建立水稻幼苗期耐旱相關外表性狀指標

劉佩欣[1]，陳榮坤[2]，曾信嘉[1]，羅正宗[2]，黃文理[1*]

[1]國立嘉義大學農藝學系；[2]臺南區農業改良場嘉義分場
*通信作者信箱：wlhuang@mail.ncyu.edu.tw

摘　　要

本研究選取120個水稻(*Oryza sativa* L.)種原，以水耕栽培模式於幼苗期階段調查各種原之地上部與根部性狀，進一步於木村氏水耕液中添加20% PEG模擬乾旱處理，依IRRI標準觀察葉片捲曲程度判斷對乾旱逆境之耐受性。試驗結果顯示幼苗期之根數、根長、根鮮重、根乾重、地上部鮮重與乾重等農藝性狀在不同水稻參試種原間均呈現顯著差異。依在PEG處理下之反應，可明顯區分出不同種原幼苗對乾和逆境之耐受性明顯不同。以逆境處理下葉片捲曲程度到達7時所需天數(Days to IRRI7; DIR7)，作為幼苗期之耐旱指標，顯示DIR7與幼苗之根數、根鮮重、根乾重及地上部乾重等性狀有顯著正相關。上述結果可作為水稻耐旱育種篩選系統之參考依據，此結果可作為後續耐旱關聯性QTL定位之應用。

關鍵詞：水稻、乾旱逆境、相關性分析、外表型指標、幼苗期。

前　　言

水稻(*Oryza sativa* L.)是全球最主要的糧食作物之一，據估計約有50%以上的稻作生產地區長年處於乾旱逆境之威脅(Hanson *et al.* 1990; Fukai *et al*. 1999)。臺灣地區雖雨量豐沛，但因雨量分配不平均，因缺水造成水稻休耕仍時有所聞，尤其隨著全球氣候變遷，極端乾旱與強降雨的頻率有愈來愈明顯的趨勢，影響稻作生產與糧食安全甚鉅。乾旱耐受性被認為是所有生物性與非生物性逆境中最為複雜的機制(Hanson *et al.* 1990; Mackill *et al*. 1996; Mitchell *et al.* 1998; Jongdee *et al.* 2002;

Cairns *et al.* 2009)，為一數量性狀，影響耐旱相關之數量性狀基因座(quantitative trait loci; QTL)數量眾多，已有許多與耐旱相關之QTLs被鑑定出(Steele *et al.* 2006; Qu *et al.* 2008; Lang and Buu 2008; Zhang *et al.* 2008; Cairns *et al.* 2009)，惟不同種原或篩選系統可能篩選到不同之QTLs，使得水稻耐旱品種的選育工作進展緩慢。

近年來，由於分子生物技術的快速演進，利用基因轉殖技術導入有用基因與開發重要農藝性狀相關之DNA分子標誌以輔助新品種選育，已廣泛在許多先進國家的育種計畫中施行，唯在許多消費者仍對基因改造技術存有疑慮的現今，更突顯分子標誌輔助選拔技術(marker assisted selection; MAS)的重要性與可行性。要利用DNA分子標誌輔助耐旱特性之選拔，除須先釐清種原特性外，乾旱處理之篩選系統與鑑定指標，也是影響選拔成效之重要因子。一般言之，水稻幼苗期與生殖生長期對乾旱逆境最為敏感，前者遭遇乾旱時會影響幼苗地上部與根部之生長勢及分櫱數(Mitchell *et al.* 1998; Jongdee *et al.* 2002; Gowda *et al.* 2011)。水稻不同生育階段對乾旱逆境之反應與耐受能力也不同，耐旱相關鑑定指標中，又以逆境下葉片捲曲之外表形態指標最為普遍(Standard Evaluation System: IRRI 1996; Mitchell *et al.* 1998)，常被用來表示水稻遭受乾旱逆境時的傷害指標且廣泛的運用於水稻抗旱育種的選拔系統(Mitchell *et al.* 1998；Pantuwan *et al*. 2004)。

