Abstract

This project deals with the screening of single nucleotide polymorphism (SNP) markers for drought tolerance in barley (*Hordeum vulgare* L.), the fourth largest cultivated crop worldwide. Barley seeds contain various valuable nutrients such as dietary fibers, starch, protein, free lipids, and trace amount of minerals. Barley is mainly used as animal feed and in the brewing industry. Barley plants have a good capacity of adaptation. Drought affects barley plants in different stages of its lifecycle, minimizing the overall yield of the crop. Dehydrin (*Dhn*) genes are abundant in barley, like other drought tolerant plants species. In this project, the variations in *Dhn* genes is analyzed using different molecular markers techniques. The aim is to find the suitable markers for selection of drought tolerant barley genotypes. In this study, two varieties of barley from Kazakhstan, known as Auksiniai (AUK) and Natali (NAT), as well as a collection barley cultivars were tested for genotyping of *Dhn* genes using SNP analysis. Twenty-six primers targeting two dehydrin genes, *Dhn9* and *Dhn7*, were designed using the National Centre for Biotechnology Information Genebank database and Oligocalculator. The amplification of the selected regions in two barley varieties was performed as per the standard protocol of PCR. The PCR product were visualised by 1% agarose gel electrophoresis and the purified for Sangers sequencing. The sequencing results were analysed using Chromas Lite software. Allele-specific primers (ASPs) were synthesised to identify the SNP in the studied genotypes for validation using Real-time quantitative polymerase chain reaction (qPCR). Using developed primers, clear single bands of DNA amplification were obtained. However, the Sangers sequencing showed an absence of SNPs in both of the barley varieties in three regions of *Dhn7* and *Dhn9* genes. Only one clear SNP was identified in the *Dhn7* gene among the collection of barley genotypes, while AUK and NAT remained monomorphic. All three possible variants of the SNP, both homozygous ('TT' and 'AA') and heterozygous ('AT') were detected. Amplifour-like SNP markers were developed based on the identified SNP. The distribution of FAM and HEX fluorescence in amplified PCR products of this allele (*Dhn7*-F7, F8 and R) using Real-time qPCR showed three distinct groups of barley collection with 'TT', 'AA' or 'AT' genotypes with the Amplifuor-like SNP marker application. This project has laid down the protocol to detect SNP markers in barley dehydrin genes. The method used could be applied to other plant species for screening different traits because it is suitable for high throughput analysis and is robust and reporducible. The allele-specific PCR enables homozygous or heterozygous SNP genotypes involved in drought tolerance to be easily distinguished in varieties of barley. This is a modern approach for selecting suitable barley cultivars with tolerance to dehydration stress. However, the fruitfulness of this study can only be enhanced by testing more regions of dehydrin genes in other barley varieties.