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F_2 variability study and phylogenetic analysis of *sd1* gene in rice

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Abstract
The extent of genetic variability present in any base population is most important for the success of breeding programme. The present investigation was assessed using 100 F_2 individuals and their parents (NVSR 2179 and NVSR 2803) for characters *viz.*, days to flowering, plant height, panicle length, productive tillers per plant, number of grains per panicle, grain yield per plant, straw yield per plant and harvest index. High estimates of genotypic and phenotypic variance were observed for days to flowering, plant height, grains per panicle, grain yield per plant, straw yield per plant and harvest index. High genotypic and phenotypic coefficient of variation was found in grains per panicle, grain yield per plant, straw yield per plant. Less difference between values of GCV and PCV observed for all the traits suggesting negligible role of environment in the expression of traits, therefore, improvement by phenotypic selection is possible. The characters days to flowering, grains per panicle, grain weight, grain length, grain breadth, L: B ratio, grain yield per plant and straw yield per plant exhibited very high heritability estimates while, plant height and panicle length exhibited high heritability estimates. High heritability coupled with high genetic advance (per cent) for traits plant height, grains per panicle, grain weight, grain yield per plant and straw yield per plant indicated that these characters were governed by additive gene action, selection for improvement of such traits could be rewarding. The green revolution and the breeding of hybrid rice cultivars have significantly improved rice yield by utilization of semi-dwarf genes. Along with variability study molecular dissection was also carried out to detect presence of variation for dwarfing gene *sd1* at DNA level between dwarf line NVSR 2179 and tall line NVSR 2803. Total 8 SNPs were identified having good quality score between exon3 of NVSR 2179 and NVSR 2803. The phylogenetic analysis using exon3 sequences of both lines and 25 NCBI blast sequences generated total five clusters. The allelic differences found in form of 8 SNPs may be validated in rice germplasm or populations showing variation for plant height.

Keywords: Rice, variability, heritability, genetic advance, *sd1* gene, phylogenetic analysis

Introduction
Rice (*Oryza sativa* L.) is the most important cereal crop of the world. Rice is a self-pollinated crop and diploid in nature with twelve pairs of chromosomes (2n = 24). Rice is princess among the cereals having twenty four recognized species belonging to genus *Oryza* and tribe *Oryzeae* in the family Poaceae. *Oryza sativa* L. and *Oryza glaberrima* Steud. are the only two cultivated species. The morphology, physiology, agronomy, genetics and biochemistry of *O. sativa* have been intensely studied over a long time. More than 40,000 varieties of rice had been reported worldwide (Gopalan *et al* 2007) [15].

Rice remains a staple food for about half of the global population grown in 162.56 million ha with 504.94 million tons (Mt) milled rice production (Anonymous, 2021) [4]. Asia is considered to be the 'rice bowl' of the world, and it produces and consumes more than 90 percent of world's rice. India is the largest rice growing country, while China is the largest producer of rice. Rice is the staple food of 65 percent of the total population in India. Rice constitutes about 42 per cent of the total food grain production and 45 per cent of total cereal production of our country. In India, rice is cultivated in 43.78 million hectares with production of 118.43 million tones and productivity of 2705 kg per hectare (Anonymous, 2020) [5]. Chhattisgarh is known as "rice bowl" of India, while the cultivated area of rice in Gujarat is 9.06 lakh hectares along with production 21.47 lakh tonnes and productivity of 2369.7 kg/ha (Anonymous, 2021) [6].

The existence of variability is essential for resistance to biotic and abiotic factors as well as for wider adaptability in different sets of environment. Variability refers to the presence of differences among the individuals of plant population and it results due to differences either in the genetic constitution of the individuals of a population or in the environment in which they

are grown. Selection is also effective when there is presence of ample genetic variability among the individuals in a population. Hence, insight into the magnitude of genetic variability present in a population is of paramount importance to a plant breeder for starting a judicious breeding programme. The genetic variability is calculated from phenotypic observations, which are the results of interaction of genotypes and environment. Yield enhancement is the major breeding objective in rice breeding programmes and knowledge on the nature and magnitude of the genetic variation governing the inheritance of quantitative characters like yield and its components is essential for effective genetic improvement. Direct selection based on crop yields is often a paradox in breeding programmes because yield is a complex polygenically inherited character, influenced by its component traits. A critical analysis of the genetic variability parameters, namely, Genotypic Coefficient of Variability (GCV), Phenotypic Coefficient of Variability (PCV), heritability and genetic advance for different traits of economic importance is a major pre-requisite for any plant breeder to work with crop improvement programmes.

