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Research Article

DETERMINATION OF STRESS INDICES FOR SELECTION OF SUPERIOR GENOTYPES UNDER DROUGHT SITUATION IN RICE (*Oryza sativa* L.)

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Abstract- One of priority research area in rice is identification of suitable genotypes for rain fed condition. Quantifying the drought tolerance genotypes was done with different stress indices obtained from the yield data under severe drought and irrigated condition with four backcross inbred lines derived from the cross between IR64 (drought susceptible) and Apo (drought tolerant) which carried three mega QTL in different combination of classes namely $qDTY_{2.2}$, $qDTY_{3.1}$ and $qDTY_{8.1}$. The genotype CB 229 is the highly tolerant one confirmed by tolerant and susceptible indices, which had three *DTY* QTL ($qDTY_{2.2}$, $qDTY_{3.1}$ and $qDTY_{8.1}$) combinations maximizes the yield in drought condition. Among the selection indices, YI, MPI, DRI, STI, HM, GMP, MRP and RE are the best indices to identify tolerant genotype. Clustering based on principal component analysis exhibits that CB 229, CB 193-2 and CB 193-3 falls on the quarter where tolerant indices found. Overall study shows that the selection based on stress indices may be rewarded to identify superior genotypes under severe drought condition.

Keywords- Rice, Drought, Backcross Inbred Lines, Stress Indices and Quantitative trait loci

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Introduction

Rice production is affected by drought, which is one of the major abiotic stresses in rain fed areas. Recent trends in climate change have predicted a further increase in drought intensity, making the development of new drought-tolerant rice cultivars critical to sustain rice production in this ecosystem [1]. Rice production heavily depends on water availability while, drought is one of the most important constraint adversely affecting the yield in rain fed upland cultivation. India has witnessed severe drought in the year 2002, 2009 and 2012 which caused reduction of yield (21.5 in 2002 and 10.02 in 2009) million tonnes [2]. The timing of drought, early season, mid-season or terminal stage, has a major influence on how much yield loss occurs [3]. Variability of drought and yield attributing characters are prerequisite for the identification of drought tolerant high yielding genotypes. Rice is particularly sensitive to drought stress during reproductive growth, even under moderate drought stress [4, 5]. In rice, moderate stress can be broadly characterized by 31 to 64% loss in grain yield as compared with non-stress conditions [6]. The ability of crop cultivars to perform reasonably well in drought-stressed environments is paramount for stability of production. The relative yield performance of genotypes in drought-stressed and non-stressed environments can be used as an indicator to identify drought-tolerant varieties for drought-prone environments. Several drought indices have been suggested on the basis of a mathematical relationship between yield under drought conditions and non-stressed conditions. These indices are based on either drought tolerance or drought susceptibility of genotypes [7]. Therefore, in order to quantify the drought tolerance in rice genotypes and contribution of yield components due to water availability of upland rice with different drought tolerance indices obtained from the yield data under rain fed upland stressed and irrigated non stressed condition experiments.

Materials and Methods

This present study was conducted at Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore during *Kharif, 2015*. Paddy Breeding Station is located at latitude of 11° N and longitude of 77° E and an altitude of 426.7 m above MSL. Backcross Inbred Lines of IR64 (four lines) developed from the cross combination of IR64 X APO (BC_1F_5) along with the parents were raised under rainout shelter (stress experiment) and flooded condition (irrigated experiment) in randomized block design with four replication. Apo, drought tolerant upland variety, developed at IRRI, recommended for cultivation under aerobic condition was used as donor parent. Owing to its drought tolerance nature and good performance under aerobic conditions, they serve as important source for mining drought tolerant QTLs. IR64 used as recipient parent is a medium duration and high yielding variety but highly prone to drought. The four BIL lines *viz*., CB 193-1 ($qDTY_{2.2}$ and $qDTY_{3.1}$), CB 193-2 ($qDTY_{2.2}$ and $qDTY_{8.1}$), CB 193-3 ($qDTY_{3.1}$ and $qDTY_{8.1}$) and CB 229 ($qDTY_{2.2}$, $qDTY_{3.1}$ and $qDTY_{8.1}$) were used to study the profound expression of QTL under severe drought condition.

