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Molecular screening of yield component QTLs for strong culm, grain number and grain width using gene specific markers in *indica- tropical japonica* derived rice lines

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ABSTRACT

Rice varieties with 20% yield advantage over present day popular varieties have to be developed in order to meet the growing demand. Yield is a polygenically controlled complex trait. Three major characters like high grain number, grain weight and strong culm are necessary in order to increase the yield potential of rice. In this study, ninety five indica-tropical japonica derived rice lines were screened for the presence of yield QTLs, the main objective being identification of donor lines to improve the popular varieties by marker-assisted breeding programme. Three functional markers namely Gn1a, GW2 and SCM2 were used to validate the presence of the yield QTLs. Thirty six genotypes namely CR2674-24-1-1-1, CR2674-24-1-2-3-1, CR2688-6-3-1-1-2, CR2667-1-9-5-1-1, CR2667-1-9-5-2-1, CR2667-9-2-4-1-1, CR2667-9-2-4-1-1-2, CR2683-46-5-6-1-1-1, CR2683-46-5-6-1-1-1-2-1, AC38700, AC 38790, AC38687, CR-978-3-2, CR-2080-16-17-6-2-1, CR-2251-3-4-3-1, CR-2608-5-3-1-1, CR-2683-45-1-2-2, CR3813-3-3-1-1-1, CR3820-1-7-1-1-1, CR3820-5-3-1-1-1, CR2683-45-1-2-1-1, CR2683-45-1-2-1-2, CR2682-1-1-5-1-2, CR2683-46-5-8-3-3, CR2683-46-5-8-3-1, CR2683-46-5-8-3-2, CR 3696-5-5-2-2-1, CR 2682-1-1-5-1-1, CR2687-13-5-7-1-2, CR 2681-5-2-1-1-1, CR2683-46-5-2-1-1, CR 3818-1-1-1-1-1, CR3598-1-4-2-1-1, CR 2678-2-1-1-1-1, CR3696-5-5-2-1-1 and CR2683-46-5-9-1-1 showed the presence of three yield QTLs for high grain number (Gn1a), strong culm (SCM2) and grain weight and width (GW2). These three QTLs may contribute significantly towards yield enhancement of rice. However,phenotypic confirmation is required because of wide allelic variations in the traits under study.

Key words: Yield potential, yield QTLs, QTL - validation

Rice is the staple food for the largest number of people on Earth. The global rice production has to be enhanced by 70% by 2050 to meet the demand of growing population (FAO 2009; NRRI 2013). The current rice production of India is 105 million tons per annum that needs to be enhanced to 150 million tons by coming 30 years (NRRI, 2013). To achieve the target yield level, rice varieties with a yield advantage of about 20% over widely grown varieties must be developed with higher yield potential. The yield potential is defined as the yield of a variety when grown in environments to which it is most adapted, with nutrients and water non limiting and pests and diseases and other stresses effectively controlled (Evans, 1993).

Yield is a polygenically controlled complex trait. A large number of yield QTLs have been identified during last few decades of which more than 20 QTLs directly affecting rice grain yield and its components have been cloned (Bai *et al.* 2012). The cloned QTLs are grain number (*Gn1a, Ghd7, DEP1, DEP3 and Ghd8/DTH8*), grain weight (*GS3* and *GW2*), grain size (*GS3, GS5, GW2, SRS3, SP1* and *GW5*), grain filling (*GIF1, FLO2*), panicle number (*DEP1* and *WFP*), culm strength (*SCM2*), harvest index (*APO1*), plant architecture (*OSSPL14*) and Narrow leaf (*Nal1/2/3/*

5). The functional analysis of *Nal1/2/3* showed that they play a role in regulating vein patterning (Ishiwata *et al.*2013) and adventitious root development at the transcriptional level (Sung-Hwan *et al*. 2014). *Nal1* otherwise known as SPIKE has been reported to increase grain yield in *indica* cultivars (Fujita *et al.* 2013). It controls leaf width and plant height through its effects on cell division (Jiang *et al.* 2015). Attempts are to be made for accumulation of these QTLs in a single background to study their interaction in positive or negative direction. Ashikari *et al*. (2005) were able to increase grain number by 45% while reducing plant height by 20% by combining the grain number QTL (*Gn1a*) and the semi dwarfing gene (*sd1*) using QTL pyramiding strategy. Accumulation of the favorable yield QTLs in a better genetic background would be a way forward for rice journey.

