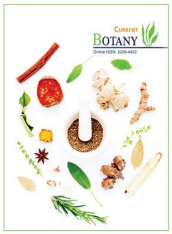
Current Botany 2024, 15: 120-126
doi: 10.25081/cb.2024.v15.9122
https://updatepublishing.com/journal/index.php/cb

Research Article



ISSN: 2220-4822

Analysis of inter-varietal hybridization in rice (*Oryza sativa* L.) indicate transgressive segregation

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Received: August 22, 2024
Revised: October 19, 2024
Accepted: October 20, 2024
Published: November 09, 2024

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ABSTRACT

Traits under continuous stabilizing selection may yield transgressive phenotypes in suitable cross combinations. Farmers maintained Njavara (*Oryza sativa* L. landrace Njavara), a poor yielding medicinal rice landrace in Kerala state over centuries for medicinal applications using short duration (60-70 days) as a selection criterion. This implies that the heading date together with several of the yield related traits, which are co-controlled by pleiotropism, were under constant stabilizing selection in Njavara. We examined the level of transgression in 13 yield related traits in F2 populations raised from crosses between Njavara and a high yielding hybrid variety Jyothi. The cross yielded transgressive phenotype in all of the 13 traits in F2 populations. Difference in the genetic architecture between Njavara and Jyothi due to the difference in the selection pressure experienced by them may have contributed to the excessive transgression observed in the study. The results highlight the value of unattractive poor yielding land races in providing alleles to lift yield barrier in high yielders, and suggest the need to examine the genetic architecture of the trait of interest in parental lines for better genetic advancement in crop improvement programmes.

KEYWORDS: Stabilizing selection, Transgressive segregation, Genetic distance, Genetic architecture, Medicinal rice

INTRODUCTION

Njavara (*Oryza sativa* L. landrace Njavara) is an ancient short duration (60-70 days) medicinal rice landrace geographically restricted to Kerala, south India (Raj *et al.*, 2024 and references therein). Njavara represents a rare example in traditional agriculture. Farmers maintained the genetic distinctness of this cultivar over centuries by on farm selection using short duration as a selection criterion (Sreejayan *et al.*, 2011). Thus, the heading date, and likely to be several of the yield related traits as well, as both share a common genetic control (Xue *et al.*, 2008; Wei *et al.*, 2010; YuShan *et al.*, 2010; Yan *et al.*, 2011; Cai *et al.*, 2012), experienced a long history of stabilizing selection in Njavara populations.

Those traits that have a long history of stabilizing selection are likely to show transgressive segregation, the phenomenon of individuals in segregating hybrid populations expressing traits that are extreme relative to those of either parental line (Albertson & Kocher, 2005; Stelkens & Seehausen, 2009; Stelkens *et al.*, 2009; Koide *et al.*, 2019). The stabilizing selection, which allows independent genetic divergence between populations while maintaining their phenotype, may generate alleles with complementary effects capable of transgression upon hybridizations (Rieseberg *et al.*, 2003; Albertson & Kocher, 2005; Stelkens & Seehausen, 2009). Thus, the type of selection experienced by a population, genetic distance between them or difference in their genetic architecture are all variously indicated as good predictors of transgression (Rieseberg *et al.*, 2003; Albertson & Kocher, 2005; Stelkens & Seehausen, 2009; Stelkens *et al.*, 2009).

Many authors have highlighted the potential of transgressive segregation in crop improvement programmes (Rieseberg *et al.*, 2003; Zhao *et al.*, 2011; Mao *et al.*, 2011; Mackay *et al.*, 2021). While heterosis, which is pronounced in F1 hybrid, has been exploited in crop improvement programmes over a century now (Goff, 2011), little effort has been made so far to exploit transgression in crop improvement programmes. Most of the useful transgressions are reported in interspecific hybridizations (Brondani *et al.*, 2002; McCouch *et al.*, 2007; Gutiérrez *et al.*, 2010) and not in intraspecific hybridizations. In this study, we evaluated the level of transgressive segregation in yield related traits in an intraspecific cross between Njavara and an improved hybrid Jyothi.