Direct seeding was done in drought experiment, while transplanting was done in irrigated one on same dates. The irrigated experiment was considered to be a favorable condition so that plots were watered at planting, tillering, heading, flowering and grain filling stages. Nitrogen and phosphorus Potash fertilizers were applied at the rate of 100:60:40 Kg/ha. Half dose of nitrogen + full doses of phosphorus, Potash fertilizers was applied at the time of sowing and remaining half dose of nitrogen in two equal doses at the time of tillering and panicle initiation stages of crop growth were applied. The grain yield was measured by harvesting five single plants from each replication at maturity. The grain yield data were recorded for each genotypes at both environment (non stress-irrigated and drought stress) and both were subjected to estimate stress indices. The stress indices were calculated using the following formulas given bellow

Difference in plant height (DFPH)	Plant height under irrigated treatment - Plant height under stress condition
Difference in panicle length (DFPL)	Panicle length under irrigated treatment – Panicle length under stress treatment
Drought susceptibility index (S) [8]	((Yi)ns – (Yi)s)/ (Yi)ns
Drought response index (DRI) [9]	$(Y_{si} - \hat{Y}_{si})$ / Std. error of $\hat{Y}_{si}$ Y_{si}- actual stress yield $\hat{Y}_{si}$- Estimated stress yield based on regression analysis
Yield index (YI) [10]	$\mathrm{YI} = \frac{(Yi)s}{(Y)ns}$
Yield stability index (YSI) [11]	$\mathrm{YSI} = \frac{(Yi)s}{(Yi)\mathrm{ns}}$
Relative Drought Index [8]	$\mathrm{RDI} = \frac{(Yi)s}{(Yi)ns} / \frac{\mathrm{Ys}}{\mathrm{Yns}}$
Relative Efficiency (REI) [12]	$\mathrm{REI} = \frac{(Yi)s}{Ys} \mathrm{X} \frac{(Yi)ns}{Yns}$
Mean Relative Performance (MRP) [12]	$\mathrm{MRP} = \frac{(Yi)s}{Ys} + \frac{(Yi)ns}{Yns}$
Mean Productivity Index (MPI) [12]	$\mathrm{MPI} = \frac{(Yi)ns \text{ and } (Yi)s}{2}$
Harmonic mean (HM) [13]	$\mathrm{HM} = \frac{2((Yi)s \text{ X } (Yi)ns))}{(Ys + Yns)}$
Geometric Mean of Productivity (GMP) [14]	$\mathrm{GMP} = \sqrt{(Yi)s \text{ X} (Yi)ns}$
Stress Tolerance Index (STI) [15]	$\mathrm{STI} = \frac{((Yi)ns \text{ X } (Yi)s)}{Yns^2}$
Drought tolerance efficiency (DTE) [3]	$\mathrm{DTE}\ (\%) = \frac{\mathrm{Ys}}{\mathrm{Yns}} \text{ X } 100$
Drought resistance Index (DI) [16]	$\mathrm{DI} = \frac{(Yi)s \text{ X } \left(\frac{(Yi)s}{(Yi)ns}\right)}{\mathrm{Ys}}$
Relative decrease Yield (RDY) [17]	$\mathrm{RDY} = 100 - \left(\frac{(Yi)s}{(Yi)ns} \text{ X } 100\right)$
Stress Susceptibility Index (SSI) [8]	$\mathrm{SSI} = \frac{\left(1 - \frac{(Yi)s}{(Yi)ns}\right)}{\mathrm{SI}}$
Schneider's Stress Severity Index (SSSI) [18]	$\mathrm{SSSI} = \left(1 - \frac{(Yi)s}{(Yi)ns}\right) - \left(1 - \frac{Ys}{Yns}\right)$
Stress Susceptibility Percentage Index (SSPI) [19]	$\mathrm{SSPI} = \frac{(Yi)ns - (Yi)s}{2(Y)ns} \text{ X } 100$
Stress Tolerance (TOL) [13]	TOL = $(Y_i)_{ns}$-$(Y_i)_s$
Abiotic Tolerance Index (ATI) [19]	$\mathrm{ATI} = \frac{(Yi)ns - (Yi)s}{\frac{Yns}{Ys}} \text{ X } \sqrt{(Yi)ns \text{ X } (Yi)s}$

(Y_i)s and $(Y_i)_{ns}$ denotes the yield of the i^{th} genotype under drought stress and non-stress (irrigated) condition. Y_s and Y_{ns} represent the yields of all genotypes evaluated under drought stress and irrigated conditions, respectively.

