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Research Article

Assessment of heterotic potential of *indica* rice hybrids derived from KMR 3/*O. rufipogon* introgression lines as restorers

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Abstract
Present investigation was carried out to assess the heterotic yield potential of 162 *indica* rice hybrids derived from twenty six KMR3/*O. rufipogon* introgression lines (ILs) and six cytoplasmic male sterile (CMS) lines. The derived hybrids and testers were phenotyped for days to 50% flowering, plant height, number of productive tillers and yield per plant. The performance of hybrids was estimated based on standard heterosis over the hybrid check KRH2 along with analysis of variance for combining ability. Among these 3 lines and 20 testers were identified as good general combiners and significantly contributed to yield per plant. Nine out of 162 hybrids viz., IR58025A x IL458, IR58025A x IL467, IR58025A x IL473, DRR9A x IL106, DRR9A x IL109, DRR9A x IL117, DRR9A x IL410, DRR9A x IL478 and IR68897A x IL410 showed significantly high specific combining ability (SCA) and standard heterosis over KRH2. These hybrids are recommended for large scale field evaluation for yield.

Key words
Heterosis, Introgression line, Rice hybrid, Parental line, Wild rice

Introduction

The world population will reach 9.15 billion by 2050 and rice production must increase considerably to meet the demands of increasing population (Brar and Khush, 2013; Li *et al*., 2014). Shrinking cultivable rice area, biotic, abiotic stresses and future climate change pose a challenge to rice breeders for increasing rice production further. Hybrids can offer substantial yield increase especially in irrigated favorable ecosystems. In India 75 rice hybrids are released from public and private sectors, in this only 15-20 are widely grown. They occupy about 3.5% of the total hybrid rice area (2.8 million hectare) in India (AICRIP, 2015). The development of modern rice varieties and hybrids depends on continued availability of genetic diversity.

Wild species are known to possess wide genetic diversity for yield related traits, biotic and abiotic stress tolerance which can be utilized to improve the parental lines of rice hybrids to break yield barriers (Rangel *et al*., 2013; Gaikwad *et al*., 2014; Thalapati *et al*., 2014, 2015; Hu *et al*., 2016; Adedze *et al*., 2016). Progress has been made in molecular breeding using wild rice for the improvement of quantitatively inherited traits like yield (Marri *et al*., 2005; Luo *et al*., 2011; Bai *et al*., 2012). For example, *O. rufipogon* introgressions increases yield in varieties across several genetic backgrounds and environments (Marri *et al*., 2005; Sudhakar *et al*., 2012) but very few were reported in hybrids (Gaikwad *et al*., 2014; Thalapati *et al*., 2015; Adedze *et al*., 2016). The ILs derived from the cross 9311/ *O. rufipogon* had favourable yield enhancing QTLs *qyld1.1* and *qyld2.1* on chromosome 1 and 2 which increases the yield by 18 and 17% respectively (Liang *et al*., 2004). Likewise, Luo *et al*. (2011) identified heterotic loci associated with six yield related traits from the introgression lines of Guichao 2 (*O. sativa* L.) and *O. rufipogon*. Similarly the restorer line Q611 had two yield enhancing QTLs *qyld1.1* and *qyld2.1* from *O. rufipogon* and the derived hybrid J23A/ Q611 also showed high standard heterosis of 35 percent (Yuan, 1994). In addition, *O. rufipogon* has not only been used as a donor parent for several yield related QTLs like grain number (*Gnla*), grain weight (*gw9.1*) plant height (*ph1*) (Ashikari *et al*., 2005; Xie *et al*., 2006, 2008) but also for candidate genes like *OsCKX2* (Ashikari *et al*., 2005) *LRK* (He *et al*., 2007) and *Os11GSK* (Thalapati *et al*., 2012).
It was hypothesized that improvement of the restorer lines using wild alleles would enhance the yield of derived hybrid and would further help in boosting hybrid yields. Hence, the present study was undertaken to identify high yielding restorer KMR3 ILs with introgressions from *O. rufipogon* at *qyld2.1* along with six cytoplasmic male sterile (CMS) lines

to obtain higher yielding hybrids. The combining ability and heterosis of introgression lines (ILs) was also estimated for yield and yield related traits. Combining ability and heterosis in 162 hybrids including 156 hybrids developed using six CMS lines and twenty six high yielding KMR3/*O. rufipogon* ILs and 6 control hybrids without *O. rufipogon* introgression along with the restorer line KMR3 are reported.

