficiency in peanut (*Arachis hypogoea* L.). *Aust. J. Plant Physiol.*, 15: 799-813.

Khanzada, A.N., S.S.M. Naqvi and R. Ansari. 1986. Proline content in relation to salt tolerance in wheat. In: *Proc. National Symp. Environmental Stress and Plant Growth*. (Eds.) S.S.M. Naqvi and R. Ansari. AEARC, Tandojam, Pakistan. p.9-18.

Lin, G. and Leonel da S.L. Sternberg. 1992. Effect of growth form, salinity, nutrient and sulfide on photosynthesis, carbon isotope discrimination and growth of red mangrove (*Rhizophora mangle* L.). *Aust. J. Plant Physiol.*, 19: 509-517.

Lu, Z., J. Chen, R.G. Percy, M.R. Sharifi, P.W. Rundel and E.Zeiger. 1996. Genetic variation in carbon isotope discrimination and its relation to stomatal conductance in pima cotton (*Gossypium barbadense*).

Aust. J. Plant Physiol., 23: 127-132.

Meinzer, F.C., N.Z. Saliendra and C.H. Crisosto. 1992. Carbon isotope discrimination and gas exchange in *Coffea arabica* during adjustment of different soil moisture regimes. *Aust. J. Plant Physiol.*, 19: 171-184.

Nageswara Rao, R.C., M. Udaykumar, G.D. Farquhar, H.S. Talwar and T.G. Parasad. 1995. Variation in carbon isotope discrimination and its relationship to specific leaf area and ribulose-1, 5-biphosphate carboxylase content in groundnut genotypes. *Aust. J. Plant Physiol.*, 22: 545-551.

Stewart, G.R., M.H. Turnbull, S. Schmidt and P.D. Erskine. 1995. ^{13}C natural abundance in plant communities along a rainfall gradient: a biological integrator of water availability. *Aust. J. Plant Physiol.*, 22: 51-55.

Walker, C.D. and R.C.M. Lance. 1991. Silicon accumulation and ^{13}C composition as indices of water-use efficiency in barley cultivars. *Aust. J. Plant Physiol.*, 18: 427-434.

Wright, G.C., K.T. Hubick and G.D. Farquhar. 1988. Discrimination in carbon isotope of leaves correlate with water use efficiency of field grown peanut cultivars. *Aust. J. Plant Physiol.*, 15: 815-825.

(Received for publication 23 September, 1997)