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Molecular, anatomical and physiological diversity for water use efficiency traits in rice (*Oryza sativa* L.)

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Abstract
This study was conducted to evaluate the genetic diversity of 48 rice genotypes for water use efficiency related traits in relation with molecular diversity to identify water stress tolerant genotypes. The results indicated that water stress tolerant varieties had lower number of stomata with increase in the size of guard cells and increased distance between stomata, lesser carbon isotope discrimination, and higher leaf relative water content and with less specific area than water stress susceptible varieties. Significant negative relationship between carbon isotope discrimination and stomata size and distance between stomata reveals that lesser ^{13}C discrimination. Significant negative relationship between relative water content and stomata number on leaf surface indicates maintenance of water status under stress conditions. There is no significant relationship between specific leaf area and relative water content, specific leaf area and stomata characters. Molecular diversity using SSR markers provided the dendrogram with four clusters showing the differentiation in genotypes for water stress tolerance. This implies the use of member of these clusters as parents to build up population for selection of transgressive segregants against water stress in subsequent generations and for the development of QTLs related with water stress tolerance.

Keywords: Carbon isotope discrimination, relative water content, specific leaf area and SSR markers

Introduction
Rice (*Oryza sativa* L.) is one of the major staple food crops for over half of the world's human population. The production and productivity of rice is constrained by many abiotic and biotic stresses. Moisture stress is one of the major abiotic stresses that need much attention from scientists all over. Rice, being a semi-aquatic crop consumes approximately 3000 to 5000 litres of water to produce one kilo gram of paddy, which is about 2 to 3 times more than to produce 1 kilogram of other cereals such as wheat or maize Bouman *et al*. (2002) [1]. Hence, the projected demand of the food production should be met with less water and land. The water use efficiency (WUE) is a most sought after trait to consider for designing of rice varieties, especially for rainfed ecosystems. Water use efficiency (WUE) is the ability of the crop to produce biomass per unit of water transpired. Water use efficiency was considered as an important component of adaptation to water stress, where the identification of genotypes with high yield and high WUE is important. Water use efficiency is a complex trait determined by many morphological and anatomical characters besides governed by many genes.
Assessment of the genetic diversity is a pre-requisite for any crop improvement program as it helps in estimating and establishing of genetic relationship in germplasm. Besides, study of genetic diversity facilitates identification of diverse parental combinations to create maximum transgressive segregants and introgressing desirable genes from diverse germplasm (Thompson J.A *et al*. 1998 and Islam M.R *et al*. 2012). The aim of present study is to evaluate genetic diversity of rice germplasm for water use efficiency related traits employing physiological, anatomical and molecular markers.

Material and Methods
Plant material
A set of 48 rice genotypes comprising of drought tolerant lines, NERICA (New RICe for Africa) lines, landraces, derived lines of Moroberekan and Vijetha cross and modern cultivars have been used in the present investigation (Table 1). The genotypes under study were kept for germination in petri plates and 14 days old germinated seedlings were planted in pots (32cm x

36cm) filled with potting mixture of wetland soil and farmyard manure in 3:1 proportion along with 0.5 g urea, and 4 g 15–15–15 (N–P–K) fertilizer. At the 5-leaf stage, only one plant with vigorous growth was retained in each pot and extra plants were removed. Plants were irrigated at certain intervals to maintain a shallow water layer. The experiment was conducted at the Institute of Biotechnology, ANGRAU, Hyderabad. The leaves of the rice genotypes were collected and characterized based on the number of stomata, distribution and size of stomata and distance between the stomata using Scanning Electron Microscope (SEM – Model: JOEL- JSM 5600) at 8 KV and magnification at 1000X and 2500X as per the standard procedure at RUSKA Lab, College of Veterinary Science, Sri Venkateswara Veterinary University, Rajendranagar, Hyderabad. Rice genotypes also screened for carbon isotope discrimination (CID), relative water content (RWC) and specific leaf area (SLA) and molecular diversity analysis using following procedures. Carbon isotope discrimination (Δ) is a character to indicate amount of 13C depleted by photosynthesis mechanisms and carried at National Facility for Stable Isotope Studies in Biological Sciences, Department of Crop Physiology, University of Agriculture Science (UAS), GKVK campus, Bangalore, India. Carbon isotope discrimination $\Delta^{13}C$ values were computed using following formula. Farquhar G.D. *et al*. (1989).

$$\Delta^{13}C = [\delta^{13}C_a - \delta^{13}C_{lb}] / [1+ (\delta^{13}C_{lb}/1000)]$$

Where, $\Delta^{13}C$ = Carbon isotope discrimination; $\delta^{13}C_a$ =Carbon isotope composition of the air and $\delta 13C_{lb}$ = Carbon isotope composition of bulk leaf matter.

