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POLYMORPHIC SSR MARKERS AMONG *ORYZA SATIVA* CV. SWARNA AND ITS DERIVED ADVANCED BACKCROSS LINES WITH WILD INTROGRESSIONS FROM *O. NIVARA*

N.P.S. DE SILVA[1,3], B. KAVITHA[2], M. SURAPANENI[2], A.K. RAJU[2],V.G. SHANKAR[1], D. BALAKRISHNAN[2], S. NEELAMRAJU[2], S.N.C.V.L PUSHPAVALLI[1] AND D. SRINIVASA CHARY[1]

[1]Professor Jayashankar Telangana State Agricultural University, Hyderabad, 500030
[2]ICAR - Indian Institute of Rice Research, Hyderabad, 500030
[3]Regional Rice Research & Development Centre, Bombuwela, Kalutara, 12000, Sri Lanka

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ABSTRACT

Wild species derived introgression lines are good source of novel genes for improving complex traits like yield. Identification of molecular markers revealing genotypic polymorphism is a prerequisite for mapping QTLs and genes for target traits. Polymorphism between parental lines of mapping populations involving Swarna, 166s and 148s was identified in this study. Swarna is a high yielding mega variety, while, 166s and 148s are advanced backcross introgression lines (BILs) developed from a cross between Swarna x *O. nivara.* A total of 530 randomly selected SSR markers were used to identify the polymorphism between Swarna and BILs 166s and 148s. Eighty eight markers (16.66%) which showed distinct alleles between Swarna and 166s were again used to study the polymorphism between Swarna/148s and 166s/148s. Out of 88 markers, 50 were (56.81%) polymorphic between Swarna/148s and 54 markers (61.36%) were polymorphic between 166s /148s. Some of these polymorphic SSR markers were already reported as linked to yield traits in previous studies. These identified markers will be useful in further QTL mapping of the populations derived from the parental lines used in this study.

Rice (*Oryza sativa* L.) is one of the most important food crops in the world and to meet the growing demand from the increasing human population, rice varieties with higher yield potential and greater yield stability need to be developed. To meet the demand of estimated 8.8 billion population in 2035 and raising the rice productivity from the current 10 to 12 tons ha^{-1} are posing a major challenge to rice scientists (Kaur *et al*., 2018). In the domestication process, the strong directional selection reduced the genetic variability in existing cultivated rice genotypes. Due to a great loss of allelic variability or "genetic erosion", the improvement of rice yield became a challenging part in rice breeding programmes.

As a primary gene pool, wild relatives of rice contain unexploited genes which can help in improving rice yield, through broadening the genetic variation in modern rice breeding. Introgression lines (ILs) having genomic fragments from wild rice can be used to develop improved cultivars. The wild relatives of rice have been utilized extensively for introgression of major genes for disease and insect resistance, but their utilization in enhancing yield and yield-related traits has remained limited (Neelam *et al*., 2016). There are several studies to improve the rice yield using high yielding varieties of cultivated rice (Sharma *et al*., 2011; Marathi *et al*., 2012; Okada *et al*., 2018; Yu *et al*., 2018; Ma *et al*., 2019) by improving plant architecture (San *et al*., 2018) or increasing photosynthesis rate (Takai *et al*., 2013). As well, there are records on increasing rice yield by introgression of QTLs or genes of wild rice into cultivated rice (Rangell, 2008; Fu *et al.*, 2010; Srividhya *et al*., 2011; Thalapati *et al*., 2012; Luo *et al*., 2013; Yun *et al.,* 2016; Nassirou *et al*., 2017; Kaur *et al.*, 2018; Kemparaju *et al.*, 2018).

Grain size is a major determinant for domestication and one of the important components determining grain yield in rice. Grain size is considered as the main breeding target due to its effect on both yield and quality. Therefore, it is important to study the traits *viz*., grain size and grain weight for simultaneous improvement of yield and quality (Yu *et al*., 2018; Feng *et al*., 2020).

