# Characterization of $GA_3$ Insensitive Reduced Height Mutant of Emmer Wheat Var. NP200 (*Triticum Dicoccum*)

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**Abstract**

A γ-ray induced semi-dwarf mutant was obtained in tall emmer wheat variety NP200. The mutant was insensitive to externally applied gibberellin. An allele specific marker for dwarfing gene *Rht-B1b* was used to check the status of dwarfing gene in the mutant, semi dwarf and tall emmer and semi dwarf durum varieties. The primer showed amplification of *Rht-B1b* gene in semi dwarf durum and emmer varieties. The parent NP200 showed presence of wild type allele (*Rht-B1a*) with the primer pair BF-WR1. All semi dwarf emmer varieties showed a band of 237bp with primer pair BF-MR1showed presence of *Rht-B1b*. However, the mutant showed absence of amplification for both *Rht-B1a* and *Rht-B1b* alleles with respective primer pairs. The results indicated that the reduced height mutant carried a mutation different from the known allele *Rht-B1b*.

**Keywords:** *Triticum dicoccum*; $GA_3$ insensitivity; Reduced height mutant; *Rht* genes; Yielding ability; Allele specific primer

## Introduction

Emmer wheat (*Triticum dicoccum* Schubler) is cultivated in parts of peninsular India. Emmer wheats are reported to contain high amounts of protein and dietary fibre [1,2] and hence are being recommended for inclusion in diet [3]. *T. dicoccum* possesses better resistance to wheat rust and have more tolerance to high temperature than other species of wheat [4,5]. Traditional varieties of emmer are tall, susceptible to lodging and low yielding [6]. Tall Emmer variety NP200 is a selection from local wheat. Introduction of semi dwarf stature has resulted in improvement in harvest index and yield in bread wheat. Till date, 21 height reduction genes are known in wheat including two major genes *Rht-B1b* and *Rht -D1b* which are present in 90% of the semi-dwarf cultivars [7]. Search for alternative height reducing genes led to the discovery of two genes *Rht-B1d* and *Rht 8* (located on 2DS) from varieties Saitama-27 and Akakomughi respectively. Introduction of dwarfing gene *Rht-B1b* has also shown improvement in emmer wheats [8]. It seems possible to generate variability for reduced height genes so that they can be used to increase option for the breeder to improve the emmer wheats [9]. A more comprehensive understanding of how the *Rht-1* genes confer dwarfism will allow the development of novel alleles with improved specificity for agronomic traits [10,11]. Novel genetic variants of GA-insensitive *Rht-1* genes in hexaploid Wheat characterized and their agronomic value have also been reported [12]. In this study, a γ-ray induced short statured mutant HW1095 was taken which is $GA_3$ insensitive and was characterized using molecular markers.

## Materials and Methods

An emmer wheat variety NP200 was used in this study. Seeds were subjected to 100, 200, 300 and 400 Gy of γ-rays. The treated seeds were space planted in $M_1$

## Results and Discussion

The plant height of tall parent variety NP200 varied from 97.0 cm to 120 cm with a mean of 110 cm. The reduced height mutant showed mean height of 71.0 cm and showed 35.4 percent reduction in height over parent in $M_2$ generation. The dwarf dicoccum mutant bred true to type in $M_3$

The specificity of these markers has been validated on bread and durum wheat varieties (Figure 1B). Use of the allele specific markers has not been reported on emmer wheats.

In this study, the allele specific markers were used to check the status of *Rht* genes in tall emmer varieties (NP200 and NP201), five semi dwarf emmer varieties (DDK1009, DDK1025, HW5013, HW5301 and MACS2961) and the newly developed dwarf mutant (HW1095) (Figure 1A and 1B). The validity of primers in tall emmer varieties for *Rht-B1a* and semi dwarf emmer varieties for *Rht-B1b* was confirmed. All semi dwarf emmer varieties showed a band of 237bp with primer pair BF-MR1 for presence of *Rht-B1b*. The parent variety NP200 showed amplification of wild type allele (*Rht-B1a*), however, the mutant (HW1095) showed absence of amplification for both *Rht-B1a* and *Rht-B1b* alleles with respective primer pairs. The PCR amplification was repeated on DNA extracted from next generation seedlings and results were found to be repeatable. This indicated that the mutant carried a different mutation than the existing allele (*Rht-B1b*). This was further confirmed by amplifying with another primer pair which covers a longer stretch of DNA segment (Figure 1C). In the mutant, this primer amplified a product of about 400bp as compared to 237bp with perfect primer pair (BF-MR1). Since the primer amplified the mutant allele, it is possible that mutation does not involve a large deletion. The amplification will enable further characterization of induced mutation. A variation was also observed in case of tall durum cultivar Bijaga Yellow where a band of 237 bp was expected with the primer pair BF-WR1, however, no amplification was observed with either primer pairs (Figure 1A and 1B).

This indicated that the mutant carried a different mutation than from the existing major gene mutations (*Rht-B1b*). The $GA_3$ sensitivity test showed that the two tall emmer varieties responded to externally applied gibberellin resulting in significant increase in seedling height. The semi dwarf emmer varieties and the new mutant showed no significant difference in mean seedling height for the water grown control and the $GA_3$ treatment.

A comparative yield trial, which included eight released varieties of emmer wheat, one bread, wheat and one macaroni wheat variety as control, NP200 parent and the mutant HW1095, was conducted in five diverse locations. The results showed that the mutant gave highest yield in three of the five locations among all the varieties. The mutant gave higher yield than the parent and other emmer wheat varieties at all the locations (Table 2). For mean of all the locations the mutant was at second place in ranking at 40.0 qt/ha compared to the bread wheat variety MACS 2496 which gave highest average yield of 40.4 qt/ha. These results showed that the mutant has high yielding potential in the tested locations.



**Figure 1:** PCR analysis of *T.dicoccum* varieties and *T.dicoccum* (mutant) and *T. durum* varieties. Lane M: 100 bp ladder; Lane 1 & 3: *T.dicoccum* tall varieties-NP200 & NP201; Lane 2: *T.dicoccum* mutant HW1095; Lanes 4-8: *T.dicoccum* dwarf varieties - DDK1009, DDK1025, HW5013, HW5301, MACS2961; Lanes 9 & 10: *T.durum* tall varieties- A-9-30-1 & Bijaga Yellow; Lanes 11-13: *T.durum* dwarf varieties-HD4502, HD4530 and MACS2846: with primer BF-WR1 for Rht-B1a (A), BF-MR1 for Rht-B1b (B) and new primer pair (5' CTCCTCC CTCCCCACCCCAAC-3') and (5'-CATCCCCATGGCCATCTCGAGCTA-3') (C)

The study reported here showed induction of a mutation resulting in about 30% reduction in height. The mutant was insensitive to externally applied gibberellin. The absence of amplification with primers specific to wild type allele or the known allele causing height reduction and insensitivity to externally applied gibberellin indicated that there was a different mutation at the *Rht-B1* locus.

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