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The Occurrence of Kranz Type Species Among the Noxious Weeds on Cultivated Land of Taiwan and Their Biochemical Subdivision

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ABSTRACT

One hundred and one noxious weeds on cultivated land of Taiwan were investigated for the occurrence of "Kranz" leaf anatomy and activities of PEP carboxylase and C_4 acid decarboxylating enzymes : NADP-malic enzyme, NAD-malic enzyme, PEP carboxykinase. Based on the leaf anatomy and a/b chlorophyll ratio, twenty-seven species exhibit "Kranz" type leaf anatomy, and seventy-four species were found without it. Among the species investigated, *Digitaria radicosa* (Presl) Miq., *Leptochloa chinensis* (L.) Nees, and *Sporobolus fertilis* (Steud.) W.D. Clayton in the Gramineae were first recorded as C_4 plants. Twenty-seven species of "Kranz" type leaf anatomy, include those of monocotyledon ; sixteen species in Gramineae, six species in Cyperaceae. Those of dicotyledon ; two species each in Euphorbiaceae and Amaranthaceae and one species in Portulacaceae. The subtype of fourteen previously uninvestigated species among twenty-seven species were further determined. The properties of the three decarboxylating enzyme from representative species were also characterized.

Based on primary initial CO_2 fixation product, higher plants can be divided into three main groups, namely C_3, C_4 and CAM plants (Edwards and Walker, 1983). Each group of plants is usually adapted to a particular environment, due to their distinct photosynthetic characteristics. For example associated with C_4 plants is a specialized form of leaf anatomy (Kranz type) with two types of leaf cells adopting specialized functions in the C_4 pathway of photosynthesis. The coordination of these two photosynthetic cells in carbon, nitrogen and sulfur metabolism as well endows C_4 plants with a specially high potential for growth and productivity accompanied by a more efficient use of water and nutrients, particularly under high light and high temperature conditions. While these features make some C_4 plants as important agronomic crops, they also make them prone to be noxious weeds. This is attested by the fact that C_4 plants dominated the list of the world's worst weeds based on costs in terms of damage or control. A recent listing by Holm *et al*. (1977) showed that fourteen of the world's worst eighteen weeds are C_4 plants.

Among the 165 weedy species (in 42 families) identified on the cultivated land of Taiwan (Chiang and Leu, 1981) sixty-two species are considered as common weeds and thirty species as notorious weeds (Lin, 1980). The occurrence of C_4 plants among these weedy species on this subtropical island (24°N) has not been examined previously.

In a survey with 101 species on leaf anatomy and chlorophyll a/b ratio, twenty-six species were listed as having Kranz anatomy (Lin, C.H., manuscript submitted to Weed Sciences). How-

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ever, leaf anatomy is not a definite diagnostic criterion for classifying species as C_4 plants (Edwards and Ku, 1987). In a follow up study, twenty-seven species were investigated for the activities of PEP carboxylase and three C_4 acid decarboxylating enzymes (Tai *et al.*, manuscript submitted to Australian Journal of Plant Physiology) including the subtypes of fourteen previously uninvestigated C_4 species (Elmore and Paul, 1983). The properties of the three decarboxylating enzymes from representatives species were also characterized.

The cross sectional structure of bundle sheath, chlorophyll a/b ratio of 101 weed species on cultivated land of Taiwan were investigated. Twenty-seven species were listed for having "Kranz" arrangement, and seventy-four species were found without it. Those twenty-seven species of "Kranz" arranged plants include monocotyledon ; sixteen species in Gramineae, six species in Cyperaceae. Those of dicotyledon, two species each in Euphorbiaceae and Amaranthaceae and one species in Portulacaceae.

The leaf anatomy gives the first indication of whether a plant takes the C_3 or the C_4 photosynthetic pathway. Based on leaf anatomy alone, eighty percent or more of the C_4 plant species can be verified. The number and concentration of chloroplasts, mitochondria and peroxisomes in the bundle sheath cell is a reliable anatomical criterion for determining the photosynthetic capacity of a given plant (Black and Mollenhauer, 1971).

Based on the leaf anatomy, here we report the first investigation of its kind in the area (24 N in latitude). Twenty-seven species in five families are listed as having "Kranz" anatomy. Among the twenty-seven species, twenty-four species were listed by and agree well with the conclusion of others (Downton, 1975 ; Hattersley and Watson, 1976 ; Welkie and Caldwell, 1970). In those three species not previously been listed as C_4 plant, the characteristic anatomy of a C_4 plant in *Digitaria radicosa* (Presl) Miq. (Fig. 1), *Leptochloa chinensis* L. Nees (Fig. 2) and *Sporobolus fertilis* (Steud.) Clayton (Fig. 3) were evident.