MATERIALS AND METHODS

Njavara accessions N8, N9 and N14 representing the short black, short yellow and long yellow morphotypes respectively (Sreejayan *et al.*, 2011) were crossed with Jyothi in both direct and reciprocal directions. The accessions from the fourth morphotype of Njavara, intermediate yellow, was excluded in the study as this class is likely to be a product of natural introgressions (Sreejayan *et al.*, 2011). Jyothi is an improved hybrid developed from a cross between PTB 10 × IR8, with crop duration of 110-125 days, released by Kerala Agricultural University (http://www.kau.edu/varieties.htm). IR8 (Peta × Dee-geo-woo-gen) is an improved hybrid released by International Rice Research Institute, Manila while PTB 10 is a selection from local landrace Thekkan cheera, released by Kerala Agricultural University. One of the F1 progenies each obtained from N8 × Jyothi and Jyothi × N8 combinations was self-pollinated and raised the F2 populations.

In order to assess the level of genetic divergence, the four cultivars used in the study were genotyped using 71 microsatellite loci. DNA was isolated using the GenElute Plant Genomic DNA Kit (Sigma) in accordance with the supplier's instruction. PCR analysis was carried out according to Schuelke (2000). The alleles were separated on an automated DNA sequencer (ABI Prism 3730). The genetic distance based on microsatellite data was computed between the four cultivars according to C.S. Chord distance (Cavalli-Sforza & Edwards, 1967).

The parental lines, the F1 and the F2 were raised in pots and were evaluated for 13 quantitative traits. The quantitative traits included: leaf length (LL; cm), leaf width (LW; cm), culm length (CL; cm), culm number (CN), panicle length (PaL; cm), 100 grain weight (100 GW; g), grain length (GL; cm), grain width (GW; cm), panicle number (PaN), grain number per panicle (GN), flag leaf length (FlL; cm), flag leaf width (FlW; cm) and stem girth (SG; cm). The morphological data were scored according to the 'descriptors for wild and cultivated rice' (Biodiversity International *et al.*, 2007).

The extent of deviation in the value of quantitative traits between F1 and the parental lines was assessed using mid-parent heterosis (MPH) and better-parent heterosis (BPH). The parameters were computed using the formulae: MPH = ((F1 – MP)/MP) × 100 and BPH = ((F1- BP)/BP) × 100, where MP = (P1 + P2)/2, P1 and P2 are the mean value of the first and second parent respectively and BP = mean value of the better of the two parents used in the cross. One sample *t* test was used for testing the significance of MPH and BPH. Normality condition was assumed for the one sample *t* test. Data analyses were performed using the statistical packages SAS 9.2. As individual plants were analyzed, the morphological data of the F2 plants were not subjected to statistical treatments. Instead for the F2 segregants, if a trait showed a value 15% greater than that of the value of the parent which produced highest value of the respective trait, we arbitrarily treated the trait as transgressive in nature.

RESULTS

All of the 71 loci analyzed were homozygous in all the cultivars (Supplementary Table S1). Out of the 71 loci analyzed, 55 loci yielded distinct alleles in Jyothi as compared to Njavara lines while 45 loci were polymorphic between the three Njavara lines. The C. S. Chord distances between Jyothi and N8, N9 and N14 were 0.7931, 0.7989 and 0.8116, respectively. Between Njavara lines the distances were: 0.5521 (N8 and N14); 0.3239 (N8 and N9); 0.5199 (N9 and N14) (data not shown).

The statistics of parental and F1 morphology is summarized in Table S2. Of the 13 traits examined, Jyothi had significantly ($P<0.05$) higher values than Njavara lines in eight traits (CN, PaL, 100GW, GL, GW, PaN, GN, FlW and SG). For one trait, CL, the value was significantly lower in Jyothi than Njavara lines. N14 recorded the highest value for LL and LW. The value for FlL was almost similar between Jyothi, N8 and N14, but was significantly low in N9.

