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CARBON ISOTOPE DISCRIMINATION AS A SELECTION TOOL FOR HIGH WATER USE EFFICIENCY AND HIGH CROP YIELDS

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Abstract

CARBON ISOTOPE DISCRIMINATION AS A SELECTION TOOL FOR HIGH WATER USE EFFICIENCY AND HIGH CROP YIELDS.

Results of back-up research conducted at the FAO/IAEA Agriculture and Biotechnology Laboratory in support of the FAO/IAEA Co-ordinated Research Programme on the Use of Isotope Studies on Increasing and Stabilizing Plant Productivity in Low Phosphate and Semi-arid and Sub-humid Soils of the Tropics and Sub-tropics, are presented here. This work mainly focused on field validation, using neutron probes, of the recently developed ^{13}C discrimination (Δ) technique, considered a possible future tool for screening and selection of crop species for high water use efficiency (WUE) and high yields. ^{13}C discrimination in leaves is a plant trait indicative of drought adaptability. Both field and glasshouse experiments were carried out involving food crops and tree species. In addition to experiments solely conducted by the research staff of the laboratory, research fellows themselves as part of their training at the laboratory, carried out experiments which have generated very valuable information.

Neutron probe measurements confirmed the earlier reports of a strong correlation of Δ with grain yield and water use efficiency of wheat. High soil gypsum content and soil salinity, a wide spread problem in soils of arid and semi-arid climatic zones, do not interfere with the association of Δ with crop yields, provided plants are grown in similar soil water status and soil fertility level. Results of a glasshouse experiment using selected cowpea genotypes showed that Δ values measured at flowering stage positively correlated with total dry matter production and percent N_2 derived from atmosphere (%Ndfa), contributing to an earlier report from the laboratory that it may be possible to use Δ values for screening of leguminous crops for high N_2 fixation potential. ^{13}C isotope discrimination in the leaves of *Gliricidia sepium* was measured to examine if the technique could be extended to studies with trees. Results of a glasshouse experiment with 18 provenances of *Gliricidia sepium* showed highly significant correlations of Δ with total dry matter production, water use efficiency and total N accumulated through biological nitrogen fixation. Although the correlations of Δ with water use efficiency and dry matter yield are reatively clear and better understood, the correlation with nitrogen fixation still needs a closer examination under different environmental conditions and with different species. While ^{13}C isotope discrimination may be a valuable tool for identifying annual crops with high water use efficiency and high yield potential, it may be more attractive for tree species considering the long growth periods taken for trees to produce economic yields either for food, fodder or for fuelwood.

1. INTRODUCTION

Identifying genotypes with high water use efficiency and high yield has become an important issue as environmentally friendly and sustainable agricultural practices receive high priority in all

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countries. For the initial screening process, rapid but reliable greenhouse or field methods for assessing the yield potential, particularly under water limited environments, would be invaluable [1]. Water stress is the most important limitation to crop productivity in water scarce arid and semi-arid regions of the world. Although agronomic practices are important under water deficit agricultural areas, cultivar improvement is usually seen as the most promising approach to increase yields [2]. Plant breeders and plant physiologists believe that better adapted and high yielding varieties could be bred more efficiently and effectively if plant attributes which are indicative of high yields under water limited conditions could be identified and used as selection criteria [3]. Therefore identification of plant attributes contributing to superior performance of crop plants under drought conditions has been a long-term goal of plant scientists. Water use efficiency is a trait which can contribute to crop productivity in the areas where water resources are scarce [4] because it is considered an important component of adaptation to drought [5]. Water use efficiency, in general terms, is the ratio of productivity to water loss by a plant. It is defined as the molar ratio of photosynthesis to transpiration (short-term measurements) or as the ratio of biomass produced to water consumed (long-term measurements) [6].

One of the difficulties in using plant water use efficiency as a trait for identifying genotypes superior in performance under drought conditions is the difficulty of accurately assessing water use efficiency in large scale field experiments. Faquhar et al. [6] have shown that genotypic variability in intrinsic water use efficiency (mole of C fixed per mole of water transpired) is closely associated with ^{13}C discrimination (Δ) in C_3 plants. Subsequent studies demonsted that the extent of ^{13}C isotope discrimination is a reliable indicator of water use efficiency and that substantial genotypic variability exits in this character [6]. In wheat [1,7, 8], cowpea [9], peanut [10], cotton [11], tomato [12], grasses [13, 14], and in upland rice [15], ^{13}C discrimination has shown a negative correlation with field water use efficiency (dry matter produced per unit amount of water used). It was also shown that Δ correlates significantly with grain yield of wheat and barley [1, 16, 17], and dry matter yield of cowpea [9], and forage grasses [13]. The carbon isotope discrimination has also shown a close association with other plant characteristics other than the yield attributes. For example, Masle and Farquhar [18] reported that Δ correlates positively with the leaf mineral content in C_3 species, and negatively in sorghum, a C_4 species. Kumarasinghe et al. [19] found a negative correlation of Δ with biological nitrogen fixation in soybeans.

Although there are reports on the potential use of Δ as a screening tool for annual crops for high water use efficiency and high crop yields in drought conditions, very little information is available at present (with the exception of the work of Meinzer et al. [20]) on the possible uses of this technique for identifying tree species with high water use efficiency and high biomass production. Meinzer et al. [20] demonstrated a close correlation between Δ and yield of coffee. Studies conducted under an IAEA Technical-Co-operation project in Sri Lanka (SRL/5/026) have shown that ^{13}C isotope discrimination in leaves of coconut is negatively correlated with water use efficiency and also with dry matter yield. These preliminary studies indicate that ^{13}C isotope discrimination could be used as a valuable tool to select coconut cultivars efficient in water use and high in yield so that they could be recommended for cultivation in areas with low water resources. The methods also has the additional advantage in that the long experimental periods commonly required for tree crops like coconut, oil palm, rubber, tea, and coffee can be substatially reduced saving considerably on experimental inputs including labour.

The Soil Fertility, Irrigation and Crop Production Section of the Joint FAO/IAEA Division of of Nuclear Techniques in Food and Agriculture implemented a Co-ordinated Research Program on the Use of Isotope Studies on Increasing and Stabilizing Plant Productivity in Low Phosphate and Semi-arid and Sub-humid Soils of the Tropics and Sub-tropics which included a major component on water use efficiency studies. The validity of the ^{13}C isotope discrimination technique was investigated in greenhouse and field experiments involving annual crops and perrenial tree species. The program was funded by the Swedish International Development Authority (SIDA), in Sweden. Back-up research at the FAO/IAEA Agriculture and Biotechnology Laboratory in Seibersdorf was conducted by the staff as well as by IAEA Fellows. Highlights of the research carried out in the laboratory are presented here.

