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RESEARCH ARTICLE

Identification of alternate dwarfing gene sources to widely used Dee-Gee-Woo-Gen allele of *sd1* gene by molecular and biochemical assays in rice (*Oryza sativa* L.)

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Abbreviations: DGWG: Dee-Gee-Woo-Gen
GA: gibberellic acid
PCR: polymerase chain reaction
sd1: semi dwarf-1

After the success of IR8 and TN1, breeders depended heavily on these two rice cultivars for source of short stature led to the narrow genetic base to majority of present day rice varieties, as far as *sd1* (semi-dwarf1) gene is concerned. In addition, analysis of genetic lineage of the majority of the cultivated rice varieties in tropical Asia reveals that *sd1* from DGWG (Dee-Gee-Woo-Gen) is the source of dwarfing gene. Such high amount of genetic homogeneity renders rice plants vulnerable to epidemic of diseases and insect pests. In the current study, we made an attempt to identify the alternate sources of DGWG allele of *sd1* gene by characterizing 29 induced and 3 spontaneous dwarf accessions employing marker for DGWG allele of *sd1* gene and exogenous application of gibberellic acid (GA_3). When occurrence of DGWG allele of *sd1* gene and GA_3 response were analyzed together, existence of two kinds of dwarfs was noticed viz., dwarf accessions with DGWG allele and dwarf accessions without DGWG allele of *sd1* allele exhibiting varying responses to GA_3. As many as 22 of 32 dwarf accessions showed absence of DGWG allele of *sd1* gene with varying response to GA_3 could be used as excellent alternate sources for DGWG allele of *sd1* gene. These dwarf accessions could be used for broadening the genetic base for the plant height and thereby minimize the risk of genetic vulnerability. Our strategy of combining molecular and biochemical assays can be efficiently used for identifying alternate dwarfing gene sources to the Green Revolution gene *sd1*.

Rice is the staple food for more than one half of the world's population. Tropical Asia accounting for over 90% of production and consumption of rice has been growing tall statured lodging prone varieties of very low yields until the advent of the non lodging high yielding semi-dwarf varieties in sixties. The short statured varieties developed using Dee-Gee-Woo-Gen (DGWG), a dwarf of spontaneous origin, have enabled many countries in the region to achieve self-sufficiency in rice in a short span of 15 years (Sasaki et al. 2002; Spielmeyer et al. 2002). Initial attempts to study the genetics of semi-dwarfism using crosses of traditional talls with semi-dwarf varieties indicated that it was controlled by a single recessive gene

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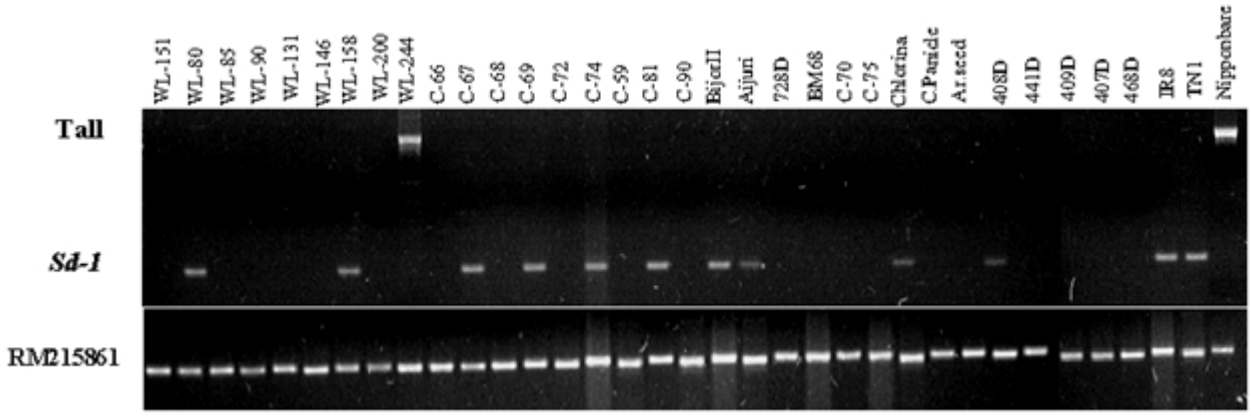

Figure 1. Characterization of dwarf accessions at *sd1* locus. A 731bp product is there in all tall rice cultivars. 348bp product is there in all accessions with DGWG allele of *sd1* RM215861 marker was used as positive control for DNA amplification of all rice accessions.

