their genome and optionally is grown directly from the whole seed or part of a seed without a callus stage.

[0009c] In another example, there is provided a method of effecting a genetic alteration in the genome of embryonic plant tissue, comprising bombardment of the embryonic plant tissue with a polynucleotide composition comprising a particle complexed with a ribonucleoprotein (RNP) comprising an RNA-guided nuclease and at least one guide RNA (gRNA) having a nucleotide sequence designed to alter a target nucleotide sequence in the genome, thereby effecting the genetic alteration.

[0009d] In one aspect, the present disclosure refers to a method of delivering a guide RNA (gRNA) to a non-epidermal plant cell, wherein the non-epidermal plant cell is in a plant or part of a plant, wherein the plant is a maize, rice, wheat, tomato, or soybean plant, wherein the gRNA has a nucleotide sequence designed to alter a target nucleotide sequence in the non-epidermal plant cell, and wherein the gRNA is provided as a polynucleotide composition comprising:

(i) a CRISPR RNA (crRNA) that includes the gRNA, or a polynucleotide that encodes a crRNA, or a polynucleotide that is processed into a crRNA; or

(ii) a single guide RNA (sgRNA) that includes the gRNA, or a polynucleotide that encodes an sgRNA, or a polynucleotide that is processed into an sgRNA;

wherein the delivery of the polynucleotide composition comprises at least one treatment selected from the group consisting of: microinjection and mechanical cell wall breakage; and wherein the non-epidermal plant cell, or the plant or plant part in which the non-epidermal plant cell is located, is subjected to heating or heat stress;

whereby the gRNA is delivered to the non-epidermal plant cell and the target nucleotide sequence in the non-epidermal plant cell is altered.

[0009e] In another example, there is provided a non-epidermal plant cell comprising an altered target nucleotide sequence, provided by the method as described herein, or a plant grown or regenerated from the non-epidermal plant cell comprising an altered target nucleotide sequence, wherein the plant comprises cells having the altered target nucleotide sequence, or seed or plant parts of the plant.

[0009f] In another example, there is provided the doubled haploid cell that is homozygous for the altered target nucleotide sequence, provided by the method as described herein.

[0009g] In another example, there is provided the regenerated doubled haploid plant provided by the method as described herein, or seed or plant parts of the regenerated doubled haploid plant, or a hybrid plant having at least one parent plant that is the regenerated doubled haploid plant.

[0009h] In another example, there is provided a plant grown or regenerated from the embryonic plant tissue having the genetic alteration in its genome provided by the method as described herein, or a seed of the plant, wherein the plant or seed comprises cells comprising the genetic alteration in their genome and wherein the plant optionally is grown directly from the whole seed or part of a seed without a callus stage.

What is claimed:

1. A method of delivering a guide RNA (gRNA) to a non-epidermal plant cell,
wherein the non-epidermal plant cell is in a plant or part of a plant,
wherein the plant is a maize, rice, wheat, tomato, or soybean plant,
wherein the gRNA has a nucleotide sequence designed to alter a target nucleotide sequence in the non-epidermal plant cell, and wherein the gRNA is provided as a polynucleotide composition comprising:
(i) a CRISPR RNA (crRNA) that includes the gRNA, or a polynucleotide that encodes a crRNA, or a polynucleotide that is processed into a crRNA; or
(ii) a single guide RNA (sgRNA) that includes the gRNA, or a polynucleotide that encodes an sgRNA, or a polynucleotide that is processed into an sgRNA;
wherein the delivery of the polynucleotide composition comprises at least one treatment selected from the group consisting of: microinjection and mechanical cell wall breakage; and wherein the non-epidermal plant cell, or the plant or plant part in which the non-epidermal plant cell is located, is subjected to heating or heat stress;
whereby the gRNA is delivered to the non-epidermal plant cell and the target nucleotide sequence in the non-epidermal plant cell is altered.
2. The method of claim 1, wherein the plant or part of a plant is selected from the group consisting of a plant tissue, a whole plant, an intact nodal bud, a shoot apex or shoot apical meristem, a root apex or root apical meristem, lateral meristem, intercalary meristem, a seedling, a whole seed, a halved seed or other seed fragment, an embryo, and callus.

