PERFORMANCE OF BACKCROSS INBRED LINES OF RICE (*ORYZA SATIVA* L.) ACROSS DIFFERENT WATER REGIMES AND THEIR GENOME ANALYSIS

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ABSTRACT
The present investigation focused to introgress semi-dwarfism (*sd1* locus) from TKM 9 (drought susceptible, donor parent for semi-dwarfism) to Norungan (drought resistant, recurrent) background to develop drought resistant genotypes with improved yield under water limited situations. Stability analysis for grain yield of selected BILs performed under three different moisture regimes were analyzed through Eberhart and Russell (1966) model, revealed that both linear and non-linear components was significantly contributed to grain yield. Six backcross inbred lines recorded higher mean yield than the population mean, regression coefficient (b) around unity and deviation from regression (S^{2}d) nearer to zero, which was non-significant. All the BILs were also genotyped for 167 microsatellite (SSR) markers including gene specific markers of *sd1* locus to confirm the introgression to drought resistant background of Norungan and the marker data was used to establish Graphical GenoTypes for each stable individuals using GGT ver.2.0. The result indicated that the stable genotypes of BILs recovered most of the genome (above 90 %) from the recurrent parent Norungan which exhibits high adaptability across environments and had useful introgression for *sd1* locus which harness semi-dwarfism from the donor parent TKM 9 on chromosome 1 in all identified genotypes responsible for stable and improved grain yield across varied water regimes.

INTRODUCTION

Rice (*Oryza sativa*) is one of the most important cereal food crops which are cultivated in all parts of the world and staple food for more than one half of the world populations. Rice is a semi-aquatic plant that is commonly grown under flooded conditions. However, about half of the rice area in the world does not have sufficient water to maintain flooded conditions and yield is reduced to some extent by drought, defined here as a period of no rainfall or no irrigation that affects crop growth (Hanson *et al.*, 1990). Over 50% of the world's rice area is rainfed and which contributing to only a quarter of total rice production (McLean *et al.*, 2002). While the total area of upland rice is much smaller than that of rainfed lowland rice, drought physiology research has been concentrated mostly on upland rice to identify responses of various growth processes to water stress. Asia is the home of rice as more than two billion people are getting 60 to 70 % of their energy requirement from rice and its derived products (Seema *et al.*, 2014). About 90 % of total rice is grown and consumed in Asia. In several breeding programmes, the study and consideration of the amount of adaptability of crops in relation to different environmental conditions have a special importance. There is a prime need for improving suitable varieties more adaptable to different geographical areas. To meet these goals, estimation of genotype × environment interaction is extremely imperative.

Recommendation of crop cultivars with wide adaptability is essential for countries with diverse environments or agro-ecological regions and seasons. Under such situations, it is important to account for the differential response of different crop varieties to different environments; *i.e.* G × E interaction has to be studied to evaluate yield stability and adaptability of crop varieties over diverse environments and seasons (Simmonds, 1991). Genotype × environment interaction and yield-stability analysis has continued to be important in measur-ing varietal stability and suitability over different locations, agro - ecological zones and seasons. The prime and foremost objective of the study is to develop semi-dwarf, drought resistant genotype by introgression of *sd1* region from TKM 9 to the drought resistant landrace Norungan. The rice semi-dwarf 1 (*sd-1*) gene is well known as the "green revolution gene". This gene has contributed to the signiûcant increase in crop production seen in the 1960's and 1970's, especially in Asia. This gene, originally derived from the Chinese cultivar Dee-Geo-Woo-Gen (DGWG), provides rice cultivars with short, thick culms, raises the harvest index and improves lodging resistance and responsiveness to nitrogen fertilizer, resulting in high yields without aûecting panicle and grain quality (Futsuhara and Kikuchi, 1997). These introgression patterns were studied at molecular level by using three gene specific

markers (GA20Oxi_1, GA20Oxi_2 and GA20Oxi_3) linked to the trait semi-dwarfism as foreground selection and several other random SSR markers for background selection.

