Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

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Studies on early backcross generation inbred lines of Rice (*Oryza sativa* L.) for yield under stress evaluated under different moisture regimes

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Abstract
Rice under rainfed condition faces frequent moisture stress. Recent studies at IRRI have shown moderate to high heritability of grain yield under stress. QTLs were introgressed through backcrossing of Apo (donor) and IR 64 (recipient) lines. Evaluation was done for reproductive stage moisture stress tolerance in severe moisture stress and well irrigated condition to study the effect of the introgressed QTLs in BILs. Set of 85 backcross inbred lines (BILs) at BC_1F_3 (81) and BC_2F_3(4) generations developed through MAS (Marker Assisted Selection) bearing one at a time, two and three *DTY* (yield under drought) QTLs (Quantitative Trait Loci) for yield under stress donated by Apo. High heritability for yield and predominant association was seen between spikelet fertility, harvest index and grain yield per plant. Single QTLs *DTY*2.2 performed best for grain yield and also showed least drought susceptibility index (DSI). *DTY* (2.2 + 8.1) performed better in traits like grain yield, plot yield and spikelet fertility and harvest index than expected three QTL line. QTLs bearing BILs were advantageous over IR 64 under severe moisture stress with respect to higher 1000 grain weight and spikelet fertility %.

Keywords: Drought tolerance, Quantitative Trait Loci, Yield under stress, Backcross Inbred lines

Introduction
Rice is a wonder crop which can come up in varied ecosystem of this globe and is most grown and consumed crop of the world. There is evidence suggesting that rice was the first crop domesticated by humans in the Yangtze river basin as early as 13000 years ago (Lu *et al*., 2002). It is cultivated on all the continents except Antarctica, over an area of more than 150 million ha, but most rice production takes place in Asia (Akhtar *et al*., 2010). In Asia, more than two billion people are getting 60-70% of their energy requirement from rice and its derived products. In India, the area under rice cultivation is 43 m ha with an annual production of 102 million tonnes and an average productivity of 1.9 t ha^{-1}. Strictly less than half of the world's rice area is irrigated; the rest of the rice area relies on rainfall for its water requirement. Drastic climatic changes due to anthropogenic activities have resulted in shifts in rainfall patterns and crop experiences frequent drought spells. Upland rice, produced by smallholder farmers in India, is the lowest-yielding rice production system. Under severe stress, yield reduction in rice is 65-85% compared with that in non-stress conditions (Kumar *et al*., 2008). Rice is particularly sensitive to drought stress during reproductive growth, even under moderate drought stress (O'Toole, 1982). Most of the high-yielding varieties like IR 36, IR 64, Swarna, ADT 45 and Sambha Mahsuri grown in rainfed areas are varieties bred for irrigated ecosystems and they were bread for higher grain yield but not for moisture stress tolerance. Farmers of moisture stress-prone areas require varieties that provide them with high yield in years of good rainfall and sustainable good yield in years with moisture stress. Recent studies at IRRI have shown moderate to high heritability of grain yield under moisture stress (Bernier *et al*., 2007; Venuprasad *et al*., 2007; Kumar *et al*., 2008), thus opening area for direct selection for grain yield instead of secondary traits. Further, direct selection for grain yield under moisture stress has been reported effective (Kumar *et al*., 2008; Venuprasad *et al*., 2008) and the feasibility of combining high yield potential with good yield under moisture stress has been demonstrated beyond doubt. By employing direct selection for grain yield under stress, several promising breeding lines for rainfed lowlands and rainfed uplands have been identified recently (Verulkar *et al*., 2010; Mandal *et al*., 2010).

Similarly, at the molecular level, recent reports indicate that QTLs with large effects on yield under stress may not be uncommon. DNA markers especially SSR markers have been identified that co-segregate with QTLs for yield under stress in many populations. Bernier *et al*., 2007 reported a QTL on chromosome 12 in the Vandana/Way Rarem population

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explaining about 51% of the genetic variance for yield under severe upland drought stress. Kumar *et al.*, (2007) reported a major QTL for grain yield under lowland drought stress in the CT9993/IR62266 population on chromosome 1 explaining 32% of the genetic variance. Venuprasad *et al.*, (2009) identified a major QTL for grain yield on chromosome 3 that explained 36% of the genetic variance. Venuprasad *et al.*, (2011) identified qDTY6.1 on chromosome 6 which had a large effect on grain yield under favorable aerobic condition in three different backgrounds viz., Apo/2*Swarna, Apo/2*IR 72, and Vandana/IR 72, using bulked segregant analysis (BSA) particularly in Apo/2* Swarna with an value of 66%. The identification of QTLs with major effect on grain yield raises new hopes of improving grain yield under moisture stress through marker-assisted breeding. In the present experiment we are aiming to introgress the reported QTLs for yield under drought (DTY) from the tolerant variety Apo into IR 64 through marker assisted back cross selection and to evaluate the early backcross inbreed lines for the effect of QTLs on yield and its contributing traits in the background of IR 64, a major elite variety but, susceptible to moisture stress situations, otherwise high yielding with fine grain quality.