Relative water content (RWC) indicates the ability of plants to keep their water status at a reasonable level when they experience water stress and was calculated using the formula suggested by Gonzaleg and Gonzaleg-Viar (2001) [5].

$$RWC\ (\%) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

For specific leaf area (SLA) ($cm^2\ g^{-1}$), the leaf area was estimated using a leaf area meter (LICOR model-3100). The leaves were dried in a hot air oven at 80 ℃ and dry weight was recorded (Laza *et al*. 2006 and This *et al*. 2010). The formula used was SLA = LA/LDW; Where LA = Leaf area (cm^2); LDW = Leaf dry weight (g). For marker genotyping and diversity analysis, DNA isolation was carried out as per the standard CTAB method (Murray and Thompson 1980) [9]. A total of 35 SSR markers, which are already reported to be linked to QTLs of various WUE related traits were used for screening all 48 rice genotypes. Microsatellites or SSRs have a high level of allelic diversity and is possible to tag markers adjacent to the targeted gene or QTL for the trait of interest when two alleles (*i.e.* a marker and the target gene) are more or less likely to appear together. Present study the markers were genotyped using traditional agarose gel electrophoresis system. The PCR was performed with 10 µl final volume containing 25-50 ng of genomic DNA, 10X buffer, 2.5 mM dNTPs, 0.2 µM of each forward and reverse primer, and 5U of *Taq* DNA polymerase. The PCR was set up with an initial denaturation of 94 ºC for 5 min, followed by 35 cycles of denaturation at 94 ºC for 45s, annealing at 55 ºC for 45s, extension at 72 ºC for 1min, followed by the final extension of 72 ºC for 7 min. The amplified products were run on 3% agarose gel electrophoresis system and visualized in gel documentation system (Bio-Rad, CA, USA). The gels were scored and represented by their allele sizes as allelic data. Clearly resolved, unambiguous polymorphic bands were scored visually for their presence or absence. The scores were obtained in the form of a matrix with '1' and '0', which indicate the presence and absence of bands in each genotype respectively. Using the DARWIN 5.0 software (http://darwin.cirad.fr/) Perrier X. *et al*. (2005) [12], a simple matching dissimilarity index was calculated from the allele-size data set with 100 bootstraps and this matrix was then subjected to Neighbour-Joining analysis to construct the dendrogram.

Table 1: Variation in stomatal characters of 48 rice genotypes

S. No.	Genotype	Sub population	Upper Surface			Lower Surface		
			Stomata number (USN)	Dist b/w stomata (µm) (USD)	Size of Stomata(µm) (USS)	Stomata number (LSN)	Dist b/w stomata(µm) (LSD)	Size of Stomata(µm) (LSS)
1	BPT5204	*Indica*	5.00	30.46	13.85	6.00	30.50	14.63
2	Swarna	*Indica*	5.66	32.93	14.83	5.00	28.38	14.82
3	MTU1010	*Indica*	4.66	36.27	14.39	4.66	35.41	14.20
4	Solumpiket	*Indica*	2.66	41.85	20.40	3.66	46.36	17.61
5	Annada	*Indica*	3.66	31.69	13.39	4.00	29.40	16.42
6	Vandana	*Indica*	3.00	41.26	16.68	3.33	43.97	13.95
7	Bala	*Indica*	2.33	39.76	18.18	3.66	46.08	14.45
8	Kalinga 3	*Indica*	4.66	36.9	15.01	4.00	26.01	15.68
9	INRC10192	*Indica*	2.33	41.73	21.03	3.33	47.02	19.52
10	Lalnakanda	*Indica*	3.33	38.76	18.15	4.66	42.30	16.74
11	IR64	*Indica*	6.33	28.44	14.29	8.33	24.35	12.49
12	NLR145	*Indica*	4.00	40.10	19.87	3.33	50.56	19.68
13	NLR34242	*Indica*	3.00	24.15	15.95	4.00	22.59	14.45
14	NLR3010	*Indica*	3.66	15.20	15.03	4.00	25.06	14.28
15	NLR33671	*Indica*	3.66	28.55	16.62	4.00	47.83	17.97
16	Tellahamsa	*Indica*	5.33	30.79	17.12	6.66	23.26	15.76
17	Acharmati	*Indica*	4.00	33.46	18.03	5.00	22.57	15.17
18	Azucena	*Japonica*	4.00	39.91	18.39	3.66	48.30	18.81
19	N22	*Japonica*	3.00	38.08	20.11	4.00	43.16	17.22
20	Moroberekan	*Japonica*	3.30	38.70	19.00	4.00	40.20	18.50
21	NL 1	*Os x Og*	3.66	31.06	16.41	3.66	42.20	16.43
22	NL2	*Os x Og*	4.66	37.21	14.14	4.33	40.05	13.95