The F1s resulting from all cross combinations showed significant positive MPH ($P< 0.01$) and BPH ($P<0.01$) in LL, LW, CN PaL, GN, and FlL and significant negative BPH ($P<0.01$) in 100GW, GL and GW, except for GW in N14 × Jyothi and Jyothi × N14 (Supplementary Table S2). Significant positive MPH and BPH was recorded in FlW in all combinations except Jyothi × N14. While CL showed significant positive BPH ($P<0.01$) for all combinations except N14 × Jyothi and Jyothi × N14, SG and PaN showed BPH only for two combinations (N9 × Jyothi and N14 × Jyothi) and one combination (Jyothi × N8) respectively. In all cases yielding positive BPH, the value in F1 significantly outstripped the value of respective trait in Jyothi, irrespective of whether Jyothi was the better parent or not. In the case of negative BPH, the F1 value was significantly lower than that of the better parent Jyothi (Table S2).

The F2 segregation of the morphological traits was evaluated in two populations, one consisting of 308 individuals and the other consisting of 327 individuals, derived by self-fertilizing one of the F1 progenies of N8 × Jyothi and Jyothi × N8 crosses respectively. The range of morphological variations observed in F2 individuals in both the crosses are given in Table 1. Of the 308 F2 individuals derived from the N8 × Jyothi, 305 individuals (99%) had transgressive phenotypes (Figure 1a). The number of transgressive phenotypes per individual varied from 1 in 20 individuals to 11 in 3 individuals with the highest number of 55 individuals with 5 traits. Transgression occurred at a lower level in the F2 individuals derived from Jyothi × N8 cross. Of the 327 F2 individuals of Jyothi × N8 cross, only 257 (78%) had a transgressive phenotype (Figure 1a). Similarly proportion of F2 individuals with transgression for multiple traits was also very low in Jyothi × N8 populations. Out of the 257 individuals with transgressive phenotype, 132 had transgressive phenotype for only one trait.

Transgressive expression varied greatly between the 13 traits examined. PaN recorded the highest frequency in N8 × Jyothi population with transgression in 268 of the 308 individuals, followed by FlL in 252 individuals, LL in 226, LW in 189, CL

Table 1: Range, mean and standard deviation (SD) for the 13 quantitative traits in F2 populations derived from Jyothi × N8 and N8×Jyothi, together with the parental values

Trait	Jyothi	N8	F2 population (Jyothi × N8)		F2 population (N8 × Jyothi)	
	Mean±SD	Mean±SD	Range	Mean±SD	Range	Mean±SD
LL (cm)	48.7±3.05	36.7±3.32	13.6-66.1	34.32±8.74	20.2-86.88	55.75±11.27
LW (cm)	1.23±0.08	0.9±0.04	0.78-1.5	1.10±0.12	0.74-1.72	1.29±0.18
CL (cm)	69.09±3.41	80.98±3.96	19.8-104.9	58.69±18.04	27.9-143.5	81.02±24.72
CN	20.5±1.72	11.8±1.69	4.0-29.0	12.09±4.43	5.0-38.0	17.93±5.55
PaL (cm)	25.55±0.91	22.58±1.03	13.1-93.6	20.97±6.33	12.2-98.6	24.54±5.79
GW100	2.67±0.12	1.64±0.04	1.1-2.7	2.04±0.28	1.1-3.1	2.04±0.30
GL (mm)	0.91±0.01	0.74±0.02	0.65-1.0	0.79±0.05	0.61-0.94	0.77±0.06
GW (mm)	0.28±0.01	0.24±0.01	0.20-0.33	0.26±0.02	0.2-0.31	0.25±0.02
PaN	20.5±1.72	17.7±3.13	7-65	25.87±10.22	11-63	31.14±9.72
GN	178.7±12.26	81.6±7.65	34.0-226	92.1±29.64	40-324	154.3±52.67
FIL (cm)	40.92±2.73	40.82±4.82	19.2-55.8	34.39±6.78	20.42-84.56	49.81±10.27
FIW (cm)	1.59±0.05	1.11±0.08	0.92-1.88	1.33±0.15	0.94-2.12	1.56±0.2
SG (cm)	2.31±0.11	1.72±0.09	1.12-2.44	1.82±0.21	0.94-2.62	2.03±0.22