2. ANNUAL CROPS

2.1. Wheat

2.1.1 Carbon isotope discrimination at vegetative stage as indication of yield and water use efficiency of spring wheat

A.R.A.G. Mohamed, Atomic Energy Authority, Nuclear Research Center, Cairo, Egypt: A field experiment was conducted to investigate if Δ measured at vegetative stage of spring wheat (*Triticum turgidum* L. var. *durum*) is related with the yield and field water use efficiency at repining. A line source sprinkler irrigation system exposed the wheat genotypes to different levels of watering, from rainfed to full irrigation. The results of Δ values measured at late stem elongation stage, 60 days after planting, shows strong positive correlation with total dry matter yield (Fig. 1), and highly significant negative correlation with water use efficiency measured at ripening 105 DAP (Fig. 2). The data (also published elsewhere [1]), suggest that the imprints of Δ measured at vegetative stage persists throughout the entire growth period, until maturity

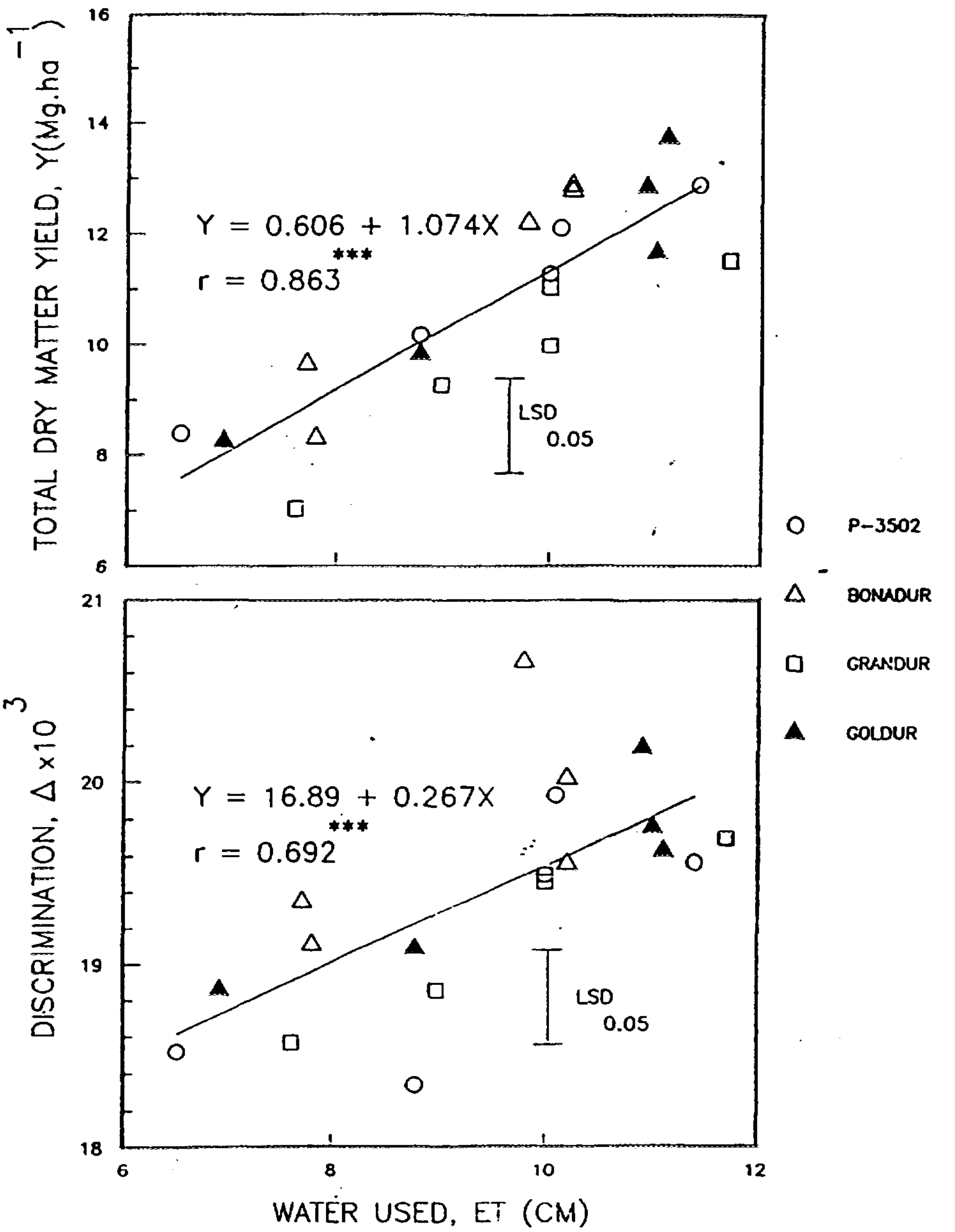

FIG. 1. Total dry matter yield of spring wheat (Triticum turgidum L. var. *Durum) genotypes at ripening stage (105 DAP) and the carbon isotope discrimination, measured at 60 DAP, are positively correlated with plant water consumption (ET) during the early growth period until 60 DAP (After Kirda et. al. [1]).*