3. The method of claim 1, wherein:
(a) the polynucleotide composition optionally comprises an RNA-guided nuclease, or a polynucleotide that encodes the RNA-guided nuclease; or
(b) the method further includes the step of providing to the non-epidermal plant cell an RNA-guided nuclease or a polynucleotide that encodes the RNA-guided nuclease; or
(c) the non-epidermal plant cell comprises an RNA-guided nuclease or a polynucleotide that encodes the RNA-guided nuclease.
4. The method of claim 1, wherein
(a) the polynucleotide composition further comprises a chemical agent or a physical agent or a combination of both chemical and physical agents, or
(b) the method further comprises the step of treating the plant cell with a chemical agent or a physical agent or a combination of both chemical and physical agents;
wherein the chemical agent is at least one selected from the group consisting of solvents, fluorocarbons, glycols or polyols, surfactants; primary, secondary, or tertiary amines and quaternary ammonium salts; saponins; organosilicone surfactants; lipids, lipoproteins, lipopolysaccharides; acids, bases, caustic agents; peptides, proteins, or enzymes; cell-penetrating peptides; RNase inhibitors; cationic

branched or linear polymers; dendrimers; counter-ions, amines or polyamines, osmolytes, buffers, and salts; polynucleotides; transfection agents; antibiotics; non-specific DNA double-strand-break-inducing agents; antioxidants; and chelating agents; and

wherein the physical agent is at least one selected from the group consisting of particles or nanoparticles, magnetic particles or nanoparticles, abrasive or scarifying agents, needles or microneedles, matrices, and grids.

5. The method of claim 1, wherein the crRNA, the polynucleotide that encodes a crRNA, the polynucleotide that is processed into a crRNA, the sgRNA, the polynucleotide that encodes an sgRNA, or the polynucleotide that is processed into an sgRNA further comprises one or more additional nucleotide sequences selected from the group consisting of an aptamer or riboswitch sequence, a nucleotide sequence that provides secondary structure, a nucleotide sequence that provides a sequence-specific site for an enzyme, T-DNA sequence, a DNA nuclear-targeting sequence, a regulatory sequence, and a transcript-stabilizing sequence.
6. The method of claim 1, wherein the crRNA, the polynucleotide that encodes a crRNA, the polynucleotide that is processed into a crRNA, the sgRNA, the polynucleotide that encodes an sgRNA, or the polynucleotide that is processed into an sgRNA comprises: (a) double-stranded RNA, (b) single-stranded RNA; (c) chemically modified RNA; (d) a combination of (a) – (c).
7. The method of claim 3, wherein the RNA-guided nuclease or polynucleotide that encodes the RNA-guided nuclease is provided:
   (a) as a ribonucleoprotein complex comprising the crRNA and the RNA-guided nuclease;
   (b) as a complex comprising the RNA-guided nuclease or polynucleotide that encodes the RNA-guided nuclease and at least one peptide selected from the group consisting of a cell-penetrating peptide, viral movement protein, or transfecting peptide;
   (c) as a fusion protein comprising the RNA-guided nuclease and at least one peptide selected from the group consisting of a cell-penetrating peptide, viral movement protein, or transfecting peptide;
   (d) on a carrier molecule or a particulate;
   (e) in a liposome, micelle, protoplast or protoplast fragment;
   (f) using a combination of any of (a) – (e).
8. The method of claim 3, wherein the RNA-guided nuclease is provided:
   (a) by contacting the non-epidermal plant cell with the RNA-guided nuclease or polynucleotide that encodes the RNA-guided nuclease;
   (b) by transporting the RNA-guided nuclease or polynucleotide that encodes the RNA-guided nuclease into the non-epidermal plant cell using a chemical, enzymatic, or physical agent;
   (c) by bacterially mediated transfection with a polynucleotide encoding the RNA-guided nuclease; or
   (d) by transcription of a polynucleotide that encodes the RNA-guided nuclease.

9. The method of claim 1, wherein the polynucleotide composition comprises a liquid, a solution, a suspension, an emulsion, a reverse emulsion, a colloid, a dispersion, a gel, liposomes, micelles, an injectable material, an aerosol, a solid, a powder, a particulate, a nanoparticle, or a combination thereof.
10. The method of claim 1, wherein the non-epidermal plant cell is haploid, wherein the method further comprises the step of chromosome doubling in the non-epidermal plant cell comprising the altered target nucleotide sequence to produce a doubled haploid cell that is homozygous for the altered target nucleotide sequence.
11. The method of claim 10, further comprising regeneration of a doubled haploid plant from the doubled haploid cell, wherein the regenerated doubled haploid plant comprises cells that are homozygous for the altered target nucleotide sequence.

12. The method of claim 3, wherein the RNA-guided nuclease is selected from the group consisting of an RNA-guided DNA endonuclease, a type II Cas nuclease, a Cas9, a type V Cas nuclease, a Cpf1, a CasY, a CasX, a C2c1, a C2c3, an engineered nuclease, and a codon-optimized nuclease.
13. The method of claim 1, wherein the genetic alteration is heritable to succeeding generations.