designated as *sd1* (Singh et al. 1979; Cho et al. 1994). The *sd1* gene has been reported to reduce plant height by 25% through approximately proportional reductions in lengths of the top five internodes; with practically no effect on panicle length (Rutger, 1984). Later, three research groups have mapped *sd1* gene independently to chromosome 1 and shown to encode a GA_{20}oxidase (GA20ox) (Monna et al. 2002; Sasaki et al. 2002; Spielmeyer et al. 2002), an enzyme that catalyzes the conversion of GA_{53} to GA_{20} in gibberellic acid (GA) biosynthesis. Semi-dwarf rice cultivars possessing the *sd1* allele from DGWG are characterized by a 383bp deletion in the GA20ox gene that introduces a stop codon resulting in a highly truncated and inactive enzyme. Thus the normal height of the semi-dwarf rice is restored by exogenous application of GA (Ellis and Spielmeyer, 2002; Sasaki et al. 2002; Spielmeyer et al. 2002).

The success of DGWG gene based varieties such as IR8 and Taichung (Native) 1 has made breeders allover to depend excessively on these two rice cultivars for source of short stature. With over 90% of the high yielding varieties in cultivation today having DGWG gene (Kikuchi and Ikehashi, 1984; Cho et al. 1994; Spielmeyer et al. 2002), the genetic base, as result is quite narrow, as far as *sd1* gene is concerned. Apprehending that genetic homogeneity to that high extent might render a vital character like plant type genetically vulnerable to sudden outbreak of diseases and insect pests, many efforts have been made since last two or three decades for broadening the genetic base through identification and use of alternate sources of dwarfing gene (Reddy and Padma, 1976; Singh et al. 1979). Genetic analysis of a large number of dwarfs of spontaneous and induced origin has revealed occurrence of dwarfs non-allelic to *sd1* appears to be rare. Past efforts to identify dwarfing gene sources alternate to *sd1* gene by conventional allelic relationships failed to yield desired results on account of difficulties experienced in phenotyping the segregating population and hybrid sterility in cross between dwarfs of *indica* and *japonica* origin.

In rice, as many as 61 dwarfing genes designated as *d1* to *d61* have been identified (Cho et al. 1994; Ashikari and Matsuoka, 2002). Even though many different dwarf accessions of mutant and spontaneous origin have been tried as alternate sources for developing semi-dwarf varieties, none other than *sd1* locus of DGWG source proved to be of practical value. Different alleles of this locus have been used by scientists of Asia and America (Asano et al. 2007). Several of the dwarf mutants accumulated over a period have been characterized at molecular level and their dwarfness has been a attributed to defective signal transduction molecules like heterotrimeric-G protein (Ueguchi-Tanaka et al. 2000), homeobox like *OSH15* (Sato et al. 1999), brassinosteriods (Yamamuro et al. 2000) and various GA biosynthesis genes (Sasaki et al. 2002; Itoh et al. 2004). Realizing the importance of broadening the genetic base for plant height, the present study was initiated to identify alternative dwarfing gene source(s) among a collection of induced and spontaneous mutants using molecular markers linked to *sd1* locus that can distinguish DGWG allele type from the other alleles and exogenous application of GA.

MATERIALS AND METHODS

Details of 34 rice genotypes comprising 29 induced and 3 spontaneous dwarf accessions, and two DGWG based semidwarf cultivars *viz.,* TN1 and IR8 and screened to identify alternate sources to *sd1* are summarized in Table 1. One tall variety (Nipponbare) was included as a check for PCR amplification of *sd1* allele.

DNA extraction

DNA of the experimental material was isolated following the modified potassium acetate method (Dellaporta et al. 1983).