本研究擬利用關聯性定位(association mapping)之研究策略(Tuberosa and Salvi 2006)進行後續耐旱相關QTLs篩選，試驗首先需建立耐旱相關辨別指標。以臺南區農業改良場所蒐集的120個不同稻種原為材料，以水耕栽培模式調查各種原3-4葉齡幼苗之農藝性狀，並以水耕液中添加聚乙二醇(polyethylene glycol; PEG)滲透調節劑模擬乾旱處理(Michel and Kaufmann 1973; Hardegree and Emmerich 1990)，比較各種原幼苗期階段之耐旱性，試驗以IRRI葉片捲曲指標延伸而來的DIR7(Days to IRRI 7)為耐旱之判斷指標，進一步利用相關性分析(correlation analysis)釐清幼苗期階段農藝性狀與乾旱逆境耐受性之相關性。

材料與方法

(一)試驗材料

本試驗以臺南區農業改良場所提供之120個水稻與陸稻種原作為試驗材料 (如表1)。種原包括國內栽培粳稻、秈稻、及糯稻、嘉義茶山地區、山美地區收集之陸稻品系、及種原庫中收集自IRRI、日本地區、美國及中國大陸之種原等。

(二)植株栽培

本試驗利用木村氏水耕液系統進行水稻幼苗之栽培。參試種原種子於35℃水浴槽內浸種48 h，各種原挑選催芽種子移至不鏽鋼網架上，置於27℃、高光強度(20000 lux)、相對溼度65%之植物生長箱中進行水耕栽培，三重複。每隔3天更換水耕液，約14天後，幼苗可達3~4葉齡，進行農藝性狀調查。

(三)農藝性狀調查

將120個水、陸稻試驗種原以水耕栽培至3~4葉齡時，分別調查幼苗地上部與根部特性，包括地上部鮮重、乾重，根部之根長、根數、根鮮重、及根乾重，每種原至少調查5單株，試驗重複三次。

(四)PEG滲透壓處理與外觀觀察

將上述生長至3~4葉齡之水稻幼苗移至含20% PEG 6000木村氏水耕液中進行模擬乾旱處理。參考國際水稻研究所訂定之水稻乾旱分級標準(Standard Evaluation System: IRRI 1996)，每日觀察並依水稻葉片捲曲程度分為0、1、3、5、7及9等不同等級進行目視調查(如Fig. 1)，紀錄各品系水稻幼苗葉片之捲曲程度(leaf rolling score)，本試驗以葉片捲曲程度到達7時所需天數(Days to IRRI7; DIR7)，作為幼苗期之耐旱指標。

(五)統計分析

以SAS (Statistical Analysis System) 9.2版本之套裝軟體進行DIR7耐旱指數與上述幼苗期調查之各農藝性狀間相關性分析。

表 1. 本試驗之 120 個水稻品種(系)

Table 1. 120 Rice lines used in this study.

No.	Variety	Origin	Type	No.	Variety	Origin	Type
1	H91-38-1	Japan	Japonica	31	Nato	USA	Japonica
2	H91-38-3	Japan	Japonica	32	KFAW	Taiwan	Native germplasm
3	Ochikara	Japan	Japonica	33	NHAW	Taiwan	Native germplasm
4	H87-43	Japan	Japonica	34	TNAW	Taiwan	Native germplasm
5	D6	Taiwan	Native germplasm	35	Ku 2986	Taiwan	Native germplasm
6	D8	Taiwan	Native germplasm	36	Kabasico	Philippines	indica
7	Milyang 23	China	Indica	37	Azucena	Philippines	Japonica
8	KHS 139	Taiwan	Japonica	38	Khao-lo-1	Taiwan	Native germplasm
9	TYW 2	Taiwan	Glutinous	39	Ku79-2	Taiwan	Native germplasm
10	TCW 70	Taiwan	Glutinous	40	Hseng-Ma-Tsam	Taiwan	Native germplasm
11	TNGS14	Taiwan	Indica	41	Hung-Mi-Hsiang-Ma-Tsam	Taiwan	Native germplasm
12	TNGSY2414	Taiwan	Indica	42	Basmati 370	India	Indica
13	IR72	Philippines	Indica	43	Jasmine 85	Thai	Indica
14	IR65598-112-2	Philippines	Indica	44	DYAJ	Taiwan	Native germplasm
15	IR66159-189-5-5-3	Philippines	Japonica	45	Aromatic Lemont	USA	Indica
16	IR68003-45-2-2	Philippines	Japonica	46	NBW	Taiwan	Native germplasm
17	IR61608-3B-20-2-2-1-1	Philippines	Indica	47	JPAW	Japan	Glutinous
18	B6490	Philippines	-	48	CYPAW	Taiwan	Native germplasm
19	BSI324	Philippines	Japonica	49	CKRW	Taiwan	Native germplasm
20	BSI325	Philippines	Japonica	50	CPRW	Taiwan	Native germplasm
21	BSI328	Philippines	Japonica	51	TDGW	Taiwan	Native germplasm
22	H3340	Philippines	Japonica	52	Pankaj	Taiwan	Native germplasm
23	H3308	Philippines	Japonica	53	Ching Chueh Chin yu	Taiwan	Native germplasm
24	IR71694-39-2-5-2	Philippines	Indica	54	IR76-83-3-1	Philippines	Indica
25	IR76904-7-19	Philippines	Indica	55	TL3	Taiwan	Native germplasm
26	IR75286-AC5	Philippines	Indica	56	FKR 19	Philippines	Indica
27	IR61608-3B-20-2-2-1-2	Philippines	Indica	57	Guizhao	China	Indica
28	R95-899	Philippines	Indica	58	NER 10A KEAN 2	Africa	NERICA
29	Annada	Japan	Japonica	59	RASI	Africa	NERICA
30	Lemont	USA	Japonica	60	WAB 56-50	Africa	NERICA

