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Molecular breeding of "Swarna," a mega rice variety for lodging resistance

Girija Rani Merugumala · Satyanarayana P.V · Chamundeswari Narne · Ravikumar B.N.V.S.R · Ramana Rao P.V · Pavani L. · Deepika V

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Abstract Lodging of the crop is one of the major constraints in cyclone-prone coastal rice areas. The feasibility of conventional breeding approaches for lodging resistance is limited due to high influence of structural and weather parameters. Stem lodging is the major risk for irrigated low land areas. In the present study, F_2 population derived from the cross between lodging-susceptible Swarna variety and lodging-resistant advanced breeding line MTUII 110-9-1-1-1-1 was used for QTL mapping. A total of five QTLs were identified for culm diameter, culm thickness, culm strength, bending stress, and panicle length. The phenotypic response and QTLs identified from the above population elucidate that culm diameter and culm thickness play key role in lodging resistance. Gene prediction in the mapping region of culm diameter and thickness on chromosome 6 revealed that gene sets of microtubule-based movement LOC_0s06g45900 and potassium transporter LOC_0s06g45940 might be putative candidate genes responsible for lodging resistance. Near isogenic line (NIL) with wider and thick culm in Swarna background was developed using marker-assisted backcross breeding. Developed lodging resistant line in the background of widely adopted mega rice variety Swarna would enhance the rice productivity even under adverse climatic conditions.

Keywords Quantitative trait loci · *Oryza sativa* L. · Lodging resistance · Marker-assisted breeding · Crop productivity

G. R. Merugumala (✉) · S. P.V · C. Narne · R. B.N.V.S.R · R. P.V · P. L. · D. V
Regional Agricultural Research Station, Acharya N G Ranga Agricultural University, Maruteru-534122, West Godavari District, AP, India
e-mail: girija_aprri@yahoo.co.in

Introduction

Rice (*Oryza sativa* L.) is an important staple food for more than half of the world population especially in South Asia. Realization of sustained yields under changing climatic conditions with shrinking resources to feed ever-increasing population is a major challenge to the rice scientists. Among the abiotic stresses, lodging of the crop at reproductive and maturity stages in cyclone-prone areas is one of the major problems hampering rice productivity. Lodging can be defined as displacement of stem from vertical position; it is either permanently or partially reversible based on extent of bending. Stem lodging is a major risk in irrigated low land ecosystem. Two types of stem lodging exist and they are the breaking and bending type. Breaking type lodging is most prevalent in coastal irrigated ecosystem where lodging occurs due to breakage of lower internodes and bending type of lodging exists in low land rice areas (Hirano et al. 2017). Lodging in rice plants not only reduces grain yield, but also deteriorates grain quality (Setter et al. 1997) and up to 40% yield loss was reported (Nishiyama 1986).

Breeding for lodging resistance using conventional methods is quite difficult as it is influenced by both structural properties of the stem and weather parameters. Crop management practices like excess nitrogen fertilizer application enhances lodging (Hashem et al. 2016) and results in reduction in structural carbohydrates of culm and also lignin deposition in secondary cell wall of lower internodes of culm (Zhang et al. 2016). Incidence of brown planthopper (BPH) and sheath blight weakens the lower internodes (Wu et al. 2012). Climate changed conditions like higher CO_2 enhances the risk of stem lodging (Zhu et al. 2013).

In the early 1960s, incorporation of *sd-1* gene from the Chinese variety Dee-geo-woo-gen in traditional varieties minimized the lodging risk to certain extent (Khush 1999). But still it remains a major problem even in semi-dwarf varieties because of weak culm characteristics viz. narrow culm, and elongated internodes. For instance, semi-dwarf mega rice variety Swarna is highly vulnerable to lodging by virtue of lower culm diameter (Girija Rani et al. 2017).

Although the traditional target was reduction of height, the other important traits contributing to lodging resistance are culm characteristics viz. basal internodal length, culm diameter at 4th internode, culm wall thickness, bending stress, culm strength, leaf sheath wrapping and thickness, and linear density of culm (Chang and Vergara 1972; Matsuda et al. 1983; Kashiwagi and Ishimaru 2004; Zhong et al. 2007; Zhu et al. 2016; Rao et al. 2017). Breaking type lodging resistance was improved due to increased lignin accumulation and culm diameter (Okuno et al. 2014). Silicified cells provide strength to culm by upregulation of cinnamyl alcohol dehydrogenase (CAD), a key gene responsible for lignin biosynthesis (Dorairaj and Ismail 2017). The physical strength was positively and highly significantly correlated with the total amount of potassium and silicon in culm during grain filling, and total amount of soluble sugars in culm at the full heading and milky stages (Zhang et al. 2010; Yan-Hua et al. 2011). Re-accumulation of stem non-structural carbohydrates (NSC) in the later period of grain filling related to slower senescence and is correlated with lodging resistance (Kashiwagi et al. 2006; Takashi et al. 2017). Thickened secondary cell walls with higher cellulose levels in the mature plants enhances the mechanical strength (Fan et al. 2017). Leaf sheath can delay the aging of stem and increase the strength of the stem (Kashiwagi et al. 2008). Involvement of epsistatic gene interactions for lodging resistance–related traits was reported (Girija Rani et al. 2015) and marker-assisted selection would help in fixation of favorable alleles involved in epsistatic interactions (Fethi et al. 2011). Phenotypic evaluation for lodging resistance requires visual estimates in plots of breeding lines and genetic variability is too high in early generations.