23	NL3	*Os x Og*	3.66	50.03	19.88	4.00	29.50	17.50
24	NL 5	*Os x Og*	5.00	36.54	14.23	5.50	33.35	14.86
25	NL7	*Os x Og*	3.66	42.85	18.54	5.60	33.86	16.60
26	NL9	*Os x Og*	3.00	34.10	17.33	5.60	27.46	15.48
27	NL16	*Os x Og*	4.66	36.46	16.55	5.33	36.75	15.10
28	NL22	*Os x Og*	3.30	40.20	18.90	2.30	39.10	19.70
29	NL24	*Os x Og*	3.33	42.06	18.31	5.00	35.69	17.41
30	NL32	*Os x Og*	4.00	31.50	12.70	3.00	28.68	15.19
31	NL34	*Os x Og*	4.33	35.23	14.36	6.30	33.30	13.70
32	NL42	*Os x Og*	3.33	45.61	18.68	3.66	37.66	15.78
33	NL44	*Os x Og*	4.60	48.73	12.70	4.30	27.40	14.50
34	NL45	*Os x Og*	4.00	36.83	21.75	4.60	29.10	20.80
35	NL48	*Os x Og*	4.60	31.30	14.00	5.00	34.40	13.60
36	NL50	*Os x Og*	3.30	31.00	14.70	3.60	35.70	17.00
37	NL52	*Os x Og*	5.00	36.12	14.78	4.30	31.60	14.80
38	NL60	*Os x Og*	5.66	37.15	16.73	4.30	37.90	17.10
39	NL61	*Os x Og*	4.00	35.54	15.76	4.66	40.13	18.02
40	MT1	M x V	4.33	36.01	16.11	2.33	37.20	15.01
41	MT2	M x V	4.60	39.10	11.10	6.60	30.50	13.20
42	MT3	M x V	4.33	37.08	12.42	4.33	23.49	10.25
43	MT4	M x V	4.30	25.00	13.30	5.30	29.20	14.00
44	MT 5	M x V	4.33	37.27	15.42	6.33	38.44	14.56
45	MT 6	M x V	3.33	39.85	18.04	4.00	29.89	13.43
46	MT7	M x V	2.33	33.76	18.14	4.33	41.26	16.68
47	MT 8	M x V	4.33	35.67	17.12	6.00	23.10	13.67
48	MT 9	M x V	5.33	33.97	14.25	6.33	46.03	12.17
	Mean±SD		3.9±0.8	35±6.1	16.3±2.5	4.5±1.1	35±8.1	15.6±2.1

Note: Values are averaged over 4 microscopic imaged areas of each 10 µm for each genotype
NL- Nerica Lines derived from cross between *Oryza sativa* (*Os*) x *Oryza glaberrima* (*Og*)
MT- RILs derived from cross between Moroberekan x Vijetha (MxV)

Results

Assessment of genetic diversity is the key for designing varieties to sustain the food security. The physiological, anatomical and molecular diversity analysis offers immense scope for selection of donors for WUE traits in rice breeding. The present study revealed the apparent natural variability in WUE related traits in rice genotypes studied. (Table 2 & Figure 1). All the traits showed continuous variation except for specific leaf area and stomata number on upper surface. The traits stomata number and size of the stomata followed bimodal distribution, which means possibility of involving two major genes in controlling these two traits (Figure 1).