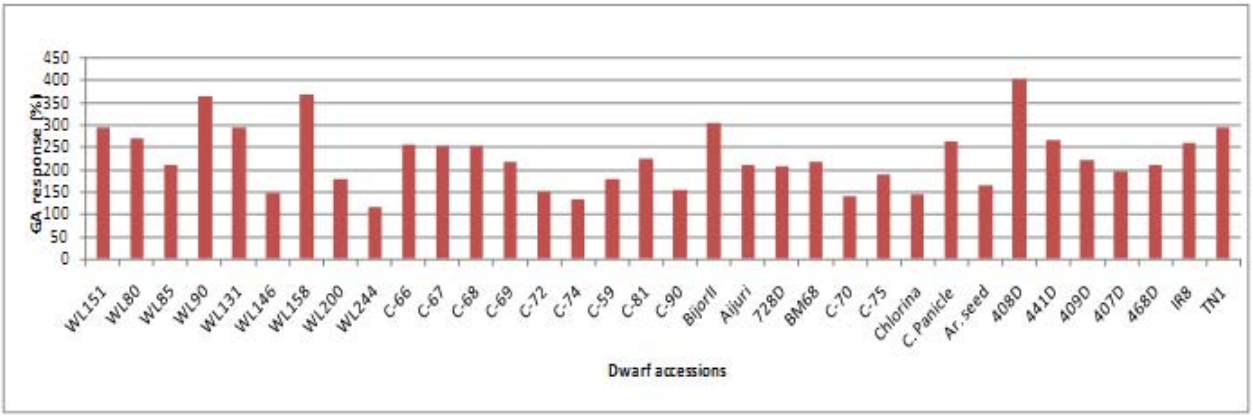

Figure 2. Characterization of dwarf accessions using GA responsiveness. GA response = (GA response in dwarf/ GA response in control)*100.

Detection of *sd1* allele

Based on the 383bp deletion in *sd1* allele of DGWG, reported PCR primers were used for detection of *sd1* allele (Ellis and Spielmeyer, 2002). *Sd1*- F: 5'- CAC GCA CGG GTT CTT CCA GGT G -3'. *Sd1*- R: 5'- AGG AGA ATA GGA GAT GGT TTA CC- 3'.

Amplification of DNA was performed in 10 mM Tris-HCl pH 9.0, 50 mM KCl, 1.5mM $MgCl_2$, 0.2 mM dNTPs, 1 U of Taq DNA polymerase (Bangalore Genei, India) and 30 ng of DNA per 20-ml reaction using a GeneAmp PCR system 9700 (PE Applied Biosystems, USA). After initial denaturation for 5 min at 94ºC, five cycles at each cycle comprised 30 sec denaturation at 94ºC, 30 sec annealing at 55ºC, 1 min and 30 sec extension at 72°C, 30 cycles at each cycle comprised 20 sec denaturation at 94ºC, 20 sec annealing at 55ºC, 1 min extension at 72ºC with a final extension for 10 min at 72ºC. Amplified products were mixed with bromophenol blue (gel loading dye) and were electrophoresed on a 2% agarose gel using 1 x Tris Borate buffer pH 8.0. The gels were stained in ethidium bromide and were documented using Alpha Imager 1220 (Alpha Innotech, USA).

Gibberellic acid (GA_3) treatment

Seeds of the experimental material were surface sterilized with 2.0% $HgCl_2$ solution for 30 min and then washed with water. They were placed in petri dishes lined with wet filter paper and then kept in dark at 30ºC for two days for germination. Elongation of shoot was quantified by microdrop method (Mitsunaga et al. 1994). Ten uniformly germinated seeds were placed on a 1% agar plate-five for gibberellic acid treatment and five as controls and allowed to grow at 25ºC under fluorescent light till emergence of second leaf sheath. After two days, 1 µl of a solution of GA_3 (10mg/ml) in ethanol was applied to coleoptile of rice seedlings. Three days after this treatment, the length of the second-leaf sheath was measured and average of 5 plants was taken to calculate GA_3 response (GAR) as under: GAR= (GA_3 treatment/control)*100.

Plant growth conditions

The genomic DNA was analyzed using RAPDs molecular markers (Williams et al. 1990; Schnell et al. 1995). We standardized the DNA amplification conditions by assessing the following: DNA concentration, primer concentration; different brands, concentrations and storage time of the Taq polymerase, as well as temperatures for PCR amplification 25 µl-reactions of PCR contained: 25 ng of DNA, 0.25 mM of dCTP, dGTP, dATP and dTTP respectively; 2.5 mM of MgCl, 1 U of Taq polymerase Invitrogen® and Promega® and 7 µM of primer. Thermocycler (Biorad MyCycler®) was programmed for 34 cycles, with a denaturating step of 30 sec, 94ºC, an annealing temperature of 40ºC for 30 sec, and an extension step at 72ºC for 1 min.

