



## Economizing marker assisted selection through cost-effective assay of *sd1* gene in rice (*Oryza sativa*)

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### ABSTRACT

PCR amplification and detection of GC rich sequences in DNA is a challenge due to formation of secondary structures which resist denaturation, thereby stalling Taq DNA polymerases as well as affecting primer annealing. Presently, high fidelity Taq DNA polymerase is used for amplifying long and GC-rich fragments, while dimethyl sulfoxide (DMSO) has also been suggested as an additive in Polymerase Chain Reaction (PCR) mix to avoid formation of secondary structures in templates containing high GC content. In the present study, the amplification efficiency of normal Taq DNA polymerase with 5% DMSO as compared to high fidelity Taq DNA polymerase has been applied for better amplification of fragments with high GC content of *sd1* gene in rice. Normal Taq DNA polymerase with 5% DMSO displayed better and reproducible results as compared to platinum DNA polymerase for the amplification of gene *sd1*, thereby successfully detecting the dominant wild type allele governing tall plant height from the recessive semi-dwarf allele. There were no adverse effects of 5% DMSO in PCR amplification and the amplified fragments improved significantly indicating the improvement in amplification efficiency. The modified PCR protocol including DMSO provides a cost effective and cheaper alternative for reliable assessment of the genomic differences in GC-rich regions of the gene. This will help in eliminating the need for costly high fidelity Taq DNA polymerase, thereby helping in economizing marker assisted selection.

**Keywords:** Amplification efficiency, DMSO, GC-rich region, Polymerase chain reaction, Taq DNA polymerase

PCR Amplification of Guanine-Cytosine (GC) rich in genomes is challenging, as such DNA samples forms complex secondary structures, or extend hydrogen bonding between cytosine and guanine (Musso *et al.* 2006). Such complex structures resist denaturation, and also affect primer annealing affecting amplification (Shore and Paul 2010).

Taq DNA polymerase is deficient in proofreading activity, which results in low fidelity (Cline *et al.* 1996) and decrease yields of amplicons >1 kb in size. DNA polymerases with high fidelity (Pfu Turbo DNA polymerase, Platinum Taq polymerase and Platinum Pfx polymerase) were identified to address this issue, which could amend better specificity, yield, and amplification of larger fragments than the conventionally used Taq DNA polymerase.

However, with increase in length of amplified fragment or GC content, these DNA polymerases show significantly

lower efficiency in amplifying regions with 78% GC content as compared to a region having 45% GC (Arezi *et al.* 2003). Alternately, the use of different organic constituents (called as additives) such as DMSO, betaine, glycerol, formamide and 1,2-propanediol have been explored in order to increase the amplification efficiency of GC-rich sequences. Among these, DMSO is very economical and the most widely used for successful amplification of the complete gene fragment, as it unfolds secondary structures, promote polymerase stability, processivity and reduces non-specific amplification. Among the important genes cloned in rice, *sd1* sourced from Dee-geo-woo-gen (DGWG) is the most common height reducing gene used in rice improvement, which spurred the green revolution. The *sd1* gene is rich in GC content (Spielmeyer *et al.* 2002). PCR based functional marker targeting the 383 bp deletion in *sd1* from DGWG amplifies 731 bp long fragment with a GC content of 64.56%. Therefore, it necessitates the use of high fidelity Taq DNA polymerases for amplifying the target allele for marker assisted selection (Ellis and Spielmeyer 2002).

The main objective of present investigation was to develop and validate an alternative methodology for the amplification of high GC containing sequence of *sd1* gene using DMSO as an additive along with normal Taq DNA

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polymerase, and compare its relative efficiency with high fidelity Taq DNA polymerase.

## MATERIALS AND METHODS

**Plant materials and genomic DNA extraction:** The plant materials included an F<sub>2</sub> population from the cross, Pusa Basmati 1 (semi-dwarf) Basmati rice variety, and Nagina 22 (tall *aus* rice) variety grown during *kharif* 2020 at ICAR-IARI, New Delhi. DNA was extracted using CTAB method and its quality was checked on 0.8% agarose gel and quantified using a NanoDrop Spectrophotometer.

**PCR reactions and conditions:** PCR reactions were performed using three different types of DNA polymerases namely, normal Taq DNA polymerase (Genie™, Bangalore, India, Cat # 0601600051730, Lot No # 51097C), High-Fidelity platinum DNA polymerase (Invitrogen, CA; cat # 10966-026; lot 1169610 and normal Taq DNA polymerase with Dimethyl sulfoxide (DMSO) 0.48 µl of 100% DMSO as an additive. The primer sequences used for assessing the polymorphism of the GC-rich region based on the 383 bp deletion in *sd1* gene were, sd1-F: 5'-CACGCACGGGTTCTTCCAGGTG-3' and sd1-R: 5'-AGGAGAATAGGAGATGGTTTACC-3' (Ellis and Spielmeier 2002). PCR amplification of the samples was carried out as described in Dhawan *et al.* (2021) and the products were resolved on 3.5% agarose gel and visualized in a gel documentation system (BioRad, USA).