表 1. (續)

Table 1. (Cont.)

No.	Variety	Origin	Type	No.	Variety	Origin	Type
61	WAB365-B-1-1-H1-HB	Africa	NERICA	91	SM 1	Taiwan	Native germplasm
62	WAB450-1-B-P-163-4-1	Africa	NERICA	92	SM 2	Taiwan	Native germplasm
63	WAB450-11-1-1P31-HB	Africa	NERICA	93	SM 3	Taiwan	Native germplasm
64	TS 1	Taiwan	Indica	94	TS 1	Taiwan	Native germplasm
65	GY 1	Taiwan	Native germplasm	95	CD-13	Taiwan	Native germplasm
66	KHS 143	Taiwan	Japonica	96	CD-2	Taiwan	Native germplasm
67	TC 191	Taiwan	Japonica	97	CD-3	Taiwan	Native germplasm
68	STW	Taiwan	Indica	98	CD-1	Taiwan	Native germplasm
69	G 9	Taiwan	Indica	99	CD-20	Taiwan	Native germplasm
70	A 23	Taiwan	Native germplasm	100	SM 5	Taiwan	Native germplasm
71	TK 2	Taiwan	Japonica	101	SM 6	Taiwan	Native germplasm
72	TK 4	Taiwan	Japonica	102	SM 7	Taiwan	Native germplasm
73	TK 8	Taiwan	Japonica	103	SM 8	Taiwan	Native germplasm
74	TK 9	Taiwan	Japonica	104	SM 9	Taiwan	Native germplasm
75	TK 11	Taiwan	Japonica	105	SM 10	Taiwan	Native germplasm
76	TK 14	Taiwan	Japonica	106	SM 11	Taiwan	Native germplasm
77	TK 16	Taiwan	Japonica	107	SM 12	Taiwan	Native germplasm
78	TNG 71	Taiwan	Japonica	108	TS 2	Taiwan	Native germplasm
79	TY 1	Taiwan	Japonica	109	TS 3	Taiwan	Native germplasm
80	TN 11	Taiwan	Japonica	110	TS 4	Taiwan	Native germplasm
81	TT 30	Taiwan	Japonica	111	TS 5	Taiwan	Native germplasm
82	TKW 3	Taiwan	Glutinous	112	TS 6	Taiwan	Native germplasm
83	TNW 10	Taiwan	Glutinous	113	TS 7	Taiwan	Native germplasm
84	TNG 67	Taiwan	Japonica	114	TS 8	Taiwan	Native germplasm
85	TNGW 73	Taiwan	Glutinous	115	TS 9	Taiwan	Native germplasm
86	TCS 10	Taiwan	Indica	116	TS 10	Taiwan	Native germplasm
87	Mudgo	India	Indica	117	TS 11	Taiwan	Native germplasm
88	SM 4	Taiwan	Native germplasm	118	TS 12	Taiwan	Native germplasm
89	Kasalath	India	Indica	119	TS 13	Taiwan	Native germplasm
90	YM	Taiwan	Native germplasm	120	IR64	Philippines	Indica