Materials and Methods

Development of experimental hybrids Twenty six ILs which were phenotypically better in terms of high number of productive tillers, and grain yield per plant compared to KMR3 were selected. These were crossed with six WA-based CMS lines viz., IR58025A, IR79156A, APMS6A, DRR9A, IR68897A and PUSA5A in line x tester mating design which resulted in 156 test hybrids with *O. rufipogon* introgression and 6 control hybrids without *O. rufipogon* introgression. The heterotic performance of hybrids were tested along with corresponding restorers (ILs) for yield and related traits with four popular hybrids, KRH2, DRRH3, PA6129, NK5251, and popular high yielding varieties Jaya, IR64 and Annada used as standard checks. Twenty five days old seedlings were transplanted and each entry was planted in two rows with 15x20 cm spacing at IIRR (Indian institute of rice research) farm station (lat 17°32' N, 78°39' E) Hyderabad, during kharif 2014. Single seedling was transplanted per hill and recommended packages of practices were followed.

Twenty six ILs and their hybrids were grown in two replications in randomised complete -block design and observations were taken for plant height (PH), number of productive tillers per plant (NT), days to 50% flowering (DFF) and grain yield per plant (YLDP) from middle 5 plants of each replication using standard evaluation system for rice (SES), international rice research institute (IRRI).

DNA was isolated from the young leaves of 26 ILs, and parents KMR3 and *O. rufipogon* using CTAB (Cetyl Trimethyl Ammonium Bromide) method (Rogers and Bendich, 1988) followed by PCR amplification (Chen *et al*., 1997) using 14 SSRs and one gene specific markers, which includes RM262, RM263 flanking markers of *qyld2.1* and 8 subQTL markers, RM3666, RM1303, RM3688, RM3762, RM3874, RM3515, RM6318, RM1920. In addition one gene specific marker NSH9 (*remorin*), on chromosome 2 and 2 SSRs RM3412, and RM8094 within *saltol* QTL on chromosome1 were used to analyse the marker segregation in ILs. Two fertility restoration linked markers RM6100 for *Rf4* on chromosome 10 and RM10313 for *Rf3* gene on chromosome 1 were also used to confirm the fertility restoration gene introgressions from KMR3.

Combining ability was determined to obtain the genetic value of inbreds, to identify superior cross combinations and to assess the gene action involved in expression of various quantitative characters. The mean data of experimental hybrids was analyzed for combining ability (Kempthorne, 1957). Superior parents with good general combining ability (GCA) and cross combinations with good specific combining ability (SCA) were determined using genotypic means using Linear Mixed Model Fit by Restricted Maximum Likelihood method (Wu and Matheson, 2001; Mohring *et al*., 2011). Analysis of variance (ANOVA) and standard heterosis was estimated as percent gain in yield per plant over the standard hybrid were calculated using PB tools (2014) (Version 1.4, http://bbi.irri.org/products) and TNAUSTAT software (Manivannan, 2014).