Sixty Operon® RAPD's primers were evaluated: Kit A (1-20), Kit B (1-20), OPAA6, OPAB11, OPAB14, OPAC4, OPAC7, OPAC10, OPAD1, OPAD4, OPAD10, OPAD14, OPAG6, OPAG8, OPAH5, OPAH10, OPAM4, OPAM10, OPAM13, OPAN5, OPAN8 and OPAN17. Two individuals from each department were taken to select the primers that generated a good polymorphism level. A negative control and two positive controls were included (*Curatella americana* and a rice variety). From the 60 primers that were evaluated in the six populations, 10 did not amplify, 18 produced a low amplification, 11 primers were monomorphic, and 16 were polymorphic. Five (5) primers were selected from the 16 primers that were polymorphic (Table 1) and were therefore launched in the populations. The amplification products were run in 1.5% agarose gels prepared in TBE 1 X, stained with ethidium bromide [0.5

Table 1. Details of the dwarf accessions included in the study

S.No.	Name of the accessions	Source of the accessions	Nature of the accession
1	Cigar Panicle (CRRI No. 35833)	Central Rice Research Institute (CRRI),Cuttack	Induced (Gamma rays)
2	Ratna Chlorina (CRRI No. 35835)	Central Rice Research Institute (CRRI),Cuttack	Induced (Gamma rays)
3	BM 68 (IC-144565)	Indian Agricultural Research Institute (IARI), New Delhi	Induced (Gamma rays)
4	WL 51	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
5	WL 80	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
6	WL 85	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
7	WL 90	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
8	WL 131	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
9	WL 146	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
10	WL 158	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
11	WL 200	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
12	WL 244	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
13	C-59	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
14	C-66	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
15	C-67	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
16	C-68	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
17	C-69	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
18	C-70	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
19	C-72	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
20	C-74	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
21	C-75	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
22	C-81	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
23	C-90	Bhabha Atomic Research Centre (BARC), Mumbai	Induced (Gamma rays)
24	407D	Punjab Agricultural University (PAU), Ludhiana	Induced (Gamma rays)
25	408D (BSDM ACC3019 SC459)	Punjab Agricultural University (PAU), Ludhiana	Induced (Gamma rays)
26	409D	Punjab Agricultural University (PAU), Ludhiana	Induced (Gamma rays)
27	441(DM24 ACC3251 SC165-B)	Punjab Agricultural University (PAU), Ludhiana	Induced (Gamma rays)
28	468D	Punjab Agricultural University (PAU), Ludhiana	Induced (Gamma rays)
29	728D	Punjab Agricultural University (PAU), Ludhiana	Induced (Gamma rays)
30	Bijor II	Assam Agricultural University (AAU), Titabar	Spontaneous
31	Aijuri	Assam Agricultural University (AAU), Titabar	Spontaneous
32	Area old seed	Assam Agricultural University (AAU), Titabar	Spontaneous
33	IR8	Directorate of Rice Research, Hyderabad	Semi-dwarf
34	TN1	Directorate of Rice Research, Hyderabad	Semi-dwarf

µg/ml] and a voltage of 4-5 V/cm was applied to the electrophoretic horizontal camera (Gibco® Horizon 20-25) for 2 hrs. Subsequently, the amplified products were visualized and photographed with the Gel Doc XR System (BioRad®).

Observation on the number of internodes, length of internodes and plant height

Number of internodes and length of each of the internodes were recorded for at least five plants. Plant height was measured by actual measurement (cm) from soil surface to tip of the tallest panicle (awns excluded) from at least five plants.

RESULTS

In the present study, phenotypic data of 32 dwarf accessions and two semi-dwarf varieties were collected.

Phenotypic characterization of the dwarf accessions

Number of internodes ranged from 2 to 4. Unlike the typical semi-dwarf varieties (4), most of the dwarf accessions (20) included in the study has 3 internodes (Figure 3A and Supplementary Figure 1). Two dwarf accessions viz., C-74 and C-81 have two internodes whereas remaining all (10) having four internodes like semi-dwarf rice varieties. Length of the first (top most internode in the plant) internode was found to account for more than 60% of the plant height in the accessions WL200, C-74, 408D and 468D (genotypes with two internodes were not considered). Only in two genotypes viz., WL90 and C-70 length of the second internode was more than the first internode. Length of the third and fourth internodes was highly variable ranging from 3.4 to 25.6% and 2 to 10%, respectively (Figure 3A). Plant height of the 32 dwarf accessions ranged from 30 cm to 96 cm. The height of the genotypes with four and three internodes ranged from 42 cm to 89 cm and 30 cm to 96 cm respectively. The height of the genotypes with two internodes ranged from 33 cm and 44 cm (Figure 3B).