Grain weight is one of the most important components of grain yield and is controlled by quantitative trait loci (QTLs) derived from natural variations in crops. Song *et al.* (2007) reported cloning and characterization of *GW2* QTL that controls rice grain width and weight. *GW2* encodes a RING-type protein with E3 ubiquitin ligase activity, which is known to function in the degradation by the ubiquitin-proteasome pathway. Loss of *GW2* function increased cell numbers, resulting in a larger (wider) spikelet hull, and it accelerated the grain milk filling rate, resulting in enhanced grain width, weight and yield.

Higher yields of rice have always been a predominant goal in rice breeding techniques. Three genes *Gn1a* for grain number per panicle and *GS3* and *GW2* for grain weight have been shown a potential in improving the rice yield level (Yan *et al*. 2009). Grain weight is a major determinant of crop grain yield and is controlled by naturally occurring quantitative trait loci (QTLs). *Gn1a* is a gene for cytokinin oxidase/ dehydrogenase (OsCKX2), an enzyme that degrades the phytohormone cytokinin. Reduced expression of OsCKX2 causes cytokinin accumulation in inflorescence meristems and increases the number of reproductive organs, resulting in enhanced grain yield (Ashikari *et al*. 2005). Ookawa *et al*. (2010) used chromosome segment substitution lines to identify the QTL *SCM2*. A near-isogenic line carrying *SCM2* showed enhanced culm strength and increased spikelet number because of the pleiotropic effects of the gene.

The identification of the genotypes possessing lodging-resistance (*SCM2*), grain number (*Gn1a*) and grain width (*GW2*) genes will be useful to develop improved lines through marker assisted backcross breeding to increase the overall productivity in rice cultivars. The present study aims to identify potential donor lines having *SCM2*, *Gn1a* and *GW2* genes, which in turn can be used for developing lodging resistant varieties having better yield potential.

MATERIALS AND METHODS

Plant materials

Ninety five *indica-tropical japonica* derivatives of rainfed lowland rice preserved in rice gene bank of "National Rice Research Institute" were grown in big trays in RGA-cum-Phytotron facility chamber under controlled condition. Thirty days old seedlings were transplanted in field and grown following standard agricultural practices.

DNA Isolation

Leaves were collected from 30 days-old seedlings to extract genomic DNA for molecular screening of yield QTLs among the genotypes. Total genomic DNA was extracted after crushing the sample in liquid nitrogen in microfuge tubes using Cetyltrimethyl ammonium bromide (CTAB) extraction buffer (100mM Tris–HCl pH 8, 20mM Ethylene diaminetetra acetic acid (EDTA) pH 8, 1.3MNaCl, 2% CTAB) and by chloroform–isoamyl alcohol extraction followed by RNase treatment and ethanol precipitation (Murray and Thompson 1980). Agarose gel electrophoresis was used to estimate DNA concentration and each sample was then diluted to approximately 30ng/µl.

PCR amplification and visualization of markers linked to yield QTLs

PCR amplification of rice varieties were done with gene specific primers for the QTLs *SCM 2*, *Gn1a* and *GW2*. DNA amplification reaction was performed in a volume of 20 µl containing 1.5mM Tris–HCl (pH 8.75), 50mM KCL, 2mM MgCl2, 0.1% Triton X-100, 200mM each of Deoxyadenosine triphosphate (dATP), Deoxycytidine triphosphate (dCTP), Deoxythymidine triphosphate (dTTP), Deoxyguanosine triphosphate (dGTP), 4 pmol of each forward and reverse primers (Table 2), 1 unit of Taq Polymerase and 30ng of

Table 1. List of primers used for the study

Trait	Marker name	Sequence	Annealing temp.	Amplicon size
High grain Number	Gn1aM2(F) Gn1aM2 (R)	5' TGAGGATGCCGTGGAAGACGA 3' 5' TTCGTGTTCGCGCAGGACGT 3'	55	180bp
Grain Width and Weight	GW2(F) GW2 (R)	5' CCAATAAAGATGTCCATTCTGTTA 3' 5' GCTCTTCCTGTAACACATATTATG 3'	55	180bp
Strong Culm	SCM 2 (F) SCM 2 (R)	5' ATTCAGATCAATAGGTTGAGTGT 3' 5' TGCTATGTATATCCTATCGGTTC 3'	58	172bp