**Amplification efficiency:** Amplification efficiency (%) of PCR was calculated as:

$$\text{Amplification efficiency (\%)} = \frac{\text{Number of specific amplicons}}{\text{Number of total amplicons}}$$

The cost per sample for carrying out PCR was calculated using the prevailing rates of Taq DNA polymerases and other ingredients of PCR.

## RESULTS AND DISCUSSION

The *de novo* synthesis of GC-rich sequences is a challenge owing to its liability to form complex secondary structure and mispriming in nucleic acid, which hinders the analysis of essential genes and their introns (Jensen *et al.* 2010). Eventhough web-based softwares and tools enable optimization of codons in difficult genomic regions, there are limitations, mainly the cost for precise amplification of GC-rich sequences in important genic regions in crops. In order to overcome this limitation, we assessed the use of DMSO as an additive in the conventional PCR and compared it with already available standard protocols for detecting polymorphism in *sd1* gene of rice. The use of normal Taq polymerase without DMSO (nPCR) either failed to amplify any fragment or resulted in non-specific amplification with multiple fragments from the GC rich region of *sd1* gene fragment following standard PCR protocol. Large number of high fidelity DNA polymerases with higher amplification efficiency than Taq polymerases for GC-rich gene segments in plants are commercially available (Spangler *et al.* 2009). The low proof reading activity of these high fidelity DNA

polymerases aids in increasing the yield of PCR products, amplification of lengthy target sequences, and better fidelity as compared to Taq DNA polymerase alone (Arezi *et al.* 2003). In the present study, the high fidelity Platinum Taq polymerase was successful in amplifying the target GC-rich region in the samples.

Optimizing the PCR through use of additives such as DMSO, glycerol, etc., helps in preventing pre-PCR mispriming. DMSO is usually applied to open secondary structures, when high GC containing templates amplified (Hardjasa *et al.* 2010). Montgomery and Wittwer (2014) reported improved target product specificity and yield with the use of 5% DMSO with normal Taq DNA polymerase (0.5 U/µl) as compared to platinum Taq polymerase. In the present study, the GC-rich region of *sd1* gene was successfully amplified through the use of 0.48µl DMSO in the PCR mixture with normal Taq DNA polymerase in all the individuals of the F<sub>2</sub> population without any change in the PCR conditions. DMSO prevents the formation of secondary structure, facilitating annealing of primers to the template DNA resulting in better amplification efficiency (Simon *et al.* 2009).

A comparison of the amplification efficiencies of various PCR and their cost per sample for nPCR (Taq DNA polymerase without DMSO), hfPCR (high fidelity platinum Taq DNA polymerase) and dimePCR (Taq DNA polymerase with DMSO) is presented in Table 1. nPCR could not amplify any of the samples tested, whereas the dimePCR could amplify the target fragments in all the samples under similar PCR conditions. The amplification of the target fragments in hfPCR was also good in all the samples, except some nonspecific amplicons, especially in the heterozygous samples. dimePCR could clearly differentiate the homozygotes from the heterozygotes in the F<sub>2</sub> population. In contrast, the amplification effectiveness and fidelity of Taq DNA polymerase formulations with DMSO was maximum in dimePCR (100%) as compared to no amplification in nPCR and 75% amplification efficiency in hfPCR (Table 1). Economically, hfPCR was the costliest (₹60.67/sample) owing to higher cost of high fidelity Taq polymerases, while the cost of dimePCR was significantly lower (₹5.70/sample) and was almost equal to nPCR (₹5.65/sample), thereby reducing the cost per sample to about 1/10<sup>th</sup> of hfPCR.

Amount of PCR products is usually the most limiting factor when standardizing a PCR protocol to amplify high GC-rich regions. Despite its importance, there are few studies on assessing the efficiencies Taq polymerases for

Table 1 Comparison of amplification efficiency and cost with different PCR mixtures

| Parameter                    | PCR Mixture      |       |         |
|------------------------------|------------------|-------|---------|
|                              | nPCR             | hfPCR | dimePCR |
| Amplification efficiency (%) | No amplification | 75    | 100     |
| Cost per sample (₹)          | 5.65             | 60.70 | 5.70    |

effective amplification. In the present study, we successfully optimized a reliable PCR assay using DMSO as a PCR additive, to amplify the GC-rich DNA templates, which can be used to amplify GC rich genic regions in plants, while minimizing the cost of PCR assays of such genic regions. The modified PCR protocol including DMSO helps in economizing marker assisted selection for *sd1* gene in rice.

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