Marker-assisted breeding is one of the viable strategies to develop lodging-resistant rice varieties. Quantitative trait loci (QTL) having major effects for culm traits viz. basal culm thickness, culm length and culm strength (Mu et al. 2004), pushing resistance (Kashiwagi and Ishimaru 2004), and basal culm traits (Hu et al. 2008; Zhu et al. 2008) would be useful in selection of non-lodging lines in early generations. Effective quantitative trait loci for culm diameter, *Strongculm2* (*SCM2*) on chromosome 6 was identified (Ookawa et al. 2010) and this QTL has role in gene regulation on vascular development and first branch of panicle to affect the yield (Terao et al. 2010). QTL for leaf sheath length in vicinity of *SCM2* (Liu et al. 2011); QTLs for strong culm on chromosome 1, 5, 6, 8, and 11 (Yamamoto et al. 2013); physical strength of the upper culms (Kashiwagi 2014), Strongculm 3(*SCM3*), and conferring culm strength (Yano et al. 2015) were identified by earlier workers. Leaf star rice variety with superior lodging resistance possessing gold hull and internode2 (*gh2*) on chromosome 2 was developed by Ookawa et al. (2014). QTL preventing culm strength deterioration after grain filling *BSUC11* (Kashiwagi et al. 2016) and two major effect QTLs, *qCD1.1* and *qCS1.1*, on chromosome 1 (Yadav et al. 2017) were associated with lodging resistance. QTLs for the density of hemicellulose, cellulose, and holocellulose cell wall materials in *japonica* varieties contributing to increased bending stress were reported by Mulsanti et al. 2018.

The present study aimed to identify new QTLs for lodging resistance traits and to incorporate strong culm traits in highly susceptible mega rice variety Swarna using marker-assisted breeding besides adopting phenotypic techniques for lodging resistance.

Materials and methods

Selection of the parents and generation of mapping population

Swarna, a mega rice variety developed at Regional Agricultural Research Station (RARS) is being cultivated nearly in 5 million ha worldwide. Swarna is an *indica* variety developed by pedigree method using Vasista and Mahsuri as parents. It is a semi-dwarf erect plant type with 110 cm plant height, dark green foliage, completes its life cycle in 150 days, medium slender brown glume grain, intermediate amylose content, and is widely adopted by farmers because of its high yielding capacity under low input management. Swarna, a highly lodging-susceptible variety was used as recipient parent and an advanced breeding line MTUII 110-9-1-1-1-1 (Girija Rani et al. 2017) was used as donor for lodging resistance. Swarna was crossed with MTUII 110-9-1-1-1-1 and F_1 was selfed to generate F_2 population. One hundred eighty individual F_2 plants were selfed to generate respective F_3 families. Simultaneously, some of F_1 plants were backcrossed with the recurrent parent Swarna three times followed by four generations of selfing to generate NILs of Swarna with lodging resistance (Fig. 1).

F_1 and backcross F_1 seedlings (25 days old) were transplanted to with spacing of 30 cm between rows and 30 cm between plants to assess maximum expression of traits, whereas F_2, F_3 families, and advanced backcross progenies were transplanted at 20 cm between rows and 15 cm between plants. Fertilizer application of 90:60:60 kg/ha of nitrogen, phosphorous, and potassium was practiced. Plant protection against sheath blight, BPH, and stem borer was practiced.

Phenotypic evaluation for lodging resistance

The present study was carried out at RARS, Maruteru, Andhra Pradesh, India, located in typical coastal irrigated ecosystem where cyclones are most prevalent especially during the maturity to harvesting stages. The rainfall data at reproductive stage from 2012 to 2017 was furnished in Table 1.

A total of 180 F_2 individual plants and respective F_3 families pertaining to Swarna/MTUII 110-9-1-1-1-1 cross were evaluated for different traits contributing to lodging resistance viz. culm diameter (mm), thickness (mm), basal internodal length at 4th internode from top (cm) at 20 days after heading and traits culm strength (score 1–9), bending stress (g $stem^{-1}$) and percent of lodging just before harvesting. The culm internodes were cut transversely at 4th internode from the top at 20 days after heading with a scalpel to measure the inner and outer diameters of the internode with a vernier calipers. The averaged culm wall thickness was then calculated by the following equation:

$$\text{Culm wall thickness} = (\text{outer diameter} - \text{inner diameter})/2$$

Bending stress was measured at the time of harvesting by pushing hill at 20 cm above the ground at 45° angle using a prostrate tester (DIK 7401, Daiki Rika Kogyo Co. Ltd., Tokyo, Japan), and it was expressed in g $stem^{-1}$ using the following formula as per Bhagat et al. (2011).

$$\text{Bending stress} = [(\text{test reading}/40) \times (1000/\text{number of tillers})]$$

At the time of harvesting stage, culm strength scores and percent of lodging were recorded as per SES, IRRI 2002.