genomic DNA. Amplification was performed in a Programmable Thermal Cycler (Veriti; Applied Biosystems, Life technologies, Singapore). The reaction mixture was first denatured for 4 min at 94ºC and then subjected to 35 cycles of 1 min denaturation at 94ºC, 1 min annealing at 55-58ºC (Table 1) and 1 min extension at 72ºC, and then a final extension for 10 min at 72ºC. Aliquots of 10 µl of DNA products from PCR amplification were loaded in 2.5% agarose gel containing 0.8 µ g/ml Ethidium Bromide for electrophoresis in 1X Tris-Borate-EDTA (TBE) (pH 8.0). A 50 bp DNA ladder was used for determination of size of amplicons. The gel was run at 120v (2.5 V/cm) for 3 hour and photographed using a Gel-Doc System (SynGene).

Data analysis

The data were scored as 1/0 for presence/ absence of the target genes for each primer-variety combination. Data were analyzed and similarity matrix was constructed from binary data with Jaccard's coefficients and cladogram was generated with unweighted pair group method arithmetic average (UPGMA) algorithm using FreeTree software (Hampl *et al*. 2001; Pavalicek *et al*. 1999) and the dendrograms were visualized by Treeview 32 software (Page 1996).

RESULTS AND DISCUSSION

The amplification of genomic DNA of ninety five genotypes was performed using gene based direct markers for grain number, strong culm, grain width and weight. Gene specific marker *Gn1a* screens for the presence of higher grain number in a rice genotype. *SCM2* indicates the presence of strong culm in the studied genotypes. *GW2* amplification shows presence of low grain width. Thirty six genotypes namely CR2674-24-1-1-1, CR2674-24-1-2-3-1, CR2688-6-3-1-1-2, CR2667-1-9-5-1-1, CR2667-1-9-5-2-1, CR2667-9-2-4-1-1, CR2667-9-2-4-1-1-2, CR2683-46-5-6-1-1-1, CR2683-46-5-6-1-1-1-2-1, AC38700, AC 38790, AC38687, CR-978-3-2, CR-2080-16-17-6-2-1, CR-2251-3-4-3-1, CR-2608-5-3-1-1, CR-2683-45-1-2-2, CR3813-3-3-1-1-1, CR3820-1-7-1-1-1, CR3820-5-3-1-1-1, CR2683-45-1-2-1-1, CR2683-45-1-2-1-2, CR2682-1-1-5-1-2, CR2683-46-5-8-3-3, CR2683-46-5-8-3-1, CR2683-46-5-8-3-2, CR 3696-5-5-2-2-1, CR 2682-1-1-5-1-1, CR2687-13-5-7-1-2, CR 2681-5-2-1-1-1, CR2683-46-5-2-1-1, CR 3818-1-1-1-1-1, CR3598-1-4-2-1-1, CR 2678-2-1-1-1-1, CR3696-5-5-2-1-1 and CR2683-46-5-9-1-1 exhibited specific bands and may possess all the three QTLs namely *Gn1a*, *GW2* and *SCM2* which have the potential to be used as donors in molecular breeding programme (Table 2).

Grain number is an important trait for higher grain yield in rice. The QTL *Gn1a* enhances grain yield significantly whenever the allele present in the rice genotype. Hence, in the present study, it was taken up for surveying its presence in the study materials. The QTL *Gn1a* is a functional gene which have been cloned and functionally characterized by Song *et.al.* (2007). The distribution of *Gn1a* allele in the population is very important as it increases grain yield and finally total rice production. The present study revealed that 43.2% of the genotypes *i.e.* 41 genotypes out of 95 in the studied material were having the said QTL *Gn1a* (Table 2; Figure 1a,b). Earlier study of Yan *et.al.* (2009) indicated that presence of *Gn1a* allele contributed 54% in *indica* and 21% in *japonica* cultivars towards variance of the trait.

Besides grain number, grain width also plays important role for increasing grain yield in rice. High grain width increases yield while low grain width increases grain quality of rice. Here, in the present experiment, *GW2* is a functional gene which indicates the presence of low grain width in rice. In the present

Table 2. Response of genotypes to the gene based markers used for different QTLs for yield component traits.