In the present study, 32 dwarf accessions have screened for presence of DGWG allele of *sd1* gene and GA response (Table 1). The dwarf accessions exhibited both DGWG allele of *sd1* gene and moderate response to GA treatment were considered to be allelic to *sd1* gene from DGWG and those behaving otherwise as alternates to DGWG allele of *sd1* gene.

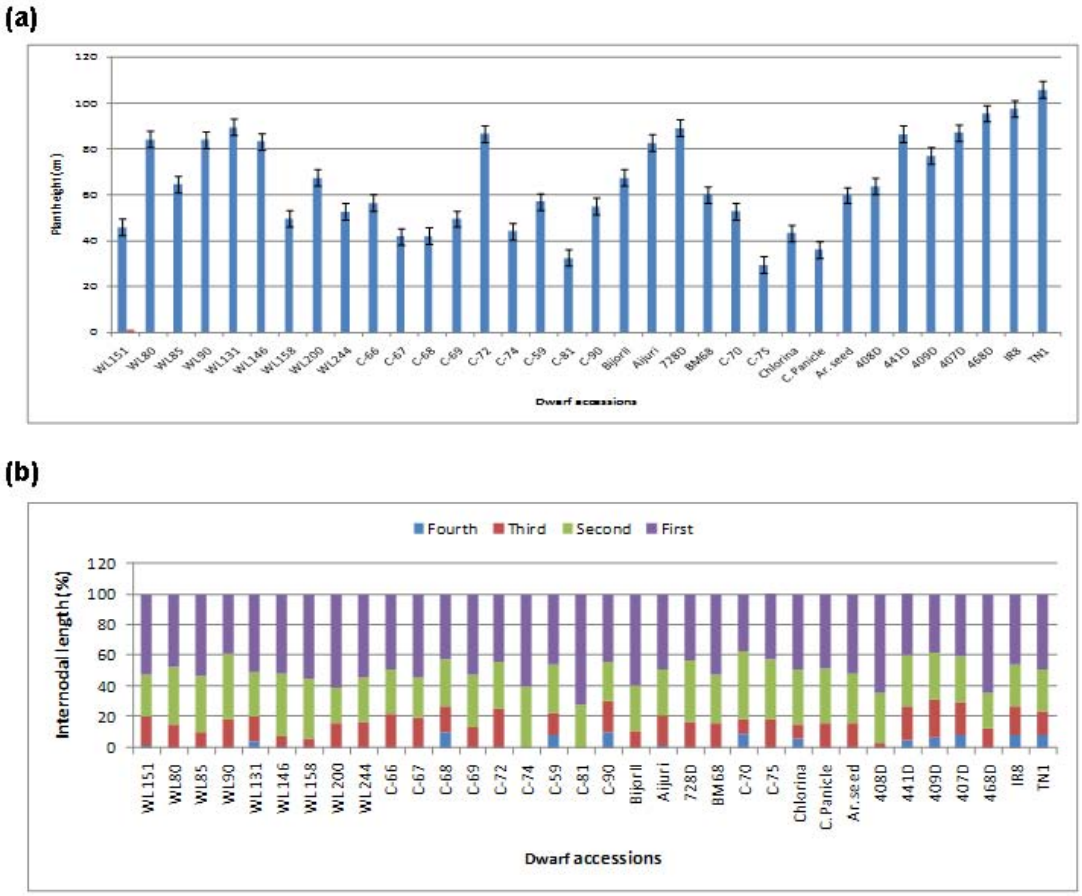

Figure 3. Characterization od dwarf accession using intermodal length (a) and plant height (b).