MATERIALS AND METHODS

The experimental material for the present investigation comprised of 101 genotypes of Backcross Inbred Lines (BILs) developed by two parents *viz.*, Norungan (drought resistant, recurrent) and TKM 9 (drought susceptible, donor). This study clearly investigated the Genotype X Environment for the grain yield across three water regimes during February 2009 to June 2009 (Irrigated-E1), September 2009 to January 2010 (Severe stress under Rain out Shelter-E2) and October 2009 to February 2010 (Irrigated-E3) at Paddy Breeding Station, Coimbatore. The Rain out Shelter is erected on 25 m high space frame structure fitted with >80% transparent clear polycarbonate sheet as the permanent roof. The open but birds protected sides offer free air circulation not letting the air temperature inside the Rain out Shelter to maintain as that prevails outside. The stable performances of the genotypes were estimated using the method developed by Eberhart and Russell (1966). For determining adaptability and stability of genotypes in this method, parameters like mean genotypes yield, regression line slope (b_i) and variance of deviation from regression (S^2di) were used. In this model various amounts of b_i *i.e.* $b_i = 1$, $b_i < 1$ and $b_i > 1$ are expressing medium, high and low stability, respectively. According to this model, a genotype is encountered as the most stable that its regression line slope is equal to unit, variance of deviation from regression is the least (non-significant with zero) and its average yield is highest.

Graphical genotyping

A graphical representation of the molecular marker data has relevance in studying the genome constitution of the recombinant population or backcross inbred populations. The SSR genotypic data generated in the population were analysed using the software, GGT ver. 2.0 (acronym for Graphical GenoTypes). GGT 2.0 deals with visualization and analyses that involve molecular marker scores. Common input data consist of a matrix of marker scores with markers arranged in rows and genotypes arranged in columns. In most cases, GGT 2.0 was used to visualize data of markers with known map positions on a genetic map, allowing GGT 2.0 to display estimated lengths of genomic compositions as colored chromosome bar segments.

RESULTS AND DISCUSSION

The mean data averaged over replication for genotypes from three environments (Table 1) for plot yield were subjected to pooled analysis. The mean squares due to genotypes, environments and G X E were tested against mean sum of square due to pooled deviation and pooled error and they were highly significant ($p < 0.05$, 0.01) for plot yield and satisfied the requirement of stability analysis. The variance due to G X E was partitioned into linear (*i.e.* variance due to G X E) and nonlinear components (*i.e.* variance due to pooled