The scanning electron microscopy (SEM) analysis of rice genotypes revealed that the stomata number on upper surface is ranged from 2.33 (INRC10192 and MT7) to 6.33 (IR64) while the range is 2.30 (NL22) to 8.33 (IR64) on lower surface. In case of distance between stomata, the range is 15.2 um (NLR3010) to 50.03 um (NL3) on upper surface and 22.57 um (Acharmati) to 50.56 um (NLR145) on lower surface. The NERICA line NL45 showed larger stomata size in both upper (21.75um) and lower (20.8um) surfaces whereas MT2 (11.1um) and MT3 (10.25um) exhibited smaller stomata size on upper and lower surfaces, respectively (Table 1 & Figure 2). In the present study, NERICA line, NL45 showed large stomatal size in both upper and lower surfaces. Generally, the varieties consisting of lower number of stomata with increase in the size of guard cells, increased pore size and increased distance between stomata on upper leaf surface were responsible for higher water use efficiency by reducing the evapotranspiration losses. The presence of abundant, closely spaced, smaller size stomata is responsible for higher evapotranspiration losses (Kulkarni *et al.*, 2008) [7]. Based on the above criteria that INRC10192 having 2.33 stomata per 10 µm leaf area with, stomata size of 21.03 µm, and the distance between stomata is 41.73 µm, followed by Solumpiket (2.66, 20.40 µm and 41.85 µm), Bala (2.33, 18.18 µm and 39.76 µm) and Lalnakanda (3.33, 18.15 µm and 38.76 µm) respectively, exhibited similar arrangement. Hence, these genotypes can be treated as higher water use efficient types. Whereas, the modern cultivars like IR64 (Figure 4.3) having (6.33, 14.29 µm and 28.44 µm), BPT5204 (5.00, 13.85 µm and 30.46 µm), Swarna (5.66, 14.83 µm and 32.93 µm) and NLR3010 (3.66, 15.03 µm and 15.20 µm) exhibited higher number of stomata with smaller size and closely spaced arrangement respectively, may results in higher evapotranspiration losses. Hence, these genotypes can be considered as lower water use efficient types. Yu *et al.* (2013) reported that the transgenic rice plants overexpressed with Arabidopsis *AtEDT1/HDG11* (Homeodomain glabrous11) gene exhibited reduced stomatal density with increase in the stomatal size apparently contributes to the reduced rate of water loss and consequently increases the WUE compared to control plants. Stomatal pores on the aerial surface of rice leaves facilitate gas exchange between plant and its environment and these stomatal characters are also reported to be positively associated with the yield related traits. The selection criterion in the current study for identifying the donors for high WUE based on the stomatal parameters is similar to the ideotype model proposed by Kullarni *et al.* (2008).

Among physiological traits, the carbon isotope discrimination (CID) can be used as surrogate for water use efficiency in many crops as WUE is associated with the differences in the ability of plants to discriminate against ^{13}C compared with ^{12}C during stomatal CO_2 diffusion and enzymatic fixation Shangguan Z.P *et al.* (2000) [17]; Yin Z.H *et al.* (1998) [24] and Dercon G *et al.* (2006). The CID values for the rice genotypes studied in the present study, ranged from 19.81% (INRC10192) to 23.03% (MT9). Among all the rice genotypes screened, some of the *indica* (INRC10192-19.81%, NLR145-19.91%, Lalnakanda-19.93%), *japonica* (Azucena-19.92%)and NERICA lines (NL5-19.85%, NL32-19.88%, NL34-19.99%, NL44-19.94% and NL48-19.96%) showed

lesser leaf $\Delta^{13}C$ values indicating that these genotypes are higher water use efficient as CID is inversely proportional to WUE. The result obtained in the present study are in agreement with earlier study by Xu Y. *et al.* (2009). Reduction in CID was due to an increase in stomatal resistance and consequently a reduction in the ratio of intercellular and atmospheric CO_2 partial pressure (Pi/Pa) Farquhar G.D *et al*. (1984).

The relative water content (RWC) is one of the appropriate measures which gives an idea of leaf water status and considered as the sensitive index of plant water content Painawadee M. *et al.* (2009). The RWC of all 48 rice genotypes ranged from 78.50% (NL2) to 96.75% (MT3). Among the tested genotypes, N22 (95.20%), INRC10192 (94.06%), Solumpiket (95.03%), Azucena (92.12%), and Vandana (92.40%) maintained higher tissue water content.

The genotypes maintaining higher RWC accumulates more solutes and also show higher photosynthesis. The genotypes having higher RWC would be preferable to maintain higher water balance and plays a central role in stabilizing the various plant processes and finally influence the yield. A possible explanation of variation among varieties for RWC is that they vary in leaf thickness and stomatal conductance. Hence, enhanced RWC helps the plants to perform the physiological processes like stomatal conductance, photosynthesis, transpiration biochemical metabolism more effectively even under low moisture condition Rekika *et al.* (2000) [14].