Results and Discussion

Comparison of genotypes based on stress indices

Breeding drought-tolerant rice has always been a tough challenge for plant breeders. The use of grain yield as a selection criterion has proved to be a boon in this area of research. Several large-effect QTL for grain yield under drought with effects across genetic backgrounds and environments have been reported in the recent past. The identification of drought yield (*DTY*) QTL has opened a way for the development of drought-tolerant versions of popular varieties. Each of the individual *DTY* QTL showed a yield advantage of 300−500 kg ha^{-1} under moderate to severe drought conditions [20]. This yield advantage needs to be more to make an impact on a commercial level where performance of one QTL is enhanced in the presence of the other [1]. Also, IR64 lines with two and three pyramided QTLs have shown a yield advantage of 1.2−1.5 t ha^{-1} over IR64 under moderate to severe drought conditions, while maintaining similar yield potential under normal irrigated conditions [21]

To determine the most desirable drought tolerant genotype by considering all the calculated stress indices [Table 1a and 1b]. The indices were grouped into two categories *viz.,* tolerant indices and susceptible indices. With respect to tolerant indices, the BIL CB 229 (3 QTL line) had recorded higher value in most of the tolerant indices *viz*., DRI (4.97), YI (0.83), RE (1.22), MRP (2.21), MPI (24.17), HM (26.13), GMP (23.98) and STI (0.87). These findings were in line with [20]. This was followed by the line CB 193-3 (2 QTL line) which recorded slightly lower stress indices of RE (1.12), MRP (2.12), MPI (23.20), HM (24.03), GMP (23.00), STI (0.80) and DI (0.84). The tolerance indices used [22] such as, RDI, STI, YSI, SSPI, and MSTI for screening tolerance in bread wheat landraces. There was also a report by [7] that, drought yield index provides a more effective assessment as it is calculated after accounting for a significant genotype x stress-level interaction across environments. For rain fed areas with variable frequency of drought occurrence, Mean yield index (MYI) along with deviation in performance of genotypes from currently cultivated popular varieties in all situations helps to select genotypes with superior performance across irrigated, moderate and severe reproductive-stage drought situations.

Among the genotypes evaluated, the susceptible parent IR64 had registered lower tolerant indices. Based on the susceptible indices of DSI, RDY, SSI and SSSI, the BIL CB 229 (3 QTL) had registered lesser susceptible indices whereas the line CB 193-2 had lesser values of susceptible indices namely SSPI (11.23), ST (5.77) and ATI (92.35). These results indicated that, CB229 and CB193-3 exhibited higher tolerance and lesser susceptibility among evaluated genotypes under severe stress condition. On the basis of grain yield, the genotype CB229 (21.20 g) recorded higher grain yield under stress condition than the tolerant parent Apo (19.93 g). The drought resistant genotype had highest drought tolerance efficiency, minimum drought susceptible index and minimum reduction in grain yield due to moisture stress was reported by [23].

Table-1a *Drought tolerant indices of Backcross Inbred lines harboring different combination of drought yield QTL and parents under severe moisture stress condition*