In each generation of development of NILs, confirmed positive plants using foreground markers were phenotypically evaluated for lodging resistance traits. At BC_3F_2 stage onwards, yield parameters like days to 50% flowering, plant height (cm), ear-bearing tillers, panicle length (cm), and grain yield per plant were measured along with lodging-related parameters for confirmed lines.

Genotyping of mapping populations using SSR markers and QTL mapping

Leaf tissue was collected from all the individual plants in all the generations. The genomic DNA was isolated as per the protocol of Zheng et al. 1995. Quality and quantity of DNA was assessed using a nanodrop eight-channel spectrophotometer (Thermo Fisher Scientific). Polymerase chain reaction was performed in 10 μL final reaction volume comprising of 1 μL of 10X Taq buffer A with 15 mM $Mgcl_2$, 0.5 μL of 2.5 mM dNTPs, 1 μL each of 5 μM forward and reverse primers, 1 U Taq polymerase, 2.5 μL of genomic DNA (30 ng/μL), and 3 μL of sterile distilled water. Polymerase chain reactions were carried out using an Eppendorf master cycler

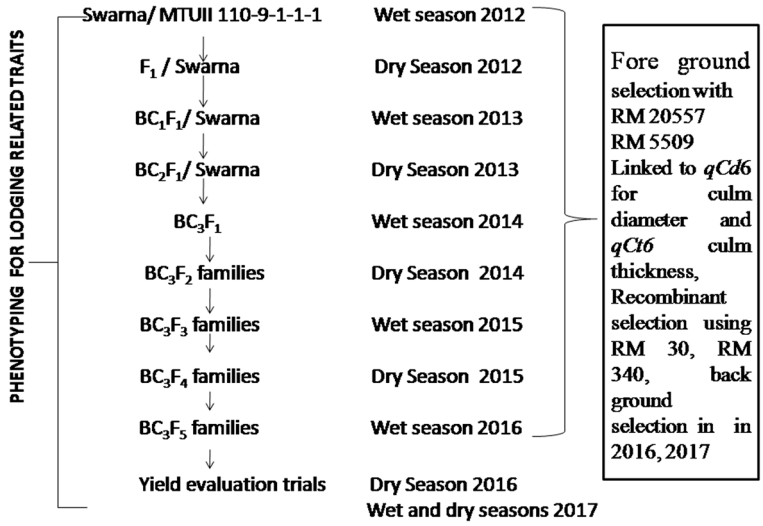

Fig. 1 Schematic representation of development of lodging-resistant introgression lines in the genetic background of Swarna

gradient with amplification profile of 94 °C for 5 min for initial denaturation, 35 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. PCR products were subjected to a 3% high-resolution metaphor Agarose (Lonza), and gel images were captured under UV light using the syngene gel documentation system.

Statistical analysis

The means and variances of means for F_1, F_2 generations were computed using individual plant data and F_3 generation on family basis. Identified 5 NILs were evaluated along with two parents in three replications in randomized block design, and data was recorded on 5 randomly selected plants. Statistical analysis was performed using Cropstat 7.2 version. Graphical genotyping was performed for selected NILs to assess maximum genome recovery of recurring parent Swarna using GGT 2.0 (Van Berloo 1999). A Win QTL cartographer (Batsen et al. 2005) was used to detect QTLs by using composite interval mapping with 1000 permutations.

Results

QTL mapping

Recurrent parent Swarna as well as donor parent MTUII 110-9-1-1-1-1 were evaluated for different traits for lodging resistance and there was significant variation between the parents, F_1, F_2, F_3, generations for most of the traits during wet season of 2013 (Table 2, Fig. 2). Parental polymorphism survey was carried out between Swarna and MTUII 110-9-1-1-1-1 using 576 SSR markers covering 12 chromosomes and identified 104 polymorphic markers were used to genotype 180 F_2 individual plants and F_3 families.

Table 1 Data on rainfall during cyclones at the time of reproductive phase of the rice crop at RARS, Maruteru from 2012 to 2017

Name of the cyclone	Stage of the crop	Month	Rain fall (mm)	Rainy days
Neelam	Milky stage	November 2012	416.4	4
Philon, Helin	Flowering stage	October 2013	565.2	15
Heavy rains	Grain hardening stage	May 2014	170.0	5
Hudhud	Flowering stage	October 2014	125.8	7
Heavy rains	Milky stage	November 2015	135.6	6
Heavy rains	Grain hardening stage	November 2016	4.0	1
Heavy rains	Grain hardening stage	May 2017	12.0	1
Heavy rains	Flowering stage	October 2017	124.0	9

Table 2 Phenotypic estimates of means and standard errors for lodging-related traits among parents, F_1, F_2, and F_3 during wet season of 2013

Parents/generations	Culm diameter (mm)	Culm thickness (mm)	Culm strength	4th Basal inter nodal length (cm)	Bending stress (g stem^{-1})	% Lodging
Recurrent parent Swarna	3.91 ± 0.07	0.72 ± 0.02	8.67 ± 0.22	7.87 ± 0.28	27.42 ± 2.96	100.00 ± 0.00
Donor MTUII 110-9-1-1-1-1	8.03 ± 0.16	0.94 ± 0.03	2.00 ± 0.30	4.73 ± 0.30	62.06 ± 1.52	0.00 ± 0.00
F_1	7.07 ± 0.16	1.28 ± 0.09	3.10 ± 0.34	6.28 ± 0.80	31.12 ± 2.87	10.00 ± 6.88
F_2	5.81 ± 0.09	1.15 ± 0.04	5.94 ± 0.19	10.55 ± 0.31	63.27 ± 2.47	51.11 ± 3.74
F_3	6.76 ± 0.44	1.12 ± 0.24	5.20 ± 0.96	7.96 ± 0.80	33.69 ± 5.94	48.30 ± 5.31