The SLA is often considered as an indirect measure of leaf expansion. The SLA was negatively related to WUE and was positively related to harvest index, suggesting that selection for low SLA might result in the production of more dry matter with minimal influence on pod weight (Wright *et al.*, 1988, 1993) [21]. In the present study, SLA of all the rice genotypes ranged from 49.50 cm^2 g^{-1} (NL50) to 242.30 cm^2 g^{-1} (Acharmati). The genotypes that exhibited less leaf area are INRC10192 (50.41 cm^2g^{-1}), Solumpiket (56.84 cm^2g^{-1}), NL48 (58.00 cm^2g^{-1}) and NL60 (54.40 cm^2g^{-1}) (Table 2). Hence, these genotypes can be ascertained not only photosynthetically efficient but are also high WUE types. Genotypes with low SLA (thick leaves) were also known to have more photosynthetic machinery *i.e.*, more chlorophyll content. Genotypes with lower SLA and high SCMR (SPAD chlorophyll meter reading) values recorded higher yield and suggested to use for screening drought tolerance (Sudhakar *et al*., 2006 and Renuka devi *et al*., 2009) [15].

Table 2: Phenotypic performance of the genotypes for Water Use Efficiency related traits

S. No.	Genotype	CID (‰)	RWC (%)	SLA (cm^2/g)
1	BPT 5204	20.78	85.03	98.20
2	Swarna	20.45	90.30	76.30
3	MTU1010	20.48	92.06	110.00
4	Solumpiket	21.85	95.03	56.84
5	Annada	20.72	88.45	168.53
6	Vandana	21.95	92.40	90.23
7	Bala	20.20	88.96	88.12
8	Kalinga3	20.62	83.53	95.00
9	INRC10192	19.81	94.06	50.41
10	Lalnakanda	19.93	88.07	94.25
11	IR64	20.55	88.89	78.30
12	NLR145	19.91	95.65	108.80
13	NLR34242	20.23	90.00	60.40
14	NLR3010	21.67	95.97	82.20
15	NLR33671	21.28	87.07	128.30
16	Tellahamsa	20.71	83.87	122.20
17	Acharmati	20.29	91.16	242.30
18	Azucena	19.92	92.12	113.80
19	N22	22.16	95.20	109.70
20	Moroberekan	21.88	91.40	118.30
21	NL1	20.90	83.03	113.00
22	NL2	22.67	78.50	120.20
23	NL3	20.36	87.61	116.00
24	NL5	19.85	85.58	70.25
25	NL7	20.73	92.23	78.00
26	NL9	21.53	84.18	120.00
27	NL16	20.65	90.18	67.00
28	NL22	21.35	88.56	69.00
29	NL24	20.54	85.62	58.90
30	NL32	19.88	88.20	98.00
31	NL34	19.99	79.53	69.50
32	NL42	20.53	90.27	78.30
33	NL44	19.94	86.40	136.40
34	NL45	22.79	81.50	92.00
35	NL48	19.96	89.81	58.00
36	NL50	21.59	93.27	49.50
37	NL52	21.76	80.95	128.90
38	NL60	20.64	87.07	54.40
39	NL61	20.29	91.51	160.00
40	MT1	22.04	85.50	68.90
41	MT2	21.27	80.41	75.50

42	MT3	21.78	96.75	153.50
43	MT4	20.48	94.62	75.20
44	MT5	22.22	93.81	75.30
45	MT6	21.13	88.62	87.00
46	MT7	20.42	95.45	120.60
47	MT8	20.38	90.00	68.60
48	MT9	23.03	86.92	132.20
	Range	19.81 – 23.03	78.50-96.75	49.50-242.30
	Mean ± SD	20.90 ± 0.8	88.65±4.65	97.63±36.4

Note: NL- Nerica Lines derived from cross between *Oryza sativa* (*Os*) x *Oryza glaberrima* (*Og*)
MT- RILs derived from cross between Moroberekan x Vijetha (MxV)
CID- Carbon isotope discrimination, RWC- Relative water content, SLA- Specific leaf area

Correlation analysis

Phenotypic correlation analysis of the rice genotypes (Table 3) revealed that CID showed significant and negative association with USS (-0.218*), LSD (-0.225*), LSS (-0.244*). RWC also exhibited significant and negative associations with both the surfaces of stomata number while positively associated with USD and USS. Stomata number on upper surface (USN) is negatively associated with many of the other stomatal traits such as USS, LSD and LSS except with LSN. Contrary to this, USD is positively associated with USS, LSD and LSS. The stomata size of the upper surface (USS) is negatively associated with LSN while positively with LSS. LSN is significantly negatively associated with the traits LSD and LSS whereas LSD is positively and significantly associated with LSS.