Sl.no.	Genotype name	*SCM 2*	*Gn1a*	*GW2*
1	CR2674-24-1-1-1	+	+	+
2	CR2674-24-1-2-3-1	+	+	+
3	CR2673-4-1-1-5-1	+	-	+
4	CR2687-9-2-1-1-1	+	-	+
5	CR2688-6-3-1-1-2	+	+	+
6	CR2688-6-3-1-1-3	+	-	+
7	CR2667-1-9-5-1-1	+	+	+
8	CR2667-1-9-5-2-1	+	+	+
9	CR2667-1-9-5-1-2	+	-	+
10	CR2683-46-5-5-2-1	+	-	+
11	CR2667-9-2-4-1-1	+	+	+
12	CR2667-9-2-4-1-1-2	+	+	+
13	CR2683-46-5-6-1-1-1	+	+	+
14	CR2683-46-5-6-1-1-1-2-1	+	+	+
15	CR2683-46-5-6-1-1-1-2-2	+	-	+
16	CR2688-2-3-1-1-2	+	-	+
17	CR2673-4-1-1-4-1	+	+	-
18	AC38700	+	+	+
19	AC 38790	+	+	+
20	AC38687	+	+	+
21	AC 38605	+	+	-
22	AC38599	+	+	-
23	AC38586	+	+	-
24	CR-978-3-2	+	+	+
25	CR-780-1937-1	+	-	+
26	CR-2080-16-17-6-2-1	+	+	+
27	CR-2251-1-1-1-1-1-1-1	+	-	+
28	CR-2251-3-4-3-1	+	+	+
29	CR-2274-13-1-2-2-1	+	-	+
30	CR-2274-2-3-1	-	-	+
31	CR-2304-5-7-2-3-1	+	-	+
32	CR 3815-1-1-1-1	+	-	+
33	CR-2340-4-1-1-1	+	-	+
34	CR-2608-5-3-1-1	+	+	+
35	CR-2652-14	+	-	-
36	CR-2675-10-2-1-1	+	-	+
37	CR-2676-15-10-3-2-2	+	-	+
38	CR-2676-19-10-3-2-1	+	-	-
39	CR-2681-23-3-1-1	+	-	-
40	CR- 2681-23-1-1	+	-	-
41	CR-2681-2-1-1-1-2	+	-	-
42	CR-2682-7-1-1-1(MS)	+	-	+
43	CR-2682-7-1-2-1	+	-	+
44	CR-2682-7-1-1-1(MB)	+	-	+
45	CR-2682-4-10-5-2	+	-	+
46	CR-2682-7-2-1	+	-	+
47	CR-2683-2-1-1	+	-	-
48	CR-2683-4-5-1-2	+	-	+
49	CR-2683-10-9	+	-	+
50	CR-2683-13-5-3-1	+	-	+
51	CR-2683-35-2-1-1	+	-	+
52	CR-2683-28-45-1-4	+	-	+
53	CR-2683-15-5-3-1	+	-	-
54	CR-2683-28-12-1-4	+	-	+
55	CR-2683-35-1-1-1	+	-	-
56	CR-2683-45-1-2-2	+	+	+
57	CR-2683-45-1-2	+	-	+
58	CR-2683-48-1-2	+	-	+
59	CR-2687-4-3-5-2-1	+	-	-
60	CR-2690-6-1-1-1-2	+	-	+
61	CR2274-2-1-1-1-1	+	-	+
62	CR3813-4-3-2-1	+	-	+
63	CR3813-5-2-1-1	+	-	+
64	CR3697-4-4-2-3-1	+	-	+
65	CR 3813-4-4-4-3-1	+	-	+
66	CR 3697-3-2-2-1-1	+	-	+
67	CR3813-3-3-1-1-1	+	+	+
68	CR3697-4-2-4-1-5	+	-	+
69	CR3697-2-2-5-1-1	+	-	+
70	CR3697-4-7-1-1-1	+	-	+
71	CR 3813-4-10-1-1-1	+	-	+
72	CR3820-1-2-1-2-1	+	-	-
73	CR3820-1-2-1-1-1	+	-	+
74	CR3820-1-7-1-1-1	+	+	+
75	CR3820-5-3-1-1-1	+	+	+
76	CR3820-2-1-5-1-2	+	-	+
77	CR2683-45-1-2-1-1	+	+	+
78	CR2683-45-1-2-1-2	+	+	+
79	CR2682-1-1-5-1-2	+	+	+
80	CR2683-46-5-8-3-3	+	+	+
81	CR2683-46-5-8-3-1	+	+	+
82	CR2683-46-5-8-3-2	+	+	+
83	CR 3696-5-5-2-2-2	+	-	-
84	CR 3696-5-5-2-2-1	+	+	+
85	CR 2682-1-1-5-1-1	+	+	+
86	CR2687-13-5-7-1-2	+	+	+
87	CR 2681-5-2-1-1-1	+	+	+
88	CR2683-46-5-2-1-1	+	+	+
89	CR 3605-4-2-1-1-1	+	-	+
90	CR 3818-1-1-1-1-1	+	+	+
91	CR3598-1-4-2-1-1	+	+	+
92	CR2682-1-1-5-1-2	+	-	+
93	CR 2678-2-1-1-1-1	+	+	+
94	CR3696-5-5-2-1-1	+	+	+
95	CR2683-46-5-9-1-1	+	+	+