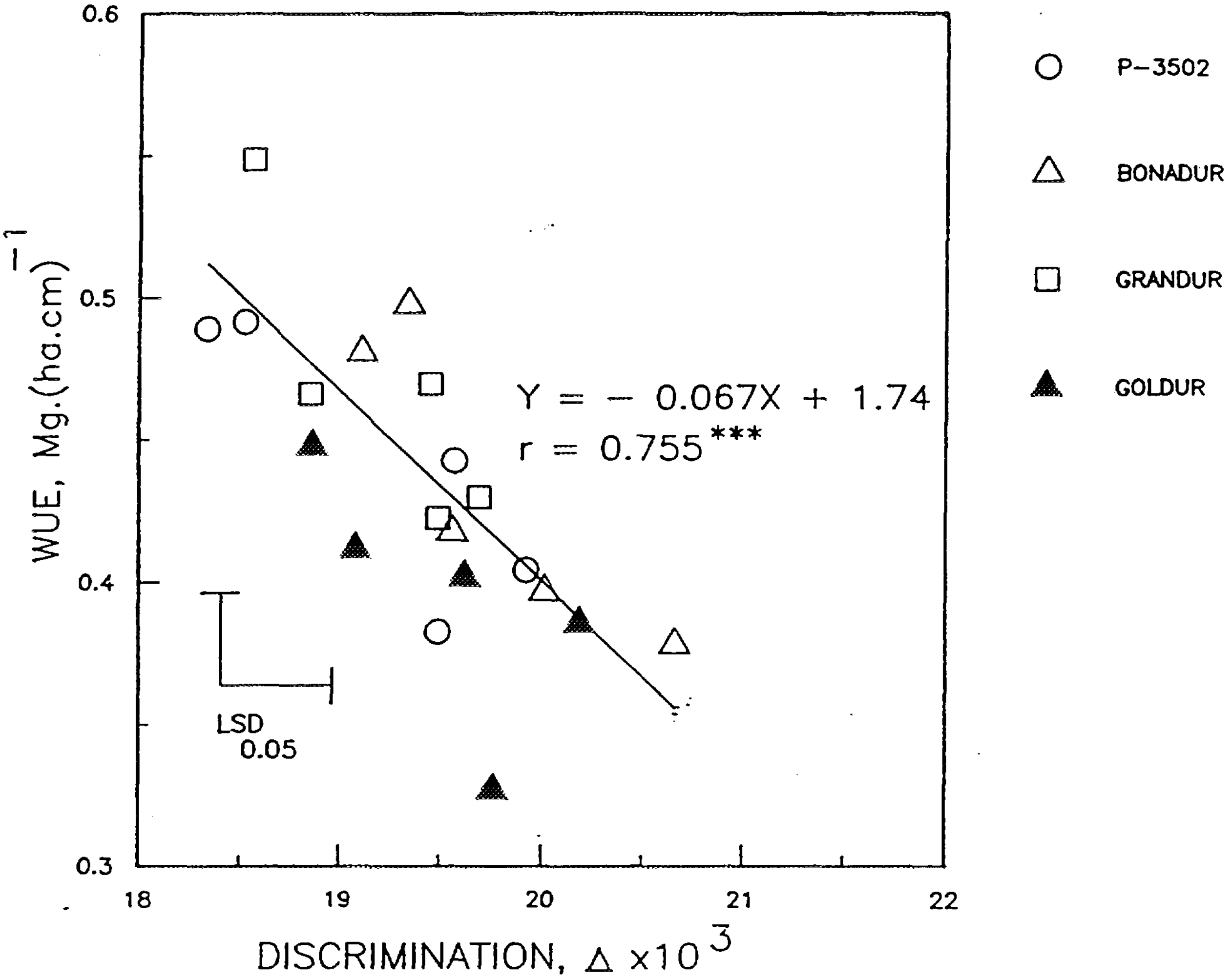

FIG. 2. Water use efficiency of four spring wheat (Triticum turgidum L. var. *Durum) genotypes at ripening (105 DAP) gives strong association with cabon isotope discrimination measured at 60 DAP (After Kirda et al. [1]).*

2.1.2 *Carbon isotope discrimination as an indicator of yield and water use efficiency of spring wheat grown in salt-affected gypsiferous soils*

A. Arslan, Syrian Arab Republic, Atomic Energy Commission, Damascus, Syria: In a pot study, the relationship between Δ and water use efficiency of six wheat cultivars grown in Syria (Buhuth 1, Daki, L43, Cham 3, Cham 4, and L92-6) was examined in salt affected gypsiferous soils, common and wide spread in the Euphrates basin. Soil treatments were of a non saline gypsum-free soil (S0G0), and the same soil but amended with gypsum (S0G1), with salts alone (S1G0), and with gypsum and salts (S1G1). Treatments with gypsum (S0G1 and S1G1) had 14 % industrial gypsum. Saline treatments (S1G0, S1G1) were prepared using either NaCl or $CaCl_2$ at a rate of 9 g salt per kg of soil sample. Some key data on chemical analysis of the experimental soils are given in Table 1. The results showed that neither gypsum nor salinity affected potential use of Δ for screening for high crop yields and water use efficiency. Figure 3 shows a significant negative correlation between Δ and water use efficiency as commonly reported [1, 7, 8]. A negative correlation also existed with dry matter production and Δ (Figure 4) indicating that biological yield of wheat varieties tested was predominantly controlled by different photosynthetic capacities [1, 2]. The existence of a positive correlation in Δ and water use efficiency in wheat has been previously shown by Hubick et al [21]. Farquhar and Richards [7] also reported a similar negative correlation between Δ and dry matter production under wet soil conditions where plants were not exposed to water stress as was the case in this work.

TABLE I. CHEMICAL ANALYSIS OF THE EXPERIMENTAL SOILS

	Treatments			
Measurement	S0G0	S1G0	S0G1	S1G1
OM (%)	2.5	1.9	1.7	2.1
EC_e (dS.m-1)	0.8	8.9	2.6	10.3
pH	7.7	7.5	7.5	7.4
$CaCO_3$ (%)	5.2	4.7	3.2	4.7
Gypsum (%)	T	T	14.8	13.3
Cations ($me.l^{-1}$)	10.1	104.7	46.0	137.8
SAR	0.38	16.19	0.18	11.21

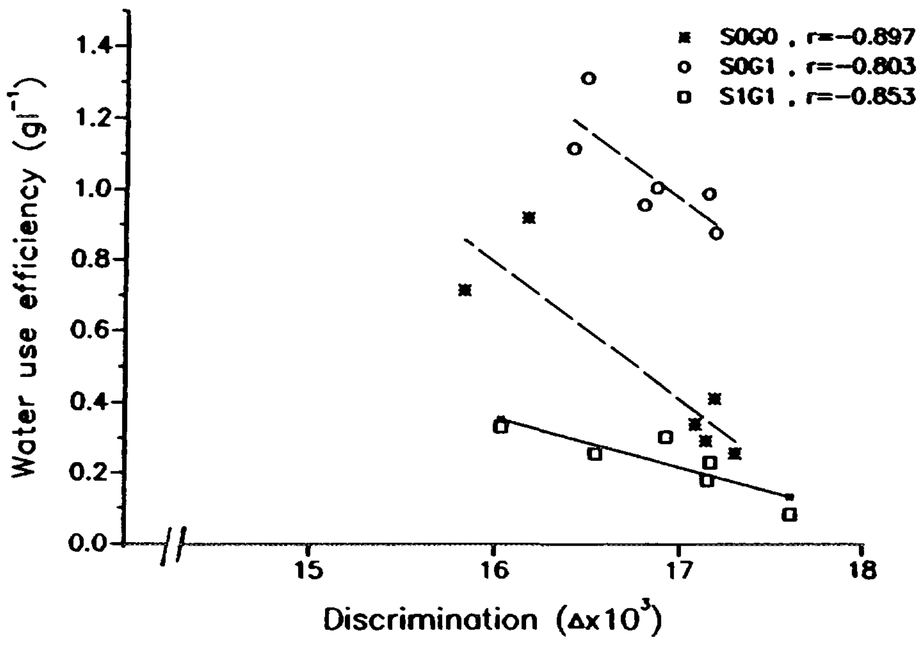

FIG. 3. Regression lines for water use efficiency versus Δ as influenced by soil salinity and soil gypsum content.

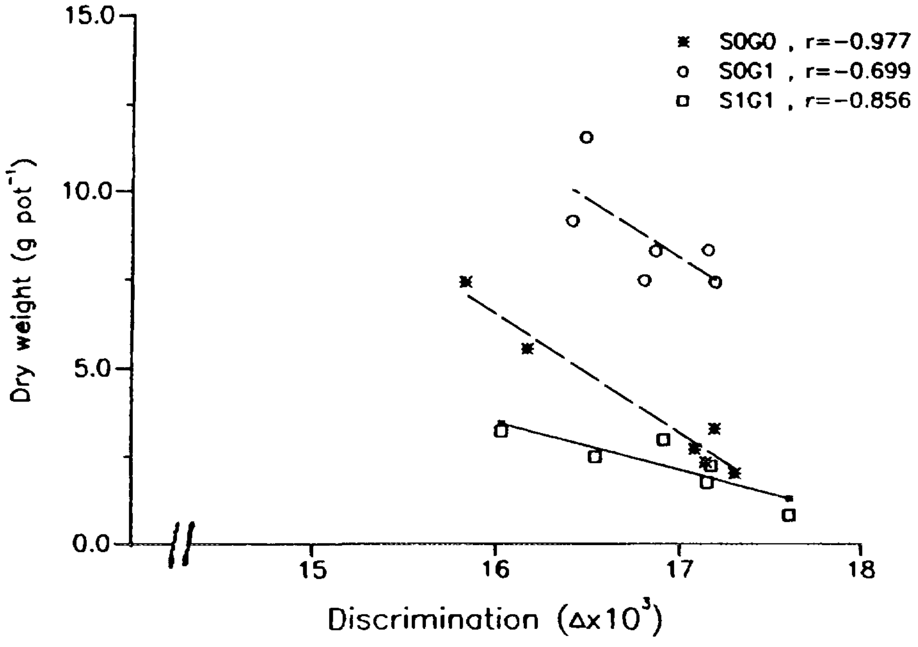

FIG. 4. Regression lines for total dry matter production versus Δ as influenced by soil salinity and soil gypsum content.