Significant and negative association between USN and USS, LSN and LSS, USN and USD, LSN and LSD indicates that varieties with lower number of stomata were responsible for higher water use efficiency by reducing evapotranspiration losses. In the present study the stomata number showed negative correlation with stomata size in both upper and lower surfaces. Similar results were obtained by Ohsuni *et al*. (2007) [10]. Contrary to this, Sarwar *et al*. (2013) [16] reported that stomatal size does not vary with the change of stomatal density.

Table 3: Correlation coefficients among the water use efficiency and stomatal traits of 48 genotypes

	CID	RWC	SLA	USN	USD	USS	LSN	LSD	LSS
CID	1.000								
RWC	-0.064	1.000							
SLA	0.061	-0.048	1.000						
USN	0.061	-0.328*	-0.128	1.000					
USD	-0.148	0.397*	-0.105	-0.178	1.000				
USS	-0.218*	0.265*	-0.088	-0.569**	0.399*	1.000			
LSN	0.009	-0.432**	-0.052	0.505**	-0.213*	-0.342*	1.000		
LSD	-0.225*	0.120	0.123	-0.333*	0.320*	0.362*	-0.381*	1.000	
LSS	-0.244*	0.290	-0.092	-0.396*	0.273*	0.727**	-0.460**	0.386*	1.000

* = p<0.05; **= p<0.01. CID- Carbon Isotope Discrimination, RWC-Relative water content,
SLA- Specific Leaf Area
USN-Stomata number on upper surface, USD-Distance between stomata on upper surface, USS- Stomata size on upper surface, LSN- Stomata number on lower surface, LSD-Distance between stomata on lower surface, LSS- Stomata size on lower surface.

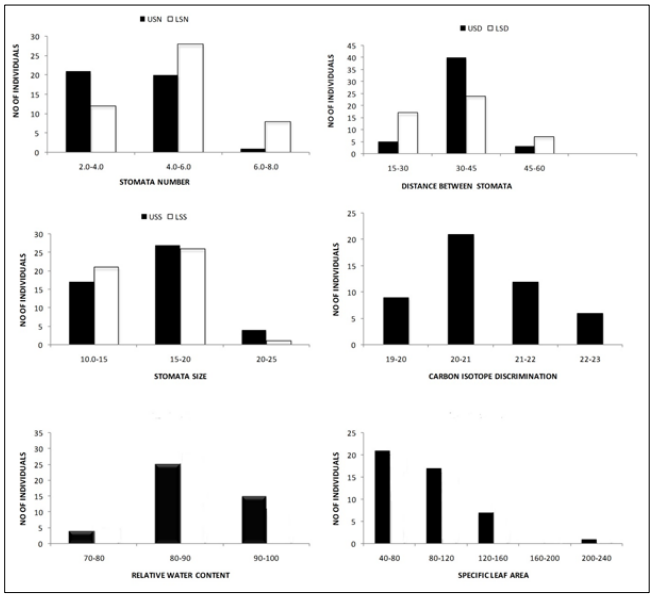

Fig 1: Frequency distribution of Water Use Efficiency related traits in 48 germplasm lines

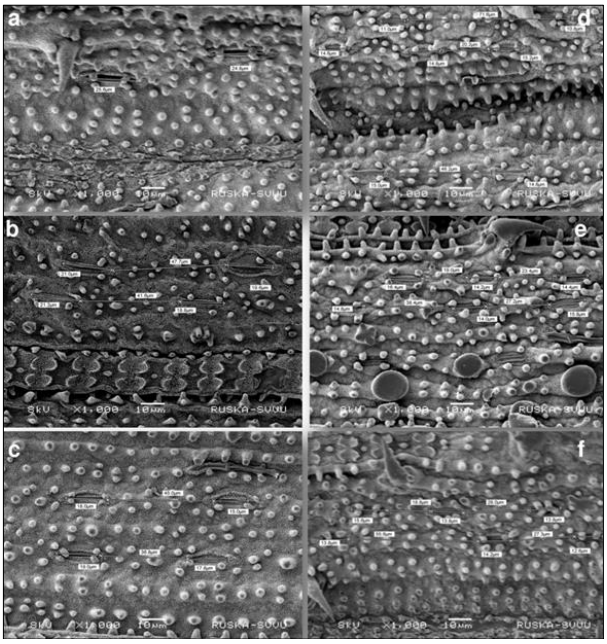

Fig 2: Stomatal characters of Genotypes measured using SEMb a) INRC10192 b) Solumpiket c)Azucena d) BPT5204 e)Swarna f) IR64

