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Title: Unraveling novel and rare mutations for alpha-amylase and key transcription factors in EMS-

induced wheat mutants for amylose by TILLING

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mutations for alpha-amylase Kevwords:

EMS-induced wheat mutants amylose by TILLING

Issue Date: 2022

Publisher: Springer Nature

Citation: Molecular Biology Reports, 35092561.

Abstract:

Background TILLING (Targeting Induced Local Lesions in Genomes) is a reverse-genetic strategy that is used to locate an allelic series of induced point mutations in genes of interest. Highthroughput TILLING allows the rapid and cost-effective detection of induced point mutations in populations of chemically mutagenized individuals. Grain amylose content is the major constraints for its nutritional quality and have drawn research interest. Identification of allelic variations in genes involved in starch biosynthesis in wheat endosperm is pre-requisite to amenable for nutritional quality improvement. Methods and results In this study, 44 EMS-induced (M4 generation) mutant lines having variation for amylose content were used for TILLING sequencing. Overall 2098.08 kb of the sequence was analyzed, and the average mutation density was 1/65.56 kb. In analysis, at the high depth score a total of 32 variations were identified including three natural variations, 76% transitions, 10% transversions, and 14% InDels respectively. The substitutions led to intronic variants, UTRs and up-downstream gene variants in Alpha-amylase, TabZIP77.1, TabZIP1 and Myb respectively. In the Myb transcription factor two missense mutations recorded namely Myb 7B c.680G > A and c.1358 T > C led to p.Gly227Asp and p.Met453Thr and c.1390G > A one substitution in Myb_7D led to p.Val464Ile. Conclusion The identified missense substitutions were predicted to affect the protein function; hence they may have a probable role in context to the amylose content in mutants. The mutations ascertained in the current study will help in gene discovery in wheat and identified mutants can be used as genetic resources to improve nutritional quality of wheat.

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URI: https://doi.org/10.1007/s11033-022-07155-0 (https://doi.org/10.1007/s11033-022-07155-0)

http://hdl.handle.net/123456789/4837 (http://hdl.handle.net/123456789/4837)

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