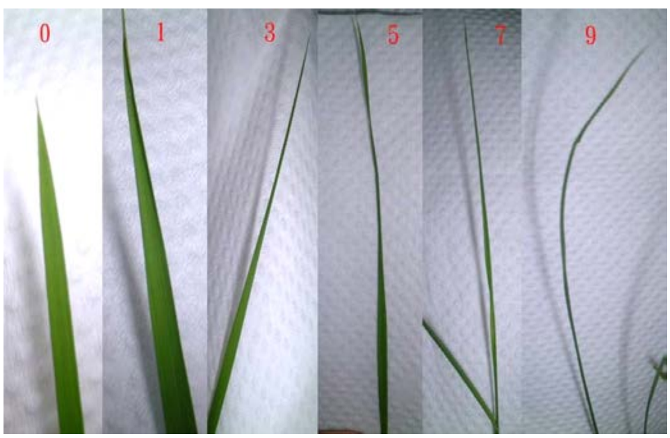

圖 1. 乾旱逆境處理下水稻幼苗葉片捲曲程度等級

本等級主要參照IRRI於1996年所訂定之水稻標準評估系統(Standard Evaluation System for Rice, SES)。

三、結果與討論

(一)不同水稻種原幼苗期之農藝性狀

將水耕栽培120個種原3~4葉齡之幼苗取出，進行各項幼苗形態觀察與性狀調查，結果如表2。試驗結果顯示不同品種間根長、根數、根鮮重、根乾重、地上部鮮重與乾重均呈現顯著差異，其中又以地上部與根部鮮重在各品種間之變異範圍最大；此外，部分種原之根數超過10個(Fig. 2 A, B)、部分則僅有5~6個根(Fig. 2 C, D)，顯示幼苗農藝性狀為各種原之重要品種特性，值得進一步分析其與幼苗期耐旱能力之相關性。

表 2. 水稻參試種原幼苗地上部與根部農藝性狀之調查

Table 2. Agronomic characters of shoot and root in different rice germplasms seedling

	Root length (cm)	Root number	Root fresh weight (mg)	Root dry weight (mg)	Shoot fresh weight (g)	Shoot dry weight (mg)
Mean	10.26	9.00	33.70	5.00	226.14	52.68
S.E	2.83	2.52	16.10	1.60	92.18	17.95
C.V	27.6%	28.0%	47.8%	32%	40.8%	34.1%
Range	2.95~18.71	3.0~16.0	3.50~72.70	0.40~9.40	65.2~451.2	7.23~79.60

(二)水稻不同種原幼苗以PEG模擬乾旱處理之比較

於木村氏水耕液中添加20% PEG -6000模擬乾旱處理，比較對不同水稻種原3-4葉齡幼苗之影響，結果顯示處理兩天後，有5個品種葉片捲曲程度達index 7，4天後有10個品種葉片捲曲已達到index 9 (Fig. 3)，將其歸為乾旱敏感型品種。反之，有12個參試種原在含PEG水耕液中處理14天後，葉片捲曲指數仍維持在index 5，呈現乾旱耐受型(如Fig. 3)。較耐旱品種(系)中，以國內收集之陸稻居多；而較不耐旱之品種(系)中，則以糯稻為主。