Standard error of means at 0.05 significant levels

The QTL analysis using F_2 population of Swarna/MTUII 110-9-1-1-1-1 resulted in identification of five QTLs on chromosome 2, 6, and 7 (Table 3, Fig. 3). Among them, three QTLs were identified on chromosome 6 viz. *qCd6* for culm diameter, *qCt6* for culm thickness, and *qPl6* for panicle length. QTL for culm diameter was located in the marker interval between RM 20547 and RM 20557 with LOD score of 6.07 and phenotypic variance of 10.39% while the QTL *qCt6* for culm thickness was a major QTL with a LOD score of 3.06 and phenotypic variance of 13.04%. QTL for culm strength *qCs7* was mapped on chromosome 7 in the chromosomal region between RM 418 and RM 320 with LOD score value of 3.13 explaining phenotypic variance of 11.19%. A major significant QTL for panicle length (*qPl6*) was mapped on chromosome 6 with 3.55 LOD score and 16% phenotypic variation. Bending stress QTL (*qBs2*) was identified on chromosome 2 with LOD score value of 3.14 and R^2 value of 2.36%. In all the detected QTLs, the donor MTUII 110-9-1-1-1-1 alleles had increasing effect. Validation of identified QTLs using linked markers among F_3 families of Swarna and MTUII 110-9-1-1-1-1 cross revealed co-segregation for respective lodging-related traits. Out of five QTLs, culm diameter, culm thickness-linked markers RM 20557 and RM 5509 were found to be co-segregated

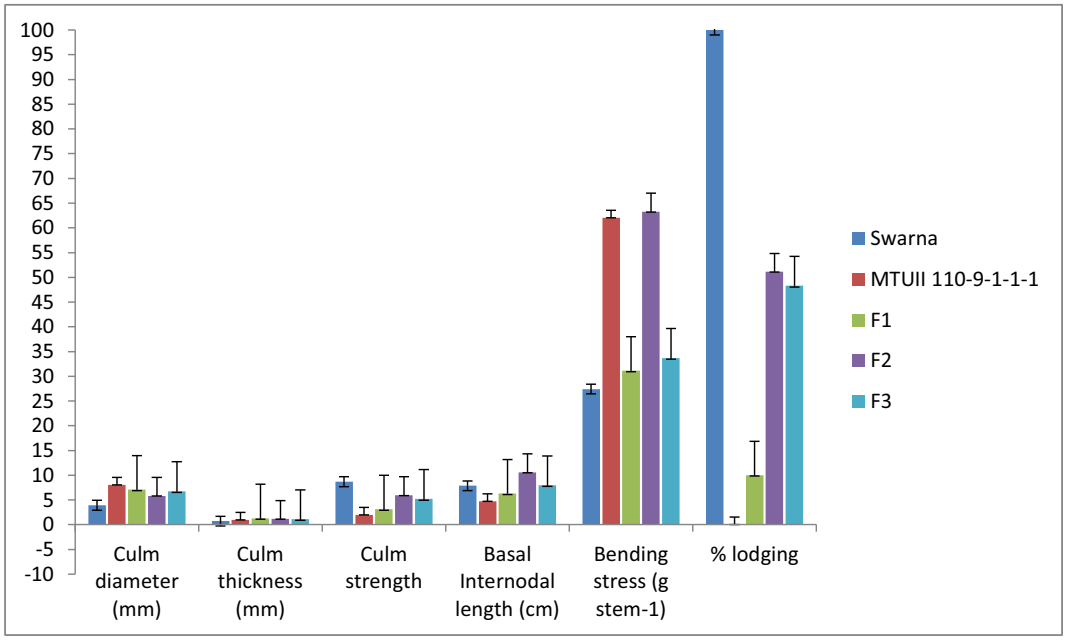

Fig. 2 Comparison of lodging-related traits among parents, F_1, F_2, and F_3 generation during wet season of 2013

Table 3 QTLs identified for lodging resistance-related traits in the Swarna/MTUII 110-9-1-1-1-1

Trait	QTL	Chromosome	Marker interval	LOD	Phenotypic variance (%)	Additive effect
Culm diameter	*qCd6*	6	RM20547-RM20557	6.07	10.39	1.89
Culm thickness	*qCt6*	6	RM5509-RM20583	3.06	13.04	0.45
Culm strength	*qCs7*	7	RM418-RM320	3.13	11.19	1.089
Panicle length	*qPl6*	6	RM30-RM20547	3.55	16.00	1.69
Bending stress	*qBs2*	2	RM12569-RM3865	3.14	2.36	2.26

with lodging resistance–related traits in another mapping population of Indra/BPT 2270 at F_2 generation and it indicated that RM 20557 and RM 5509 markers can be used to transfer wider and thick culm traits though marker-assisted breeding.