Table 2. Classification of dwarf accessions based on DGWG allele of sd1 gene and GA_3 response

GA_3 response	Dwarfing gene	Accessions
Medium	sd1	WL80, C-67, C-69, C-81, Aijuri
High	*sd1*	WL158, BijorII, 408D
Low	*sd1*	C-74, Chlorina
Medium	asd1	WL151, WL85, WL131, C-66, C-68, 728D, MB68, C-75, C.Panicle, 441D, 409D, 407D and 468D
High	*asd1*	WL90
Low	*asd1*	WL146, WL200, C-72, C-59, C-90, C-70, Ar.seed and WL244

asd1: alternate semi-dwarfing gene

Characterization of dwarf accessions at *sd1* locus

Based on the presence of DGWG allele of *sd1* gene (348bp PCR product), 10 dwarf accessions *viz*., WL80, C-67, C-69, C-81, Aijuri, WL158, BijorII, 408D, C-74, and Chlorina were identified to be allelic to DGWG allele of *sd1* gene and the rest as non-DGWG allele type of *sd1* gene (Figure 1).

Characterization of "null alleles" in dwarf accessions at *sd1* locus

To characterize the "null allele" in 21 accessions that did not give any PCR product at the *sd1* locus, we have used seven rice microsatellites (www.gramene.org) in upstream and two in downstream region of the *sd1* gene with an average distance between markers is approximately 125kb (Supplementary Table 1). Although all induced dwarf accessions are treated with same mutagen *i.e*, gamma rays we have noticed different lengths of the sequence deletion around the *sd1* gene. Of 32 dwarf accessions, it is surprising to note that, as many as 18 dwarf accessions showed the expansion of the deletion around the *sd1* gene ranging from 59.82kb to 783.11kb (including the *sd1* gene region). In some dwarf accessions viz., WL190, C-68 and 409D, the deletion is restricted to *sd1* gene only. In remaining dwarf accessions deletion is restricted to DGWG allele of *sd1* gene (383bp deletion) except WL244 in which full length *sd1* gene is present. Either length of the expansion or presence or absence of the DGWG allele of the *sd1* not matching to the response of the GA treatment. For instance, C-59 and 469D the length of the deletion is 192.56kb but their response to GA treatment is low and medium respectively.

DISCUSSION

Apprehending the genetic vulnerability of the present day semi-dwarf rice cultivars on account of their having one and the same dwarfing gene (*sd1*) for plant height (Cho et al. 1994; Spielmeyer et al. 2002) and keeping in view the need for alternate dwarfing gene sources of still higher physiological efficiency, the present attempt was made to identify alternate sources to DGWG allele of *sd1* by characterizing of 29 induced (gamma rays) and 3 spontaneous dwarf accessions at physiological and molecular levels. Molecular assay was done using precise marker available for identifying DGWG allele of *sd1* gene while physiological assay by sensitiveness to exogenously applied GA_3. Generally, rice varieties with DGWG allele of *sd1* gene reportedly exhibit moderate response to exogenously applied GA_3 (Kumar and Singh, 1984; Hedden, 2003). When specific amplification of *sd1* allele and GA_3 response were analyzed together, existence of two kinds of dwarfs were noticed viz., dwarfs having *sd1* allele and those characterized by absence of *sd1* allele but with varying responses to GA_3. Presence of DGWG allele of *sd1* allele in the genotypes irrespective of their GA_3 response as observed in the present study suggest involvement of various other GA biosynthesis related gene(s) in determining plant height. Mapping analysis of four GA 20ox-like genes viz., *OsGA20ox1*, *OsGA20ox2*, *OsGA20ox3* and *OsGA20ox4* reveal them to be located on chromosomes 3, 1, 7 and 5 respectively and *OsGA20ox2* is identical to the rice green revolution gene *sd1* (Sakamoto et al. 2004). OsGA20ox1 encoding an isoform of gibberellin 20-oxidase has been found to regulate of plant stature in rice (Oikawa et al. 2004). Other than GA 20 oxidase genes, ent-kaurene oxidase (KO) has been found to be implicated in determining semi-dwarf nature of Tan-Ginbozu (*d35*)

(Itoh et al. 2004) and involvement of GA 3 oxidase-2 which encoded by the dwarf gene (*d18*) was reported to cause dwarf nature.

Though these dwarf mutants could be defective at one or the other steps in the GA biosynthetic pathway, which could be rescued by the application of GA. However, dwarfness need not always as a result of deficiency of GA-like substances but of other factors. For instance, the d_1 type dwarf was reported to be defective in α-subunit of the heterotrimeric G protein affects gibberellin signal transduction (Ashikari et al. 1999; Ueguchi-Tanaka et al. 2000). Likewise, loss of function mutation in the rice homeobox gene *OSH15* was also reported to affect the architecture of internodes resulting in *d6*dwarf plants (Sato et al. 1999). Characterization of dwarfs of *d61* mutants reveals a protein kinase with high similarity to a putative brassinosteroid receptor in Arabidopsis to be linked to prevention of internode elongation (Yamamuro et al. 2000). A brassinosteroid-deficient mutant, ebisu dwarf d_2 has been reported to be caused by loss of function of a new member of cytochrome P450 (Hong et al. 2002).