The green revolution and the breeding of hybrid rice cultivars have significantly improved rice yield by utilization of dwarf or semi-dwarf genes. The plant height is one of the key agronomic traits, which determine the grain yield in rice (Kadambari *et al*., 2018) [18]. The short statured rice varieties developed using Dee-Gee-Woo-Gen (DGWG) have enabled many countries to achieve self-sufficiency in rice production in a short span of 15 years (Spielmeyer *et al.*, 2002) [25]. Tall rice varieties showed very low potential for yield increase due to their poor response to fertilizers and sensitivity for lodging. Chinese rice breeders first proposed the strategy of breeding for dwarf rice. In the late 1950s, successful breeding of Taichung Native 1 (TN-1), Aijiaonante and Guangchangai, which were high-yielding dwarf rice varieties, marked a new epoch in dwarf rice breeding. Later, the International Rice Research Institute (IRRI) cross-bred the Dee-gee-woo-gen rice as a dwarf-gene source with tall Peta rice. The dwarf variety IR-8 known as miracle rice was developed in year 1964. The yield per unit area was increased over 30% by the dwarf rice varieties. The successful breeding of dwarf rice varieties was considered as just the beginning of the global "green revolution".

The study of genetics of semi-dwarfism using crosses of traditional tall with semi-dwarf varieties indicated that it was controlled by a single recessive gene designated as *sd1* (Singh *et al*., 1979) [24]. The success of DGWG gene-based varieties such as IR-8 and Taichung Native-1 (TN-1) has made rice breeders to depend excessively on these two rice cultivars for source of short stature. Semi-dwarfism is one of the most important traits in cereal crops, including rice and proven successful in increasing grain yield since advent of 'green revolution'. Hence, the present investigation was conducted on variability study and to dissect the variation at DNA level for *sd1* gene along with its phylogenetic analysis.

Materials and Methods

Variability study

Crossing program was conducted at Main Rice Research Station, Navsari Agricultural University, Navsari in summer, 2019 to get F_1s for development of F_2 mapping population. The female parent naming NVSR2179 was crossed with a male parent NVSR2803 to develop F_1 seeds. The F_1 plants were confirmed for heterozygosity by phenotypic observation and at molecular level using SSR marker. Parents along with F_2 mapping population were sown during summer 2020 for evaluation. Phenotypic data for various traitsviz., days to flowering (DF), plant height (PH), productive tillers per plant (PT), panicle length (PL), grains per panicle (GPP), 100 grain weight (100GW), grain length (GL), grain breadth, L: B ratio, grain yield per plant, straw yield per plant and harvest index were recorded from both the parents and 100 randomly selected plants of F_2 mapping population. Genotypic and phenotypic coefficients of variability were computed according to the method suggested by Burton (1952) [10]. Heritability in broad sense was calculated as per the formula given by Allard (1960) [2]. Range of heritability was categorized as suggested by Robinson *et al*., (1949) [23]. Genetic advance and it's classification into high, moderate or low genetic advance were made as per the method suggested by Johnson *et al*., (1955) [17].

Molecular dissection

The total genomic DNA was isolated from parents (NVSR2179 and NVSR2803) with the protocol of Doyle and Doyle, 1990 with some modifications. The extracted DNA was quantified and *sd1* gene (Exon 3) was amplified using gene specific primers (Table 2). PCR mix for one reaction (volume 20 µl) contained 2 µl DNA, nuclease free water12 µl, 2.5 µl 1x taq buffer + $MgCl_2$, 1 µl dNTP, 1 µl of each forward and reverse primers, and 0.5 µl Taq DNA polymerase. PCR amplification was performed with the following steps: pre-denaturing at 95℃ for 5min, followed by 35 cycles of 95℃ for 30 sec., 52-58.5℃ for 45 sec. and 72℃ for 1 min, and last step for 10 min at 72℃. Amplified products were analyzed using 2% agarose gel. The gel along with the DNA samples was stained with green satin (5 µl/100 ml). Electrophoresis was carried out for 1 hr at 90 volts and visualized under UV-transilluminator. PCR amplified fragments were sequenced for further characterization using Sanger sequencing. Amplicon sequence data obtained were then processed by using Bioedit software and phylogenetic tree was constructed using neighbor joining tree function of MEGA (Molecular Evolutionary Genetic Analysis) Software.