Marker-assisted backcross breeding

Culm diameter and culm thickness QTL-linked markers RM 20557 at 27.45 Mb and RM 5509 at 27.82 Mb on chromosome 6 were identified as foreground markers for traits conferring lodging resistance in new donor MTUII 110-9-1-1-1-1. Polymorphic markers RM 30 located at 26.86 Mb and RM 340 at 28.49 Mb were selected as recombinant selection markers. Based on the phenotyping and genotyping, positive plants were selected in each generation and were utilized in developing NILs of Swarna (Table 4). Three positive F_1 plants for foreground markers and non-lodging traits were used for generation of BC_1F_1. Natural screening for lodging at BC_1F_1 generation with the occurrence of Neelam cyclone during wet season of 2013 was depicted in Fig. 4. Out of 32 BC_1F_1 plant genotyped, 6 positive plants with lodging resistance were selected for generation of BC_2F_1. To generate BC_3F_1s, eight plants conferring positive for foreground markers RM 20557 and RM 5509 besides lodging resistance traits were selected out of 329 BC_2F_1 plants genotyped. At BC_3F_1 generation, 51 positive plants confirmed for strong culm traits were selfed to study BC_3F_2 generation. Grain yield (> 15 g/plant), wider culm diameter (> 7.0 mm), culm thickness (> 1.5 mm), stronger culm (scores 1 or 3), basal internodal length (< 5 cm), bending stress (> 40 g $stem^{-1}$), and percent of lodging (< 25%) with flowering duration matching with recurring parents were used as selection criteria to select advanced breeding lines with high yield potential from BC_3F_2 generation onwards besides genotypic confirmation. This resulted in selection of 99 advanced progenies with Swarna characters out of 3672 plants of BC_2F_2 genotyped. At BC_3F_3 generation, 65 plants selected by genotypic and phenotypic selection of 1980 plants pertaining to 99 families. Five NILs were selected out of 65 progenies at BC_3F_4 generation. Based on the phenotyping, foreground, recombinant, and background selections, five NILs of Swarna with lodging resistance were generated. Results of yield evaluation of NILs over three seasons were furnished in Table 5 and three seasons mean for each trait was compared with pooled analysis of variance of three seasons. There is significant variation for the all traits except days to 50% flowering and ear-bearing tillers. Among the five NILs, NIL 2, NIL 1, and NIL 3

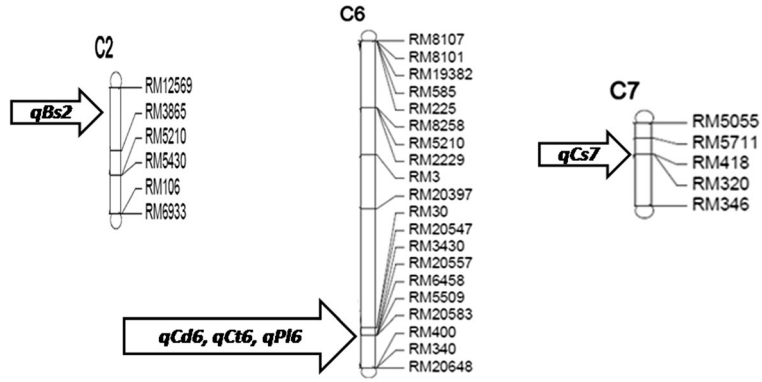

Fig. 3 QTLs identified for bending stress *qBs2* on chromosome 2, culm diameter *qCd6*, culm thickness *qCt6*, panicle length *qPl6* on chromosome 6 and culm strength *qCs7* on chromosome using Swarna and II 110-9-1-1-1-1 mapping population

Table 4 Number of plants selected in each generation through marker-assisted breeding in Swarna/MTUII 110-9-1-1-1 cross

Generation	No. of plants studied	Number of positive plants selected
F_1	30	3
BC_1F_1	32	6
BC_2F_1	329	8
BC_3F_1	283	51
BC_3F_2	3672	99
BC_3F_3	1980	65
BC_3F_4	65 Progenies	5
BC_3F_5	5 Progenies	1

expressed yield increase of 51.21%, 49.63%, and 45.56% over recurrent parent Swarna with lodging-resistant traits. Out of the above three high-yielding NILs, NIL 1 was found to be the best with wider (6.37 mm) and thick (1.29 mm) culm, increased panicle length (27.47 cm), bending stress (46.19 g/stem), desirable mean culm strength score of 2.67 and minimum percentage of lodging (1.01%). Basal internodal length of NIL1 is on par with Swarna. Background selection was carried out for five NILs using 73 markers covering 12 chromosomes to assess maximum recovery of Swarna. Results of graphical genotyping revealed that NIL1 expressed maximum recovery 94.1% of Swarna (Table 6). Developed NIL1 has distinguishable characters such as wider and thick culm, long panicles with 3–5 days earlier in duration than recurrent parent Swarna and quality parameters are on par with Swarna (Fig. 5, Table 7).