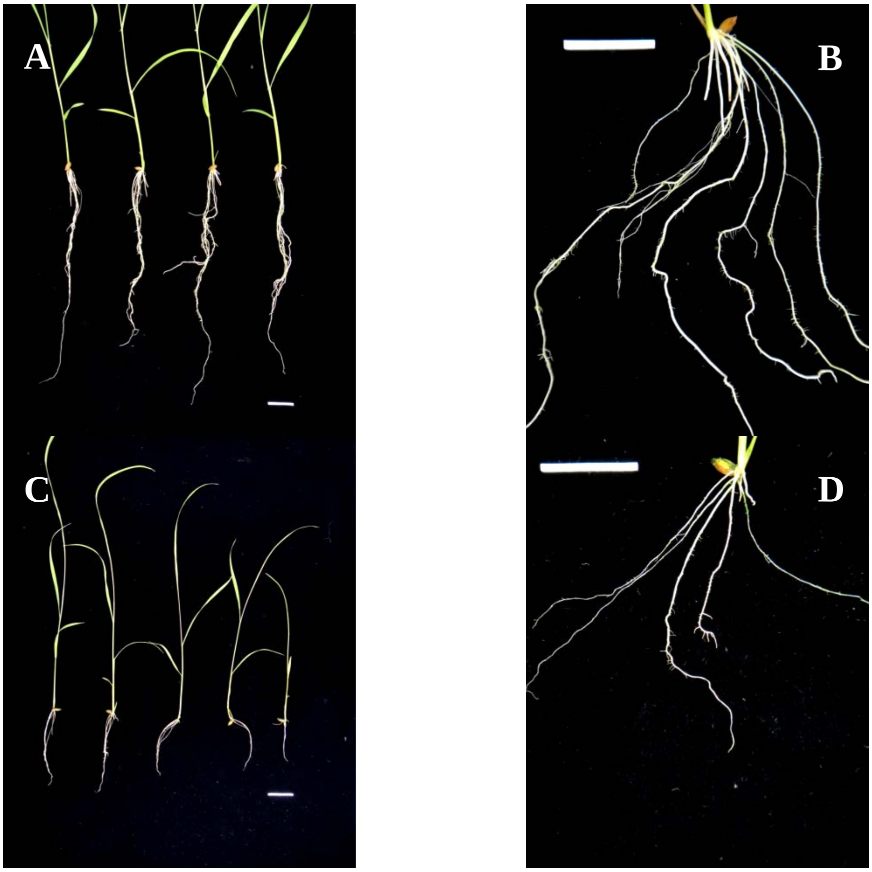

圖 2. 不同水稻種原水耕栽培下之幼苗性狀比較

Fig. 2. Phenotype of rice seedling with hydroponic culture. A and B, CD-20; C and D, DYAJ. Bar = 2 cm

(三)水稻幼苗期農藝性狀與DIR7耐旱指數之相關性

本試驗分別調查120水稻品系之幼苗期根長、根數、地上部鮮重、地上部乾重、根部鮮重及根部乾重，並以乾旱處理後葉片捲曲達到IRRI指標7的天數(Days to IRRI 7; DIR7)作為耐旱評估指標。針對PEG處理後葉片捲曲之反應，進一步選取上述乾旱敏感型與耐受型水稻種原之幼苗農藝性狀與DIR7耐旱指數進行相關性分析，結果如表2所示。分析結果顯示DIR7耐旱指標與根數、根鮮重、根乾重及地上部乾重均呈現顯著相關，這些性狀極有潛力可作為水稻幼苗期耐旱性之重要參考。