Genetic polymorphism is defined as the occurrence of a various allelic forms of specific

email:pradeepa_dsilva@yahoo.com

chromosomal regions in the germplasm, as two or more discontinuous genotypic variants. Many researchers preferred SSRs or microsatellites for genotyping due to their advantages in molecular breeding (Varshney *et al*., 2005). SSRs remained as the most popular and preferred marker for a wide array of plant genetic analysis in past few decades. SSRs are the most widely used markers for genotyping plants because they are abundant, co-dominant, efficient, highly reproducible, requiring less quantity of DNA, robust amplification, polymorphic potential, multi-allelic in nature, wide genomic distribution and cost-effective (Li *et al.,* 2004; Varshney *et al*., 2005; Parida *et al*., 2006; Miah *et al*., 2013; Daware *et al*., 2016; Usman *et al*., 2018; Chukwu *et al*., 2019). The microsatellites are found in both coding and non-coding regions and have a lower level of mutation rate (10^{-2} and 10^{-4}) per generation (Chandu *et al*., 2020). The studies on population structure, genotype finger printing, genetic mapping, MAS, genetic diversity studies and evolutionary processes in crop plants are conducted with SSR markers. They have great potential to help breeders by linking genotypic and phenotypic variations and speed up the process of developing improved cultivars (Edwards and Batley, 2010; Gonzaga *et al*., 2015). This study aimed to identify informative polymorphic markers between the parental lines of mapping populations as the primary step for QTL mapping.

MATERIAL AND METHODS

The experimental material consisted of Swarna, 166s and 148s rice lines. Swarna is a semi-dwarf, low-land, high yielding, *indica* rice variety, which matures between 140-145 days with an average yield of 6.5 t ha^{-1}. 166s and 148s are advanced backcross introgression lines (BC_2F_8 lines) derived from a cross between Swarna and *Oryza nivara* and are early (148s) and late (166s) duration genotypes. 166s has strong culm strength, high grain number and panicle weight and 148s with high grain weight were obtained from crop improvement section, IIRR, Hyderabad.

The genomic DNA of Swarna, 166s and 148s was extracted from the young leaves of the plants by CTAB method (Doyle and Doyle, 1987). In summary, 0.1g of leaves was weighed and DNA was extracted with DNA extraction buffer (2% CTAB, 100 mM Tris HCl, 20 mM EDTA, 1.4 M NaCl, 2 % PVP) pre-heated at 60^0C. The extracted DNA was estimated by measuring the OD values at 260 nm/280 nm and 260 nm/230 nm for quality and quantity using a Nano Drop, ND1000 spectrophotometer. The estimated DNA quantity ranged between 88 to 964.4 ng/µl with 1.75-2.38 OD at 260 nm/280 nm. Five hundred and thirty randomly selected SSR markers were used to identify polymorphism between Swarna and 166s. Out of them, 88 SSRs markers showed polymorphism between Swarna and 166s and were used to screen the alleles between Swarna/148s and 166s/148s. PCR was carried out in thermal cycler (Veriti Thermal cycler, Applied Biosystems, Singapore) with a final reaction volume of 10 µl containing 50ng of genomic DNA (2µl), 10X assay buffer (1µl), 10 mM dNTPs (0.1µl), 5µM forward and reverse primers (0.5µl), 1 unit of Taq DNA polymerase (Thermo Scientific) (0.1µl) and MQ water (6.3µl). PCR cycles were programmed as follows: initial denaturation at 94° C for 5 min followed by 35 cycles of 94° C for 30 sec, 55° C for 30 sec, 72° C for 1 min, and a final extension of 10 min at 72°C. Amplified products were resolved in 1% agarose gel prepared in 1X TBE buffer and electrophoresed at 150 V for 10 minutes to fasten the DNA movement from the wells, followed by 120 V for 1-2 hrs until the bands are clearly separated. Gels were stained with Ethidium bromide and documented using a gel documentation system (Syngene, Ingenious 3, USA).