Material and Methods
The experiment was carried out in paddy breeding Station, centre for plant breeding and genetics, TNAU. Experiment conducted during 2013 Kharif included two treatments with

two replications for each of the treatment namely Stress experiment (withholding irrigation) in rain out shelter and Control experiment (irrigated) in open field. The material for the study consisted of a set of 86 back cross inbred lines of IR 64 which were introgressed with QTLs for yield under stress (located on chromosomes 2, 3 and 8), one at a time and combinations of two and three. The QTLs were originally derived from Apo, an Indica cultivar. IR 64 and Apo was crossed and F_1s selfed to get F_2 which were evaluated under severe moisture stress at reproductive stage to identify genotype performing with good seed set, grain yield and screened through MAS to derive QTL (yield under stress) positive lines. Recombinant inbred lines of IR 64 and Apo in F_4 generation with three QTLs for yield under stress in the background donated by Apo were used as male for backcrossing with IR 64 in present experiment to generate BC_1F_1 and were selfed till BC_1F_3 (fig 1). 8 BC_1F_1's were identified out of 120 progenies of cross and these were selfed to get 422 plants. Thirty four lines were identified which were homozygote for RM71 (DTY2.2); 9 lines homozygotes at RM520 (DTY3.1); 15 homozygotes for RM256 (DTY8.1); eight lines for both RM71 and RM520 [DTY(2.2+3.1)]; 15 lines for both RM71 and RM 256 [DTY(2.2+3.1)]; and only five plants were recovered which were homozygotes for all the three markers RM71, RM520 and RM256 [DTY (2.2+3.1+8.1)].
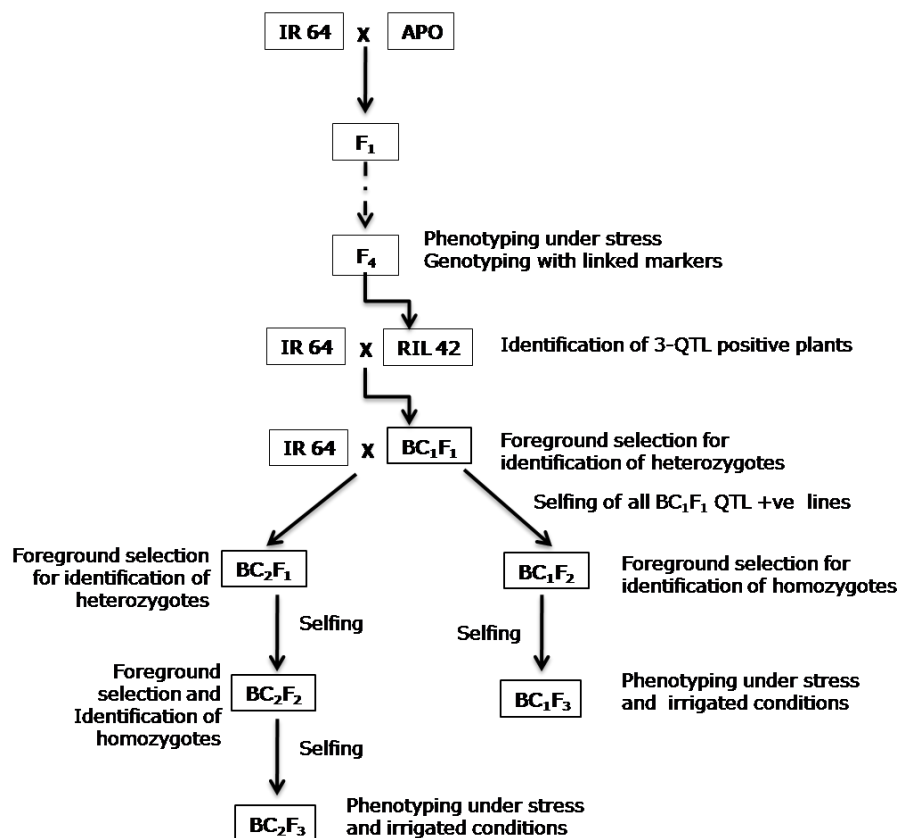

Fig 1: Introgression of DTY QTLs in to IR64

Marker assisted selection
Selection of QTL positive lines was carried out by screening polymorphic primers for fore ground selection (table 1 and fig 2) at every back cross generation. The parents were surveyed with 10 SSR markers reported by venuprasad *et al.* (2009) three markers namely RM71, RM520, RM256 exhibited polymorphism between the parents IR 64, Apo (figure 2).