Difference in plant height in general between tall and semidwarf types in particular is mainly due to differences in the length of the first (subtending panicle) and the second (subtending flag leaf/stem) internodes (Harada and Vergara, 1972). In the present study, internodal length has been found to vary with genotypes. Some genotypes like WL131, C-68, C-59, C-70 and Aijuri are characterized by the noticeably shortened lower internodes without significant reduction in the length of panicle. Such plant types among the dwarfs studied might be value as potential genetic resources for evolving non lodging ideotypes of high productivity. The pattern of internodal elongation is like that of *sd1* but with twin advantages as of having their centre of gravity relatively at lower position thereby enabling the plant to withstand lodging and the long upper internode causing least reduction in panicle length and facilitating better panicle emergence. Though dwarf mutatnts of japonica could be classified based on the elongation pattern of upper four internodes into *dn*, *dm*, *d6*, *sh* and *nl* types (Takeda, 1977; Sato et al. 1999), the dwarf genotypes included in the present study could not be classified into such groups, as their tall parent of the genotypes (unknown for majority of them) are not included. However, the following two specific patterns were noted other than normal ratio, viz., (i) length of second internode exceeding that of the first internode in accessions like WL90, C-70 and (ii) Excessive shortening of internodes below the second internode in accessions like C-70.

Genetic dwarfing is generally associated with reduction of internode length or decrease in internode number or both (Futsuhara et al. 1967). The present finding based on a variety of dwarf accessions of both induced and spontaneous origin suggest that number of internodes was not as much associated as length of internodes with plant height.

Most of the dwarf mutants identified in rice are not used in crop improvement because they are associated with one or other undesirable traits like severe dwarfism, sterility, or abnormal plant height and grain development etc. Some of the dwarf mutants in the present study have been found to be of good phenotypes characterized by inhibition of elongation of lower internodes, least reduction of length of top two internodes and hence normal panicle size, erect foliage etc (Supplementary Figure 1). They could be useful profitably be exploited in breeding for alternate ideal plant types, if the severity of their dwarfism were controlled.

In summary, dwarfing appears to be caused by different mechanisms, as observed in the present study as well as from earlier reports (Huang et al. 1996; Sakamoto et al. 2003). The success of *sd1* of DGWG was attributed to right combination of the defective *OsGA20ox2* to reduce plant height and expression of other GA 20-oxidase (s) including *OsGA20ox1* to balance normal development or reproduction related systems (Sasaki et al. 2002). Presence of *sd1* allele in genotypes differing in their level of GA_3 response such as WL158, BijorII, 408D, C-74 and Chlorina found in the present study suggest involvement of various other genes that are not included in the GA biosynthesis in determining the plant height. Although the dwarf accessions belonging to this group are allelic to *sd1*, they might be associated with modifier genes of different strengths in biosynthesis of GA. In addition, most of the accessions 22 of 32, showed absence of DGWG allele of *sd1* gene with varying response to GA_3. The dwarf accessions without *sd1* gene but with responding variedly to GA treatment could be used as excellent alternate sources for *sd1* gene tentatively designated as "*asd1*". Even though one dwarf accession *i.e*, WL244 produces functional protein of *OsGA20ox-2*, its dwarfness appears to be controlled by genes which are not involved in pathway of GA biosynthesis. When amplification of the entire *sd1* gene and its surrounding region tried, many dwarfs failed to give amplified PCR products indicating the 383bp deletion (DGWG allele) of *sd1* gene is expanded downstream and upstream of the *sd1* gene and this expansion of sequence deletion ranging from 59.82kb to 783.11kb. This clearly suggests that the *sd1* locus is either highly mutable or it might have undergone high selection pressure since beginning of green revolution on account of the extensive continuous and excessive cultivation of *sd1* gene based varieties since their advent in the mid sixties. Contrary to the generally held view, spontaneous dwarf mutants are largely allelic to *sd1* of DGWG (Singh et al. 1979), in the present study, one of the spontaneous dwarf accessions *i.e*., Area old seed could be non-allelic to *sd1*. Although further experiments are required to study the mode of inheritance of the newly non-allelic dwarfing genes, our strategy of combining molecular and biochemical assays can be efficiently used for identifying new alternate dwarfing gene sources to the Green Revolution gene *sd1*. Use of identified new sources of semi-dwarfism along with DGWG gene in future breeding programme could help greatly broaden the

genetic base for the plant height and thereby minimize the risk of genetic vulnerability.