RESULTS AND DISCUSSION

The genomic DNA of the two parents Swarna and 166s was screened using 530 rice microsatellites (RM markers) distributed over the twelve chromosomes of rice. Out of 530 RM markers, 88 were identified as polymorphic between Swarna and 166s. These 88 markers were further used to confirm the polymorphism among the Swarna and its derived BILs 166s and 148s, and identified 50 and 54 polymorphic markers for Swarna/148s and 166s/148s respectively (**Table 1**) and the list of polymorphic markers between these three sets of parents with their respective chromosome numbers are presented in **Table 2**.

The per cent of polymorphism was 16.66% between Swarna/166s and the markers were distributed across all the twelve chromosomes. Among these, the highest number (18) of polymorphic markers were identified on chromosome 2 and the lowest number (3) of polymorphic markers were identified on

Table 1. Polymorphic markers identified on each chromosome among the parents Swarna, 166s and 148s

S.No.	Chromosome	Number of polymorphic markers identified		
		Swarna/166s	Swarna/148s	166s/148s
1	1	9	9	10
2	2	18	13	12
3	3	6	3	4
4	4	6	-	-
5	5	10	4	7
6	6	6	6	5
7	7	3	2	5
8	8	6	3	3
9	9	6	-	-
10	10	7	4	2
11	11	7	4	5
12	12	4	2	1
	Total	**88**	**50**	**54**

Table 2. List of polymorphic markers identified between Swarna /166s, Swarna /148s and 166s /148s crosses

S.No.	Chr.number	Polymorphic SSRs identified between		
		Swarna × 166s	Swarna ×148s	166s × 148s
1	**1**	RM495	RM 140	RM 495
		RM140	RM 490	RM 488
		RM8004	RM 488	RM 8004
		RM5638	RM 495	RM 6738
		RM2318	RM 5638	RM 140
		RM6738	RM 8004	RM 5638
		RM237	RM 6738	RM 490
		RM5310	RM 237	RM 1287
		RM 488	RM 1	RM 237
				RM 1
2	**2**	RM279	RM 279	RM 13599
		RM13599	RM 6375	RM 13616
		RM13616	RM 14001	RM 13630
		RM13630	RM 13599	RM 3774
		RM13641	RM 13616	RM 250
		RM14102	RM 13641	RM 106
		RM12729	RM 3774	RM13641
		RM6375	RM 250	RM 279
		RM424	RM106	RM6375
		RM2634	RM424	RM7485
		RM 5430	RM 13630	RM424

S.No.	Chr.number	Polymorphic SSRs identified between		
		Swarna × 166s	**Swarna ×148s**	**166s × 148s**
		RM3515	RM 13452	RM2634
		RM106	RM 2634	
		RM14001		
		RM250		
		RM3774		
		RM7485		
		RM154		
3	**3**	RM3372	RM14303	RM231
		RM7197	RM489	RM7197
		RM231	RM 231	RM232
		RM232		RM14303
		RM426		
		RM489		
4	**4**	RM7585	-	-
		RM16335		
		RM6314		
		RM3708		
		RM17377		
		RM6909		
5	**5**	RM17962	RM 3170	RM 17962
		RM5874	RM 3437	RM 3170
		RM18614	RM 164	RM 5874
		RM164	RM 334	RM 164
		RM3437		RM 3437
		RM430		RM 169
		RM3664		RM 334
		RM5907		
		RM169		
		RM3170		
6	**6**	RM6467	RM 7583	RM 1369
		RM1369	RM 20377	RM 6467
		RM253	RM 1369	RM 253
		RM19620	RM 19620	RM 19620
		RM30	RM 253	RM 20377
		RM 7583	RM 6467	
7	**7**	RM3859	RM 11	RM 11
		RM346	RM 346	RM 21975
		RM 21975		RM 346
				RM 3859
				RM 21975
8	**8**	RM337	RM 23182	RM 23182
		RM152	RM 337	RM 152
		RM22837	RM 3480	RM 3480