Discussion

Two QTLs mapped for culm diameter (*qCd6*) and culm thickness (*qCt6*) were in congruent with previously reported Strong culm2 (*SCM2*) linked to culm diameter possessing pleiotropic effect with APO (Apparent panicle organization) by Ookawa et al. (2010) indicating donor MTUII 110-9-1-1-1-1 possesses alleles of strong culm and therefore the linked markers RM 20557, RM 5509 can be utilized for marker-assisted breeding for the development of lodging-resistant lines. The QTL *qPl6* for panicle length identified in the chromosomal region between RM 30 and RM 340 on chromosome 6 was very close to the QTL related to panicle length (q*Pl6*) identified by Zhu et al. 2008 in the RIL population of the cross between Lemont (*japonica*) and Teqing (*indica*) and Zhang et al. 2015 using backcross population of Nipponbare (*japonica*) and WS 3(*indica*).

Fig. 4 Evaluation of BC_1F_1 generation under Neelam cyclone during wet season of 2013 (**a**) Before cyclone, (**b**) After cyclone, lines of Swarna/MTUII 110-9-1-1-1-1/*[1] Swarna

Table 5 Evaluation of NILs of Swarna for grain yield and lodging-related traits during 2016 and 2017

Entry	Season of testing	Days to 50% flowering	Plant height cm	Ear bearing tillers per m^2	Culm diameter mm	Culm thickness mm	Basal internodal length cm	Panicle length cm	Bending stress g/stem	Culm strength	% of lodging	Grain yield kg/ha
NIL 1	Dry season 2016	99	96.8	321	6.15	0.99	4.33	26.79	53.97	2	0	5344
	Wet season 2017	107	118	290	6.65	1.5	5.62	29.35	42.76	3	3.03	5463
	Dry season 2017	112.5	102.5	396	6.3	1.38	7.53	26.26	41.85	3	0	5182
	Mean	106.17	105.77*	335.67	6.37*	1.29*	5.83	27.47*	46.19*	2.67*	1.01*	5329.67*
NIL 2	Dry season 2016	97	94.2	318	5.46	0.81	6.17	24.58	55.08	5	0	5161
	Wet season 2017	105	120.2	317	6.32	1.41	6.41	27.94	43.68	3	0	5896
	Dry season 2017	106.5	105	413	6.12	1.14	7.57	25.16	49.30	3.8	0	5098
	Mean	102.83	106.47*	349.33	5.97*	1.12*	6.72	25.89*	49.35*	3.93*	0*	5385*
NIL3	Dry season 2016	97	91.65	309	5.66	0.91	6.52	25.08	53.02	5	0	4564
	Wet season 2017	107	114.8	323	6.1	1.63	8.87	25.49	47.76	3	3.03	5842
	Dry season 2017	102	102.4	389	6.02	1.44*	7.3	25.6	50.80	5	3.75	5148
	Mean	102.00	102.95*	340.33	5.97*	1.33	7.56*	25.39	50.57*	4.33*	2.26*	5184.67*
NIL 4	Dry season 2016	103	94.05	345	6.54	1.27	4.27	23.36	45.07	5	0	3653
	Wet season 2017	109	118.2	294	6.28	1.39	5.38	26.94	75.90	3	4.84	4549
	Dry season 2017	116	106.3	380	5.61	1.15	5.96	25.34	51.90	7	51.25	3889
	Mean	109.33	106.18*	339.67	6.14*	1.27*	5.20	25.21	57.62*	5.00*	18.69*	4030.33
NIL 5	Dry season 2016	98	90.7	333	4.47	0.82	4.4	20.67	56.38	6	0	4579
	Wet season2017	107	115.6	300	5.28	0.9	5.93	25.95	52.66	7	38.48	4821
	Dry season 2017	108.5	104.7	409	5.4	0.9	6.11	27.57	54.60	8	97.75	4640
	Mean	104.5	103.67*	347.33	5.05	0.87	5.48	24.73	54.57*	7	45.41*	4680*
Swarna	Dry season 2016	107	97.1	315	4.45	0.64	3.19	23.57	33.78	9	95	3425
	Wet season 2017	108	122.8	310	4.17	0.71	7.16	23.76	48.93	9	97.72	4737
	Dry season 2017	115	105.7	393	4.4	0.5	6.21	24.99	28.40	7.6	97.87	2523
	Mean	110.00	108.53	339.33	4.34	0.62	5.52	24.11	37.04	8.53	96.86	3561.67
MTUII 110-9-1-1-1-1	Dry season 2016	100	134.5	303	8.14	1.64	6.92	28.49	67.42	3	0	4521
	Wet season 2017	118	161.2	284	7.67	1.7	7.4	28.37	79.36	1	0	4951
	Dry season 2017	109	152.4	389	5.92	0.95	13.15	29.59	74.95	3.6	3.12	3302
	Mean	109.00	149.37*	325.33	7.24	1.43	9.16	28.82	57.24	2.53	1.04	4258.00
	SEM±	NS	0.8	NS	0.21	0.06	0.505	0.59	3.24	0.41	2.05	167.664

*Significance at 0.05 probability

Table 6 Details of SSRs used for background selection of NIL1 in comparison with Swarna