	Tolerant Indices														
Genotypes	(Yi)ns	(Yi)s	DFPH	DFPL	DRI	YI	YSI	RDI	RE	MRP	MPI	HM	GMP	STI	DI
IR 64	27.18	11.05	3.75	2.40	-6.85	0.43	0.41	0.57	0.64	1.66	19.12	13.64	17.33	0.46	0.24
Apo	23.16	19.93	4.83	1.55	-0.12	0.78	0.86	1.20	0.98	1.99	21.55	20.97	21.49	0.70	0.93
CB 229	27.13	21.20	7.58	-1.38	4.97	0.83	0.78	1.09	1.22	2.21	24.17	26.13	23.98	0.87	0.90
CB 193-1	24.84	18.12	1.17	3.13	-3.37	0.71	0.73	1.02	0.96	1.95	21.48	20.45	21.22	0.68	0.72
CB 193-2	25.46	19.69	-0.75	0.33	3.69	0.77	0.77	1.08	1.06	2.06	22.57	22.77	22.39	0.76	0.83
CB 193-3	26.24	20.16	-2.08	0.38	1.37	0.79	0.77	1.07	1.12	2.12	23.20	24.03	23.00	0.80	0.84

Table-1b *Drought susceptible indices of Backcross Inbred lines harboring different combination of drought yield QTL and parents under severe moisture stress condition*

	Susceptible indices								
Genotypes	(Yi)ns	(Yi)s	DSI	RDY	SSI	SSSI	SSPI	ST	ATI
IR 64	27.18	11.05	0.59	59.36	2.08	0.31	31.43	16.14	199.98
Apo	23.16	19.93	0.14	13.94	0.49	-0.15	6.29	3.23	49.64
CB 229	27.13	21.20	0.22	21.86	0.77	-0.07	11.55	5.93	101.73
CB 193-1	24.84	18.12	0.27	27.06	0.95	-0.01	13.09	6.72	102.01
CB 193-2	25.46	19.69	0.23	22.66	0.80	-0.06	11.23	5.77	92.35
CB 193-3	26.24	20.16	0.23	23.15	0.81	-0.05	11.83	6.08	99.94

Comparison of genotypes based on rank

The tolerant genotype should have low rank sum in the drought tolerant indices; should have high rank sum among the drought susceptible indices [Table 2a and 2b]. For the susceptible genotype this scenario is reversed. With respect to rank mean and rank sum of tolerant indices, the genotype CB229 recorded lower rank mean and rank sum of 1.62 and 3.00, which was followed by CB1193-3 (2.85 and 4.06). The susceptible parent recorded higher rank mean and rank sum of 5.38 and 6.71 with respect to tolerant indices. On considering susceptible indices rank sum and rank mean of BILs, CB229 and CB193-2 recorded higher rank sum and rank mean of 4 and 5.04 respectively.

Table-2a *Rank (R), Rank Mean (RM), Standard Deviation of Ranks (SDR) and Rank Sum (RS) of drought tolerant indices*

	Tolerance Indices															
Genotypes	DFPH	DFPL	DRI	YI	YSI	RDI	RE	MRP	MPI	HM	GMP	STI	DI	Rank Mean	SD of ranks	Rank sum
IR 64	3.00	2.00	6.00	6.00	5.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	5.38	1.33	6.71
Apo	2.00	3.00	4.00	3.00	1.00	1.00	4.00	4.00	4.00	4.00	4.00	4.00	1.00	3.00	1.29	4.29
CB 229	1.00	6.00	1.00	1.00	2.00	2.00	1.00	1.00	1.00	1.00	1.00	1.00	2.00	1.62	1.39	3.00
CB 193-1	4.00	1.00	5.00	5.00	4.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	4.54	1.13	5.67
CB 193-2	5.00	5.00	2.00	4.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	4.00	3.38	0.87	4.25
CB 193-3	6.00	4.00	3.00	2.00	3.00	4.00	2.00	2.00	2.00	2.00	2.00	2.00	3.00	2.85	1.21	4.06

Table-2b *Rank (R), Rank Mean (RM), Standard Deviation of Ranks (SDR) and Rank Sum (RS) of drought susceptible indices*

	Susceptible indices									
Genotypes	DSI	RDY	SSI	SSSI	SSPI	ST	ATI	Rank Mean	SD of ranks	Rank sum
IR 64	1	1	1	1	1	1	1	1	0.00	1.00
Apo	5	6	6	6	6	6	6	6	0.38	6.24
CB 229	4	5	5	5	4	4	3	4	0.76	5.04
CB 193-1	2	2	2	2	2	2	2	2	0.00	2.00
CB 193-2	3	4	4	4	5	5	5	4	0.76	5.04
CB 193-3	3	3	3	3	3	3	4	3	0.38	3.52