S.No.	Chr.number	Polymorphic SSRs identified between		
		Swarna × 166s	**Swarna ×148s**	**166s × 148s**
		RM149		
		RM3452		
		RM3480		
9	**9**	RM23861	-	-
		RM23736		
		RM24372		
		RM24382		
		RM24430		
		RM5526		
10	**10**	RM216	RM 258	RM 25262
		RM6737	RM 25262	RM 258
		RM258	RM6100	
		RM304	RM228	
		RM6100		
		RM228		
		RM 25262		
11	**11**	RM286	RM 206	RM 27154
		RM26249	RM 27154	RM 286
		RM26513	RM 26652	RM 26652
		RM26652	RM 26998	RM 26998
		RM206		RM 206
		RM26998		
		RM27154		
12	**12**	RM3747	RM 19	RM3747
		RM27564	RM 3747	
		RM247		
		RM 19		

chromosome 7. The polymorphism between Swarna and 148s was 56.81% and the highest number (13) of polymorphic markers were on chromosome 2 and the lowest number (2) of polymorphic markers were identified on chromosome 7 and 12. The per cent of polymorphism between 166s and 148s was 61.36% and the highest number (12) of polymorphic markers was on chromosome 2 and the lowest number (1) of polymorphic markers were identified on chromosome 12.

Frequency distribution of markers explained that a greater number of polymorphic markers were

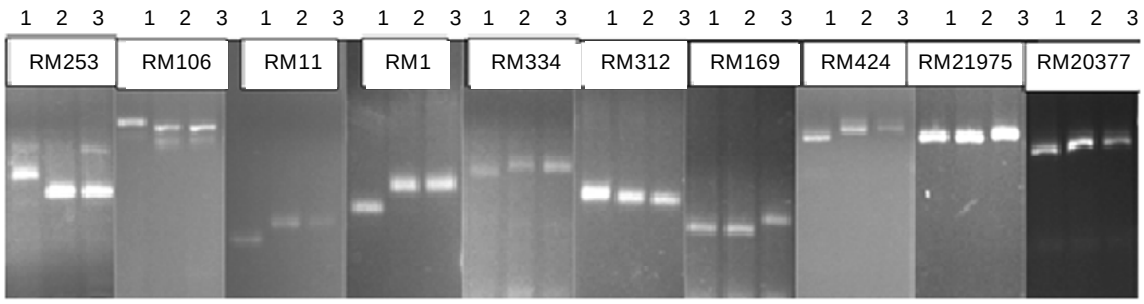

Figure 1. 10SSR markers showing polymorphism between 148s (1) Swarna (2) and 166s (3) on 1% agarose gel