Results and Discussion

Combining ability is an effective approach in hybrid breeding. The analysis of variance for combining ability revealed significant differences among the parents and crosses for all 4 traits - plant height, number of productive tillers, days to 50% flowering and grain yield per plant and descriptive statistics are summarized in Table 1 and Table 2. GCA is a measure of additive genetic variance. The estimates of GCA effects showed that parents with high GCA differed for various traits. Among the 6 CMS lines DRR9A contributes favourably for grain yield, plant height and number of productive tillers per plant, whereas IR79156A, PUSA5A, DRR9A and IR68897A contribute to lowering days to 50% flowering. Likewise, 12 testers IL 106, IL 109, IL 117, IL 194, IL 242, IL 409, IL 410, IL 431, IL 458, IL 463, IL 467 and IL 478 showed good GCA for yield per plant. Among this IL 410 contributes high yield per plant and lowering the plant height and days to 50% flowering than remaining ILs (Table 3). In our previous study we showed that 3 CMS lines APMS6A, IR79156A and CRMS32A and 3 testers IL 86-18, IL 50-12 and IL 50-7 are good general combiners for yield/plant and 1000 grain weight (Thalapati *et al*., 2015). In current study it was confirmed IR79156A and two additional CMS lines DRR9A and IR68897A and 20 out of 26 testers were good general combiners for at least one of the traits of plant height, number of productive tillers and days to 50 % flowering and grain yield per plant. Interestingly, four of this ILs 194, 381, 106 and198

showed higher net photosynthetic rate and total dry matter than parents KMR3 and *O. rufipogon* (Haritha *et al*., 2017). Indicating introgression segments from wild species contributed positively for higher level of heterosis for yield and yield contributing traits in hybrid background.

In any hybrid development study, selection of appropriate parents is of prime importance but performance of hybrid is not always interpreted from the *per se* performance of parents or their combinations, since phenotypically superior lines may show poor recombination (Tiwari *et al*., 2011). Parents with low GCA effects also generated hybrids with high SCA, indicating that non-additive gene actions including dominance and epistasis might be primary factors in controlling the gene expression of hybrids (Zhang *et al*., 2015). In current study, hybrids with high and significant SCA were produced from all parental combinations having high x low GCA. Only 19 hybrids out of 162 showed significantly (P<0.01) high SCA for yield per plant and gave more than 40g yield/plant. Thalapati *et al*. (2015) showed 7 hybrids out of 30 gave high yield of more than 40 g/plant. The cross PUSA5A x IL 242 showed highest SCA (125.83) for yield followed by DRR9A x IL 117 (95.68), IR58025A x IL 473 (82.91) and DRR9A x IL 109 (69.85) suggesting their potential for use in heterosis breeding. Even low x low GCA combinations produced hybrids with high SCA and this could be attributed to over dominance or epistatic gene action. In case for some traits parents with high GCA may produce hybrids with low SCA due to lack of complementation of parental genes whereas in some hybrids eg. PUSA5A x IL 242 it was vice-versa. It indicates that SCA of hybrid combinations is not the direct representation of GCA of parents.

The comparative estimates of variances due to GCA and SCA revealed that SCA variances were higher than GCA variances for all traits. 53 out of 156 hybrids showed high SCA and showed better yield over respective controls (CMS x KMR3-R), Similarly 63 cross combinations out of 156 showed high mean grain yield per plant over their respective controls. Of these, 9 hybrids showed significantly high SCA and positive standard heterosis over KRH2 for grain yield per plant (Table 4). The standard heterosis ranged from 2 (IR58025A x IL 473) to 36 percent (DRR9A x IL 117). The interaction due to line x tester was significant (P<0.05) for all traits indicating that combining ability contributed more to the expression of these traits. All six hybrid checks used in current study are of mid early duration and grown widely in India. KRH2 is a high yielding (7.44-7.90 t/h) and most popular hybrid since 1996 (Chamling and Basu, 2012), whereas hybrid DRRH3 is a medium duration hybrid released from IIRR, Hyderabad, India. PA6129 is popularly used as control in hybrid testing. The released hybrids showed a mean grain yield of 6-8 t/ha with 15-30 percent yield superiority over high yielding varieties (FAO, 2014) (http://www.fao.org/3/a-i4395e.pdf).