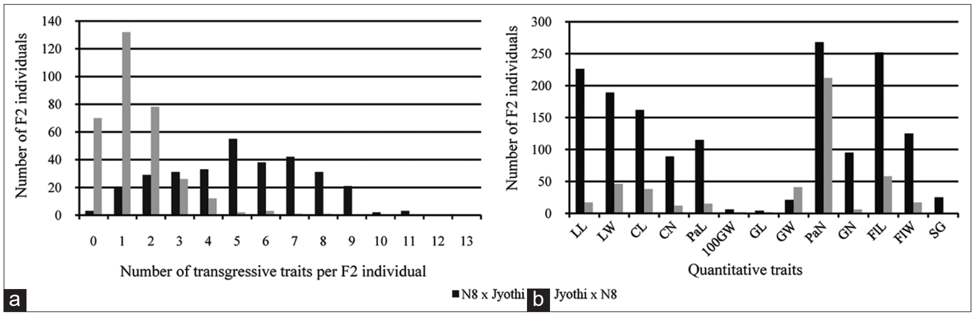

Figure 1: Extent of transgressive expression in 13 quantitative traits in F2 populations derived from N8 × Jyothi and Jyothi × N8: a) Number of transgressive traits per individuals and b) Number of individuals showing transgressive segregation for each of the quantitative traits. See materials and methods for trait abbreviations

in 162, FlW in 125, PaL in 115 GN in 95 and CN in 89, all yielding a value significantly higher than the respective value in Jyothi (Figure 1b). Whereas GW, GL and 100GW that yielded a negative BPH in F1 recorded the lowest frequency of transgression in F2. Similarly, SG that yielded poor heterosis in F1 also produced a lower frequency of transgression in F2 (Figure 1b).

The frequency of transgressive expression was substantially low in all of the traits in F2 population derived from the Jyothi × N8 cross. However, the trend of transgression in Jyothi × N8 population was same as that in N8 × Jyothi population. The traits that displayed positive BPH in F1 yielded varying frequency of transgression in Jyothi × N8 population while the traits that displayed negative BPH and SG that showed poor F1 heterosis yielded very low frequency of transgression.

DISCUSSION AND CONCLUSION

Transgression reported in rice, mostly in crosses between *indica* and *japonica* sub-species, are accidental findings in standard breeding programmes and not the outcome of designed crosses aiming at liberating transgressive phenotype (Xiao *et al.*, 1996; Liu *et al.*, 2010; Mao *et al.*, 2011). Here we designed sexual hybridizations between Njavara, an *aus* and Jyothi, and *indica* (Dinesh, 2014; Jose *et al.*, 2018), to evaluate the emergence of transgressive phenotype in the resulting F2 populations. All of the 13 traits evaluated in the study showed transgressive phenotypes, although the frequency of transgression varied greatly between the traits. The data hold significance considering the results of the two meta-analyses conducted earlier, which found transgression only in 44% and 36% out of the 1229 (Rieseberg *et al.*, 2003) and 687 (Stelkens & Seehausen, 2009) traits examined, respectively.

Genetic and molecular studies increasingly show that the heading date and several of the yield related traits such as CL, CN, PaL, GN, FlL, FlW and SG are co-regulated in rice by QTLs with pleiotropic effects (Xue *et al.*, 2008; Wei *et al.*, 2010; YuShan *et al.*, 2010; Yan *et al.*, 2011; Cai *et al.*, 2012; Vishnukiran *et al.*, 2020; Lee *et al.*, 2024). Thus, the selection history of yield related traits are distinct in Njavara and Jyothi. While the yield related traits in Njavara were co-subjected to constant stabilizing selection when farmers used short duration as a selection criterion for the maintenance of Njavara over centuries, Jyothi experienced directional selection during varietal development (Sreejayan *et al.*, 2011). In hybridization, the difference in the genetic architecture between mating partners or the genetic distance between them are the two important factors contributing to transgression (Rieseberg *et al.*, 2003; Albertson & Kocher, 2005; Stelkens & Seehausen, 2009; Stelkens *et al.*, 2009). Jyothi and Njavara lines, especially N8, are highly diverged, both genetically and morphologically, as evident from the results of microsatellite and morphologically analyses (Supplementary Tables S1 & S2). As the selective forces govern genetic architecture of populations as well as the genetic distance between them (Calsbeek *et al.*, 2011), the difference in the mode of selection experienced by Njavara and Jyothi may be primarily responsible of setting the genetic architecture essential for transgression in Njavara × Jyothi hybridizations.