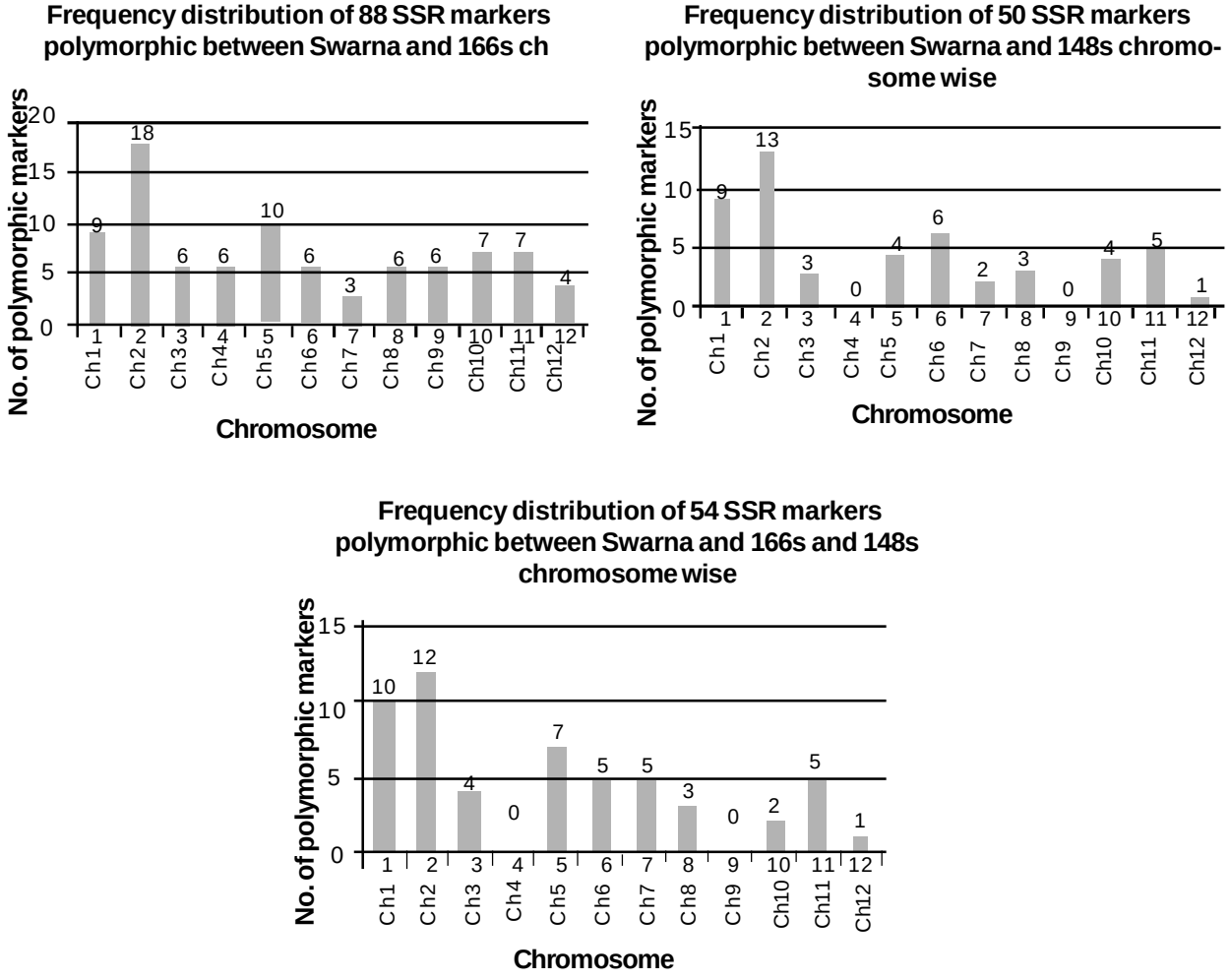

Figure 2. Frequency distribution of polymorphic markers chromosome wise in Swarna/166s, Swarna/148s and 166s/148s cosses

observed on chromosome 2 followed by chromosome 1 in all three parental combinations viz., Swarna /166s, Swarna x 148s and 166s x 148s (**Figure 2**). In case of Swarna /166s, among 88 polymorphic SSRs higher number of markers were distributed on chromosome 2 with 18 SSRs followed by 10 SSRs on chromosome 5 and 9 SSRs on chromosome 1. Chromosome 10 and 11 were observed to have 7 of polymorphic markers and chromosome 3, 4, 6, 8, 9 showed 6 polymorphic markers. Chromosome 12 was with 4 polymorphic markers followed by 3 polymorphic markers identified on chromosome 7.