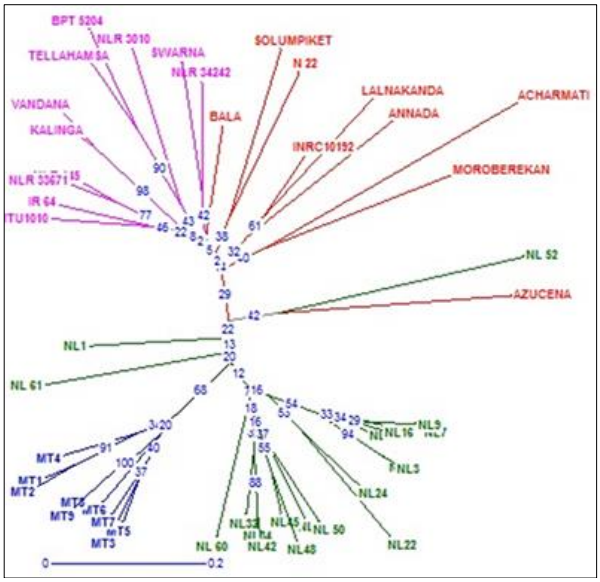

Fig 3: Dendrogram representing 48 germplasm lines

Diversity analysis at Molecular level

SSR genotyping revealed 2-4 number of alleles for all the markers studied and the allele sizes varied between 110- 280 bp. Molecular diversity analysis showed that many of the high yielding varieties were grouped into Cluster I while drought tolerant varieties and landraces were grouped to Cluster II, Moroberekan x Vijetha RILs grouped into Cluster III and all the NERICA lines grouped into Cluster IV (Figure 3). Molecular diversity employing microsatellite markers linked to the WUE QTLs also revealed the substantial genetic variability among genotypes and also revealed that clustering of water stress tolerant and susceptible genotypes separately. This is in line with the results of previous report by Reddy *et al*. (2009). The tightly linked markers can be used as foreground and background selection for marker-assisted breeding of WUE rice varieties.

Conclusion

From this study, it was demonstrated that selection based on less carbon isotope discrimination, high relative water content, less specific leaf area and lower number of stomata is the key while choosing the parents for development of WUE rice varieties. Based on stomatal characteristics, WUE traits and molecular diversity analysis INRC10192, Azucena, Soulmpiket, Lalnakanda and NL48 are identified as donors for the WUE related traits. Molecular diversity analysis using SSRs categorized the genotypes into four different clusters representing existence of substantial variation present in the genotypes. All the high WUE genotypes identified in the present study were grouped in one cluster (cluster II) whereas; the high yielding cultivars were grouped in separate cluster (cluster I). Hence, the high WUE genotypes falling in the above two clusters may be selected used as donor and recipient parents in the breeding programme for the development high water use efficient rice varieties.