Table-3a *Simple correlation coefficient among tolerant indices of Backcross Inbred lines harboring different combination of drought yi eld QTL and parents under severe moisture stress condition*

Tolerant Indices	DFPH	DFPL	DRI	YI	YSI	RDI	RE	MRP	MPI	HM	GMP	STI	DI	(Yi)ns	(Yi)s
DFPH	1	-0.231	0.032	-0.051	-0.056	-0.054	-0.020	-0.018	-0.009	-0.025	-0.048	-0.022	-0.020	0.094	-0.049
DFPL		1	-0.884*	-0.614	-0.423	-0.423	-0.763	-0.777	-0.822*	-0.768	-0.739	-0.774	-0.546	-0.378	-0.616
DRI			1	0.874*	0.750	0.753	0.924**	0.932**	0.931**	0.928**	0.922**	0.931**	0.830*	0.000	0.875*
YI				1	0.959**	0.959**	0.955**	0.956**	0.911*	0.955**	0.967**	0.954**	0.987**	0-.347	1.000**
YSI					1	1.000**	0.832*	0.835*	0.758	0.831*	0.855*	0.830*	0.989**	-0.597	0.959**
RDI						1	0.832*	0.835*	0.758	0.831*	0.855*	0.830*	0.989**	-0.597	0.959**
RE							1	0.999**	0.992**	1.000**	0.999**	1.000**	0.898*	-0.055	0.955**
MRP								1	0.992**	1.000**	0.998**	1.000**	0.902*	-0.058	0.956**
MPI									1	.992**	.985**	0.993**	0.840*	0.071	0.911*
HM										1	0.999**	1.000**	0.898*	-0.053	0.955**
GMP											1	0.998**	0.915*	-0.099	0.967**
STI												1	0.897*	-0.049	0.954**
DI													1	-0.479	0.987**
(Yi)ns														1	-0.347
(Yi)s															1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Association analysis

Correlation studies among tolerant indices of Backcross Inbred lines indicated that DI, STI, GMP, HM, MPI, MRP, RE, RDI, YSI, and YI had recorded high and significant positive correlation with yield under stress [able 3a and 3b]. Similarly among susceptibility indices, ATI, ST, SSPI, SSSI, SSI, RDY and DSI had significantly negative correlation with grain yield. Inter-correlation among susceptible indices had significant positive correlation with one another. Correlation between MP, GMP, Ys, and Yp was positive was shown by [24]. The GMP, MP, and STI were significantly and positively correlated with stress yield was reported by [25]. The observed relations were consistent with those reported by [26] in landrace wheat and [27] in durum wheat. STI, GMP indices which showed the highest correlation with grain yield under both optimal and stress conditions, can be used as the best indices for maize breeding programs to introduce drought tolerant hybrids was found by [28]. [15] believe that the most suitable indices for selection of drought tolerant cultivars are indicators which show a relatively high correlation with grain yield in both stress and non-stress conditions.

Principal Component Analysis

Principal component analysis revealed that the first component explained 81.012 % of the variation with major stress indices except DFPH and DFPL [Table-4]. Thus, the first component comprised of effective selection criteria possessing major stress indices, which can be used to cull out tolerant genotypes from susceptible ones. Furthermore, biplot graph exhibited that YI, MPI, DRI, STI, HM, GMP, MRP and RE were the best stress indices among all other indices to identify drought tolerant genotypes [Fig-1]. These findings were similar with the results of [12]. The biplot also grouped the tolerant genotypes viz., CB 229, CB193-2 and CB 193-3 in the quarter where the best tolerant indices were fall on.