In this study the subtypes of C_4 weed species were classified according to their major C_4 acid decarboxylating enzyme activity. Therefore, it is necessary to assay these enzymes at their optimum conditions with respect to pH, tempera-

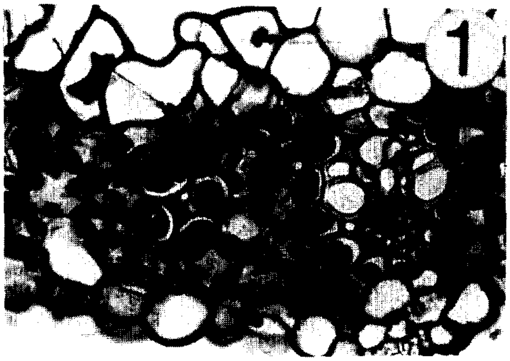

Fig. 1. Light micrograph of a *Digitaria radicosa* (Presl) Miq. leaf cross-section

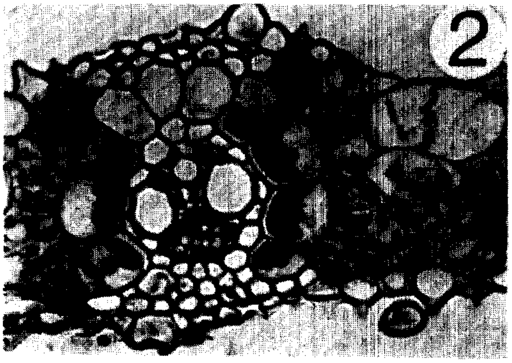

Fig. 2. Light micrograph of a *Leptochloa chinensis* L. Nees leaf cross-section.

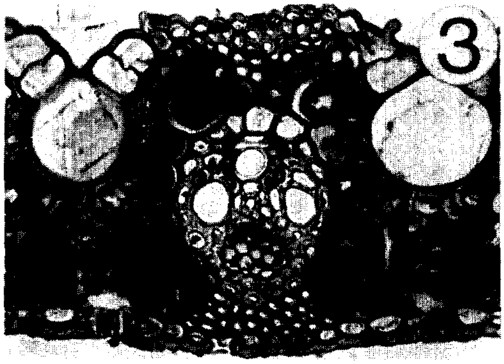

Fig. 3. Light micrograph of a *Sporobolus fertilis* (Steud.) Clayton leaf cross-section.

Table 1. A comparison of chloroplast distribution, chlorophyll a/b ratio and Kranz anatomy on 101 species of weeds on cultivated land of Taiwan.

Scientific name, Spp.	Distribution of chloroplasts within leaf cells	Chl. a/b ratio	Presence of Kranz anatomy
Ferns			
Marsileaceae			
–*Masilea quadrifolia* L.	MC	2.44	
Dicotyledons			
Leguminosae			
–*Mimosa pudica* L.	MC	2.64	
Moraceae			
Humulus scandens (Lour.) Merr.	MC	2.62	
Urticaceae			
–*Gonostegia pentandra* (Roxb.) Miq. var. *akoensis* (Yamamoto) Yamamoto & Masamune	MC	2.69	
Malvaceae			
–*Sida rhombifolia* L.	MC	2.54	
–*Urena lobata* L.	MC	2.64	
Euphorbiaceae			
–*Euphorbia hirta* L.	MC, BSC, (CP)	3.1	K
-*E. thymifolia* L.	MC, BSC, (CP)	3.0	K
–*Phyllanthus urinaria* L.	MC	2.54	
Rubiaceae			
–*Hedyotis diffusa* Willd	MC	2.50	
Ceratophyllaceae			
–*Ceratophyllum demersum* L.	MC	2.25	
Cruciferae			
–*Capsella bursa pastoris* (L.) Medic.	MC	2.86	
–*Cardamine flexuosa* With.	MC	2.61	
–*Lepidium virginicum* L.	MC	2.63	
–*Rorippa indica* (L.) Hiern.	MC	2.57	
Elatinaceae			
–*Elatine traindra* Schkuhr.	MC	2.38	
Caryophyllaceae			
-Stellaria aquatica (L.) Scop.	MC	2.60	
Portulacaceae			
Portulaca oleracea L.	MC, BSC, (CP)	2.7	K
Polygonaceae			
–*Polygonum chinense* L.	MC	2.39	
–*Polygonum lapathifolium* L.	MC	2.71	
-*P. perfoliatum* L.	MC	2.25	
-*P. pericaria* L.	MC	2.89	
-*P. plebeium* R. Br.	MC	2.60	
Chenopodiaceae			
–*Chenopodium serotinum* L.	MC	2.76	
Amaranthaceae			
–*Alternanthera nodiflora* R. Br.	MC	2.42	
–*Alternanthera sessilis* (L.) R.Br. ex Roem & Schultes	MC	2.67	
–*Amaranthus spinosus* L.	MC, BSC, (CP)	3.1	K
–*Amaranthus viridis* L.	MC, BSC, (CP)	3.0	K