We found a strong correspondence between heterosis and transgression; wherein a trait, which showed high F1 heterosis showed transgression in a higher proportion of individuals in F2 population. This indicates the possibility of heterosis and transgression sharing certain common genetic mechanisms such as epistasis as shown in rice earlier (Li *et al.*, 2008; Mao *et al.*, 2011).

As compared to N8 × Jyothi, the frequency of transgressive phenotypes were significantly low in Jyothi × N8, indicating a crucial role for N8 cytoplasm in determining transgressive segregation. Maternal effect and nuclear-cytoplasm interactions are shown to influence hybrid performance in many hybridization experiments (Han *et al.*, 2008).

To conclude, the study provides the importance of apparently poor yielding landraces in breaking the yield plateau in high yielding varieties. It also points to the value of a paradigm shift, as suggested by Tanksley and McCouch (1997), from morphological trait to the genetic architecture and selection history of the desired trait in choosing parental combinations. Further, the results highlight the value of data on the on farm evolutionary dynamics of an agronomically important trait in designing experimental crosses. Concerted hybridization experiments involving germplasm resources experiencing different evolutionary sorting may help to identify parental combinations liberating transgressive phenotypes, as being done to identify parental combinations yielding heterosis. By designing crosses between the promising transgressive segregants, it may be possible to pyramid beneficial alleles to lift yield barriers to evolve super yielders.

ACKNOWLEDGEMENTS

We thank Kreara Solutions, Thiruvananthapuram for the analysis using SAS software. D. R. R. acknowledges UGC-FDP Teacher Fellowship (No. FIP/11th Plan/KLKE002 TF03).

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SUPPLEMENTARY TABLES

Table S1: Allele configuration of the Jyothi and the Njavara lines N8, N14 and N9 at 71 microsatellite loci

Name of the microsatellite loci	Alleles (bp)				Name of the microsatellite loci	Alleles (bp)			
	Jyothi	N 8	N 14	N 9		Jyothi	N 8	N 14	N 9
RM1	80	78	70	78	RM309	0	166	166	166
RM104	236	228	228	228	RM316	196	202	202	202
RM109	91	89	91	89	RM330A	178	217	217	217
RM11	122	120	120	120	RM336	163	157	160	157
RM118	156	158	156	158	RM346	134	166	163	166
RM12	157	0	183	171	RM404	234	214	218	214
RM124	264	266	266	266	RM408	120	116	114	116
RM133	226	230	230	230	RM423	289	0	277	277
RM135	121	115	115	115	RM424	262	262	283	283
RM159	244	238	252	238	RM430	138	138	140	140
RM160	83	92	128	92	RM431	252	246	246	246
RM178	115	117	117	117	RM432	165	177	177	177
RM202	160	163	173	163	RM433	221	229	225	227
RM204	103	0	113	113	RM439	243	264	276	261
RM215	144	148	148	150	RM440	207	156	168	156
RM223	154	144	144	150	RM441	184	186	182	190
RM224	150	135	135	135	RM447	114	130	130	130
RM225	138	120	120	120	RM452	202	196	205	196
RM228	125	109	117	111	RM48	209	205	209	207
RM229	120	98	130	98	RM481	136	151	163	148
RM23	143	139	139	139	RM484	294	288	288	288
RM231	185	0	183	185	RM507	253	257	257	257
RM234	152	148	135	152	RM511	120	129	129	120
RM235	98	106	96	106	RM514	246	240	242	240
RM242	225	193	195	193	RM517	279	273	275	275
RM25	141	127	143	127	RM518	160	166	183	166
RM250	151	153	169	153	RM519	132	117	117	117
RM252	216	214	214	214	RM536	228	228	234	228
RM257	141	121	115	119	RM541	178	176	174	178
RM258	144	144	144	138	RM545	211	211	211	211
RM277	118	116	116	112	RM551	183	187	169	187
RM280	151	161	175	175	RM566	263	219	219	219
RM283	148	160	150	146	RM567	244	244	236	236
RM284	140	140	140	140	RM589	173	0	213	0
RM293	202	196	200	196	RM590	136	136	136	136
RM302	153	141	117	137					

Table S2: Parental mean and the mean, BPH and MPH of F1 hybrids for 13 morphological traits