Table 1: Grain yield of BILs under three different water regimes

BILs	E1	E2	E3	Mean	BILs	E1	E2	E3	Mean	BILs	E1	E2	E3	Mean
CB-07-701-5	280	68.3	102.5	150.3	CB-07-701-145	270	155.8	251.8	225.9	CB-07-701-233	450	23.3	243.3	238.9
CB-07-701-6	290	200.8	110.8	200.6	CB-07-701-146	315	168.5	130.0	204.5	CB-07-701-234	320	83.3	234.2	212.5
CB-07-701-7	350	151.7	70.0	190.6	CB-07-701-147	445	186.7	120.8	250.8	CB-07-701-235	375	120.0	246.7	247.2
CB-07-701-9	260	146.7	100.0	168.9	CB-07-701-148	405	115.8	288.3	269.7	CB-07-701-236	350	125.0	106.7	193.9
CB-07-701-10	300	108.3	80.0	162.8	CB-07-701-149	340	98.3	97.5	178.6	CB-07-701-238	290	111.7	193.3	198.3
CB-07-701-11	260	40.0	100.0	133.3	CB-07-701-150	305	92.5	95.8	164.4	CB-07-701-251	340	120.8	123.3	194.7
CB-07-701-12	320	117.5	55.0	164.2	CB-07-701-151	430	167.5	106.7	234.7	CB-07-701-252	370	121.7	315.8	269.2
CB-07-701-22	315	182.5	160.8	219.4	CB-07-701-153	300	105.8	80.0	161.9	CB-07-701-254	260	90.0	117.5	155.8
CB-07-701-23	295	175.8	77.5	182.8	CB-07-701-154	290	72.5	52.5	138.3	CB-07-701-255	385	184.2	146.7	238.6
CB-07-701-25	275	79.2	180.0	178.1	CB-07-701-155	300	101.7	67.5	156.4	CB-07-701-256	420	175.0	326.7	307.2
CB-07-701-27	315	60.0	142.5	172.5	CB-07-701-156	345	118.3	42.5	168.6	CB-07-701-260	315	122.5	170.0	202.5
CB-07-701-29	400	158.3	169.2	242.5	CB-07-701-161	340	221.7	100.0	220.6	CB-07-701-261	410	105.8	117.5	211.1
CB-07-701-39	255	65.0	138.3	152.8	CB-07-701-166	285	70.0	103.3	152.8	CB-07-701-262	295	150.0	281.7	242.2
CB-07-701-51	315	108.7	175.8	199.8	CB-07-701-167	280	32.5	85.0	132.5	CB-07-701-263	255	103.3	45.0	134.4
CB-07-701-53	290	62.5	254.2	202.2	CB-07-701-170	265	146.7	179.2	196.9	CB-07-701-264	325	129.2	139.2	197.8
CB-07-701-54	360	60.0	236.7	218.9	CB-07-701-174	265	205.8	72.5	181.1	CB-07-701-265	320	155.8	329.2	268.3
CB-07-701-61	370	53.3	185.0	202.8	CB-07-701-175	360	70.8	188.3	206.4	CB-07-701-266	350	82.5	159.2	197.2
CB-07-701-62	330	51.7	199.2	193.6	CB-07-701-177	450	75.0	92.5	205.8	CB-07-701-268	260	191.7	120.0	190.6
CB-07-701-66	265	60.0	176.7	167.2	CB-07-701-178	295	68.3	110.0	157.8	CB-07-701-269	310	135.8	170.8	205.6
CB-07-701-82	365	117.5	84.2	188.9	CB-07-701-179	360	138.7	105.0	201.2	CB-07-701-274	395	116.7	305.8	272.5
CB-07-701-94	290	94.2	79.2	154.4	CB-07-701-181	255	126.7	108.3	163.3	CB-07-701-278	400	150.0	67.5	205.8
CB-07-701-97	350	95.0	65.8	170.3	CB-07-701-185	290	102.5	188.3	193.6	CB-07-701-279	350	140.0	277.5	255.8
CB-07-701-98	300	142.3	42.5	161.6	CB-07-701-189	320	148.3	256.7	241.7	CB-07-701-280	330	130.0	45.0	168.3
CB-07-701-105	285	151.5	102.5	179.7	CB-07-701-199	340	153.3	309.2	267.5	CB-07-701-281	320	110.8	338.3	256.4
CB-07-701-109	310	182.5	269.2	253.9	CB-07-701-200	290	95.0	288.3	224.4	CB-07-701-282	410	93.3	259.2	254.2
CB-07-701-113	280	100.8	173.3	184.7	CB-07-701-204	270	30.0	280.8	193.6	CB-07-701-283	400	145.8	40.0	195.3
CB-07-701-114	315	70.8	106.7	164.2	CB-07-701-211	395	100.8	47.5	181.1	CB-07-701-284	375	136.7	60.0	190.6
CB-07-701-115	317	165.0	213.3	231.8	CB-07-701-217	360	97.5	60.0	172.5	CB-07-701-285	310	115.0	207.5	210.8
CB-07-701-120	260	85.0	47.5	130.8	CB-07-701-218	275	126.7	92.5	164.7	CB-07-701-286	270	102.5	288.3	220.3
CB-07-701-126	285	131.7	92.5	169.7	CB-07-701-219	360	63.3	184.2	202.5	CB-07-701-287	280	120.0	210.0	203.3
CB-07-701-128	350	187.5	160.0	232.5	CB-07-701-220	290	94.2	135.0	173.1	CB-07-701-288	285	174.2	100.0	186.4
CB-07-701-129	375	133.3	120.0	209.4	CB-07-701-224	365	99.2	60.0	174.7	Norungan	190	148.3	164.2	167.5
CB-07-701-130	400	116.7	158.3	225.0	CB-07-701-227	280	132.5	226.7	213.1	TKM 9	240	65.0	237.5	180.8
CB-07-701-141	450	105.8	110.0	221.9	CB-07-701-228	315	98.3	60.0	157.8					
CB-07-701-143	365	149.2	259.3	257.8	CB-07-701-230	380	138.3	220.8	246.4					

Table 2: ANOVA for stability as per Eberhart and Russell (1966)

Source of variation	df	SS	MS	F(div.dev)	F(div.ME)
Genotype	102	407953.3	3999.54	0.8729	18.9817**
Environment(E)+ Interaction(I)	206	3278026.00	15912.75	3.4729**	75.5214**
Environment(Linear)	1	2547136.00	2547136.00	555.8904**	12088.63**
G X E(Linear)	102	258959.30	2538.81	0.5541	12.0491**
Pooled deviation	103	471954.60	4582.08	21.7464**	
Pooled error	612	386854.80	632.1156	ME = 210.70	