experiment, 85 genotypes were observed to possess *GW2* gene in the composition of the material (Table 2; Figure 1 c,d). Zhang *et al.* (2013) and Gao *et al.* (2015) reported *GW2* controls grain width and weight in *japonica* rice. However, in *indica* rice a lot of allelic variants exist for *GW2* where many single nucleotide polymorphisms (SNPs) and Insertion-deletions (InDels) exist leading to variation in the trait (Dixit *et al.* 2013).

Strong culm is an important trait of rice plant which provides non-lodging character to plant. Any plant devoid of strong culm may not be able to bear a heavy

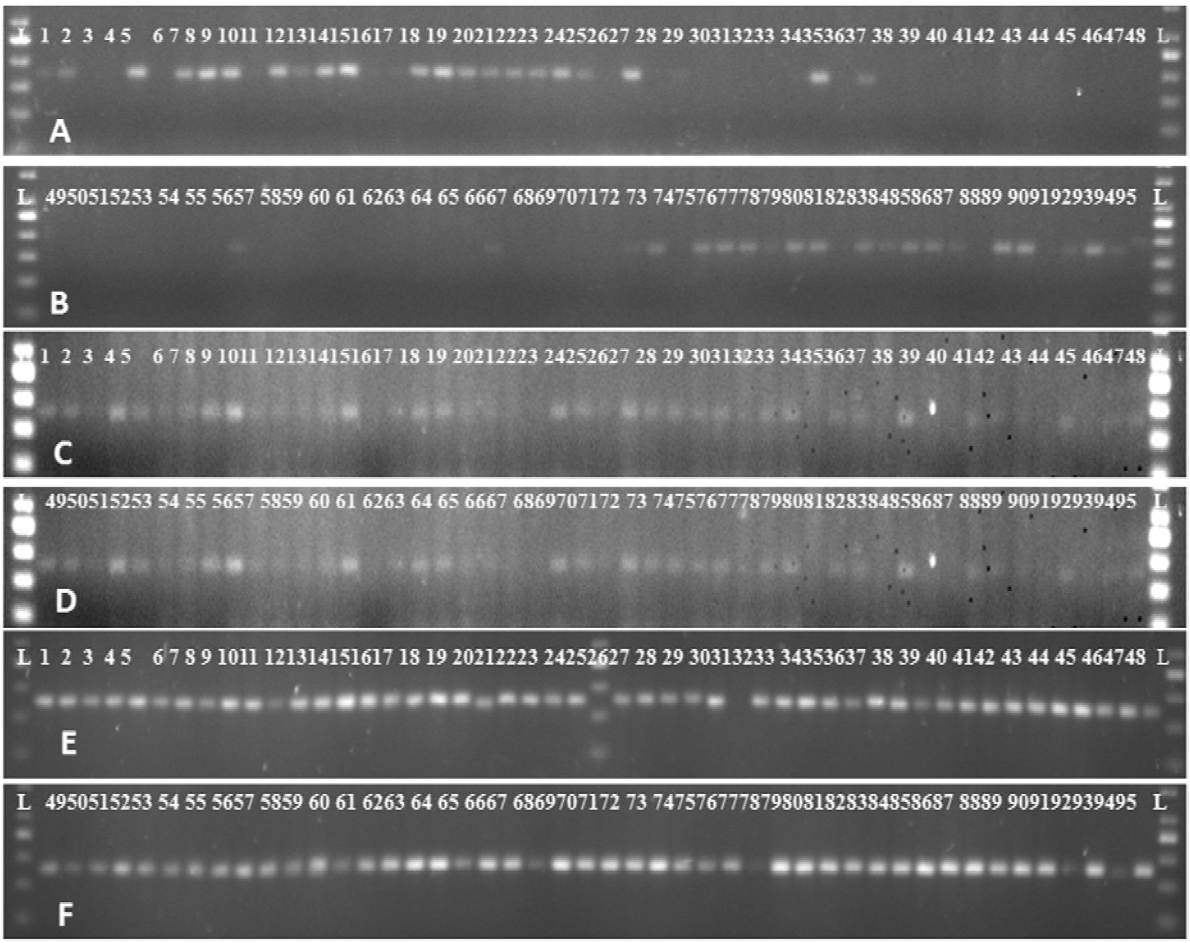

Fig. 1. Representative amplification pattern with yield QTL markers *Gn1a* (A,B), *GW2* (C,D) and *SCM2* (E,F). The numbers represent the genotyped given in Table 2. L: 50bp DNA ladder.

panicle load which ultimately may lodge leading to a great loss. The present experiment results indicated that ninety four genotypes were having *SCM2* gene (Table 2, Figure 1 e,f). Hence, our selection method for strong culm is effective as presence of *SCM2* QTL imparts sturdy culm to the plant. Ookawa *et al.* (2010) reported that the presence of *SCM2* provided strong culm to the rice genotype.

The clustering analysis based on molecular analysis could group the genotypes into five distinct clusters (Figure 2). The thirty six genotypes possessing the three genes *GW2*, *SCM2* and *Gn1a* could be grouped together in cluster III. The cluster II consisted forty three genotypes having all the QTLs except *Gn1a*, whereas cluster IV consisted only four genotypes lacking *GW2* QTL. The genotype CR2274-2-3-1 that possessed only *GW2* QTL, solely formed cluster I. Cluster V consisted eleven genotypes having only *SCM2*.

No genotype was observed that showed positive response for only *Gn1a* (Table 2 Figure 1 and 2). There may be combinatorial effect of these QTLs/ genes leading to increased yield potential of the genotype. The presence of *Gn1a* leads to increase in spikelet number (Song *et al.* 2007; Yan *et al.* 2009) that needs a strong culm to bear high number of grains. Similar correlation is expected between high grain weight and strong culm. The present investigation also confirms the idea because out of the ninety five genotypes studied, thirty six possessed *GW2+SCM2+Gn1a* combination, forty three possessed *GW2+SCM2* combination, whereas only four lines with *SCM2+Gn1a* combination. This indicates that wherever