Results and Discussion

Variability studies

Success of any crop improvement programme depends upon the variability in the base population. A large amount of variation is necessary in a population to enable the breeder to carry out effective selection. High estimates of genotypic and phenotypic variance were observed for days to flowering, plant height, grains per panicle, grain yield per plant, straw yield per plant and harvest index, whereas low estimates of genotypic and phenotypic variance were observed for productive tillers per plant, panicle length, 100 grain weight, grain length, grain breadth and L: B ratio in the present investigation (Table 1). Similar results were reported by Hefena *et al.* (2016) [16] for days to flowering, plant height, 100 grain weight, grains per panicle, panicle length, grain yield per plant; Balat *et al.* (2018) [7] for days to flowering, plant height, panicle length, productive tillers per plant and 100 grain weight; Donkor *et al.* (2021) [11] for plant height, panicle length, productive tillers per plant.

High genotypic and phenotypic coefficients of variation were found for grains per panicle, grain yield per plant and straw yield per plant. Moderate genotypic and phenotypic coefficient of variation was found for plant height, productive

tillers per plant, 100 grain weight and harvest index. Low genotypic and phenotypic coefficients of variation were found for days to flowering, panicle length, grain length, grain breadth and L: B ratio (Table 1). Similar results were reported by Farhad and Shailaja (2015) [14] for days to flowering, plant height, grain breadth and grain yield per plant; Hefena *et al.* (2016) [16] for days to flowering, productive tillers per plant, panicle length; Abhilash *et al.* (2018) [1] days to flowering, plant height, 100 grain weight, panicle length and grain yield per plant; Balat *et al.* (2018) [7] for days to flowering, plant height, straw yield per plant and grain yield per plant; Bhat *et al.* (2018) [8] for days to flowering, panicle length, grain length and grain breadth; Lingaiah *et al*. (2018) [21] days to flowering, panicle length and 100 grain weight; Bhattachraya *et al.* (2019) [9] for days to flowering, plant height, grains per panicle, straw yield per plant and grain yield per plant; Khandappagol *et al*. (2019) [20] for days to flowering, plant height, productive tillers per plant, panicle length, grains per panicle andgrain yields per plant; Nirubana *et al*. (2019) [22] for days to flowering, grains per panicle, 100 grain weight, grain yield per plant, plant height and panicle length; Swapnil *et al*. (2020) [26] for days to flowering, plant height, productive tillers per plant, panicle length, grains per plant, grain length, grain breadth, L: B ratio and grain yield per plant; Donkor *et al.* (2021) [11] for days to flowering, panicle length, productive tillers per plant and 100 grain weight.

Less difference between values of GCV and PCV observed for all the traits suggesed negligible role of environment in the expression of traits, therefore, improvement by phenotypic selection is possible (Table 1). Similar results were reported by Hefena *et al.* (2016) [16], Abhilash *et al.* (2018) [1], Balat *et al.* (2018) [7], Bhattachraya *et al.* (2019) [9], Khandappagol *et al*. (2019) [20], Nirubana *et al.* (2019) [22], Swapnil *et al.* (2020) [26] and Donkor *et al.* (2021) [11] for most of the traits.

The characters days to flowering, grains per panicle, 100 grain weight, grain length, grain weight, L: B ratio, grain yield per plant and straw yield per plant exhibited very high heritability estimates while, plant height and panicle lengthexhibitedhigh heritability estimates which, indicated that these characters are largely governed by genetic means and selection for improvement of such characters could be rewarding. There was a less scope for further improvement in traits productive tillers per plant and harvest index which exhibited low heritability estimates. High heritability coupled with high genetic advance (per cent) for traits plant height, grains per panicle, 100 grain weight, grain yield per plant and straw yielder par plant indicated that these characters were governed by additive gene action, direct selection for improvement of such traits could be rewarding (Table 1). Similar results were reported by Farhad and Shailaja (2015) [14] for days to flowering, 100 grain weight and grain yield per plant; Hefena *et al.* (2016) [16] forplant height, grains per panicle, 100 grain weight, grain yield per plant; Balat *et al.* (2018) [7] for days to flowering, plant height, panicle length,100 grain weight and harvest index; Lingaiah *et al.* (2018) [21] for plant height; Bhattachraya *et al.* (2019) [9] for days to flowering, plant height, grains per panicle, 100 grain weight, grain yield per plant, straw yield per plant and harvest index; Khandappagol *et al*. (2019) [20] for days to flowering, productive tillers per plant and grains per panicle; Nirubana *et al.* (2019) [22] for days to flowering, grains per panicle, panicle length andgrain yield per plant; Swapnil *et al.* (2020) [26] for productive tillers per plant.; Donkor *et al.* (2021) [11] for days to flowering and plant height.