DNA was extracted from fresh leaf tissue by modified CTAB protocol of (Doyle and Doyle 1987) and quality of DNA was checked on 0.8% agarose gel electrophoresis. PCR amplification of genomic DNA was done using forward and reverse microsatellite primers performed in Bio-Rad (MyCycler thermal cycler) PCR machine. Programme of PCR cycles was Initial denaturing step 94°C for 5 minutes,

Denaturing 94°C for 1 minute, Annealing 55°C for 1 minute and extension at 72°C for 2 minutes. Total of 35 cycles (34 times) and final extension 72°C for 5 minutes. Resolution for polymorphism through agarose gel electrophoresis was carried out. PCR amplified products (20.0 μl) along with 100

bp ladder were subjected to electrophoresis in a 3.0 per cent agarose gel in 1X TBE buffer at 100 Volts for 2.0 hours. The ethidium bromide stained gels were documented (Bio-Rad) and later banding pattern was scored.

Table 1: Details of Foreground markers used in marker assisted selection

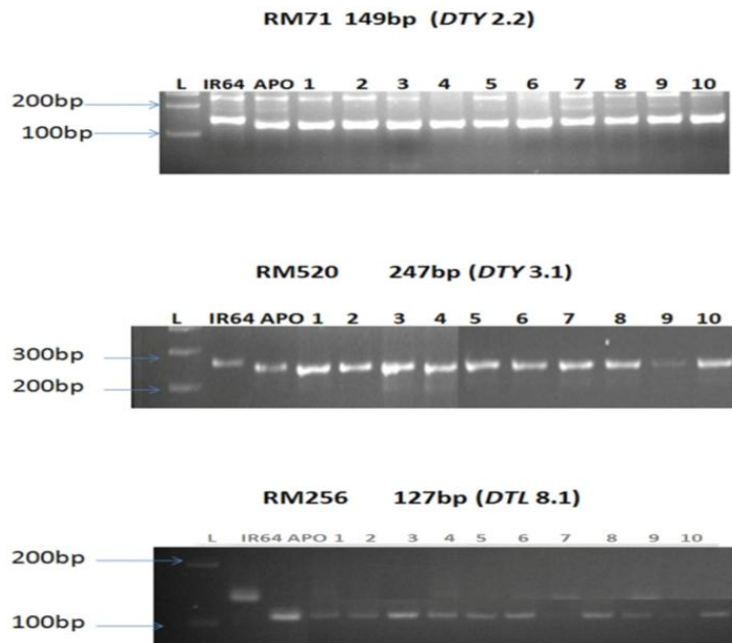| QTL	Chromosome	Position	Primer	Sequence
DTY 2.2	2	8.9 Mb	RM71	'CTAGAGGCGAAAACGAGATG' 'GGGTGGGCGAGGTAATAATG'
DTY3.1	3	30.2 Mb	RM520	'AGGAGCAAGAAAAGTTCCCC' 'GCCAATGTGTGACGCAATAG'
DTY 8.1	8	24.2 Mb	RM256	'GACAGGGAGTGATTGAAGGC' 'GTTGATTTCGCCAAGGGC'

Fig 2: Polymorphism between IR64 and Apo; 1-10: used as male parent.

Crop management in stress condition and control conditions.
The seeds of BC_1F_3 of IR 64 and parents along with check Anna 4 were sown in nursery bed and seedlings were raised. Later single seedling of 17 days old were transplanted on an experimental plot that had two rows of 2.4 m with the spacing of 20 cm between rows and 20 cm between plants within a row per genotype. Gap of 30 cm spacing was maintained between the genotypes. Recommended crop production and protection practices were followed to raise a healthy crop. Regular irrigation was given until 67 days of crop from date