2.1.3. Effects of water stress during different growth stages of wheat on N fertilizer uptake, yield and ^{13}C discrimination

E. Holmgren, Soil Science Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria: A greenhouse experiment was conducted with the objective of assessing how water stress imposed at selected growth stages of wheat would influence Δ, N fertilizer uptake and yield of 8 different wheat varieties. Correlations between Δ and water use efficiency measured with conventional methods (i.e., water balance), yield and N fertilizer uptake, measured with ^{15}N dilution technique [22], were investigated .

Eight spring wheat (*Triticum turgidum* L. var. *durum*) varieties, four from Austria (*Goldur, Bonadur, Grandur* and *P3502-85*), two from Egypt (*Sakka 8* and *Giza 163*) and two from Chile (*Naofen* and *Dalcahue*) were used. The seeds were sown in plastic pots (16.5 cm diameter, 17.5 cm deep) containing 4.5 kg of soil-sand mixture. The soil used is classified as *Typic Eutrocrets* (pH 7.4, 0.3 % N, clay loam), and it was mixed with washed sand at 2:1 soil-sand ratio. Field capacity and wilting point of the soil mixture were found to be 0.22 and 0.66 $g.g^{-1}$, respectively. Each pot had 4 plants. Ammonium sulfate solution (1 % ^{15}N atom excess) was applied to supply 100 mg $N.kg^{-1}$ soil. Four watering programs, consisting of a control (i.e. no stress) and water stress imposed at mid vegetative, flowering and at yield formation stages (Fig. 5). The treatments were imposed when half of the plots had reached the selected growth stage. Soil water content in the pots with the stressed plants was kept within the range from 25 to 40 % of field capacity; whereas, the pots of the non stressed plants had a water content of 80 to 100 % field capacity. Soil water content of the pots were controlled by weighing the pots every two days. Weighing of the pots also facilitated calculation of total water usage and thereby of measuring water use efficiency. The experiment was arranged in a completely randomized block design with three replicates. Plants were harvested 1-2 cm above ground and all relevant yield attributes were measured. Plant samples were prepared and anlyzed for ^{15}N and the Δ values.

With the provision of adequate watering during the establishment period, it was revealed that the most critical period for water deficit was the flowering stage. Both grain yield and water use efficiency were significantly reduced with water stress imposed at flowering stage. Results showed that there are highly significant genotypic differences with respect to grain yield, water use efficiency,

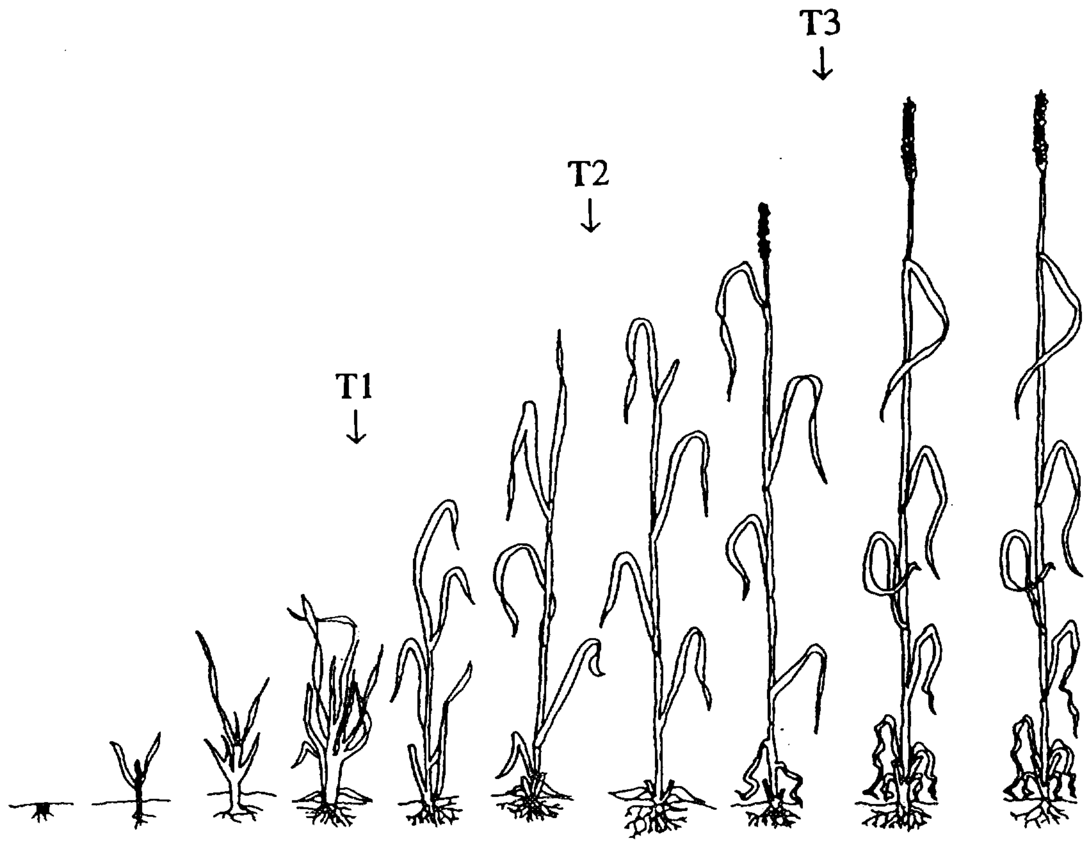

FIG. 5. Vegetative (T1), flowering(T2) and yield formation (T3) stages of wheat when water stress was imposed.

% N derived from fertilizer (% N dff) and ^{13}C discrimination (Table II). There are also highly significant negative correlations between Δ, water use efficiency and % N dff (Table III). However, no association between Δ and yield existed possibly because of very narrow range of variation observed in both yield and Δ values of the 8 wheat varieties tested.

The results obtained from this greenhouse experiment suggest that Δ measurements can usefully be used for selecting of wheat genotypes with high water use efficiency and effective use of applied N fertilizer.

TABLE II. WATER STRESS TREATMENT EFFECTS ON YIELD, WATER USE EFFICIENCY (WUE), % Ndff AND Δ VALUES OF SPRING WHEAT VARIETIES.