High heritability with low genetic advance was observed for grain length which indicated non-additive gene action. The simple selection for such trait may not be rewarding. High heritability with moderate genetic advance observed for traits days to flowering, panicle length, grain breadth and L: B ratio which indicated that the genotypes under study were diverse with immense genetic potential and further improvement in these traits could be possible by practicing simple selection technique (Table 1). Comparable results were also reported by Balat *et al.* (2018) [7] for days to flowering, kernel breadth, kernel length and breadth ratio; Bhat *et al.* (2018) [8] for grain breadth and Bhattachraya *et al.* (2019) [9] for days to flowering.

Table 1: Estimates of statistical and genetical parameters of different characters for F_2 generation of rice

Sr. No.	Traits	Range	Mean	GV	PV	GCV	PCV	Broad Sense Heritability (%)	GAM (%)
1	DF	82 - 109	91.51	32.16	34.75	6.34	6.44	96.90	12.86
2	PH	70.20 - 178.10	124.124	379.5	549.04	16.35	18.87	75.09	29.20
3	PT	2.00-14.00	8.34	0.79	4.48	18.22	25.4	51.45	26.93
4	PL	18.80 - 32.40	26.58	5.18	6.78	8.67	9.79	78.37	15.81
5	GPP	108 - 531	252.36	7480.65	8971.26	35.84	37.53	91.19	70.50
6	100GW	1.49 - 2.75	2.15	0.05	0.06	11.18	12.01	86.55	21.42
7	GL	7.12 - 9.59	8.55	0.16	0.20	4.81	5.32	81.84	8.97
8	GB	2.11 - 3.50	2.67	0.05	0.06	9.08	9.54	90.70	17.83
9	LBR	2.56 - 4.19	3.26	0.10	0.12	9.85	10.92	81.35	18.31
10	GY	2.14 - 61.42	24.79	71.36	85.59	34.09	37.31	83.45	64.15
11	SY	3.06 - 54.74	19.14	61.26	90.94	44.7	49.81	80.52	82.64
12	HI	30.67 - 68.37	57.05	7.44	61.13	10.00	13.70	53.78	15.18

Where, GV - Genotypic variance, PV- Phenotypic variance, GCV- Genotypic coefficient of variation, PCV- Phenotypic coefficient of variation, GAM - Genetic advance present of mean

Phylogenetic analysis of *SD1* gene of rice

Two rice lines NVSR 2179 and NVSR 2803 having contrasting character for plant height were studied at DNA level with the help of exon 3 specific primers of *sd1* gene using Sanger sequencing (Figure 1). Sequence alignment of both the sequences revealed presence of total 8 SNPs having good quality score between exon3 of lines NVSR 2179 and NVSR 2803 (Figure 2). Exon 3 sequences of NVSR 2179 and NVSR 2803 along with 25 NCBI blast sequences were studied for phylogenetic analysis. There total five clusters were created using MEGA software (Figure 3). The cluster 1 consisting of the dwarf line NVSR 2179 which was totally distinct from other sequences. Cluster 2 harboring two accessions AB633617.1 and AB633615.1. Cluster 3 having only one line which was NVSR 2803. One accession JN541495.1 fall in cluster 4. Cluster 5 consisting remaining 22 accessions. Based on results of phylogenetic tree, it can be concluded that line NVSR 2179 was distinct from NVSR 2083 and other accessions. Angira *et al.* (2019) [3] also characterized *sd1* gene of rice in seven haplotypes based on SNP data and groped into different clusters. Similarly, Endale *et al.* (2013) [13] carried out sequence alignment of two E. tef GA20ox (E. tef GA20ox1a and EtGA20ox1b) sequences with orthologous GA20ox gene sequences from *S. bicolor, Z. mays, O. sativa, T. aestivum, H. vulgare, L. perenne, Z. japonica* and *D. villosum.* Kenji *et al.* (2011) [19] also studied phylogenetic relations based on *sd1* genomic sequence among different species of rice.