Scientific name, Spp.	Distribution of chloroplasts within leaf cells	Chl. a/b ratio	Presence of Kranz anatomy
Lythraceae			
-*Ammannia baccifera* L.	MC	2.98	
-*Ammannia multiflora* Roxb.	MC	2.80	
-*Rotala indica* (Willd.) Koehne var. uliginosa (Miq.) Koehne	MC	2.54	
-*Rotala pentandra* (Roxb.) Blatt. & Hallb.	MC	2.59	
Onagraceae			
-*Ludwigia octovalvis* (Jacq.) Raven	MC	2.92	
-*Ludwigia peploides* (HBK.) Raven subsp. stipulacea (Ohwi) Raven	MC	2.89	
-*Ludwigia epilobioides* subsp. epilobioides Raven	MC	2.78	
Callitrichaceae			
-*Callitriche verna* L.	MC	2.62	
Umbelliferae			
-*Centella asiatica* (L.) Urban	MC	2.91	
-*Hydrocotyle formosana* Masamune	MC	2.75	
Campanulaceae			
-*Sphenoclea zeylanica* Gaerth	MC	2.93	
-*Lobelia chinensis* Lour.	MC	2.62	
Compositae			
-*Ageratum houstonianum* Mill.	MC	2.69	
-*Bidens pilosa* L. var. minor (Blume) Sherff	MC	2.45	
-*Centipeda minima* (L.) A. Braun & Ascherson.	MC	2.51	
-*Crassocephalum rabens* (Juss. ex Jacq.) S. Moore.	MC	2.46	
-*Eclipta prostrata* L.	MC	2.56	
-*Erigeron canadensis* L.	MC	2.54	
-*Gnaphalium purpureum* L.	MC	2.60	
-*Ixeris chinensis* (Thunb.) Nakai	MC	2.41	
-*Siegesbeckia orientalis* L.	MC	3.16	
-*Soliva anthemifolia* R. Br.	MC	2.23	
-*Tridax procumbens* L.	MC	2.63	
-*Youngia japonica* (L.) DC.	MC	2.09	
Solanaceae			
-*Physalis angulata* L.	MC	2.33	
-*Solanum nigrum* L.	MC	2.34	
Convolvulaceae			
-*Ipomoea cairica* (L.) Sweet	MC	2.61	
-*I. nil* (L.) Roth.	MC	2.47	
Scrophulariaceae			
-*Dopatrium junceum* (Roxb.) Hamilt.	MC	2.68	
-*Lindernia procumbens* (Krock.) Philcox ex Benth	MC	2.79	
-*Mazus pumilus* (Brum. f.) Steenis	MC	2.64	
-*Vandellia ciliata* (Colsm.) Yamazaki	MC	2.62	
-*Vandellia cordifolia* (Colsm.) G.	MC	2.68	
Oxalidaceae			
-*Oxalis corniculata* L.	MC	2.58	
-*Oxalis corymbosa* DC.	MC	2.62	
Convolvulaceae			
-*Cuscuta australis* R. Brown	-- (chloroplast not found)	0.96	