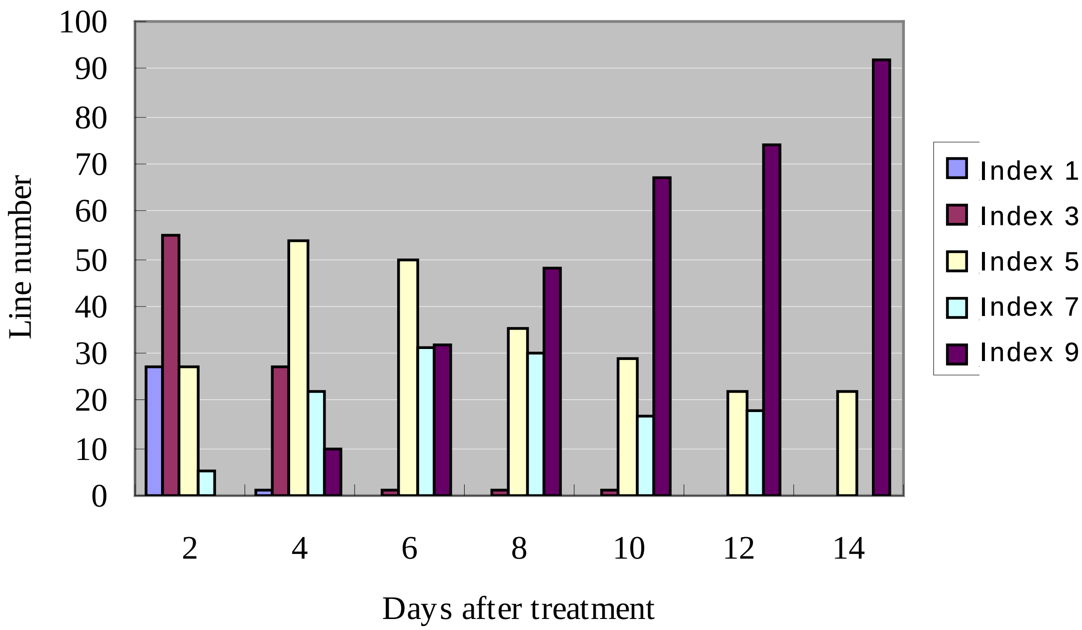

圖 3. 水稻不同種原幼苗於 20% PEG 處理下葉片捲曲程度之變化

Fig. 3. Changes of leaf rolling index in rice seedling under 20% PEG treatment

表 2. 水稻幼苗期農藝性狀與耐旱指數之相關性分析

Table 2. Correlation coefficient among seedling characters and drought tolerance index

	RL	RN	RFW	SFW	RDW	SDW	RRFW/RSFW	RRDW/RSDW	RRFW/TFW	RRDW/TDW
DIR 7	0.10526	**0.41581***	0.49846**	0.17887	0.51246**	**0.41206***	0.51459**	**0.35568***	0.53275**	**0.36232***
	0.5473	[z] **0.013***	0.0023**	0.3039	0.0016**	**0.0139***	0.0016**	**0.036***	0.001**	**0.0324***

DIR 7: Days to IRRI index 7.

RL: root length; RN: root number; RFW: root fresh weight; SFW: shoot fresh weight; RDW: root dry weight; SDW: shoot dry weight; RRFW/RSFW: root fresh weight/shoot fresh weight; RRDW/RSDW: root dry weight/shoot dry weight ; RRFW/TFW: root fresh weight / total fresh weight; RRDW/TDW: root dry weight / total dry weight.

[z] * Significant at $P < 0.05$.

** Significant at $P < 0.01$

水稻不同生育階段對乾旱逆境之反應與耐受能力也不同(Boonjung and Fukai 1996)，耐旱相關鑑定指標中，又以逆境下葉片捲曲之外表形態指標最為普遍，常被用來表示水稻遭受乾旱逆境時的傷害指標且廣泛的運用於水稻抗旱育種的選拔系統 (Mitchell *et al.* 1998；Pantuwan *et al*. 2004)。本試驗為了釐清不同生長時期、不同水稻品種間的耐旱能力是否一致，進一步將葉片捲曲指數標準設定在IRRI index 7，主要原因是多數品種在超過此指數後，持續逆境處理下就不易再恢復正常生長。相關研究也顯示，當乾旱逆境分別發生於水稻幼苗期與分蘗盛期這兩個不同時期時，水稻對於乾旱逆境的反應也不相同，幼苗期耐旱能力佳的品系，於成熟期遭遇乾旱逆境時不一定具有好的耐旱能力(Shih *et al.* 2012)。

本試驗以水耕液中添加滲透調節劑栽培系統，調查水稻幼苗期根系與地上部性狀，結果顯示幼苗期根系性狀的生長勢與根部/地上部之比值對於水稻的耐旱能力具有很大的影響。綜合上述結果，水稻利用不同篩選系統、不同生育階段對乾旱耐受性之機制可能不同，因此進行耐旱育種選拔時，應該要明確說明其篩選系統或篩選指標。本研究選取120個水稻品種(系)，於幼苗期與分蘗盛期分別進行乾旱處理，希望釐清水稻兩生育階段對乾旱耐受性之相關性，以做為耐旱育種篩選指標之依據。此外，調查120個水稻品系幼苗期農藝性狀與耐旱性之相關性，結果顯示根數、根鮮重、根乾重與地上部乾重等性狀與耐旱性(DIR7)有顯著正相關，值得進一步探討，此結果可作為水稻耐旱性育種篩選系統之依據。

誌　　謝

本試驗承蒙行政院農業委員會農糧署經費補助，特誌謝忱。

參考文獻

1. Boonjung, H. and S. Fukai. 1996. Effects of soil water deficit at different growth stages on rice growth and yield under upland conditions. 1. Growth during drought. Field Crops Res. 48: 37-45.

