During (shi et al. 2016). sequence-specific designer crispr cas9

During the last five years, enormous excitement has been witnessed withthe discovery of genome editing approaches involving sequence-specific designernucleases (ZFN, TALEN, CRISPR/Cas), which create double strand breaks in DNA (for a Review, see Gaaj et al.

, 20131). However, of thethree nucleases, CRISPR/Cas9attracted the maximum attention for developing several plant and animal specieswith desired genetic modifications through genome editing. An alternative forCas9 in the form of Cpf1 later became available giving birth to superior systemin the form of CRISPR/Cpf1, which has several advantages over CRSPR/Cas2, 3(Zetche et al., 2015; Zaidi et al.

, 2017). ZFN//TALEN/CRISPR-mediatedgenome editing has been a preferred approach over transgenics, since no foreign gene is beingintroduced, and only anexisting gene is altered, using cells own machinery involvinghomology-dependent repair (HDR) and non-homologous end joining (NHEJ). Thepreferred HDR-dependent genome editing is, however, limited by low efficiency, since NHEJ competeswith it and creates high frequency of indels and off-site alterations duringgenome editing. Also, genome editing does notallow an alteration of a specific existing base pair in a DNA molecule in a predictablemanner. In view of thefact that no foreign gene is inserted during genome editing and only anendogenous gene is altered, it has been argued that products of genomeediting technologies likeCRISPR/Cas9 should not besubjected to the regulatorysystem, which is used incase of geneticallymodified organisms (GMOs). This has madecommercialization of genome edited products easier at least in some countries. Consequently, a strain of ‘

mushroom’ with white buttons, which will notturn brown (when stored) was developed using CRISPR and commercialized in USAwithout being subjected to the regulations that are commonly applied to GMOs5, 6(Hall, 2016; Waltz, 2016); in these edited mushrooms, a gene for PPO (polyphenoloxidase) that is responsiblefor browning of mushrooms was altered, thus reducing the quantity of PPO to 30%.

A mutant corn that gave high yieldunder drought conditions has also beendeveloped through genome editing by DuPontand approved for commercial cultivation, so that it may also be used forcommercial cultivation by the farmers and may hit the market soon7 (Shi et al. 2016). Sequence-specific designer CRISPR/Cas9 system contains the following twocomponents: (i) a guide RNA (gRNA orsgRNA), and a (ii) a CRISPR-associated endonuclease (Cas protein). ThegRNA is a short synthetic RNA composed of a scaffold sequencenecessary for Cas-binding anda ~20 nucleotide spacer (upstream of PAM sequence) that defines the genomic target to be modified.

The gRNA iais designedkeeping in mind the sequence that needs to be altered, so that Cas9 binds thethe desired site and creates DSBs at specific desired site. In other words, thegenomic target for binding Cas protein and for creating alteration in aspecific region of the genome is decided by gRNA. However, outcome of CRISPR/Cas9-mediatedalteration in the genome is not precise at the individual nucleotide (base)level, and therefore it can not be used for specific alterations at the levelof single specific base. In actual practice it has been noticed that a varietyof products are obtained and a selection needs to be exercised to obtain thedesired product, which is

generally available at a frequency of not more than5%. CRISPR-Cas9 also introduces randominsertions, deletions, translocations and other base-to-base conversions, whichis another limitation associated with CRISPR/Cas9 system.