of sowing; later crop was denied irrigation for the rest of its duration. Crop for stress treatment was raised in rain out shelter to avoid rain intervention during experimental period. Same set of experimental material as said above was transplanted in same dimension of spacing and plot size in an open field and recommended crop production and protection was followed. Regular irrigation was given and flooded condition was maintained until crop is mature. Crop was harvested when the grains reached physiological maturity stage in both the experiments.
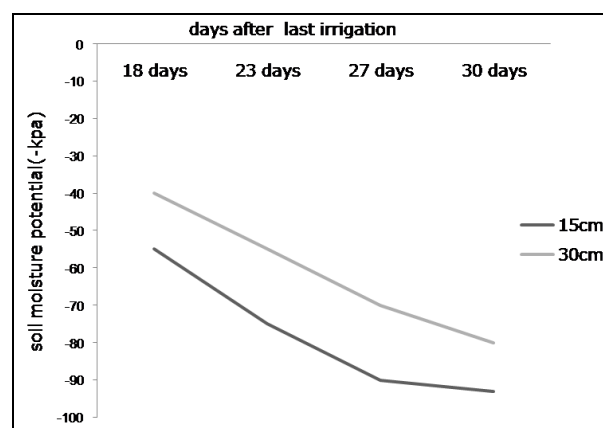

Fig 3: Comparison of dryness (pressure mounted due to water stress in terms of kilo bars) at different soil depths

Biometrical traits like grain yield and its component traits viz., plant height, days to 50% flowering, number of panicles, spikelet fertility, 1000 grain weight, harvest index and plot yield were observed and measurements recorded for statistical analysis. IRROMETER reading for measuring Soil moisture content was taken by installing four IRROMETER of two different depths viz., 30 cm, and 15 cm in stressed blocks. Each replication had two IRROMETERs of 30 cm and 15 cm respectively. Reading was taken from vacuum gauge in terms of kilobars and is represented in fig 3. The estimation of mean, variance and standard error were worked out by adopting the standard methods by Panse and Sukhatme (1961) and analysis of variance was carried out using Genres software. Heritability (h^2) in the broad sense was calculated according to Lush (1940).

Experimental Results
Analysis of variances for morphological traits among BILs
The mean values obtained for various plant growth and yield traits were analyzed individually. The genetic variability parameters of population viz., mean, range, heritability in broad sense (h^2), for the traits is presented in the table 2. While Comparing Plot yield under both water regimes though shows 80 to 90 % yield reduction in stressed condition, BILs were superior compared to susceptible IR 64. There was wide variability among BILs, parents and check used in experiment. Heritability of 72.25 % and 67.2.9 % was noticed under stress and non stress condition respectively for plot yield, where as grain yield per had high to moderate heritability of 63.7 % and 52.7%.

Table 2: Traits mean values of parents and backcross inbred lines (selected based on foreground selection) along with their range, standard deviation (SD) and critical difference (CD) at 95% and broad sense heritability (*h2*) under severe moisture stress(s) and control environments(c).

Traits	Environment	Parents			Check	BILs		SD	CD	*h2*
		IR 64	ADT 45	APO	ANNA 4	Mean	Range			
DFF	c	94.00	91.00	91.50	95.00	85.00±0.410	77.00-95.50	3.914	4.819	0.678
	s	87.50	83.50	87.00	91.50	83.32±0.373	74.05-95.00	3.562	5.226	0.571
PH	c	82.00	76.35	109.5	89.65	100.9± 1.44	72.15-130.2	13.82	14.96	0.742
	s	69.00	66.65	74.35	81.35	77.08±1.112	52.50-101.4	10.61	14.07	0.636
NOP	c	22.00	18.65	19.00	26.00	15.05±0.404	8.500-27.20	3.857	5.887	0.544
	s	9.335	8.500	5.000	9.000	8.855±0.176	3.835-13.17	1.674	2.831	0.468
1000 grain weight	c	21.27	20.11	23.07	24.14	27.99±0.256	21.75-33.39	2.442	2.373	0.786
	s	27.30	16.80	22.00	22.50	25.77±0.355	15.40-33.94	3.387	4.042	0.694
SF	c	90.70	90.20	86.93	84.74	87.88±0.541	70.37-97.03	5.165	6.039	0.705
	s	52.13	34.87	59.50	46.81	73.31± 1.383	24.17-91.21	13.194	12.45	0.797
HI	c	0.380	0.390	0.310	0.360	0.402±0.005	0.236-0.543	0.048	0.123	0.083
	s	0.095	0.045	0.160	0.180	0.235±0.010	0.015-0.485	0.097	0.134	0.610
PY	c	999.5	463.3	599.8	720.2	663.8±15.82	270.3-1136	150.9	187.8	0.672
	s	15.53	8.420	35.500	28.800	63.96±3.932	4.065-222.9	37.51	38.18	0.722
Y/P	c	39.58	33.60	43.87	41.87	43.34±1.601	25.57-126.2	15.27	23.90	0.526
	s	3.485	1.665	7.500	5.570	9.456±0.453	1.000-27.35	4.318	5.692	0.637

PH-plant height (cm); NOP-number of panicles; 1000 grain weight (g); SF-spikelet fertility (%); HI-harvest index; PY-plot yield (g); Y/P-yield per plant (g).