	Yield	WUE	% N dff	Δ
Treatment	***	***	***	***
Genotype	***	***	***	***
Interaction	NS	NS	NS	**

TABLE III. CORRELATION COEFFICIENTS BETWEEN Δ, WATER USE EFFICIENCY (WUE) AND % Ndff UNDER WATER STRESS IMPOSED AT DIFFERENT GROWTH STAGES OF WHEAT.

	No stress	Vegetative stage	Flowering	Yield formation
WUE	-0.86***	-0.74***	-0.082***	0.83***
% Ndff	-0.81***	-0.70***	-0.83***	-0.67***

2.2. Other plant species

2.2.1. *Effects of water stress and soil N status on ^{13}C discrimination in some selected plant species*

E. Holmgren, Soil Science Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria: Effects of soil N and water status on different plant species were assessed. Three plant species, millet (*Panicum milliaceum*), wheat (*Giza 157*, Egypt; *Naofen*, Chile) and barley (*Apex* and *Atem*, Austria) were grown in pots containing approximately 5 kg of soil-sand mixture. Of the 3 plant species, millet fixes CO_2 through C_4 pathway [25]. C_4 plants have low Δ values and a narrow range of variation in photosynthetic capacity. Plants were grown under two watering regimes: plant available water content (AWC) was either maintained around 80 % (no stress) or 40 % (stress). Each pot initially was planted with 12 seeds. After germination, number of plants remaining in each pot was thinned to 4 plants. Three levels of N fertilization (25 [low N], 50 [medium N] and 100 [high N] mg $N.kg^{-1}$ of soil) were considered. Fertilizer N was applied in two split applications: at planting and 30 days after planting. Plants were harvested 82 days after planting.

Figure 6 shows that plants under under water stress (with the exception of millet), have significantly lower Δ values than plants which are growing in relatively wet soil conditions. Soil N status effected Δ values, adversely. Plants growing in high soil N (100 ppm) have significantly lowerΔ values than plants growing in soils with low N (25 ppm). The results imply that plants should be grown in similar soil water status and N fertility level if ^{13}C discrimination technique is to be used as a screening tool. The ^{13}C discrimination values of millet, a C_4 plant, is insensitive to changes in both soil water and N status (Fig. 6).

2.3 Cowpea

2.3.1 *^{13}C Discrimination correlates with dry matter and N_2 fixation in selected cowpea genotypes*

E. M. C. de Bisbal, Centro Nacional de Investigaciones Agropecuarias (FONAIAP-CENIAP), Aerea Universitaria, Apartado Postal 4653, El Limón, Maracay 2101, Venezuela: Kumarasinghe et al. [19] showed evidence that Δ may be associated with biological nitrogen fixation in soybean. The objective of this work therefore was to assess whether Δ values measured in selected cowpea genotypes grown under two watering regimes show any association with N_2 fixation and thus further confirm the findings by Kumarasinghe et al. [19]. The experiment was conducted in greenhouse conditions. Plants were grown in plastic pots filled with 3 kg of soil-sand mixture at 1:1 ratio. Under one of the watering regimes, soil water status was maintained nearly at field capacity. In the second watering regimes, the soil water status was relatively dry: soil water content at planting was at field capacity; however later it was gradually allowed to decrease down to 50 % of the field capacity value (0.18 g.g-1), and maintained at that level throughout the growing period. A randomized complete block experimental design with 5 replicates was used. Statistical analysis (ANOVA) data showed that genotypic differences with respect to total dry matter production, water use efficiency and biological nitrogen fixation are significant under the two watering regimes. Table IV shows correlation coefficients of Δ with TDM and water use efficiency and % Ndfa. The Δ values are negatively correlated with water use efficiency as has been shown for other crops. On the

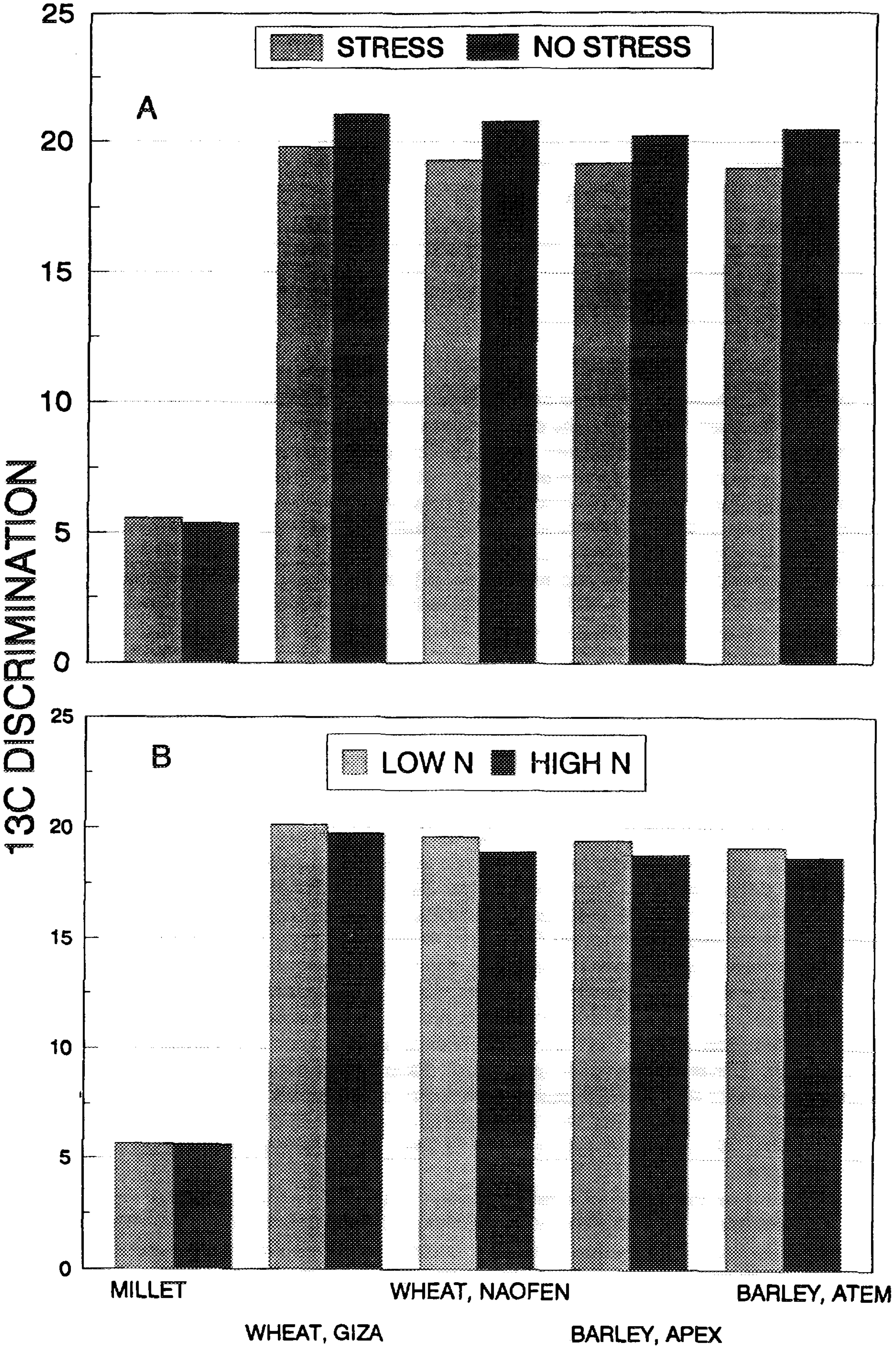

FIG. 6. Effects of water stress and soil N status on Δ values of different plant species.

