Chapter 5

Summary and Conclusion

Vitamin A deficiency (VAD) is a severe health problem that is especially common in South-East Asia and Africa. The usage of pharmaceutical supplements to treat VAD has increased recently. However, there is a chance of toxicity when using huge amounts of vitamin A pills. The biofortification of staple crops is a viable and cost-effective method of supplying micronutrients to communities that may not have easy access to a range of diets and other micronutrient therapies. One of the most significant oilseeds in tropical and subtropical nations is groundnut (*Arachis hypogaea* L.). In the globe, it ranks as the third most significant source of vegetable protein, the fourth most significant edible oil, and the thirteenth most significant food crop. Groundnut seeds have a significant biological value because of their high-quality edible oil.easily digestible protein and carbohydrates. Roughly 90% of its output comes from underdeveloped nations, and it is cultivated in almost 100 countries. India leads the world in groundnut output (18.6%) and area (32.1%), trailing only China (42.2%). Gujarat, Andhra Pradesh, Tamil Nadu, Maharashtra, Madhya Pradesh, Rajasthan, and Uttar Pradesh are the seven states in India where 75% of groundnuts are farmed. Agricultural data at a glance (2010) states that 7.17 million tons of groundnuts are produced annually on 6.16 million hectares, with an average productivity of 1163 kg/ha. With a 37.10% output share, Gujarat leads all of India, followed by Andhra Pradesh (21.62%) and Tamil Nadu (13.53%). The Saurashtra area of Gujarat produces more than 80% of Gujarat's groundnuts.

The screening of groundnut cultivars showed that the two contrasting cultivars with the lowest levels of β-carotene in their mature fruit pulp were GJG20 and GJG22. Phytoene synthase (PSY), lycopene epsilon-cyclase (LCYε), and 1-deoxyxylulose-5-phosphate synthase (DXS) are referred to as rate-limiting enzymes in the manufacture of carotenoids. While LCYε works at a branching point and redirects lycopene resources towards α-carotene, DXS supplies the substrate flow and PSY completes the first committed step towards the carotenoid biosynthesis. The groundnut genome

contains three homologs of DXS and PSY, and expression studies of these homologs were conducted in several tissues of contrasting cultivars (GJG20 and GJG22). We discovered that cultivars influenced the LCYε and BCH genes.

By using the CRISPR/Cas9 technique to modify the *phytoene desaturase* (*PDS*) gene, targeted genome editing was achieved in groundnuts. This work is supported by phenotypic evaluation (albino and variegated phenotypes) and sequencing analysis of the *PDS* gene's target area, which shows a 59% mutation frequency. Groundnut mutant lines also show a drop in chlorophyll and total carotenoid content. Stop codons were produced in *PDS* areas because of these alterations, suggesting that *PDS* protein production was terminated too soon. These findings showed that CRISPR/Cas9 genome editing is an effective method for modifying the groundnut genome. The *LCYε* gene mutation in the groundnut genome was then corrected using the well-established genome editing technique. A high mutation efficiency of (83%, or 10/12), was found in the changed Groundnut plants' *LCYε* sequence. Near the protospacer adjacent motif (PAM) region of the *LCYε* gene, three different forms of deletions (2–3 nucleotides) and one type of insertion (1 nucleotide) were discovered. Additionally, metabolite (carotenoids) profiling revealed that the seeds of modified groundnut plants had a higher β-carotene concentration (3 µg/g, or up to three times) than the non-transformed control (0.82 µg/g). On the other hand, decreased levels of lutein and α-carotene were noted.

In conclusion, the current work documents a major advancement in the creation of nutritionally enhanced bananas for South Asian nations with high VAD prevalence, such as India, using the CRISPR/Cas9 technique. Characterizing the promising line or lines for trait stability, agronomic performance, multilocation trail, and cell line/animal model-based bioavailability studies would be the work's next step.