Mean performance of BILs
Among the single QTL BILs, those possessing DTY2.2 were comparatively better than DTY3.1 and DTY8.1 in all most characters. Among two QTLs combinations, DTY(2.2+3.1) showed increased values in most of the characters than DTY(2.2+8.1), but expression of both the QTL combination lines was high over their recurrent and donor parents for the characters like panicle length, spikelet fertility, harvest index, plot yield and grain yield per plant. As expected three QTL line possessing DTY(2.2+3.1+8.1) also recorded high mean values especially grain yield, plot yield and spikelet fertility and harvest index compared to two QTL lines DTY (2.2+3.1),

DTY (2.2+8.1) and also recipient parent IR 64 and donor parent Apo (fig 4).Though BILS were obviously better than their recurrent parent IR 64 and donor parent Apo and check. Under control condition, all the 3 different QTL classes showed earliness in flowering over IR 64 and Apo. The single QTL lines also showed high values and maximum difference for characters like plant height, panicle length, harvest index, plot yield and grain yield per plant over recurrent parent IR 64 and donor parent Apo. Among the single QTL lines BILs possessing DTY2.2 comparatively performed better than DTY3.1 and DTY8.1 in most of the characters (table 3).

Table 3: Trait wise mean values of each QTL classes under stress and control conditions.

Genotypes	Envt	DFF	PH	NOP	PL	1000 Grain weight	SF	TDM	HI	PY	Y/P
IR 64	S	97.50	69.00	9.335	18.50	27.30	65.13	36.04	0.095	15.53	3.485
	C	104.0	82.00	22.00	22.00	24.27	88.70	105.7	0.38	999.5	39.58
APO	S	97.00	74.35	5.000	18.25	22.00	59.50	45.90	0.160	35.50	7.500
	C	101.5	109.5	19.00	26.50	23.07	86.93	156.9	0.31	599.8	48.48
ANNA 4	S	101.5	81.35	9.000	18.35	22.50	66.81	41.03	0.255	28.80	5.570
	C	101.5	89.65	26.00	24.50	24.14	74.74	129.7	0.36	720.2	43.87
BC₁F₃											
DTY 2.2	S	93.00	71.85	10.170	21.00	26.10	86.27	61.57	0.445	146.6	27.35
	C	103.0	114.0	22.50	26.85	27.22	90.89	279.4	0.37	794.3	103.03
DTY 3.1	S	89.50	70.70	8.500	22.85	30.00	83.18	35.55	0.385	139.9	13.82
	C	92.50	116.4	17.35	25.95	26.02	80.03	123.1	0.41	721.5	51.53
DTY8.1	S	91.50	65.65	10.00	21.45	30.90	88.17	35.74	0.380	131.9	13.42
	C	95.00	97.00	13.35	23.15	29.30	80.71	255.6	0.49	773.5	126.2

DTY2.2+3.1	S	91.00	80.65	9.500	24.00	25.44	84.88	36.36	0.470	91.09	16.79
	C	92.50	81.00	16.00	20.65	30.36	87.12	84.38	0.42	613.5	37.81
DTY2.2+8.1	S	89.00	77.50	13.17	22.15	25.12	75.18	45.82	0.445	121.4	20.57
	C	93.50	83.35	24.50	24.30	33.39	83.69	144.3	0.44	1136	62.96
DTY2.2+3.1+8.1	S	89.50	69.85	8.335	21.15	30.60	88.45	37.32	0.430	130.0	16.02
	C	92.50	104.2	15.70	24.60	28.01	82.85	96.10	0.46	887.5	44.43