TABLE IV. CORRELATION COEFFICIENTS FOR THE GENOTYPIC ASSOCIATIONS OF Δ WITH DRY MATTER YIELD (DMY), WATER USE EFFICIENCY (WUE), AND NITROGEN FIXATION (%Ndfa) USING TWELVE INDIVIDUAL VALUES FOR COWPEA GENOTYPES AVERAGED OVER FIVE REPLICATE POTS.

	DMY (g.pot-1)	WUE	% Ndfa
Δ	0.883**	-0.75**	0.642*

other hand, differences in the correlation between Δ and dry matter yield have been observed depending on the plant species and the environmental conditions. In this study, as with wheat [16] and tomato [23], correlation of Δ with TDM production was positive indicating that the Δ of the selected genotypes in this study is predominantly controlled by stomatal conductance [1] rather than by their photosynthetic capacity. Where the Δ is negatively correlated with dry matter yield, the Δ is under the control of the photosysthetic capacity (RuBP Carboxylase activity). Similar differences also appear to in existence with respect to correlation between Δ and the %Ndfa. In soybeans, Δ was negatively correlated with %Ndfa and the N yield [19] but in cowpea where the relationship between Δ and dry matter production is positive, the relationship between Δ and the %Ndfa is also positive. The genotypes giving the highest and lowest TDM production was the same as was identified with both conventional method and the Δ values. Similar results were seen for nitrogen fixation capacity; genotype 4 having the highest and genotype 6 having the lowest % Ndfa in the ranking, irrespective of the method used for the ranking (Fig. 7).

2.3.2 *Genotypic of differences of dry matter production, water use efficiency, N_2 fixation and of ^{13}C discrimination of cowpea lines*

A. Montenegro, National Institute for Agr. and Livestock Research, Exp. Station Carillanca, Temuco, Chile: A greenhouse experiment was carried out to determine if the range of differences measured in Δ values of cowpea lines are large enough and be associated with yield, water use efficiency and differences in N_2 fixation capacities. The plants were grown in plastic pots filled with soil/sand mixture at a ratio of 1:1. Experimental soil classified as *Eutric Brunusol*, was brought from Uruguay. Cowpea lines were brought from IITA, Nigeria.

Plants were grown under two watering regimes: (1) well watered and (2) water stressed conditions. Soil water status of well watered pots was maintained close to 80 % plant available water content; whereas, plant available water content for the water stressed plants was below 40 %. Initially, all pots received same level of water. Water stress condition was imposed two weeks after full emergence. Eleven cowpea lines were tested. Experimental pots received 10 mg $N.kg^{-1}$ of soil as ammonium sulfate with 10% ^{15}N atom excess to estimate biological nitrogen fixation through the use of isotope dilution technique [24]. Planting was done on 27 April 1991. Plants were harvested on 11 July 1991. Plant samples collected near to flowering stage were analyzed for both ^{15}N and ^{13}C discrimination.

The cowpea lines tested gave rather narrow range of differences, although significant ($P \leq 0.05$), with respect to total dry matter production and N_2 fixation (Table V). There were no significant differences in water use efficiency, irrespective of the watering program. Therefore differences observed in Δ values were also rather small and could not facilitate any consistent ranking, with respect to none of the plant characteristics, dry matter production, water use efficiency and N_2 fixation capacity (Table VII). Results suggest that potential use of ^{13}C discrimination as a selection tool for high yielding genotypes/varieties should be limited to varieties of completely different origin (i.e., different parents), and the technique appears to have little value as a selection tool for the selection of different lines for water use efficiency or yield within a particular progeny.