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References

1. Bouman BAM, Hengsdijk H, Hardy B, Bindraban PS, Tuong TP, Ladha JK. Water-wise rice production. Proceedings of the International Workshop on Water-wise Rice Production, 8-11 April 2002, Los Baños, Philippines. Los Baños (Philippines): International Rice Research Institute, 2002, 356.
2. Dercon G, Clymans E, Diels J, Merckx R, Deckers J. Differential ^{13}C isotopic discrimination in maize at varying water stress and at low to high nitrogen availability. Plant Soil. 2006; 282: 313-326.
3. Farquhar GD, Richards RA. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Australian Journal of Plant Physiology. 1984; 11:53952.
4. Farquhar GD, Ehleringer JR, Hubick KT. Carbon isotope discrimination and photosynthesis. Annual Review Plant Physiology. 1989; 40:503-537.
5. Gonzaleg L, Gonzaleg-viar M. Determination of Relative Water Content. Hand book of plant ecophysiology techniques, Reigosa Roger MJ (ed), Netherlands, ISBN, 2001, 207-212.
6. Islam MR, Gregorio GB, Salam MA, Collard BCY, Singh RK, Hassan L. Validation of SalTol linked markers and haplotype diversity on chromosome 1 of rice. Molecular Plant Breeding. 2012; 3(10):103-114.
7. Kulkarni M, Borse T, Chaphalkar S. Mining Anatomical Traits. A Novel Modelling Approach for Increased Water Use Efficiency under Drought Conditions in Plants. Czech Journal of Genetics and Plant Breeding. 2008; 44 (1):11-21.
8. Laza MR, Kondo M, Ideta O, Barlaan E, Imbe T. Identification of quantitative trait loci for ^{13}C and productivity in irrigated lowland rice. Crop Science. 2006; 46:763-773.
9. Murray MG, Thompson WF. Rapid isolation of high molecular weight DNA. Nucleic Acids Res. 1980; 8:4321-4325.
10. Ohsuni A, Kanemura T, Homma K, Horie T, Shiraiwa T. Genotypic variation of stomatal conductance in relation to stomatal density and length in rice (*Oryza sativa* L.). Plant Prod. Sci. 2007; 10:322-328.
11. Painawadee M, Jogloy S, Kesmala T, Akkasaeng C, Patanothai A. Identification of traits related to drought resistance in peanut (*Arachis hypogaea* L.). Asian Journal of Plant Science. 2009; 8(2):120-128.
12. Perrier X, Jacquemoud JP. DARWIN-5.0. Dissimilarity analysis and representation for Windows. User's manual. CIRAD, Montpellier, 2005.
13. Reddy ChS, Babu AP, Swamy BP, Kaladhar K, Sarla N. ISSR markers based on GA and AG repeats reveal genetic relationship among rice varieties tolerant to drought, flood or salinity. J Zhejiang Univ. Sci. B. 2009; 10:133-141.
14. Rekika AK, Matsui T, Kobayasi KM. Temperature stress induced impairment of chlorophyll biosynthetic relations in rice and wheat. Plant Physiology. 2000; 117:851-858.
15. Renuka Devi K, Rama Rao G, Reddy KB. Determination of water use efficiency in blackgram by gravimetric method and its association with physiological parameters. Annals of Plant Physiology. 2009; 23(1):17-20.
16. Sarwar AKM, Karim MA, Rana SMAM. Influence of stomatal characteristics on yield and yield attributes of rice. Journal of the Bangladesh Agrilcultural University. 2013; 11:47-52.
17. Shangguan ZP, Shao MA, Dyckmans J. Nitrogen nutrition and water stress effects on leaf photosynthetic gas exchange and water use efficiency in winter wheat. Environmental Botany. 2000; 44:141-149.
18. Sudhakar P, Babitha M, Latha P, Prasanthi L and Reddy PV. Thermostability of cell membrane and photosynthesis in blackgram genotypes differing in heat tolerance. Journal of Arid Legumes. 2006; 3(2):11-16.
19. This D, Comstock J, Courtois B, Xu Y, Ahmadi N, Vonhof WM, Fleet C. Setter T and McCouch S. Genetic analysis of water use efficiency in rice (*Oryza sativa* L.) at the leaf level. Rice. 2010; 3:72-86.
20. Thompson JA, Nelson RL, Vodkin LO. Identification of diverse soybean germplasm using RAPD markers. Crop Science. 1998; 38:1348-1355.
21. Wright GC, Hubick KT, Farquhar GD. Discrimination in carbon isotopes of leaves correlates with water use efficiency of field grown peanut cultivars. Australian Journal of Plant Physiology. 1988; 15:815-825.
22. Wright GC, Hubick KT, Farquhar GD, Nageswara Rao RC. Genetic and environmental variation in transpiration efficiency and its correlation with carbon isotope discrimination and specific leaf area in peanut. In 'stable isotopes and plant carbon water relations' (Eds JR. Ehleringer AE Hall and GD Farquhar), 1993, 247-267.
23. Xu Y, This D, Pausch RC, Vonhof WM, Coburn JR, Comstock JP and Mc Couch SR. Leaf-level water use efficiency determined by carbon isotope discrimination in rice seedlings: genetic variation associated with population structure and QTL mapping. Theoretical and Applied Genetics. 2009; 118:1065-1081.
24. Yin ZH, Raven JA. Influences of nitrogen sources on nitrogen and water use efficiency, and carbon isotope discrimination, in C_3 *Triticum aestivum* L. and C_4 *Zea mays* L. plants. Planta. 1998; 205: 574-580.