S. no	Marker	Chromosome	Position (Mb)	Swarna	NIL1
1	RM10344	1	5.77	A	A
2	RM10748	1	11.77	A	A
3	RM5919	1	24.73	A	A
4	RM 302	1	32.98	A	A
5	RM8278	1	36.62	A	A
6	RM6141	1	42.41	A	A
7	RM12569	2	4.28	A	A
8	RM3865	2	4.41	A	A
9	RM5210	2	12.66	A	A
10	RM106	2	25.14	A	A
11	RM6933	2	29.30	A	A
12	RM5303	2	29.83	A	A
13	RM5474	3	3.80	A	A
14	RM5639	3	8.20	A	A
15	RM5748	3	12.32	A	A
16	RM231	3	15.70	A	A
17	RM135	3	27.41	A	A
18	RM5924	3	30.66	A	A
19	RM1230	3	32.75	A	A
20	RM335	4	0.68	A	A
21	RM3524	4	22.70	A	A
22	RM17088	4	22.86	A	A
23	RM122	5	0.31	A	A
24	RM2010	5	1.18	A	A
25	RM163	5	19.18	A	A
26	RM3351	5	20.69	A	A
27	RM3170	5	27.95	A	A
28	RM6273	6	0.13	A	A
29	RM8107	6	0.48	A	A
30	RM8101	6	1.00	A	A
31	RM19382	6	2.53	A	A
32	RM585	6	3.16	A	A
33	RM225	6	3.41	A	A
34	RM8258	6	4.73	A	A
35	RM549	6	6.97	A	A
36	RM2229	6	15.36	A	A
37	RM3	6	19.49	A	A
S. No	Marker	Chromosome	Position (Mb)	Swarna	NIL1
38	RM 30	6	26.86	A	A
39	RM20547	6	27.41	A	B
40	RM3430	6	27.43	A	B
41	RM6458	6	27.55	A	B
42	RM20583	6	27.94	A	A
43	RM400	6	28.43	A	A
44	RM340	6	28.59	A	A
45	RM20684	6	30.17	A	A

Table 6 (continued)

S. no	Marker	Chromosome	Position (Mb)	Swarna	NIL1
46	RM5055	7	2.64	A	A
47	RM5711	7	3.11	A	A
48	RM418	7	18.13	A	A
49	RM320	7	18.69	A	A
50	RM346	7	21.57	A	A
51	RM1111	8	4.77	A	A
52	RM350	8	20.55	A	B
53	RM149	8	24.72	A	A
54	RM264	8	27.61	A	A
55	RM23865	9	6.50	A	A
56	RM5122	9	15.24	A	A
57	RM1099	9	22.15	A	A
58	RM105	9	32.10	A	A
59	RM216	10	5.35	A	A
60	RM222	10	11.30	A	A
61	RM3773	10	19.89	A	A
62	RM286	11	0.38	A	A
63	RM202	11	9.00	A	A
64	RM6293	11	28.26	A	A
65	RM5926	11	28.43	A	A
66	RM224	11	30.45	A	A
67	RM2851	12	0.75	A	A
68	RM2529	12	7.56	A	A
69	RM28073	12	14.94	A	A
70	RM28102	12	15.90	A	A
71	RM2972	12	19.13	A	A
72	RM519	12	19.90	A	A
73	RM309	12	21.45	A	A

A, Swarna allele; B, MTUII 110-9-1-1-1-1 allele

The QTL for bending stress *qBs2* identified at 4.3 Mb on chromosome 2 is within vicinity of strong culm 4 (*SCM4*) linked marker RM 3703 at 3. 86 Mb reported by Yano et al. 2015. Ookawa et al. 2016 identified a QTL for cortical fiber tissue conferring lodging resistance on chromosome 7 at 18.4 Mb which was co-localized with the QTL *qCs*7 for culm strength in the current study.

The results indicated that QTLs for culm diameter, culm thickness, culm strength and bending stress confers lodging resistance in new donor MTUII 110–9–1-1-1-1. Further it can be inferred that there was strong association between panicle length and lodging resistance traits which was in confirmation with Ookawa et al. 2010 who explained pleiotropic effect of *SCM2* with APO (Apparent panicle organization) on chromosome 6. Culm thickness is positively associated with culm diameter and culm strength. Per cent of lodging has positive correlation with culm strength as per Girija Rani and Satyanarayana 2018.

According to the rice genome annotation database (http://rice.plantbiology.msu.edu/), gene sets of micro tubule based movement LOC_0s06g45900 and potassium transporter LOC_0s06g45940 predicted in mapping region of culm diameter, culm thickness on chromosome 6. Transverse orientation of microtubules promotes cell elongation and longitudinal orientation restricts cell elongation which inturn contributes to lodging resistance. Microtuble orientation signals environmental response for strong culms (Nick 2012; Nick and Opatrny 2014). Role of microtubule in controlling cell wall properties in rice was reported by Zhang et al. 2010.