The variance of SCA was greater than that of GCA for the traits indicating predominant role of dominant gene action governing epistasis. It is therefore suggested that these traits could be improved through heterosis breeding rather than phenotypic selection. Therefore yield could be enhanced through the improvement of these component traits like plant height, number of productive tillers, days to 50% flowering and yield per plant. In rice, presence of non additive gene action for plant height, number of productive tillers, days to 50 % flowering and yield per plant was reported by Kumar *et al*. (2007) and Sharma *et al*. (2005). Thirty hybrids showed significant negative standard heterosis for plant height and these hybrids were high yielding. Mishra and Pandey (1998) reported -26.5 to 15.20 percent standard heterosis for plant height, while in current study it was -0.69 to 18.24percent over KRH2. Peng and Virmani (1991) reported -27 to 19 percent decrease for days to 50% flowering. In current study also a desirable decrease of days to 50% flowering by 0.93 to 12.15 percent was observed in most of the hybrids. Generally positive heterosis for grain yield and negative heterosis for plant height and days to 50% flowering is advantageous in developing short and early hybrids (Mishra and Pandey, 1998; Rahimi *et al*., 2010).

Heterosis of hybrids is generally expressed as heterobeltiosis, mid parent or relative heterosis and standard heterosis. Practically standard heterosis has more economical importance because of the superiority of the hybrids over the existing commercial hybrids. Estimation of standard heterosis of all hybrids for 4 traits revealed 9 hybrids IR58025A x IL 458, IR58025A x IL 467, IR58025A x IL 473, DRR9A x IL 106, DRR9A x IL 109, DRR9A x IL 117, DRR9A x IL 410, DRR9A x IL 478 and IR68897A x IL 410 out of 152 showed significant positive standard heterosis and SCA over hybrid check KRH2 for grain yield per plant. The standard heterosis ranged from 2.58 percent in IR58025A x IL 473 to 36.13 percent in DRR9A x IL 117.

Flowering synchrony between parental lines (R and CMS) is an important factor which influences the grain yield in hybrid rice (Virmani *et al*., 1998). Among the IL derived hybrids (ILH), 9 hybrids

showed high standard heterosis for yield compared to popular hybrids and KRH2 and 52 ILHs flowered earlier than the hybrid check KRH2. However, only DRR9A x IL117 showed significantly higher yield and also flowering synchrony between its parental lines. It appears to be worthy for large scale commercialization for yield.

Twenty six ILs were tested for segregation of 10 SSR markers within yield QTL *qyld2.1* and one gene specific marker and 2 markers within *saltol* QTL region and 2 markers of fertility restoration genes. All ILs showed one or the other of the 8 sub QTL marker allele introgression from *O. rufipogon*. In all, IL 50-7 was the only line which showed all homozygous *O. rufipogon* alleles for *qyld2.1* (Fig. 1). IL 198, IL 495 and IL 410 were mostly heterozygous. But, IL 198 had homozygous *O. sativa* allele at RM3666 and IL 495 had *O. sativa* alleles at RM1920 whereas IL 410 showed all heterozygous alleles except for RM1920, NSH9 and RM263 which showed homozygous *O. rufipogon* alleles. In addition, 9 ILs 50, 215, 458, 463, 467, 478, 491, and 50-7 had *O. rufipogon* allele for RM8094 and KMR3 alleles for RM3412. Likewise, all ILs had *Rf4, Rf3* alleles from KMR3 except IL 381, IL 491 and IL 501. IL 381 showed *Rf4* allele from *O. rufipogon Rf3* from KMR3, and vice versa in IL 491 and IL 501(Fig. 2). Out of 162 hybrids 9 hybrids IR58025A x IL 458, IR58025A x IL 467, IR58025A x IL 473, DRR9A x IL 106, DRR9A x IL 109, DRR9A x IL 117, DRR9A x IL 410, DRR9A x IL 478 and IR68897A x IL 410 showed high SCA for yield per plant and high standard heterosis over KRH2 and were recommended for large scale field evaluation. These ILs also had *O. rufipogon* alleles of *qyld2.1* in particular at RM3688. The simultaneous identification and transfer of yield enhancing QTLs/genes from wild rice into hybrid rice parental lines has the scope to enhance heterosis in rice hybrids by upto 36 percent. Current study confirms that subQTL3 of *qyld2.1* from *O. rufipogon* improves yield in ILs and its derived hybrids also. It is important that all these ILs had two fertility restoration genes *Rf3* and *Rf4* from KMR3 itself. In addition four ILs 458, 463, 467 and 478, out of 26 had *O. rufipogon* allele for RM8094. This is one of the important markers of *saltol* QTL reported for seedling stage salinity tolerance in rice (Krishnamurthy *et al*., 2015; Chowdhury *et al*., 2016). It is significant that these four ILs have been reported to show salinity tolerance in both seedling and reproductive stage (Ganeshan *et al*., 2016). These ILs can be used to develop hybrids for rice growing in saline regions. From current study it was revealed that, introgression segments from wild species in hybrid background contributed positively for higher level of heterosis for yield and yield traits.