Among the 50 polymorphic markers in Swarna/148s identified, 13 polymorphic markers were on chromosome 2 followed by 9 SSRs on chromosome 1, 6 SSRs on chromosome 6, 5 SSRs in chromosome 11 and chromosome 5 and 10 had 4 SSRs each, chromosome 3 and chromosome 8 with 3 SSRs, chromosome 7 with two polymorphic SSR, chromosome 12 with one SSR were found in this study. In the other set of parents 166s/148s, among all the 54 polymorphic SSRs higher number was distributed on chromosome 2 with 12 SSRs followed by chromosome 1 with 10 SSRs, chromosome 5 with 7 SSRs, chromosome 6, 7, 11 with 5 SSRs each, chromosome 3 with 4 SSRs and chromosome 8, 10, 12 distributed with polymorphic 3, 2, 1 SSRs respectively. In case of Swarna/148s and 166s/148s there was no polymorphic markers identified on chromosome 4 and 9.

Same markers were observed as polymorphic between Swarna and 148s and between 166s and 148s on chromosome 1 except RM1287 on 166s/148s. Except for RM14001 and RM13452, all the other markers showed polymorphism between Swarna and 148s on chromosome 2, along with polymorphism with 166s/148s. All the primers

Table 3. Details of polymorphic markers identified in previous studies

S.No.	Parental lines	Total no. of polymorphic markers (SSRs) identified	Polymorphic markers linked with yield traits reported previously and found polymorphic in this study	References
1.	Samba Mahsuri (BPT5204) × *Oryza rufipogon*.	149	RM5638, RM424, RM2634, RM13630, RM14303, RM232, RM206, RM11, RM3747, RM24382	Chandu *et al*., 2020
2.	Swarna × *Oryza nivara* (IRGC81832)	140	RM206, RM19, RM247, RM11, RM279, RM231, RM237, RM495	Balakrishnan *et al.,* 2020
3.	60 Rice genotypes, including 48 NPTs	66	RM154, RM489, RM6100	Donde *et al*., 2020
4.	Ali-Kazemi × Kadous	65	RM490, RM2318, RM6314, RM19	Khatibani *et al*., 2019
5.	Swarna × *O. nivara* (IRGC81832)	100	RM337, RM247	Swamy *et al*., 2018
6.	MAS25 (Aerobic rice) × IB370 (Lowland Basmati)	70	RM 154, RM424	Meena *et al*., 2018
7.	177 rice varieties	261	RM140	Liu *et al*., 2017
8.	Swarna × *O. nivara* ILs, KMR3 × *O. rufipogon* ILs, mutants of Nagina 22	49	RM489	Prasanth *et al*., 2017
9.	196 $F_{2:4}$ lines Sepidrood × Gharib (*Indica* varieties)	105	RM1, RM237, RM154	Rabiei *et al*., 2015
10.	176 RILs of Azucena × Moromutant (*O. sativa*)	26	RM152	Bekele *et al*., 2013
11.	Madhukar and Swarna	101	RM152, RM231, RM279, RM488, RM490	Anuradha *et al*., 2012

polymorphic on chromosome 5, 7 and 11 between Swarna and 148s were polymorphic between 166s and 148s also except, RM7583 on chromosome 6, RM337 on chromosome 8, RM6100 and RM228 on chromosome 10 and RM19 on chromosome 12. All other markers were polymorphic between Swarna and 148s showed polymorphism between 166s and 148s also.

Out of the nine markers on chromosome 1, showing polymorphism between Swarna and 148s, seven markers were also polymorphic between Swarna and 166s. Twelve markers on chromosome 2 and five markers on chromosome 6 were common for Swarna/148s and Swarna/166s parents. RM258, RM6100, RM228 and RM25262 on chromosome 10 and RM26652, RM206, RM26998 and RM27154 on chromosome 11, between Swarna/148s were polymorphic between Swarna/166s also.