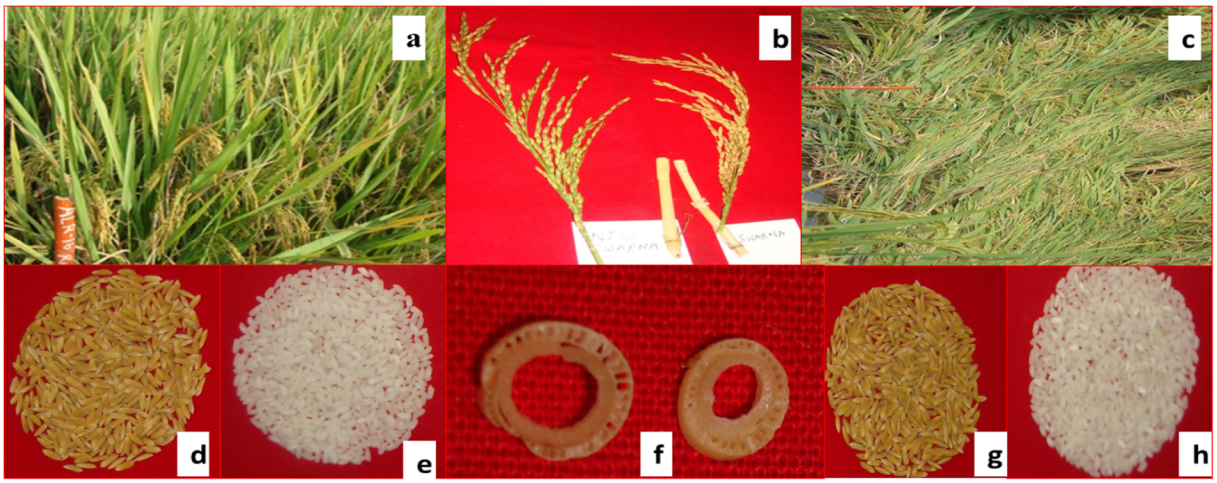

Fig. 5 Non-lodging NIL of Swarna during wet season of 2016. (**a**) Lodging-resistant NIL 1 of Swarna, (**b**) large panicle of NIL1 vs Swarna, (**c**) lodged Swarna, (**d** & **e**) grain and kernals of NIL1, (**f**) wider culm of NIL1 vs narrow culm of Swana, and (**g** & **h**) grain and kernals of Swarna

Potassium transport gene maintains cell turgidity resulting in strong culms (Wang and Wu 2015). In rice potassium culm content is directly correlated with culm mechanical strength because potassium directly associates with lignification of sclerenchyma cell and vascular bundles (De Datta and Mikkelsen 1985; Zhang et al. 2010).This indicates that microtublular movement and potassium transporter genes might be putative candidate genes responsible for strong culm traits in the mapping population.

Marker-assisted backcross breeding

Out of five QTLs, two QTLs for culm diameter and culm thickness were found to be major effect QTLs for incorporation of strong culm traits in Swarna. These markers were used as foreground markers for incorporation wider and thick culms in Swarna. Use of recombinant markers RM 30 and RM 340 limited introgression of undesirable alleles of donor MTUII 110-9-1-1-1 aided in successful introgression of genomic region of 1.86 Mb of strong culm traits in Swarna. In each generation of development of NILs, phenotypic confirmation of positive plants of RM 20557 and RM 5509 for lodging resistance related traits and use of selection criteria for selection of non-lodging lines with Swarna characters helped in identification of better NILs. Background selection resulted in identification of best NIL of Swarna (*indica*) with non-lodging traits out of 5 NILs. Earlier workers, Kashiwagi et al. (2006) identified the locus responsible for pushing resistance of the lower part of the rice plant (*prl5*) in backcross inbred lines developed from a cross between Nipponbare (*japonica*) and Kasalath (*indica*) and developed NILs of Kasalth were characterized (Kashiwagi et al. 2008) for lodging resistance. Incorporation of *SCM2* conferring for wider

Table 7 Grain quality characters of NIL1 of Swarna in comparison with parents

Characters	Recurrent parent Swarna	MTUII 110-9-1-1-1-1 Donor	NIL1 of Swarna
Kernel length (mm)	5.34	5.28	5.17
Kernel width (mm)	2.12	2.1	2.03
L/B ratio	2.51	2.51	2.54
Hulling (%)	78	74	78
Milling (%)	69	67	69
Head rice recovery (%)	58	63	59
Amylose content	Intermediate	Intermediate	Intermediate
Gelatinization temperature	Medium	Medium	Medium

culm (Ookawa et al. 2010), *SCM2*+*SCM3* pyramided for wider and strong culms (Yano et al. 2015) for the developments of NILs of Koshihikari (*japonica*) for lodging resistance.

Results of yield evaluation of NILs over 3 seasons revealed that developed best NIL1 has wider and thicker culms conferring lodging resistance with minimizing linkage drag of donor alleles. Thus molecular breeding of Swarna for lodging resistance using MTU II 110-9-1-1-1-1 as donor resulted in development of NIL with higher yield than Swarna under lodging prone conditions.

Conclusion

Identification QTLs linked to lodging resistance in new donor MTU II 110-9-1-1-1-1 helped in adoption of marker-assisted breeding for precise transmission of lodging resistance loci into mega rice variety Swarna (*indica*). Gene sets pertaining to microtubules movement and potassium transporter might be putative candidate genes responsible for strong culm traits (wider and thicker culms) in the developed mapping population. Adoption of marker assisted backcross breeding besides phenotypic selection for lodging resistance with higher yield resulted in NIL with non-lodging trait of widely grown rice variety Swarna. Identified donor MTUII 110-9-1-1-1-1 can be useful in future breeding programs, generated NIL of Swarna with lodging resistance can be released as variety after thorough multi-environment testing. Further, this material can be used as genetic stocks for future breeding programs.

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