A highly efficient CRISPR-Cas9-based gene editing system in oat (*Avena sativa*)

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Summary:

Cultivated oat (*Avena sativa*) has a unique combination of characteristics among cereals, in that it has high β-glucan and oil content, a distinctive fatty acid composition, and is gluten-free. The recent sequencing of the 12.5 Gb hexaploid oat genome (see https://wheat.pw.usda.gov/GG3/genome_browser) has provided useful information about breeding barriers caused by ancestral translocations and inversions in different chromosomes. These lead to recombination suppression and pseudo-linkage, which hinder trait introgression through conventional breeding. Over the past decade, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 system has proven useful for crop improvement and functional genomics studies in many cereals, but not oats. Major obstacles to gene editing success in oat have been 1) its large repetitive genome (which comprises three sub-genomes), 2) the lack of an efficient transformation system, and 3) gene redundancy, which makes molecular screening complex.

In this article, we report the first successful CRISPR-Cas9-based gene editing in oat of the genes *AsTLP8* (associated with regulating levels of β-glucan) and *AsVRN3* (associated with vernalization response). The efficiency of gene editing was up to 41.1%. In all of the genes studied, the gene-edited plants carried deletions and/or one-base insertions. The important role of *VRN3* in oat development was seen in further analysis of *VRN3* T_1 and T_2 mutants. Bent leaves were seen in heterozygous knockouts (AACCdD), while an extended vegetative growth phase was seen in the T_1 homozygous and biallelic mutants (aaccdd). We are confident that this highly efficient oat gene editing system will pave the way for a deeper understanding of oat genomics and create genetic diversity at recombination cold spots that will be useful for breeding.