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APPENDIX

Supplementary Table 1. DGWG allele and its flanking region in the rice genome

S. No.	Accession	RM11952	RM11971	RM11975	RM11959	RM11961	RM11963	RM11967	DGWG allele of *sd1*	RM11979	RM11997	GA response		Accession	Length of the deletion (kb)
1	WL151	480	-	-	310	190	100	190	-	-	650	M		WL131	783,11
2	WL80	450	175	200	310	-	80	190	348	330	-	M		WL146	783,11
3	WL85	-	-	-	-	-	-	-	-	-	650	M		407D	783,11
4	WL90	480	175	200	310	170	90	190	-	380	600	H		WL85	531,50
5	WL131	-	-	-	-	-	-	-	-	-	-	M		C.Panicle	490,46
6	WL146	-	-	-	-	-	-	-	-	-	-	L		C66	400,43
7	WL158	480	175	-	-	180	90	190	348	-	600	H		C59	195,69
8	WL200	450	-	-	310	190	-	-	-	370	600	L		468D	195,69
9	WL244	450	175	200	310	190	80	190	731	-	-	L		WL200	182,09
10	C66	-	-	200	-	150	80	-	-	-	-	M		C72	182,09
11	C67	474	175	-	310	170	90	190	348	380	650	M		C90	182,09
12	C68	474	175	200	-	-	100	190	-	370	600	M		C75	182,09
13	C69	450	190	200	310	190	100	210	348	330	650	M		Ar seed	182,09
14	C72	450	-	200	310	190	-	-	-	325	600	L		728D	92,68
15	C74	450	175	200	310	200	-	-	348	350	600	L		BM68	92,68
16	C59	480	-	200	310	-	-	-	-	370	600	L		C70	92,68
17	C81	450	175	200	310	170	100	210	348	370	650	M		441D	92,68
18	C90	450	175	200	310	190	-	-	-	330	650	L		WL151	59,82
19	BijorII	474	190	200	310	180	100	190	348	325	650	H		WL158	59,82
20	Aijuri	480	175	200	310	180	100	190	348	350	-	M		WL90	3,12
21	728D	-		200	310	200	80	-	-	350	600	M		C68	3,12
22	BM68	480	-	-	310	170	80	-	-	370	600	M		409D	3,12
23	C70	450	-	200	310	170	80	-	-	350	-	L			
24	C75	480	-	200	310	170	-	-	-	350	600	M			
25	Chlorina	450	175	200	310	170	90	190	348	350	650	L		H	high
26	C.Panicle	450		200	310	180	-	-	-	-	-	M		M	medium
27	Ar. seed	-	-	-	310	190	-	-	-	325	600	L		L	low
28	408D	450	175	200	-	190	80	190	348	370	-	H			
29	441D	-	-	200	310	190	80	-	-	380	650	M			
30	409D	450	190	200	310	200	80	190	-	350	650	M			
31	407D	-	-	-	-	-	-	-	-	-	-	M			
32	468D	480	-	-	310	-	-	-	-	325	600	M			
33	IR8	450	190	200	310	180	80	190	348	380	600	M			
34	TN1	450	175	200	310	170	100	190	348	370	650	M			
35	Nipponbare	500	190	200	310	190	110	200	731	400	670	L			
	Region on Chr.1	38237550	38317133	38339685	38381693	38516668	38530267	38619715	38709231	38765924	39017538				
		38237950	38317153	38339704	38382003	38516855	38530479	38619918	38.712.353	38766322	39018208				
	Interval	79,583	22,552	42,008	134,975	13,599	89,448	89,516	56,693	251,614					
		471,681	392,098	369,546	327,538	192,563	178,964	89,516		56,693	308,307				
								67,383	3.122kb	182,5					
								124,9415							

Supplementary Figure 1. Plant types of Dwarf accessions