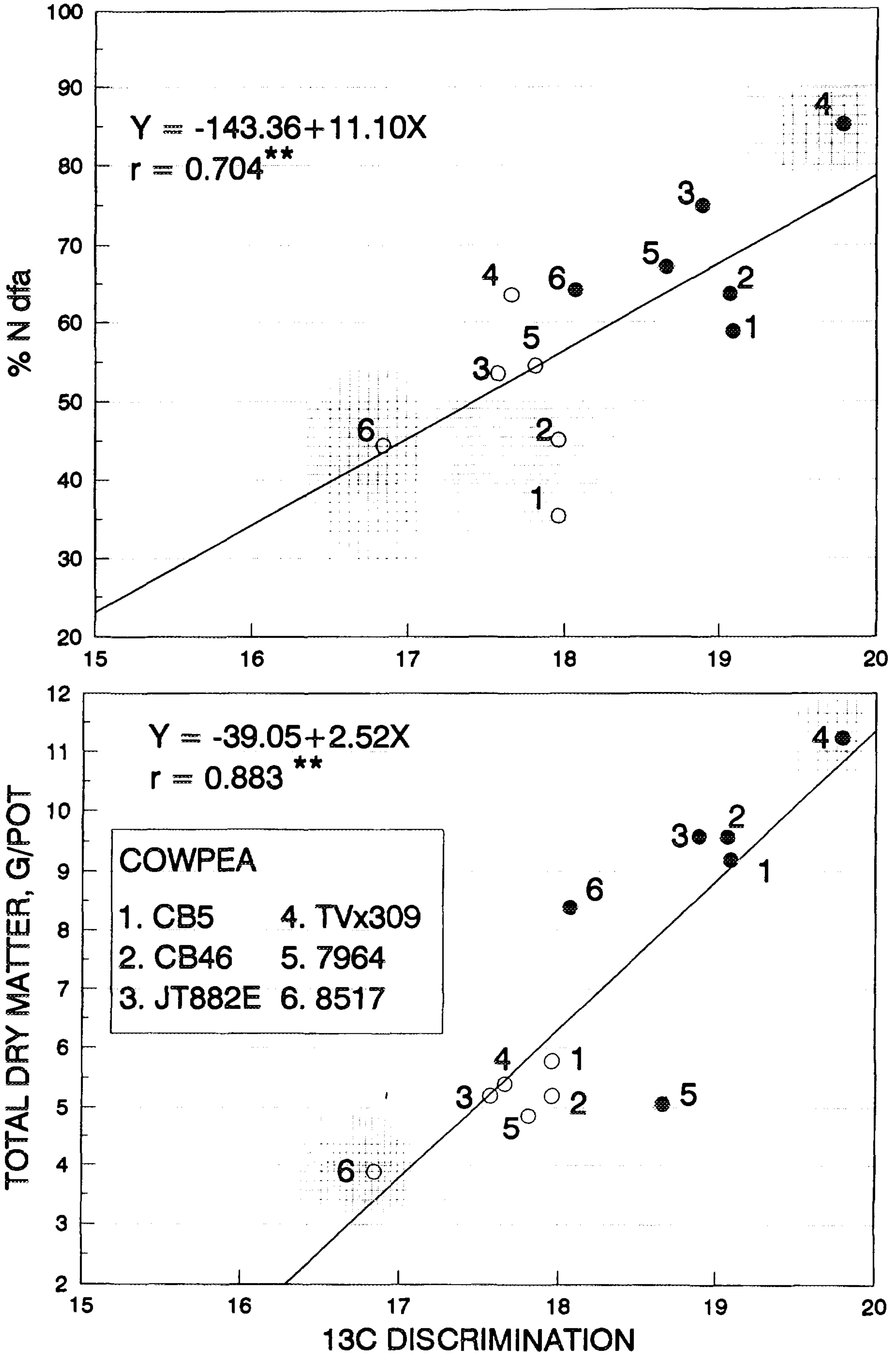

FIG. 7. Linear correlation between Δ with total dry matter (TDM) and % Ndfa of six cowpea genotypes. Shaded areas designate genotypes with highest and lowest TDM production and nitrogen fixation capacities, based on Δ and conventionally measured TDM and % Ndfa.

TABLE V. DRY MATTER YIELD (DMY), N_2 FIXATION (%Ndfa), AND Δ VALUES OF COWPEA LINES GROWN UNDER TWO WATERING REGIMES (WELL WATERED AND STRESSED CONDITIONS).

Cowpea lines	DMY (g.pot-1)		% Ndfa		Δ	
	Well watered	Stressed	Well watered	Stressed	Well watered	Stressed
T86D-627	6.84 e	4.68 ab	64.1 ab	31.3 efg	17.45 a	16.09 a
T85D-3850	9.29 d	5.46 ab	74.1 ab	58.4 ab	17.93 a	16.17 a
T86D-472	11.65 abc	4.70 ab	72.1 ab	48.8 abc	17.83 a	16.18 a
T86D-792	11.87 ab	5.44 ab	73.6 ab	36.4 bcd	17.94 a	16.34 a
T86D-1056	9.70 cd	5.40 ab	74.2 a	53.7 ab	17.75 a	16.27 a
T86D-535	8.70 de	4.88 ab	69.9 ab	25.1 fgh	17.87 a	16.74 a
T82 E-32	9.50 d	5.36 ab	67.8 ab	42.7 bcd	18.44 a	16.14 a
T86D-392	8.14 de	4.78 ab	63.4 b	19.2 gh	18.51 a	16.49 a
TUX 3236	8.15 de	4.20 ac	70.3 ab	17.9 h	17.95 a	16.09 a
T84D-448	10.09 bcd	5.79 bc	72.7 ab	49.0 abc	17.64 a	15.86 a
T84D-449	9.89 bcd	6.09 bc	68.2 ab	47.0 abc	18.44 a	15.81 a

The data in rows fallowed by the same letter are not significantly different ($P \leq 0.05$).

TABLE VI. ACCESSION NUMBERS AND COUNTRY OF ORIGIN OF *GLIRICIDIA SEPIUM* PROVENANCES

Accession No.	Countrty of origin	Accession No.	Country of origin
1. G12/86	Costa Rica	10. G16/84	Guatemala
2. G25/84	Honduras	11. G34/85	Mexico
3. G14/84	Guatemala	12. G29/84	Nicaragua
4. G14/86	Guatemala	13. G40/85	Mexico
5. G11/86	Costa Rica	14. G30/84	Nicaragua
6. G17/84	Guatemala	15. G13/86	Panama
7. G44/85	Mexico	16. G13/84	Guatemala
8. G33/85	Mexico	17. G13/86	Venezuala
9. G15/84	Guatemal	18. G13/84	Hondras

TABLE VII. CORRELATION COEFFICIENTS BETWEEN Δ, WATER USE EFFICIENCY (WUE) AND DRY MATTER YIELD BY *GLIRICIDIA* AND SPEARMAN'S COEFFICIENT OF RANK CORRELATIONS (R_s) WITH Δ

	DMY (g.plant^{-1})	WUE	N_{fixed} (mg N.plant^{-1})
Δ	-0.640**	-0.579**	-0.583**
R_s	-0.534**	-0.530**	-0.593**

3. PERENNIAL CROPS

3.1. *Gliricidia sepium*

3.1.1. *Carbon isotope discrimination of Gliricidia sepium correlates with total dry matter production, water use efficiency and nitrogen fixation*

F. Awonaike, Soil Science Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria: The experiment was conducted in a glasshouse at the FAO/IAEA Agriculture and Biotechnology Laboratory in Seibersdorf, Austria. The growth medium consisted of Seibersdorf soil (*Typic Eutrocrets*) and sand filled in pots. The soil, (pH 8.3; total N, 0.3%; plant extractable P = 55.8 ppm; and organic matter, 6.7%) was air-dried and sieved to pass through 2.0 mm sieve. Each pot had 4.5 kg of the mixture. A basal application of 20 mg P as single super phosphate, 50 mg K as muriate of potash and 1 ml of a micronutrient solution (B, 0.05 %; Mg, 0.05 %; Zn, 0.005 %; Mo, 0.005 % and Cu, 0.002 %) per kg of growth medium was applied to all pots before the planting.

Seeds of twenty provenances of *Gliricidia sepium* (kindly provided by the Oxford Forestry Institute, South Parks Road, Oxford, UK) were scarified and sterilized in concentrated H_2SO_4 for 30 minutes. They were then thoroughly rinsed with distilled water and germinated on sand. Pregerminated seedlings of identical vigor were transplanted to the soil/sand growth medium. A mixture of sp 35, sp 44 and sp 45 strains of *Rhizobium* which had been tested to effectively nodulate *G. sepium* [24] was used to inoculate the seedlings while transplanting. Each pot had two seedlings. The accession numbers and countries of origin of the 20 *G. sepium* provenances are listed in Table VI. The 20 provenances of *G. sepium* were arranged randomly in blocks and replicated twice. A single application of 10 ppm N as ammonium sulfate with approximately 10 % ^{15}N atom excess was applied to all pots as tracer to facilitate estimation of biological nitrogen fixation by *G. sepium*, using isotope dilution technique [23]. *Eucalyptus camaldulensis* was used as the reference crop.

Before transplanting, all pots were watered with dionized water to approximately field capacity of the mixture (0.22 g.g^{-1}). During the course of the experiment, the same volume of water was added to each pot. However, at least once every week, each pot was weighed and an appropriate volume of water was added to attain the original weight at the start of the experiment. The initial weight of the pots subtracted from the cumulative water added to each pot until harvest gave the total water consumption (i.e., evapotranspiration, ET) of the two trees planted in each pot. Water use efficiency of the trees (i.e., dry matter produced per liter of water used, g. l^{-1}) was calculated using the equation:

$$WUE = \frac{DM}{ET} \qquad (1)$$

where DM = total dry matter yield of the whole tree (g), and ET is water consumption (L) throughout the experiment (l).