*, ** Significant at 0.05 and 0.01 probability level

Table 3: Stability parameters of BILs and parents for plot yield under Eberhart and Russell (1966)

BILs	Mean	b	S^2dii	BILs	Mean	B	S^2dii	BILs	Mean	b	S^2dii
CB-07-701-5	150.3	1.022	0.014	CB-07-701-148	269.7	1.166	0.593	CB-07-701-252	269.2	0.917	0.733
CB-07-701-6	200.6	0.621	0.513	CB-07-701-149	178.6	1.239	0.209	CB-07-701-254	155.8	0.821	0.011**
CB-07-701-7	190.6	1.171	0.566	CB-07-701-150	164.4	1.082	0.164	CB-07-701-255	238.6	1.097	0.353
CB-07-701-9	168.9	0.688	0.327	CB-07-701-151	234.7	1.456	0.518	CB-07-701-256	307.2	0.978	0.529
CB-07-701-10	162.8	1.034	0.300	CB-07-701-153	161.9	1.042	0.290	CB-07-701-260	202.5	0.899	0.067
CB-07-701-11	133.3	1.062	0.130	CB-07-701-154	138.3	1.151	0.282	CB-07-701-261	211.1	1.537	0.201**
CB-07-701-12	164.2	1.151	0.476	CB-07-701-155	156.4	1.078	0.334	CB-07-701-262	242.2	0.502	0.517
CB-07-701-22	219.4	0.691	0.213	CB-07-701-156	168.6	1.300	0.561	CB-07-701-263	134.4	0.883	0.412
CB-07-701-23	182.8	0.790	0.579	CB-07-701-161	220.6	0.828	0.692	CB-07-701-264	197.8	0.985	0.118
CB-07-701-25	178.1	0.819	0.324	CB-07-701-166	152.8	1.040	0.020	CB-07-701-265	268.3	0.524	0.703
CB-07-701-27	172.5	1.156	0.185	CB-07-701-167	132.5	1.172	0.045**	CB-07-701-266	197.2	1.230	0.146
CB-07-701-29	242.5	1.218	0.152	CB-07-701-170	196.9	0.547	0.058**	CB-07-701-268	190.6	0.481	0.406
CB-07-701-39	152.8	0.839	0.195	CB-07-701-174	181.1	0.547	0.698	CB-07-701-269	205.6	0.828	0.022**
CB-07-701-51	199.8	0.934	0.151	CB-07-701-175	206.4	1.267	0.326	CB-07-701-274	272.5	1.08	0.683
CB-07-701-53	202.2	0.815	0.738	CB-07-701-177	205.8	1.889	0.233**	CB-07-701-278	205.8	1.431	0.613
CB-07-701-54	218.9	1.214	0.604	CB-07-701-178	157.8	1.085	0.010**	CB-07-701-279	255.8	0.825	0.490
CB-07-701-61	202.8	1.382	0.371	CB-07-701-179	201.2	1.195	0.352	CB-07-701-280	168.3	1.185	0.584
CB-07-701-62	193.6	1.156	0.481	CB-07-701-181	163.3	0.691	0.198	CB-07-701-281	256.4	0.683	0.924
CB-07-701-66	167.2	0.837	0.393	CB-07-701-185	193.6	0.804	0.258	CB-07-701-282	254.2	1.319	0.537
CB-07-701-82	188.9	1.329	0.372	CB-07-701-189	241.7	0.682	0.381	CB-07-701-283	195.3	1.495	0.730
CB-07-701-94	154.4	1.031	0.239	CB-07-701-199	267.5	0.672	0.599	CB-07-701-284	190.6	1.361	0.575
CB-07-701-97	170.3	1.359	0.358	CB-07-701-200	224.4	0.646	0.774	CB-07-701-285	210.8	0.803	0.288
CB-07-701-98	161.6	0.990	0.619	CB-07-701-204	193.6	0.771	1.015	CB-07-701-286	220.3	0.519	0.761
CB-07-701-105	179.7	0.773	0.351	CB-07-701-211	181.1	1.604	0.509	CB-07-701-287	203.3	0.655	0.302
CB-07-701-109	253.9	0.495	0.313	CB-07-701-217	172.5	1.419	0.406	CB-07-701-288	186.4	0.703	0.455
CB-07-701-113	184.7	0.785	0.200	CB-07-701-218	164.7	0.846	0.296	Norungan	167.5	0.185	0.042
CB-07-701-114	164.2	1.185	0.033**	CB-07-701-219	202.5	1.299	0.335	TKM 9	180.8	0.581	0.690
CB-07-701-115	231.8	0.690	0.106	CB-07-701-220	173.1	0.929	0.032*	Population mean	199.02		
CB-07-701-120	130.8	0.965	0.331	CB-07-701-224	174.7	1.433	0.416	SE	48.09	0.43	
CB-07-701-126	169.7	0.857	0.321	CB-07-701-227	213.1	0.560	0.336				
CB-07-701-128	232.5	0.883	0.272	CB-07-701-228	157.8	1.180	0.370				
CB-07-701-129	209.4	1.262	0.270	CB-07-701-230	246.4	1.087	0.196				
CB-07-701-130	225.0	1.382	0.039**	CB-07-701-233	238.9	1.784	0.707				
CB-07-701-141	221.9	1.794	0.278**	CB-07-701-234	212.5	0.937	0.532				
CB-07-701-143	257.8	0.904	0.352	CB-07-701-235	247.2	1.075	0.399				
CB-07-701-145	225.9	0.409	0.370	CB-07-701-236	193.9	1.186	0.280				
CB-07-701-146	204.5	0.821	0.311	CB-07-701-238	198.3	0.764	0.246				
CB-07-701-147	250.8	1.405	0.533	CB-07-701-251	194.7	1.118	0.174				