Scientific name, Spp.	Distribution of chloroplasts within leaf cells	Chl. a/b ratio	Presence of Kranz anatomy
Monocotyledons			
Hydrocharitaceae			
-*Blyxa echinosperma* (C.B. Clarke) Hook f.	MC	2.78	
Alismataceae			
-*Sagittaria pygmea* Miq.	MC	2.51	
-*Sagittaria trifolia* L.	MC	2.74	
Commelinaceae			
-*Commelina benghalensis* L.	MC	2.51	
-*Commelina diffusa* Burm. f.	MC	2.37	
Eriocaulaceae			
-*Eriocaulon cinereum* R. Br. var. sieboldianum (Sieb. & Zucc.) T. Koyama	MC	2.74	
Pontederiaceae			
-*Eichhornia crassipes* (Mart.) Solms	MC	2.62	
-*Monochoria vaginalis* (Burm. f.) Presl.	MC	2.34	
Lemnaceae			
-*Lemna perpusilla* Torr.	MC	2.23	
Juncaceae			
-*Juncus leschenaultii* J. Cay ex Laharpe	MC	2.52	
Cyperaceae			
-*Cyperus compressus* L.	MC, BSC, (RD)	2.5	K
-*Cyperus iria* L.	MC, BSC, (CF) (Double layer of BSC)	2.8	K
-*Cyperus rotundus* L.	MC, BSC, (RD) (Duble layer of BSC)	3.0	K
-*Eleocharis acicularis* (L.) Roemer & Schult.	MC	2.51	
-*Fimbristylis miliacea* (L.) Vahl	MC, BSC, (RD) (Three layer of BSC)	2.9	K
-*Kyllinga brevifolia* Rottb.	MC, BSC, (RD) (Double layer of BSC)	2.9	K
-*Pycreus polystachyos* (Rottb.) P. Beauvois.	MC, BSC, (RD) (Double layer of BSC)	2.6	K
-*Schoenoplectus juncoides* Roxb.	MC	2.45	
-*Schoenoplectus lineolatus* (Franch & Sav.) T. Koyama	MC	2.20	
-*Schoenoplectus wallichii* (Nees) T. Koyama	MC	2.35	
Gramineae			
-*Alopecurus aequalis* Sobol. var. amurensis (Komar) Ohwi	MC	3.29	
-*Brachiaria mutica* (Forsk.) Stapf	MC, BSC, (CF)	3.1	K
-*Cenchrus echinatus* L.	MC, BSC, (CF)	3.1	K
-*Chrysopogon aciculatus* (Retz.) Trin.	MC, BSC, (CF)	2.9	K
-*Cynodon dactylon* (L.) Pers.	MC, BSC, (CP)	3.0	K
-*Dactyloctenium aegyptium* (L.) Richter	MC, BSC, (CF)	2.7	K
-*Digitaria radicosa* (Presl) Miq.	MC, BSC, (RD)	2.8	K
-*Echinochloa colonum* (L.) Link	MC, BSC, (CF)	3.0	K
-*Echinochloa crusgalli* (L.) Beauv. var. oryzicola (Vasing) Ohwi	MC, BSC, (RD)	3.3	K
-*Eleusine indica* (L.) Gaertn.	MC, BSC, (CP)	2.9	K

Scientific name, Spp.	Distribution of chloroplasts within leaf cells	Chl. a/b ratio	Presence of Kranz anatomy
–*Imperata cylindrica* (L.) Beauv. var. major (Nees) C.E. Hubb. ex Hubb.	MC. BSC, (RD)	3.4	K
–*Leersia hexandra* Sw.	MC	2.34	
–*Leptochloa chinensis* (L.) Nees	MC, BSC, (RD)	3.4	K
–*Panicum repens* L.	MC, BSC, (CF)	2.9	K
–*Paspalum conjugatum* Berg.	MC, BSC, (CF)	2.8	
–*Pennisetum purpureum* Schumach	MC, BSC, (CF)	3.2	K
–*Sporobolus fertilis* (Steud.) W.D. Clayton	MC, BSC, (CF)	2.6	K

CP-Centripetal distribution of chloroplasts within the bundle sheath cells.
CF-Centrifugal distribution of chloroplasts within the bundle sheath cells.
RD-Random distribution of chloroplasts within the bundle sheath cells.
MC-Mesophyll cell.
BSC-Bundle sheath cell.
K-With Kranz type arrangement.

Table 2. Some characteristics of NADP–ME, NAD–ME and PCK in crude extracts prepared from representative species.

Characteristic	NADP–ME *Digitaria radicosa* (Presl) Miq.	NAD–ME *Leptochloa chinensis* (L.) Nees	PCK *Sporobolus fertilis* (Steud.) W.D.Clayton
Optimal pH	8.6	7.2	7.8
Optimal Temperature	51℃	41℃	41℃
Stability at 4℃	more than 8 hours	linearly decay	more than 8 hours
Apparent Km	1.21 mM Malate	*	96μ m OAA 82μ m ATP

*NAD–ME activity decayed with time ; it is difficult to determine the kinetic property accurately.

ture and substrate concentration. From an initial study, three representative species were selected for testing the optimum assay conditions of C_4 acid decarboxylases : *Zea mays* for NADP–ME, *Leptochloa chenensis* L. Nees for NAD–ME and *Sporobolus fertilis* (Steud.) W, D. Clayton for PCK. The results of the investigation are summarized and list in Table 2.

