## Abstract

Precise genome editing tools are emerging as promising technology for improving crop characteristics. The CRISPR/Cas9 as genome editing tool has proven a highly versatile and efficient for generating targeted breaks in DNA, double stranded breaks induced by this means are repaired either through error prone non homologous end joining or homology directed repair. The non-homologous end joining repair pathway cause numerous changes in DNA sequence such as substitution, insertion and deletion generating knock out or altered protein/enzyme. In contrast to the NHEJ, HDR repairs DSBs through homologous recombination provided by a donor DNA template with homologous flanking sequence, which allows knock-in (KI) of targeted locus in genome. The main objective of the present study was to validate the efficacy of CRISPR/Cas9 system in Groundnut. Globally, vitamin A deficiency (VAD) is a serious health issue. In areas where VAD is a significant issue, biofortification of staple crops is a practical and affordable way to provide micronutrients to populations with limited access to varied meals. In many developing nations, the commercial groundnut is a staple crop that ensures food security. The allotetraploid genome of groundnuts makes it challenging to introduce new features through traditional breeding. Nonetheless, provitamin A carotenoids (pVACs) in groundnuts may be improved with the application of contemporary biotechnology technologies. The Newline In this study, we used both gain-of-function (overexpression) and loss-of-function (CRISPR/Cas9) strategies to increase the content of pVACs. For genetic transformation, groundnut (GJG20) tissue culture was employed. 1-deoxy-d-xylulose-5-phosphate synthase (DXS2) was found in the investigation. Groundnut genome-editing (GE) was first developed by using CRISPR/Cas9 to target phytoene desaturase (PDS), and then it was applied to lycopene epsilon-cyclase editing. superior to the control in terms of beta carotene equivalent. In contrast, lines that overexpressed PSY1 accumulated a considerable amount of lutein (up to around 3.2 times). Compared to the control, the LCYE-edited lines displayed a roughly three-fold increase in beta-carotene content and a decrease in both lutein and beta-carotene levels. The development of a nutritionally enhanced CRISPR/Cas9 method for South Asian countries with high VAD prevalence, like India, has advanced significantly with the current study.