Based on the studies, BILs were developed from the cross between IR64 X APO with different combination of QTL which carried three mega QTL classes namely *qDTY*$_{2.2}$, *qDTY*$_{3.1}$and *qDTY*$_{8.1}$. The yield parameters recorded showed that the line CB 229 harbouring 3 QTL consistently performed better under stress condition and performed on par with IR64 under controlled condition. This was followed by CB 193-3 (*qDTY*$_{3.1}$, *qDTY*$_{8.1}$) which yielded on par with the 3 QTL line in both the condition. Drought tolerance and susceptibility index helps to screen the genotypes under stress environment for high yielding ability. In general it has been observed that the effect of QTL for yield under drought declines with decreasing severity of stress. Such pattern of effects has been seen for $qDTY_{12.1}$ [29]. From this study, it was concluded that moisture stress imposed during reproductive stage significantly reduced rice yield in all genotypes. The differential response of genotypes to imposed water stress condition indicates the drought tolerance ability of rice genotypes.

Table-3b *Simple correlation coefficient among susceptible indices of Backcross Inbred lines harboring different combination of drought yield QTL and parents under severe moisture stress condition*

Susceptible Indices	DSI	RDY	SSI	SSSI	SSPI	ST	ATI	(Yi)ns	(Yi)s
DSI	1	1.000**	1.000**	1.000**	0.999**	0.999**	0.981**	0.597	-0.959**
RDY		1	1.000**	1.000**	0.999**	0.999**	0.981**	0.595	-0.960**
SSI			1	1.000**	0.999**	0.999**	0.981**	0.596	-0.959**
SSSI				1	0.998**	0.998**	0.981**	0.595	-0.959**
SSPI					1	1.000**	0.987**	0.629	-0.947**
ST						1	0.987**	0.629	-0.947**
ATI							1	0.737	-0.887*
(Yi)ns								1	-0.347
(Yi)s									1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

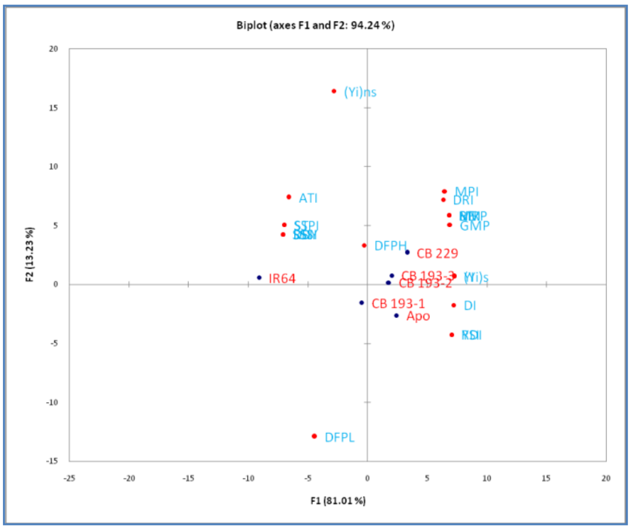

Fig-1 Graphical bi-plot display of stress indices and genotypes evaluated under stress condition

Table-4 *Principal Component Analysis of different stress indices of BILs and parents under stress condition*

Stress indices	PC1	PC2	PC3
DFPH	0.001	0.034	0.954
DFPL	0.368	0.515	0.024
DRI	0.762	0.161	0.000
YI	0.996	0.001	0.001
YSI	0.943	0.056	0.001
RDI	0.943	0.056	0.001
RE	0.882	0.107	0.004
MRP	0.885	0.106	0.003
MPI	0.795	0.193	0.005
HM	0.882	0.107	0.004
GMP	0.908	0.079	0.005
STI	0.882	0.107	0.004
DI	0.987	0.009	0.002
DSI	0.943	0.056	0.001
RDY	0.943	0.056	0.001
SSI	0.943	0.056	0.001
SSSI	0.943	0.056	0.001
SSPI	0.921	0.079	0.000
ST	0.921	0.079	0.000
ATI	0.824	0.168	0.005
(Yi)ns	0.151	0.831	0.010
(Yi)s	0.996	0.001	0.001
Eigen value	17.823	2.911	1.024
Variability (%)	81.012	13.232	4.654
Cumulative %	81.012	94.244	98.898

Values in bold correspond for each variable to the factor for which the squared cosine is the largest

Conflict of Interest: None declared

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