PH-plant height (cm); NOP-number of panicles; 1000 grain weight (g); SF-spikelet fertility (%); HI-harvest index; PY-plot yield (g); Y/P-yield per plant (g).
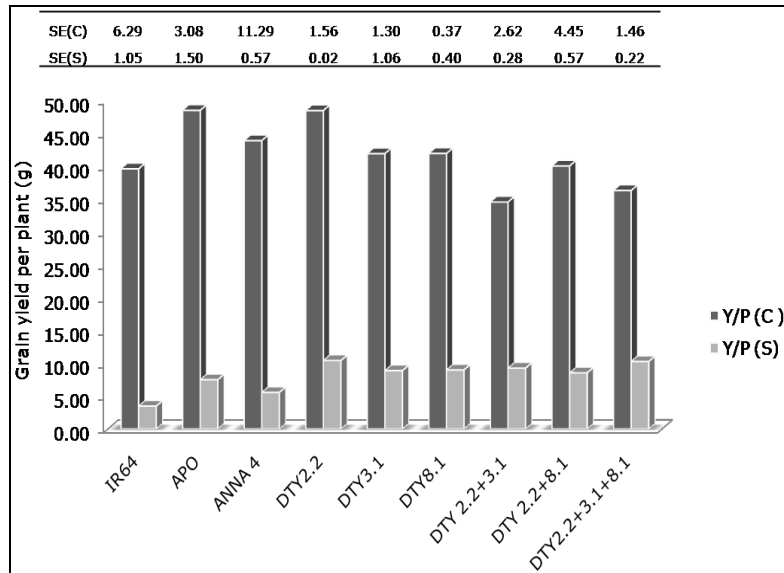

Fig 4: Comparison of grain yield per plant of parents and BILs (BC$_1$F$_3$) under control and stress conditions

Correlation studies

The results obtained and reported similar results are described below and correlation coefficients among the traits studied are presented in table 4. Spikelet fertility had positive correlation with 1000 grain weight; similarly, harvest index with 1000 grain weight and spikelet fertility which were significant, these results go along with earlier experiments (Bidinger *et al*., 1999; Pantuwan *et al*., 2002; Lafitte *et al*., 2004; Lanceras *et al*., 2004). Both plot yield and grain yield per plant had negative correlation with days to 50 % and had significant positive correlation with number of panicles, 1000 grain weight, spikelet fertility and harvest index, also there was mutual significant positive correlation between plot yield and grain yield per plant. Whereas under Control conditions both plot yield and grain yield per plant had significant positive correlation with number of panicles and panicle length, whereas grain yield per plant alone had significant correlations with total dry mater harvest index.

Table 4: Correlation coefficients among traits under stress condition.

Traits	Envt.	DFF	PH	NOP	1000 Grain weight	SF	HI	PY	Y/P
DFF	s	1.000	-0.122	0.004	-0.371	-0.472**	-0.293**	-0.394**	-0.214*
	c	1.000	0.042	0.069	-0.274**	-0.168	-0.129	0.169	0.079
PH	s		1.000	0.135	0.235**	-0.011	0.142	0.180	0.209*
	c		1.000	-0.245	0.030	-0.124	-0.349**	0.151	0.070
NOP	s			1.000	0.225**	0.278**	0.347**	0.409**	0.354**
	c			1.000	-0.137	0.046	0.065	0.499**	0.515**
1000GW	s				1.000	0.357**	0.436**	0.486**	0.312**
	c				1.000	0.098	0.142	0.135	0.022
SF	s					1.000	0.637**	0.604**	0.530**
	c					1.000	0.084	-0.066	0.033
HI	s						1.000	0.846**	0.911**
	c						1.000	0.099	0.347**
PY	s							1.000	0.779**
	c							1.000	0.395**
Y/P	s								1.000
	c								1.000

PH-plant height (cm); NOP-number of panicles;1000 grain weight (g); SF-spikelet fertility (%); HI-harvest index; PY-plot yield (g) * Significant at 0.005; ** significant at 0.001

Correlated response values were found to be high for plant height and panicle length (R^2 0.545), spikelet fertility and plot yield (R^2 0.364), harvest index and plot yield (R^2 0.716), yield per plant and plot yield (R^2 0.621) and spikelet fertility with grain yield per plant (R^2 0.817) fig 6. Proportionate effect of Apo allele at seven important marker loci on grain yield in five different moisture regimes (venuprasad *et al*., 2009) and there was significant R^2 values reported. The positive genetic correlation confirms that it is possible to select genotypes combining high non stress yield with a high degree of stress tolerance, but the relatively low proportion of stress yield variation explained by non stress performance indicates that

screening under stress is important in identifying lines with improved drought tolerance (Venuprasad, 2007).