Plants samples were dried, ground and analyzed for total N and N isotope ratios on a Carlo-Erba Analyser (N-1500) coupled to a VG-Isogas Mass Spectrometer. Percent N derived from atmosphere (% Ndfa) was estimated using the isotope dilution technique [25]. Total N fixed was calculated with the following equation

$$N_{fixed}=\frac{\%Ndfa.N_{Total}}{100} \qquad (2)$$

The stem samples from about 1 cm above the base of the shoots were analyzed for carbon isotope composition, and ^{13}C discrimination was calculated as described by Farquhar and Richards [7] and Hubick et al. [10]. Results show a highly significant correlation between carbon discrimination (Δ) and total dry matter yield, water use efficiency and total N fixed, in 6 months old *Gliricidia* (Table V). Ranking of *G. sepium* provenances simply based on Δ values gave significant Spearman's coefficient of rank correlations [26] with rankings based on conventionally measured dry matter production, water use efficiency and total N fixed (Table VII), indicating therefore the potential use of Δ as a screening tool for high dry matter production and nitrogen fixation capacity, as well as high water use efficiency. Although only a preliminary screening can be achieved using Δ values, the provenances which possess the bse characteristics for a particular trait must be subjected to further field screening with conventional methods. For example, provenences 2,3 and 10 which have a high dry matter yield also exhibited high water use efficiency charateristics (Fig. 8). These also had promising features with respect nitrogen fixation. The results reported here show that for trees, Δ of plant organs collected during crop growth can provide valuable information with respect to drought adaptability and biomass production potential. The Δ also appears to indicate the N_2 fixing ability [19]. The method, subject to further validation for other tree species, could provide a very valuable tool especially for the selection of tree provenances capable of high fuelwood production and drought tolerance.

4. CONCLUSIONS

Neutron probe measurements confirmed the earlier reports of a strong correlation of Δ with grain yield and water use efficiency of wheat. High soil gypsum content and soil salinity, a wide spread problem in soils of arid and semi-arid climatic zones, do not interfere with the association of Δ with crop yields, provided plants are grown in similar soil water status and soil fertility level. Results of a glasshouse experiment using selected cowpea genotypes showed that Δ values measured at flowering stage positively correlated with total dry matter production and percent N_2 derived from atmosphere (%Ndfa), contributing to an earlier report from the laboratory that it may be possible to use Δ values for screening of leguminous crops for high N_2 fixation potential. ^{13}C isotope discrimination in the leaves of *Gliricidia sepium* was measured to examine if the technique could be extended to studies with trees. Results of a glasshouse experiment with 18 provenances of *Gliricidia sepium* showed highly significant correlations of Δ with total dry matter production, water use efficiency and total N accumulated through biological nitrogen fixation. While the correlations of Δ with water use efficiency and dry matter yield are reatively clear and better understood, the correlation with nitrogen fixation still needs further study and confirmation under different environments and different species. ^{13}C isotope discrimination as a tool for identifying plants with a high water use efficiency and high yield potential would indeed be more attractive for tree species than for annuals considering the often long periods of time taken for trees to grow and produce economic yields either for food, fodder or for fuelwood.

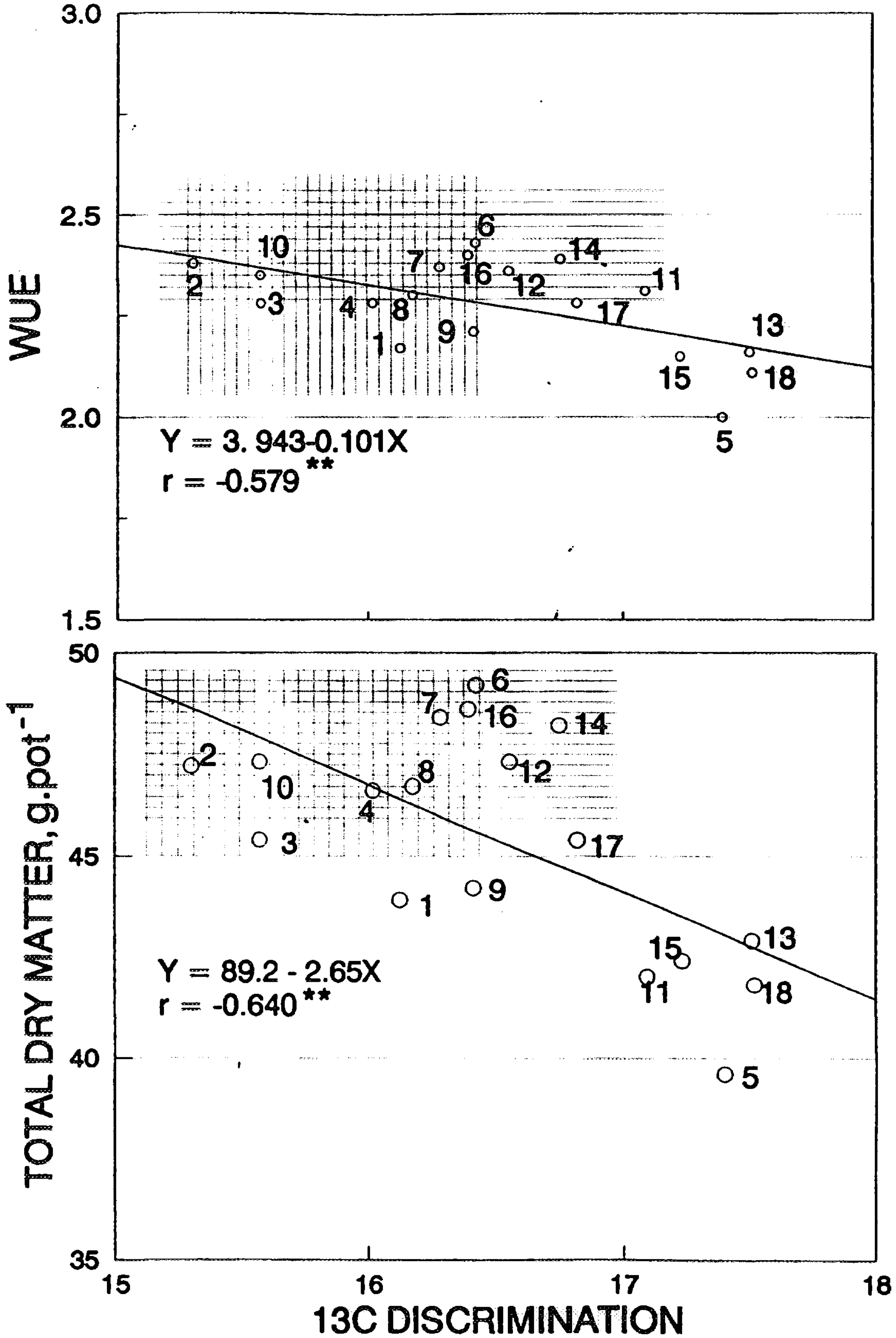

FIG. 8. Linear correlation between Δ with total dry matter (TDM) and water use efficiency of 6 months old Gliricidia species. Shaded areas designate half of the best provenances slected based on Δ and conventionally measured water use efficiency and TDM (upper and lower figures respectively). Numbers next to the data points show the provenances listed in Table VI.

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