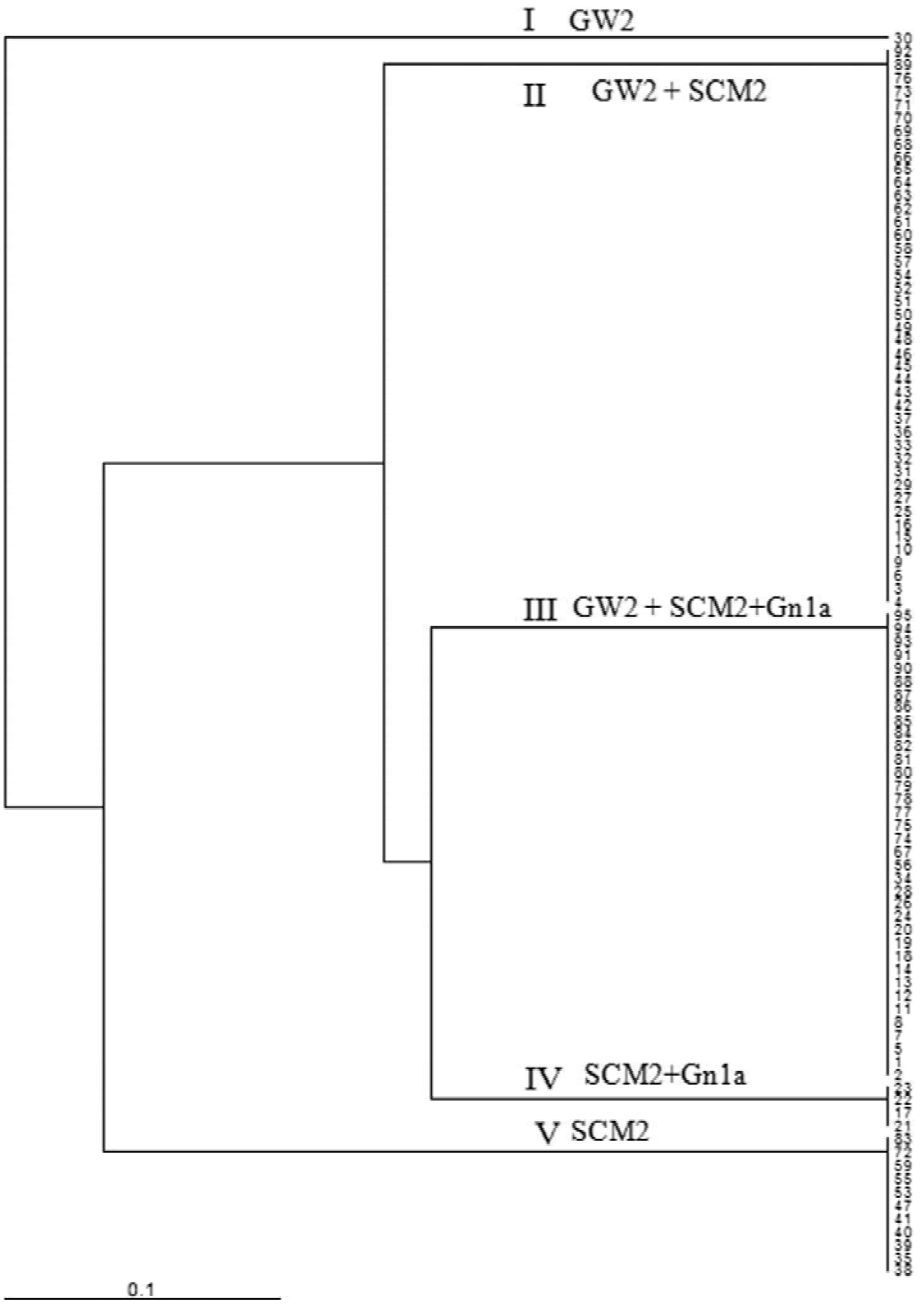

Fig. 2. Dendrogram of the ninety five genotypes with respect to the markers used for QTLs for yield component traits.

high grain weight is present, strong culm is required. In addition, if grain number is increased along with weight, then strong culm is essential. But increasing only grain number without increase in grain weight may not show a positive correlation with presence of strong culm as evident from the present study where only 0.42% genotypes showed presence of *Gn1a+SCM2*.

The genotypes CR2674-24-1-1-1, CR2674-24-1-2-3-1, CR2688-6-3-1-1-2, CR2667-1-9-5-1-1, CR2667-1-9-5-2-1, CR2667-9-2-4-1-1, CR2667-9-2-4-1-1-2, CR2683-46-5-6-1-1-1, CR2683-46-5-6-1-1-1-2-1, AC38700, AC 38790, AC38687, CR-978-3-2, CR-2080-16-17-6-2-1, CR-2251-3-4-3-1, CR-2608-5-3-1-1, CR-2683-45-1-2-2, CR3813-3-3-1-1-1, CR3820-1-

7-1-1-1, CR3820-5-3-1-1-1, CR2683-45-1-2-1-1, CR2683-45-1-2-1-2, CR2682-1-1-5-1-2, CR2683-46-5-8-3-3, CR2683-46-5-8-3-1, CR2683-46-5-8-3-2, CR 3696-5-5-2-2-1, CR 2682-1-1-5-1-1, CR2687-13-5-7-1-2, CR 2681-5-2-1-1-1, CR2683-46-5-2-1-1, CR 3818-1-1-1-1-1, CR3598-1-4-2-1-1, CR 2678-2-1-1-1-1, CR3696-5-5-2-1-1 and CR2683-46-5-9-1-1 having the QTL combination of *GW2*, *SCM2*, and *Gn1a* may be the candidate donors to be used in marker- assisted backcross breeding programmes for improving the yield component traits. However, phenotypic confirmation is required as there is a wide variation in the traits under study. The markers used in the present investigation are gene based markers and hence are very good for molecular screening and in marker-assisted breeding programme.

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