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Table 1. Analysis of variance (ANOVA) of combining ability for yield and yield components

Mean sum of squares					
Source of variation	DF	PH (cm)	NT	DFF (days)	YLDP (g)
Replication	2	632.09*	632.09*	632.09*	632.09*
Cross	161	72.98*	29.46*	28.94*	4556.80*
Line (L)	5	691.18*	261.98*	124.21*	12475.23*
Tester (T)	26	62.75*	15.94*	28.85*	4861.97*
LxT	130	51.24*	23.22*	25.29*	4191.22*
Error	322	0.099	0.099	0.099	0.099*
Total	485				

**, * significant at 0.01 and 0.05 level
L- cytoplasm male sterile lines; T- testers/restorers; L x T- line x testers (hybrids); DF-Degrees of freedom
PH-Plant height; NT-Number of productive tillers per plant; DFF-Days to 50% flowering; YLDP-yield per plant.

Table 2. Summary of descriptive statistics for yield and yield components

	PH (cm)	NT	DFF (days)	YLDP (g)
Min	63	5.4	90	92
Max	104.4	27	113	109
Mean	89.55	13.09	84.14	101.18
Std Dev	5.25	3.6	39.6	3.5
Narrow sense heritability (plot-mean based)	0.33	0.29	0.07	0.14
Broad sense heritability (plot-mean based)	1.00	0.99	1.00	0.99
Dominance Ratio	2.00	2.22	5.33	3.44

Min- minimum; Max- maximum; Std Dev- standard deviation; PH-Plant height; NT-Number of productive tillers per plant ; DFF-Days to 50% flowering; YLDP-yield per plant

Table 3. General combining ability (GCA) of parents (lines and testers)