Swamy *et al.,* 2018 identified 100 polymorphic markers between Swarna and the wild rice *O. nivara* (IRGC81832) and mapped QTLs for grain iron and zinc. They reported that RM517, RM223, RM 81A, RM256, RM264, RM287 and RM209 were polymorphic between Swarna and *O. nivara* and were also associated with grain iron and zinc QTLs. Balakrishnan *et al*., 2020 identified 140 SSRs as polymorphic among 165 SSRs for a set of 90 back cross lines at BC_2F_8 generation derived from Swarna x *Oryza nivara* (IRGC81832) and screened for yield traits and identified a set of 70 CSSLs covering 94.4% of *O. nivara* genome. Chandu *et al*., 2020 reported the SSR markers for grain iron, zinc and yield-related traits

polymorphic between Samba Mahsuri (BPT5204) and a wild rice *Oryza rufipogon*. 149 SSR markers out of 750 showed polymorphism (19%). Prasanth *et al*., 2017 reported that, nine markers (RM243, RM517, RM225, RM518, RM525, RM195, RM282, RM489, and RM570) on chromosomes 1, 2, 3, 4, 6, and 8 showed associations with six yield traits under heat stress conditions.

Surapaneni *et al*., 2017 using 94 BILs of Swarna/ *Oryza nivara* (IRGC81848) mapped QTLs associated with yield and related traits and identified fifteen QTLs. BILs were genotyped using 111 polymorphic SSRs distributed across the genome. Rabiei *et al*., 2015 constructed a linkage map using 105 SSRs and 107 AFLP markers. A total of 28 QTLs were mapped for 11 traits of yield, yield component and plant morphology. Bekele *et al*., 2013 identified SSR markers associated with grain zinc concentration and other yield-related traits using 176 RIL population and reported that RM 152 was associated with days to 50 % flowering and grain yield other than grain zinc concentration. In other study in 2012 Anuradha *et al*. identified 22% polymorphism by using 101 SSRs between parents, Madhukar and Swarna.

Among the polymorphic markers identified in present study; RM1, RM11, RM19, RM140, RM152, RM154, RM206, RM231, RM232, RM237, RM247, RM279, RM337, RM424, RM488, RM489, RM490, RM495, RM2318, RM2634, RM3747, RM5638, RM6100, RM6314, RM13630, RM14303 and RM24382 were significantly associated with QTLs for grain yield and yield-related traits (Balakrishnan *et al*., 2020; Chandu *et al*., 2020; Donde *et al*., 2020; Khatibani *et al*., 2019; Swamy *et al*., 2018; Meena *et al*., 2018; Liu *et al*., 2017; Prasanth *et al*., 2017; Rabiei *et al*., 2015; Bekele *et al*., 2013; Anuradha *et al*., 2012). The previous studies in which these SSRs were polymorphic are presented in Table 3.

Daware *et al*., 2016 validated 4048 amplified SSR markers, identified 3819 (94.3%) markers to be polymorphic and generated a high-density genetic linkage map in rice using a population of IR 64 × Sonasal *ie.,* high and low grain weight accessions respectively. Donde *et al*., 2020 reported that, out of 85 SSRs screened from a study to identify QTLs associated with grain yield and related traits 66 were polymorphic (77.65%). They identified fifteen SSRs were associated with grain yield and reported RM154 and RM489 amplified more than two alleles. In the present study RM154 showed polymorphism between Swarna/166s, RM489 was polymorphic between Swarna/148s and RM6100 showed polymorphism in both Swarna/166s and Swarna/148s parents. Meena *et al*., 2018 identified QTLs related to grain yield, *qGYP2.1* was on chromosome 2 flanked between RM154 and RM424. Khatibani *et al*., 2019 evaluated 157 RILs derived from a cross between two Iranian rice cultivars Ali-Kazemi and Kadous and constructed a linkage map and out of seven QTLs, four QTLs for two traits were consistently flanked by RM23904 and RM24432 on chromosome 9. This is confirming that polymorphic markers identified in this study will be useful to tag associated QTLs for yield and related traits in the mapping populations.

CONCLUSION

The 88, 50 and 54 polymorphic markers identified between Swarna/166s, Swarna/148s and 166s/148s on the 12 chromosomes of rice are useful in mapping QTLs for yield and any related traits in the mapping populations derived from these cross combinations.

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