deviation). The results obtained from the analysis revealed that linear and nonlinear components were highly significant for plot yield (Table 2). The stability parameters such as mean, slope (bi), deviation to regression (S^2di) and environmental indices for BILs along with their parents are presented in Table 3.

A broad adaptability of genotypes under different drought intensities varying from non-stress to stress condition is the success of breeding for moisture stress environment. Genotypes by environment (G×E) interaction are a major contributor to the phenotypic variation for most of the traits in low land rice (Cooper and Somrith, 1997; Wade *et al.*, 1999). Hence the assessment of the genotype × environment (G×E) interaction assumes greater significance during development of drought resistant cultivars. The term G×E interaction

commonly refer to the yield variation that cannot be explained by G or E alone (Yan and Hunt, 2001). A stable genotype is the one for which the regression coefficient did not show any significant difference from one and thus stability as defined as the consistency in performance of a variety over varying environments. The genotypes with unit regression value indicate that they are less responsive to environmental changes and hence showing more adaptability to all types of the environment. Thus genotype with unit regression coefficient and the deviation to regression not significantly different from zero is said to be the stable one.

The selection of stable genotypes were based on three parameters *viz.,* high mean yield of particular genotype over the population mean, regression coefficient and standard deviation to regression were considered. Among 101 BILs

studied, only six stable genotypes were selected based on the above criterion. Among them, CB-07-701-256 had significant higher mean yield (307.2 g) than the overall mean (199.5 g), b (0.978) close to 1.0 and S^2d (0.529). CB-07-701-274 recorded higher mean yield (272.5 g) than the overall mean (199.5g), b (1.08) close to 1.0 and S^2d (0.683). CB-07-701-252 shows higher mean yield (269.2 g) than the overall mean (199.5 g), b (0.917) close to 1.0 and S^2d (0.733). CB-07-701-143 recorded higher mean yield (257.8 g) than the overall mean (199.5 g), b (0.904) close to 1.0 and S^2d (0.352). On the same manner, CB-07-701-279 shows higher mean yield (255.8 g) than the overall mean (199.5 g), b (0.825) close to 1.0 and S^2d (0.49). CB-07-701-235 recorded higher mean yield (247.2 g) than the overall mean (199.5 g), b (1.075) close to 1.0 and S^2d (0.399). Similar results were also reported by De *et.al.* (1992). Yield performance of the stable genotypes in different environments could be compared with parents and presented (Fig 1). The X-axis is marked with stable genotypes and the ordinate Y-axis with their yield levels. Eberhart and Russell (1966) suggested