S. no	Lines	PH(cm)	NT	DFF (days)	YLDP(g)
L1	IR79156A	2.70 **	-1.64 **	-0.06 ns	2.04 **
L2	APMS6A	-1.57 **	-1.82 **	0.75 **	-12.57 **
L3	PUSA5A	-0.08 *	-0.57 **	-0.47 **	-12.87 **
L4	IR58025A	0.02 ns	-0.06 ns	1.79 **	11.12 **
L5	DRR9A	3.48 **	2.90 **	-0.10 **	17.17 **
L6	IR68897A	-4.56 **	1.19 **	-1.91 **	-4.90 **
SE (lines)		0.0349	0.0349	0.0349	0.0349
T1	IL 40	0.35 **	-0.20 **	-0.80 **	-8.79 **
T2	IL 50	-0.08 ns	1.98 **	-1.14 **	-17.96 **
T3	IL 50-7	0.20 **	1.81 **	0.86 **	-25.43 **
T4	IL 106	1.69 **	-1.65 **	-0.14 ns	10.66 **
T5	IL 109	2.81 **	0.05 ns	-2.80 **	6.71 **
T6	IL 117	-0.02 ns	-1.76 **	1.03 **	15.87 **
T7	IL 194	1.40 **	-0.43 **	0.70 **	5.46 **
T8	IL 198	-0.31 **	-0.22 **	1.70 **	-11.33 **
T9	IL 215	-0.71 **	1.35 **	0.36 **	-8.34 **
T10	IL 242	-5.02 **	-0.38 **	0.86 **	0.76 **
T11	IL 301	-1.46 **	0.34 **	1.86 **	-4.51 **
T12	IL 345	-2.47 **	1.42 **	0.36 **	-12.34 **
T13	IL 349	-0.21 **	0.01 ns	0.86 **	-10.46 **
T14	IL 381	0.73 **	-1.11 **	-1.30 **	-2.96 **
T15	IL 407	-0.76 **	-0.43 **	-2.30 **	-20.19 **
T16	IL 409	2.29 **	-0.03 ns	-0.97 **	11.24 **
T17	IL 410	-3.17 **	0.05 ns	-2.30 **	41.49 **
T18	IL 431	1.96 **	0.69 **	-0.64 **	22.82 **
T19	IL 458	2.64 **	-0.66 **	0.20 **	21.01 **
T20	IL 463	-1.82 **	-0.10 ns	-0.80 **	10.19 **
T21	IL 467	-0.14 ns	0.12 ns	0.20 **	23.42 **
T22	IL 473	0.01 ns	-1.18 **	1.53 **	-17.31 **
T23	IL 478	-0.19 **	-0.46 **	0.20 **	0.82 **
T24	IL 491	-2.19 **	-0.75 **	1.20 **	-26.93 **
T25	IL 495	2.48 **	0.76 **	1.53 **	-6.09 **
T26	IL 501	1.12 **	-0.00 ns	0.53 **	-6.36 **
T27	KMR3R	0.88 **	0.74 **	-0.80 **	8.57 **
SE (testers)		0.0741	0.0741	0.0741	0.0741

*, ** significant at 5 and 1 percent levels; ns- not significant (P>0.05)
PH-Plant height; NT-Number of productive tillers per plant; DFF-Days to 50% flowering; YLDP-yield per plant

Table 4. Standard heterosis and specific combining ability of best hybrids over KRH2 in crosses derived from introgression lines for yield traits

Cross	Standard heterosis over KRH2				Specific combining ability
	PH(cm)	NT	DFF (days)	YLDP (g)	YLDP (g)
IR68897A x IL 410	-24.94 **	-43.18 **	-12.15 **	16.13 **	61.13 **
DRR9A x IL 478	5.77 **	-10.23 **	-4.67 **	4.52 **	61.73 **
DRR9A x IL 410	7.16 **	-9.09 **	-2.80 **	3.23 **	19.07 **
DRR9A x IL 106	10.39 **	-7.95 **	-9.35 **	5.81 **	53.89 **
DRR9A x IL 109	11.78 **	-9.09 **	-12.15 **	13.55 **	69.85 **
DRR9A x IL 117	8.55 **	-20.45 **	-2.80 **	36.13 **	95.68 **
IR58025A x IL 467	-3.58 **	-32.95 **	-4.67 **	5.94 **	47.38 **
IR58025A x IL 473	8.55 **	-28.01 **	0.00 ns	2.58 **	82.91 **
IR58025A x IL 458	1.04 **	-35.80 **	-4.67 **	3.10 **	45.40 **

*, ** significant at 5 and 1 percent levels; ns- not significant (P>0.05),
PH-Plant height; NT-Number of productive tillers per plant; DFF-Days to 50% flowering; YLDP-yield per plant.