Discussion

Performances of BILs over susceptible parents can be explained by better values recorded by them for yield attributing characters like Spikelet fertility, harvest index which were highly significant associates of grain yield under severe moisture stress. Above all the QTLs introgressed are reported to have a strong influence on grain yield under drought stress which is evident in our study. In the five populations of IR 64*2/Azucena and Vandana/ IR 72, heritability was greater under stress than in the non stress screen no consistent reduction under severe upland drought stress relative to lowland non stress conditions (Lafitte and Courtois, 2000; Babu *et al.*, 2003; Atlin *et al.*, 2004; Lanceras *et al.*, 2004).

Traits like plant height, early flowering and Number of panicles was affected by yield under stress QTLs which was evident by observations under both conditions. Lafitte *et al.*, 2006 reported that relationship between plant height and drought resistance was due to the presence of sd-1 gene which often associated with the traits such as high tillering and shallow rooting due to their pleiotrophic effects. Profuse tillering is generally associated with dense and shallow roots while limited tillering is associated with sparse and deeper roots and this is one of the reasons why most of the cereal crop cultivars developed in dry regions shows the habit of limited tillering. Venuprasad *et al.* (2011) that Apo alleles were significantly associated with early flowering in aerobic and lowland conditions; on average, Apo allele homozygotes flowered early by about 5 days. Since the parents involved in experiment are of short duration and also highly susceptible for drought, flowering duration was much reduced in stress conditions. DTY3.1 significantly affected days to flowering, QTL related to heading date (HD) have been reported near DTY3.1 (Yano *et al.*, 2001), where as DTY2.1 effects on days to flowering were not consistent (Venuprasad *et al.*, 2009) and similarly, QTL for root and yield components under drought are also reported near DTY2.1 and DTY3.1 (Courtois *et al.*, 2000; Yadav *et al.*, 1997; Yue *et al.*, 2006). So the observed results could be either due to linkage of HD genes with other genes with effects on drought tolerance or due to pleiotrophic effects of the HD genes themselves. In our experiment also some of the individual BILs which posses DTY3.1 were earliest to flower in both stress and control condition which confirms the effect of the QTL on flowering.

In stress condition mean values of BILs had higher 1000 grain weight when compared to parental lines. Increased 1000 grain weight of BILs in stress as well as control may be attributed to bolder grains which is character donated by Apo. As expected susceptible parent IR 64 had high spikelet sterility percentage when compared to tolerant genotypes like Apo and a wide range of variability for the trait under moisture stress was noticed. Extreme differences were noticed for harvest index in both in severe moisture stress and control conditions. In this study, many of the BILs had higher harvest index than parental lines and check under severe moisture stress condition and higher value of 0.307 was expressed by 2 QTL line of BC$_2$F$_3$ DTY (3.1+8.1), this can be explained by better values of 1000 grain weight by the genotypes. Higher harvest index under stress reveals that effective utilization of photosynthates so that grain yield is not reduced.

Venuprasad *et al.*, (2009) also opined that in addition to grain yield, the seven important loci which also affected significantly were harvest index, days to 50 % flowering, and plant height under severe drought stress both in upland and lowland evaluation trials of Apo/IR 64 populations. Kumar *et al.*, (2007) noticed that the striking character of the BILs when compared to susceptible parents was bear more number of filled grains under severe stress conditions which lead to better performances of BILs in the CT 9993 / IR 62266 populations. Strong positive correlation and correlated response between single plant yield and harvest index, spikelet fertility and grain yield indicates that yield differences observed under drought were mostly the result of a large difference in the capacity of the plants to maintain seed set under stress rather than to biomass accumulation. Experiment with DH population had a non significant reduction of 1000 grain weight under stress (Babu *et al.*, 2003). Therefore the genetic improvement of harvest index would improve grain yield significantly (Fukai *et al.*, 1999; Babu *et al.*, 2003; Lancreas *et al.*, 2004).

Over all grain yield under stress condition was significantly dependent on spikelet fertility, harvest index, number of panicles and also total dry matter in stress conditions. Under control conditions grain yield was dependent strongly on number of panicles, total dry matter produced, and panicle length and harvest index. Over all comparison of trait association between stress and non stress condition shows the importance of spikelet fertility under stress to have greater tolerance of plants to stress which is shown by performing better in terms of grain yield per plant and per plot. Thus directing us to dissect these DTY QTLs with respect to genes for spikelet fertility and seed filling because trait-based approach with precise understanding will improve selection efficiency for molecular breeding strategies.

