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CRISPR/Cas in Horticultural Crops for Nutritional Security

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Worldwide each and every country is affected by various forms of malnutrition. It occurs when the body does not get enough nutrients from the food. According to WHO report, malnutrition is linked to around 3.5 million deaths of people in which 45% alone accounts for the children. Therefore, nutrient rich balanced diet is very important for good health and development of people. Several ingredients of food are very important in promoting well-being and health of an individual like vitamins, minerals, antioxidants, amino acids, fat, and dietary fiber. All these are present in different food groups like pulses, cereals, oilseeds, legumes, fruits, and vegetables. Undernutrition due to vitamin deficiency may cause severe underlying conditions. For instance, vitamin A is one of the essential nutrients for normal growth and vision, maintenance of epithelial cells, reproduction and its deficiency leads to blindness, increased risk of dying from infections, anemia, and also impairment of fetus growth in pregnant women. Over 250,000 to 500,000 children lose their eyesight due to lack of enough vitamin A in their daily diet (Boyd et al. 2022). Another important vitamin, vitamin C is responsible for healthy immune system and its deficiency weakens the immune system thereby slowing the wound healing process. Also, 30% of people worldwide are anemic due to the consumption of iron deficient foods (Kumar et al. 2022).

Fruits and vegetables play crucial role in healthful living. Daily consumption of green leafy vegetables like spinach, bathua, mint, amaranth, fenugreek leaves, roots and tubers such as carrots, potatoes can fulfill our daily dose of requirement of vitamins, minerals, proteins, antioxidants, carbohydrates, calcium, and iron (Wan et al. 2021). Currently fruits and vegetables meet the only half of the requirement of different vitamins and minerals. Thus fruits and vegetables can be fortified further with essential vitamins and minerals for sustainability of nutritional security. Apart from that, different factors such as affordability, life style, dietary habits, accessibility, and bioavailability contributes in nutritional security. With the rapid rise in population, there is a dire need to raise the production of fruits and vegetables and also concerted research should be directed towards the fortification programmes. To reach that stage where sufficient nutrients are available as recommended dietary allowance (RDA), there is a need to improve quality of fruits and vegetables via biofortification and increasing shelf life. Fortification of fruits and vegetables can be achieved by selective breeding program or via some cutting edge biotechnological approaches (Garg et al. 2018). However, breeding programs are very time consuming, laborious, and complicated. An urge to resolve the complexity and surpass the drawbacks of breeding programmes researchers developed the GM technology (Yadav et al. 2018). However, the non-acceptability of the GM technology prompted the researchers to develop

various genome editing tools like zinc finger nuclease (ZFNs), Meganucleases, TALENs (Transcriptionally activator -like effector nuclease) and clustered regularly interspaced small palindromic sequence associated nucleases (CRISPR/Cas) (Carroll, 2011; Mahfouz et al. 2011; Li et al. 2012; Gaj et al. 2013). Acceleration in sequencing technologies had provided genomic information of a number of plant species and has create ample opportunities to target desired traits using genome editing for further augmenting the crop improvement program (Priti et al. 2018).

One of the latest versatile and dynamic genome editing tool is CRISPR-Cas9, which has the potential to carry out desirable, precise, efficient, and permanent changes in the target crops. Due to its efficacy, simplicity, and accuracy CRISPR/Cas9 has become very popular among researchers (Karkute et al. 2017; Corte et al. 2019). CRISPR/Cas9 has improved hybrid-breeding processes, making it easier to eliminate undesirable characteristics or add desired features to elite varieties, allowing crop traits to be accurately modified even within a single generation. As a result, CRISPR/Cas9 has the potential to improve global food security and agriculture sustainability (Rani et al. 2016; Chen et al. 2019).

CRISPR is clustered regularly interspaced short palindromic repeats and Cas9 is an enzyme encoded by a gene adjacent to CRISPR region. CRISPR has been found in almost half of the all sequenced bacterial genomes and all the genomes of archaea. Bacteria and archaea having CRISPR in their genomes depicts the evolution of their immune system to resist themselves against viruses known as phages (Jiang and Doudna, 2017). The genetic feature of CRISPR comprises of two alternative stretches of sequences. First one is repeated sequence (23 to 47bps) of nucleotides that is already present in bacterial genomes prior to viral infection and is highly conservative for a given cluster (Figure 1) (Makarova et al. 2011). Another one is spacer sequence (21-72bps) which is highly divergent but similar in length (Makarova et al. 2011). First clue of their origin came from a bioinformatics analysis where it was found that spacer sequence was identical to the regions of known phage and plasmids and this quickly led to the idea that these sequences are involved in defense mechanism against foreign nucleic acid entering the bacterial cell. Cas9 is an endonuclease found in close association with CRISPR and contains two active domains, HNH and RuvC, for targeting individual strand of the DNA double helix (Jia and Wang, 2014). CRISPR works by acting as a pair of DNA scissors and Cas9 as the protein in CRISPR system that unzips DNA (Nekrasov et al. 2013).