In practice four enzymes (including PEP carboxylase) were characterized for each species. The stability of each enzyme in the crude leaf extract is of critical importance for assessment of enzyme activity. Activity of PEPC was stable for up to 6 hours (data not shown). NADP–ME and PCK activities held stable for more than 8 hours. NAD–ME activity declined linearly with time. Hence NAD–ME activity was given the first priority to estimate in each extraction.

Based on the optimum assay condition the biochemical subtype of twenty–seven species of noxious weeds exhibiting Kranz type leaf anatomy on cultivated land of Taiwan were investigated and listed in Table 3. Also listed in the table are three species of known biochemical characteristics as references. The three species were *Glycine max* (L.) Merrill and *Oryza sativa* L., both C_3 plants representing dicotyledons and monocotyledons, respectively ; and *Zea mays* L. representing NADP–ME subtype of C_4 monocotyledons.

Among the twenty–seven noxious weed species exhibiting Kranz anatomy, five were dicotyledons. Two of them were classified as NADP–ME subtype and three as NAD–ME subtype (Table 3). Although C–4 nature of the five dicotyledonous species has already been known, subtypes of at least three species (*Euphorbia*

Table 3. Activities of three C_4 decarboxylases and PEP carboxylase in 27 noxious C_4 weeds on cultivated land of Taiwan and representative species of C_4 and C_5 plants.

Species	Activity (μ mol mg^{-1} Chl hr^{-1})			
	NADP-ME	NAD-ME	PCK	PEPC
Dicotyledons				
NADP-ME subtype				
Euphorbia hirta L.	358	31	ND*	ND
Euphorbia thymifolia L.**	1214	11	ND	73
NAD-ME subtype				
Portulaca oleracea L.	16	196	ND	1056
Amaranthus spinosus L.**	50	146	ND	948
Amaranthus viridis L.**	49	271	ND	2090
C_3 dicotyledon				
Glycine max Merrill	12	6	ND	104
Monocotyledons				
NADP-ME subtype				
Cyperus compressus L.**	725	16	ND	877
Cyperus iria L.**	896	25	ND	2169
Cyperus rotundus L.	532	36	ND	73
Kyllinga brevifolia Rottb.	431	40	ND	92
Fimbristylis miliacea (L.) Vahl**	1405	48	ND	1055
Pycreus polystachyos (Rottb.) P. Beauv.**	933	32	ND	1007
NADP-ME subtype				
Cenchrus echinatus L.	999	10	101	828
Chrysopogon aciculatus (Retz.) Trin.**	936	19	ND	2891
Digitaria radicosa (Presl) Miq.***	665	13	62	972
Echinochloa colonum (L.) Link	853	50	304	853
Echinochloa crus-galli (L.) Beauv. var. *oryzicola* (Vasing) Ohwi	1312	57	149	2903
Imperata cylindrica (L.) Beauv. var. major (Nees) C.E. Hubb. ex Hubb. & Vaughan	1456	70	ND	1262
Miscanthus floridulus (Labill.) Warb. ex Schum. & Laut.**	1699	55	ND	1899
Paspalum conjugatum Berg.	1003	16	172	2493
Pennisetum purpureum Schumach.	1017	11	10	1529
Zea mays	1604	53	ND	2750
NAD-ME subtype				
Cynodon dactylon (L.) Pers.	43	167	ND	504
Eleusine indica (L.) Gaerth.	153	268	ND	1765
Leptochloa chinensis (L.) Nees***	54	318	ND	1576
Panicum repens L.**	67	194	ND	1160
PCK subtype				
Brachiaria mutica (Forsk.) Stapf.	13	67	772	2075
Dactyloctenium aegyptium (L.) Richter	14	161	1298	1513
Sporobolus fertilis (Steud.) W.D. Clayton	5	94	1826	1069
C_3 monocotyledon				
Oryza sativa L.	14	7	ND	99

ND : not detectable.

** These 11 species have been listed previously as C_4 plants, but have not been described for their biochemical subtypes. *** These 3 species have not previously reported as C_4 plants.

thymifolia L., *Amaranthus spinosus* L. and *A. viridis* L.) have not been reported.

For the twenty-two monocotyledonous species, fifteen are NADP-ME subtype, four are NAD-ME subtype and the remaining three are PCK subtype. Eleven out of the twenty-two species have not been reported for their subtype classification. *Digitaria radicosa* (Presl) Miq., *Leptochloa chinensis* (L.) Nees and *Sporobolus fertilis* (Steud.) W. D. Clayton in the Gramineae were first recorded as C_4 plants, and their subtypes determined.

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