Genotype	N		LL	LW	CL	CN	PL	100GW	GL	GW	PaN	GN	FIL	FIW	SG
Parents															
Jyothi	10		48.70	1.23	69.09	20.50	25.55	2.67	0.91	0.28	20.50	178.72	40.92	1.59	22.38
N8	10		36.70	0.90	80.98	11.80	22.58	1.64	0.74	0.24	17.70	81.60	40.82	1.11	17.24
N9	10		39.02	1.07	77.66	11.30	19.16	1.75	0.75	0.23	16.30	73.11	36.06	1.28	10.84
N14	10		57.84	1.27	127.80	8.20	24.08	1.90	0.71	0.26	11.50	124.98	40.46	1.27	14.09
Parental mean[$]	5		44.90	1.09	87.73	12.24	22.38	2.00	0.77	0.25	16.22	105.1	40.37	1.27	15.09
Hybrids															
N8 × Jyothi	14	Mean	66.04	1.40	110.44	29.93	29.37	2.18	0.78	0.25	22.21	228.11	54.02	1.73	22.38
		MPH	54.66**	31.46**	47.19**	85.33**	22.04**	1.16	-5.46**	-3.85	16.28	75.25**	32.18**	28.15**	12.97**
		BPH	35.61**	13.82**	36.38**	46.00**	14.95**	-18.4**	-14.3**	-10.7**	8.34	27.64**	32.01**	8.81**	0.00
Jyothi × N8	13	Mean	64.13	1.42	110.23	31.85	29.14	2.17	0.78	0.26	23.54	234.77	52.27	1.77	22.89
		MPH	50.19**	33.33**	46.91**	97.21**	21.09**	0.70	-5.46**	0.00	23.25**	80.37**	27.89**	31.11**	15.55**
		BPH	31.68**	15.45**	36.12**	55.37**	14.05**	-18.7**	-14.3**	-7.14**	14.83**	31.36**	27.74**	11.32**	2.28
N9 × Jyothi	10	Mean	68.68	1.69	113.16	35.40	28.01	2.20	0.80	0.26	22.60	272.58	50.67	2.12	24.04
		MPH	56.59**	46.96**	54.22**	122.6**	25.30**	-0.45	-3.61**	1.96	22.83*	116.5**	31.65**	47.74**	44.73**
		BPH	41.03**	37.40**	45.71**	72.68**	9.63**	-17.6**	-12.1**	-7.14**	10.24	52.52**	23.83**	33.33**	7.42**
Jyothi × N9	11	Mean	63.92	1.61	106.88	39.45	27.46	2.22	0.80	0.26	24.00	248.89	49.14	1.99	24.09
		MPH	45.74**	40.00**	45.66**	148.1**	22.84**	0.45	-3.61**	1.96	30.44*	97.67**	27.67**	38.68**	45.03**
		BPH	31.25**	30.89**	37.63**	92.44**	7.48*	-16.9**	-12.1**	-7.14**	17.07	39.26**	20.09**	25.16**	7.64
N14 × Jyothi	6	Mean	70.31	1.56	128.11	34.00	31.65	2.45	0.78	0.28	22.67	289.13	50.11	1.77	26.40
		MPH	31.99**	24.80**	30.13**	136.9**	26.78**	7.22**	-3.70**	3.70	41.69	90.41**	23.15*	23.78**	44.78**
		BPH	21.56**	22.84**	0.24	65.85**	23.88**	-8.24**	-14.3**	0.00	10.59	61.78**	22.46*	11.32*	17.96**
Jyothi × N14	10	Mean	66.26	1.50	105.89	35.90	29.66	2.51	0.81	0.27	23.80	246.94	50.14	2.88	24.04
		MPH	24.39**	20.00**	7.56	150.2**	18.81*	9.85**	0.00	0.00	48.75**	62.62**	23.22**	101.4	31.83**
		BPH	14.56**	18.11**	-17.1	75.12**	16.09*	-5.99**	-11.0**	-3.57	16.10	38.17*	22.53**	81.13	7.42

*,** Significant at $\alpha = 0.05$ and $\alpha = 0.01$ level respectively, [$]Values differs significantly between parents, Character abbreviations as given in the text, SG is recorded in mm.