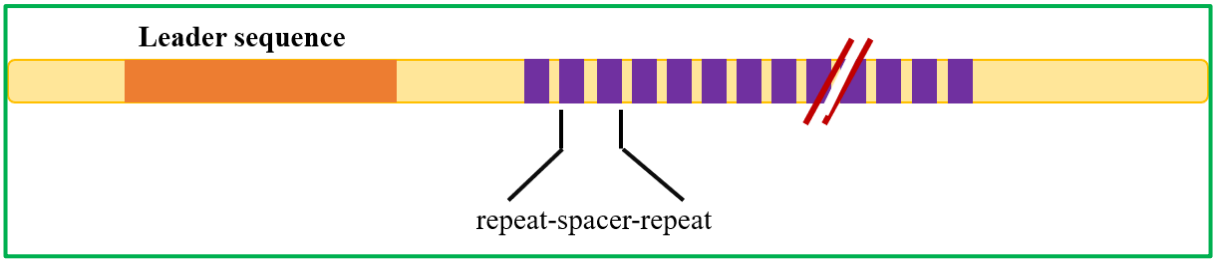

Figure 1: The organization of CRISPR locus. The repeated and spacer sequences are arranged alternatively in the genome. Proximal leader sequence often A-T rich and ~ 500 bp in length.

In 2012, two different individual laboratories at the same time came across the fact that CRISPR/Cas can be constructed *in vitro* and had biological function. This *in vitro* constructed CRISPR/Cas can also cut the individual DNA sequence (Gasiunas et al. 2012; Jinek et al. 2012). This finding laid the foundation of the fact that CRISPR/Cas can be used as a genome editing tool. Their studies in combination with some other found that to make CRSIPR/Cas editing tool functional three components are required viz. Cas enzyme, crRNA, and tracRNA. In CRISPR/Cas system, CRISPR loci transcribe in to pre-crRNA and further

processed in to each individual short crRNAs (part of guide RNA) as described by Makarova et al. (2011). Each cr RNA comprises single spacer between two half repeats and these repeats also called as 5' and 3' handles, representing the conserved part of every crRNA. Cas9 loci get translated in to Cas9, an endonuclease enzyme (Deltcheva et al. 2011). Upon infection of any virus CRISPR/Cas scan these viral DNA and if any viral DNA fragment is already present in CRISPR is detected in turn forms the complementary spacer-viral DNA pairing (Cong et al. 2013). In this way Cas9 enzyme cuts the viral DNA and confers the resistance to bacteria. The Cas9 recognizes only those viral sequences complementary to the guide crRNA, where target sequence is directly 5' to a protospacer adjacent motif (PAM). Commonly used Cas9 recognizes PAM sequence which is present 2-6 nucleotides downstream of target DNA sequence where Cas9 will make a cut. PAM is 5'-NGG-3' where N can be any nucleotide. The necessity of presence of PAM sequence may create obstacles for researchers. Thankfully, there are lot different Cas endonuclease are present from different bacterial species which can recognize different PAM which further make it a hassel free approach for genome editing.

Using CRISPR technology nutritional improvement programmes can be speeden by introducing precise and predictable mutations (insertion and deletions) directly in horticultural crops. There are several examples of improvement of various key traits like yield, growth improvement, quality, biotic and abiotic stress tolerance in a wide range of horticultural crops (Fiaz et al. 2019; Zafar et al. 2019; Ahmad et al. 2020; Wang et al. 2019). CRISPR/Cas9 can also be exploited for complete knockout of genes or silencing for the production of antinutrient compounds like lectins, oxalate, tannins which reduces the bioavailability of nutrients or can change the taste of fruits (Sinha and Khare, 2017; Sango et al. 2016) such as berries, grapes, and pomegranate (Smeriglio et al. 2017; Lamy et al. 2016). Anthocyanin (Meng et al., 2015), lycopene (Li et al., 2018), malate (Ye et al., 2017) and gama aminobutyric acid (Nonaka et al., 2017) are bioactive compounds. Editing genes regulating the metabolic pathways of these bioactive compound via CRISPR/Cas9 can increase nutrient levels in horticultural crops.

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