Comparing between QTLS

Single QTL DTY2.3 also showed a positive effect on Grain Yield under Non Stress (Palanog *et al.* 2014). The effect of this QTL was also seen on yield related traits such as number of panicle per unit area under drought and harvest index under non-stress conditions. Similar effects of this QTL have also been observed in the study conducted by Bernier *et al.* (2007) on a population derived from a cross of Vandana and Way Rarem. The QTL can play a crucial role in Marker Assisted Backcross in enhancing yield not only under stress but also under Non Stress conditions. Among two QTLs combinations, DTY (2.2+8.1) performed better and showed increased values in most of the characters than DTY (2.2+3.1) and recurrent parent IR 64. But the donor parent Apo and check Anna 4 were better performers when compared to DTY(2.2+8.1) and DTY(2.2+3.1+8.1) among the characters like number of panicles, spikelet fertility, plot yield and grain yield per plant when evaluated in control conditions. This implies that QTLs in combination were not effective under control condition in IR 64 background.

From above comparative information (table 3) we are sure that single QTL lines especial DTY2.2 did better over all other single QTL and 2, 3 QTL combined lines, though 2 and 3 QTL lines over performed against recipient parent. DTY2.2 had earlier been identified to be contributed by donor parent Aday Sel, another traditional drought-tolerant donor from India where it showed large effect in the background of IR 64 under varying severities of reproductive stage stress (Swamy *et al.*, 2013) in the advanced backcross mapping population developed by the cross of Aday Sel and IR 64. Palanog *et al.* (2014) found out that DTY2.2 also showed an effect against an MTU1010 background in BC$_1$ derived mapping

population. Such reports and our results also direct us to have deeper insight in to interaction effects of such QTLs in single background since many QTL combined lines have varied results compared to single QTL lines.

Here in our study in IR 64 back ground single QTL DTY2.2 possessing lines did better than any other combination lines as contrast to reports of Venuprasad *et al*. (2009) that DTY3.1, has been shown to have a strong influence on grain yield under drought stress in populations derived from the crosses Apo/IR 64 and IR55419/Way Rarem in IRRI locations. However the regions around loci RM572 and RM6703 on chromosome 1, RM520 on chromosome 3, RM256 on chromosome 8, RM269 on chromosome 10, and RM511 on chromosome 12 were shown to have important and consistent effects on grain yield under drought stress in the Apo/IR 64 population and thus we can expect that regions around such region may have been introgressed apart from our target regions which resulted in significant variations between and within QTL classes. Lanceras *et al*. (2004) reported that coincidence of QTL for grain yield and its components were identified in many regions especially on chromosomes 3, 4 and 8 suggesting a tight linkage or pleiotropy. There is considerable evidence from this study and two other published studies (Bernier *et al*., 2007; Kumar *et al*., 2007) to support the hypothesis that in rice a few QTL exist which have large effects on grain yield and/or flowering that are unique to particular hydrological conditions. But the reason for under performances of 3 QTL combinations DTY2.2+3.1+8.1 are not reported yet and we suspect the negative epistasis and/or the background genome and/or the environment specificity of certain QTLs to express themselves. Chakraborty *et al*. (2011) detected a total of 6 QTLs for grain yield under one environment and 5 QTLs under another. The 6 QTLs under one environment comprised of 2 QTLs from chromosome 1 and the rest from chromosome 5. Study conducted by yue *et al*. (2005) also concludes that drought tolarance mechanism at reproductive stage indeed also associated with soil types.

Conclusion
The results of this study confirm that upland rice yield under reproductive-stage drought stress is a moderately heritable trait, and that therefore direct selection for yield under stress is likely to be effective. From our study to know the effect of QTLs DTY2.2, 3.1 and 8.1 and their combinations it is much evident that QTLs have effect on yield and it's all the contributing traits whether they are present one at a time or in combinations. Though there was 80-90% yield reduction under severe moisture stress conditions when compared to well irrigated, but the QTL possessing lines over performed susceptible parents and check which confirms the advantage of these QTL introgression. Apo is reported to be the highest yielding line under lowland non stress conditions, indicating that a high level of stress tolerance can be combined with high yield potential. These results strongly support the use of donors with superior yield under stress when the objective is to develop cultivars with improved yield under severe upland drought stress. The yield advantage of stress-selected lines under stress seems to be due to better maintenance of harvest index under stress. In our experiment expectations were that QTL pyramided lines would yield better than one at a time but DTY2.2 expression was better than other single QTL and 2, 3 QTL combinations which we can attribute this type of behavior to basic characteristics of quantitative character like, negative epistasis, sensitivity towards environmental

influence, specificity of location for better expression and background of expression. In future such work demands precise knowledge of QTLs and strategy for bringing down variations in expression of these QTLs.

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