Table 2: List of primers for amplification of *sd-1* gene (Exon 3) in rice based on exons derived from previous studies

Primer No	Sequence (5'->3')	Template strand
P305	GTTTGTCCTTGTCGCGTTGCTCAG	+
P306	TCTGTTCGTTCCGTTTCGTTCCG	-

Fig 1: Bands of *sd1* (Exon 3) gene of rice in two cultivars NVSR 2179 and NVSR 2803

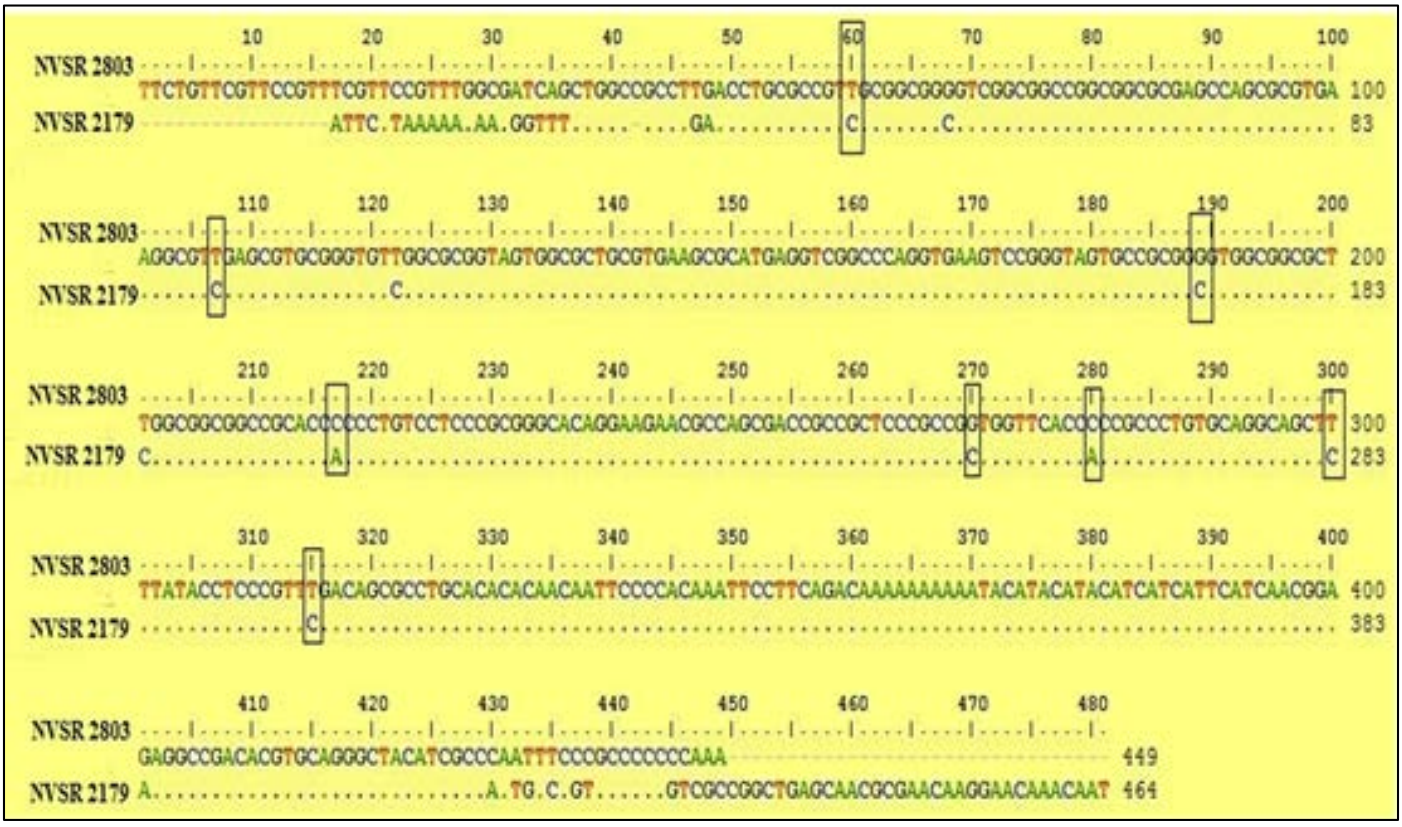

Fig 2: Sequence alignment of Exon 3 (*sd1* gene) from both the genotypes NVSR 2803 and NVSR 2179