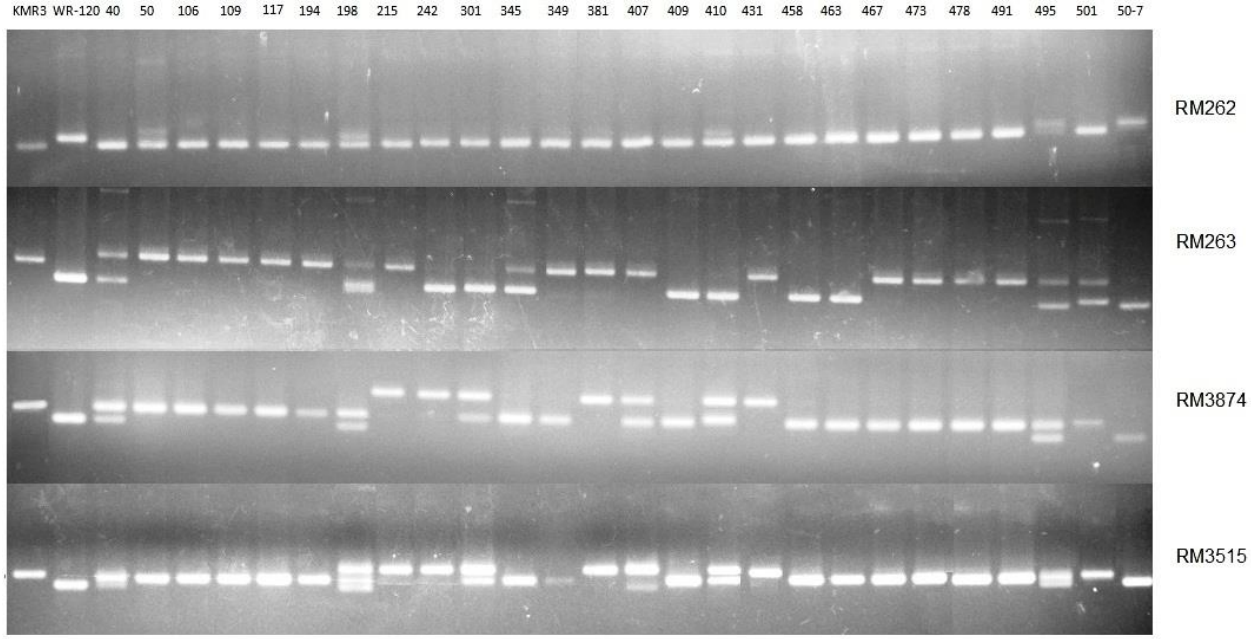

Fig. 1. SSR profile of 26 restorers (ILs) using the yield QTL *qyld2.1* flanking markers (RM262 and RM 263) and markers within *qyld2.1* (RM3874 and RM3515).

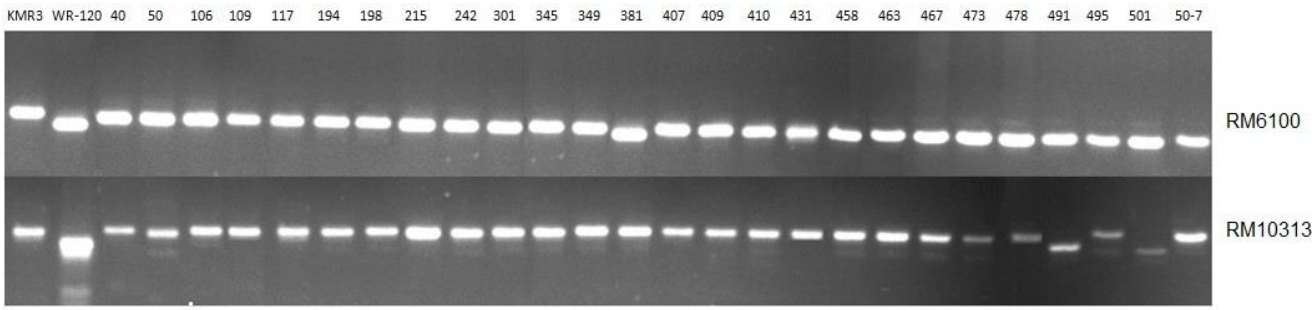

Fig. 2. Amplification pattern of the marker RM6100 (lane-1) for *Rf4* locus and RM10313 (lane-2) for *Rf3* in 26 restorer lines.