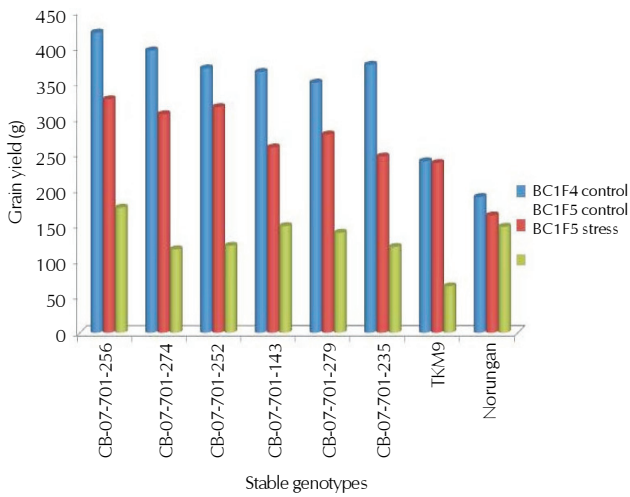that, if the traits associated with high yield show stability, the selection of genotype only for yield could be effective. A non-significant correlation between the deviation from regression (S^2di) and mean performance (xi) or regression coefficient (bi) indicated that these stability parameters might be under the control of different genes located on different chromosomes (Singh *et al.,* 1995).

The stable BILs CB-07-701-256, CB-07-701-274, CB-07-701-252, CB-07-701-143, CB-07-701-279 and CB-07-701-235 were graphically genotyped and these genotypes exhibited stable performance over different moisture regimes. All the stable genotypes had the useful introgression for semi-dwarfness on chromosome 1 which was revealed by the marker GA20Oxi-2 and the same result was reported by the earlier reporters (Spielmeyer *et al.,* 2002; Sasaki *et al.,* 2002). But most of genome was recovered from the recurrent parent Norungan. The stable genotype CB-07-701-256 was semi-dwarf in nature due to the presence of GA20Oxi-2 gene in the

Figure 1: Yield performance of stable genotype compared with parents over different environments
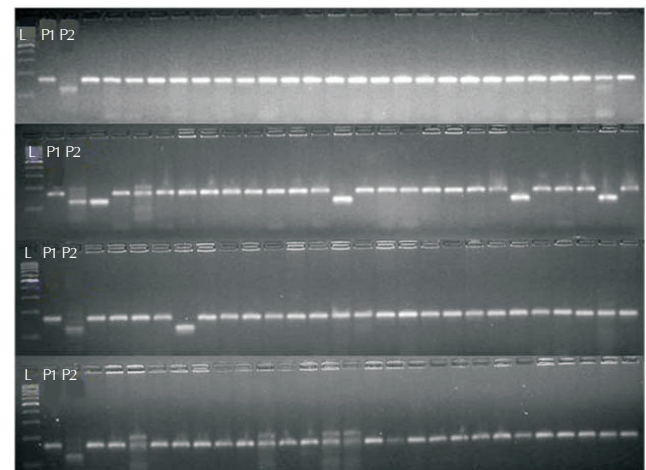
L - 100bp DNA ladder, P1 - TKM9, P2 - Norungan, Other lanes - BILs

Figure 3: BILs segregating for the maker allele amplified with the SSR primer RM302

Figure 2: Graphical genotyping of a stable genotype CB-07-701-256

genome (Fig 2). On chromosome 1, it had the useful introgression from TKM 9 but most of the genome was recovered from the Norungan which had the QTL region associated with drought resistant traits, the markers RM302 was nearer to the region of GA20 Oxi-2 (147.8 cM) which also associated with the trait plant height already reported (Fig 3). The high level of introgression was observed on the chromosome 8, 9, 10, 11 and 12 and minimum or no genome region introgression on the chromosome 2, 3, 4, 5, 6 and 7.

Similarly, the other stable genotypes viz., CB-07-701-274, CB-07-701-252, CB-07-701-143, CB-07-701-279 and CB-07-701-235 were also observed for marker trait association with all the QTLs region recovered from the recurrent parent Norungan. Low level of introgression was obtained from the donor parent TKM 9 on the chromosome 2, 3, 4, 5, 6, and 7 and high level of introgression were observed in chromosome 8, 9, 10, 11 and 12 and these introgression region also had QTL region associated with some of the useful traits like plant height and numbers of productive tillers, all the above stable genotypes showed stable yield performance across different water regimes (Deepti et al., 2013).

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