2. Cairns, J. E., A. Audebert, C. E. Mullins, and A. H. Price. 2009. Mapping quantitative trait loci associated with root growth in upland rice (*Oryza sativa* L.) exposed to soil water-deficit in fields with contrasting soil properties. Field Crops Res. 114: 108-118.
3. Fukai, S., G. Pantuwan, B. Jongdee, M. Cooper. 1999. Screening for drought resistance in rainfed lowland rice. Field Crops Res. 64, 61–74.
4. Gowda, V. R. P., A. Henry, A. Yamauchi, H. E. Shashidhar, R. Serraj. 2011. Root biology and genetic improvement for drought avoidance in rice. Field Crop Res. 122:1–13
5. Hanson, A. D., W. J. Peacock, L. T. Evans, C. J. Arntzen and G.S. Khush. 1990. Drought resistance in rice. Nature, 345:26-27.
6. Hardegree, S. P. and W. E. Emmerich. 1990. Effect of polyethylene glycol exclusion on the water potential of solution-saturated filter paper. Plant Physiol. 92: 462-466.
7. IRRI (1996) Drought sensitivity. p.37–38. *In*: Standard Evaluation System for Rice. IRRI, Los Banos, Philippines.
8. Jongdee, B., S. Fukai and M. Cooper. 2002. Leaf water potential and osmotic adjustment as physiological traits to improve drought tolerance in rice. Field Crops Res. 76: 153-163.
9. Lang, N. T. and B. C. Buu. 2008. Fine mapping for drought tolerance in rice (*Oryza sativa* L.). Omonrice 16: 9-15.
10. Mackill, D.J., W. R. Coffman, D. P. Garrity. 1996. Rainfed Lowland Rice Improvement. International Rice Research Institute, Manila, Philippines,242 pp.
11. Michel, B. E. and M. R. Kaufmann. 1973. The osmotic potential of Polyethylene glycol 6000. Plant Physiol. 51: 914-916.
12. Mitchell, J. H., D. Siamhan, M. H. Wamala, J. B. Risimeri, E. Chinyamakobvu, S. A. Henderson and S. Fukai. 1998. The use of seedling leaf death score for evaluation of drought resistance of rice. Field Crops Res. 55: 129-139.

13. Pantuwan, G., S. Fukai, M. Cooper, S. Rajatasereekul, J. C. O'Toole and J. Basnayake. 2004. Yield response of Rice (Oryza sativa L.) genotypes to drought under rainfed lowlands 4. Vegetative stage screening in the dry season. Field Crop Res 89: 281-297.
14. Qu, Y. Y., P. Mu, H. L. Zhang, C. Y. Chen, Y. M. Gao, Y. X. Tian, F. Wen, and Z. C. Li. 2008. Mapping QTLs of root morphological traits at different growth stages in rice. Genetica 133: 187-200.
15. Shih, C. L., R. K. Chen, C. N. Tsai, P. H. Liu, J. C. Lo, and W. L. Huang. 2012. Correlation analysis for drought tolerance between seedling stage and active tillering stage in rice. J. Agric. Forest., NCYU. 9(1): 55-69.
16. Steele, K. A., A. H. Price, H. E. Shashidhar, and J. R. Witcombe. 2006. Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. Theor. Appl. Genet. 112: 208-221.
17. Tuberosa, R. and S. Salvi. 2006. Genomics approaches to improve drought tolerance in crops. Trends Plant Sci. 11: 415-412.
18. Zhang, B. S., L. Yang, C. Z. Mao, Y. J. Huang, and P. Wu. 2008. Comparison of QTLs for rice seedling morphology under different water supply conditions. J. Genet. Genomics 35: 473-484.

ABSTRACT

The main objectives of this study were to establish the screening system for drought stress at seedling stage in rice with hydroponic culture. Total of 120 rice (*Oryza sativa* L.) lines provided from Tainan District Agricultural Research and Extension Station were used in this study. The agronomic characters of rice seedling including shoot fresh weight, shoot dry weight, root number, root length, root fresh weight and root dry weight, were determined. Besides, the DIR7 (Days to IRRI index 7) which based on the leaf rolling score was used as the assessment standard of drought tolerance. The 20% of polyethylene glycol was supplemented into Kimura solution to mimic the drought stress. The results showed all the seedling characters were highly variants among rice germplasms. Besides, the root number, root fresh weight, root dry weight, and shoot dry weight were highly correlated to drought tolerance index, DIR7. They are possible as the phenotypic indicators for drought tolerance screening. Our preliminary results are very useful for further study on drought tolerance QTL mapping with association mapping strategy.

Key words: *Oryza sativa L.*, Drought stress, Correlation analysis, Phenotypic indicator, Seedling stage.