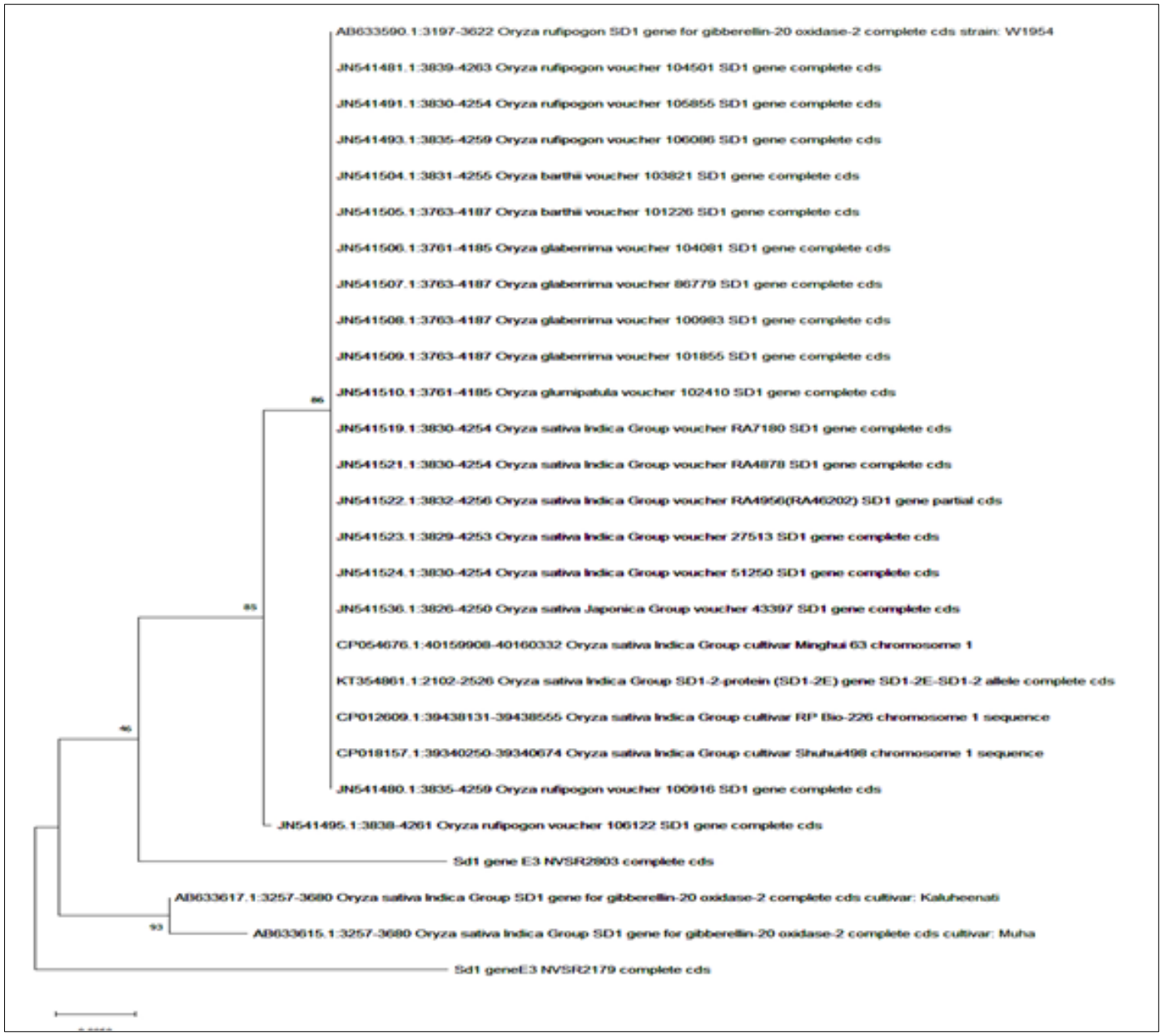

Fig 3: Phylogenetic tree of 25 accessions and 2 query sequences of rice *sd1* gene (Exon3)

Conclusion

The results from the present experiment suggest that sufficient genetic variability is present for most of the traits studied in F_2 population. Phenotypic and genotypic coefficients of variability, heritability and genetic advance revealed that plant height, grains per panicle, 100 grain weight, grain yield per plant and straw yield per plant were governed by additive gene action, simple selection for improvement of such traits could be rewarding using suitable breeding techniques. Phylogenetic analysis indicated that two phenotypic extremes for plant height utilized in the present are also clustered separately. The allelic differences found in form of 8 SNPs within *sd1* may be validated in rice germplasm or populations showing variation for plant height.

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