Geminivirus-based construct designed such that the heterologous DNA is not incorporated into the cell genome but the construct is capable of replicating and spreading within the cells of the plant grown from the seed comprising said heterologous DNA.

Any priming system and conditions as is known to a person skilled in the art can be used according to the teachings of the present invention, including systems comprising aqueous solutions with adequate osmotic potential and systems comprising solid particulates. The water potential of the medium enables water uptake that is insufficient for complete seed imbibitions, and allows only the initial stages of germination but not radicle emergence (completion of germination). The system and conditions are typically selected according to the species of the plant seed.

According to yet additional embodiments, the present invention provides a seed produced by the methods of the invention, the seed comprises a virus-based DNA construct comprising heterologous DNA, and plants or parts thereof produced from said seed.

According to certain embodiments, the plant seed is of a monocot origin. According to other embodiments, the plant seed is of a dicot origin.

Other objects, features and advantages of the present invention will become clear from the following description and drawings.

Definitions of the specific embodiments of the invention as claimed herein follow.

According to a first embodiment of the invention, there is provided a method for introducing heterologous DNA into at least one cell of a plant seed embryo comprising priming a plant seed with a priming medium containing a virus-based DNA construct comprising the heterologous DNA under conditions enabling priming and uptake of the DNA by the plant seed, thereby obtaining the seed embryo comprising the heterologous DNA.

According to a second embodiment of the invention, there is provided a seed produced by the method of the first embodiment, wherein the seed comprises a virus-based DNA construct comprising heterologous DNA.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 demonstrates the effect of seed priming on germination uniformity.

FIG. 2 is an illustration of the Geminivirus based constructs used in the seed priming experiments described herein. Fig 2A: IL-60-BS. Fig. 2B: pIR-GUS.

FIG. 3 shows the presence of GUS sequences in true tomato leaves following seed priming in the presence of the Geminivirus based constructs, detected by PCR. The dashed and solid arrows indicate the position of a size marker of 1,500 bp and the position of GUS (ca. 1,800 bp) respectively (lane 1). Lanes 8 and 12 are negative controls: PCR from plants grown from non-treated seeds and from plants grown from seeds imbibed with no DNA construct, respectively.

FIG. 4A and **4B** show cross sections of tomato leaves stained for GUS activity

Example 4: expression of specific DNA from IL-60 vectors under modified priming conditions.

Priming was conducted as in example 2 with the basic priming solution containing 5% PEG and the addition of 75% dimethylsulfoxide (DMSO) as to obtain final osmotic potential of -0.131 mPa (1.296 Atm., according to Christopher et. al Macromolecules 2003. 36:6888-6893). In this modified priming solution, the IR-GUS DNA was introduced and replicated as indicated by GUS expression as described in Examples 2 and 3 above.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means, materials, and steps for carrying out various disclosed functions may take a variety of alternative forms without departing from the invention.

The term "comprise" and variants of the term such as "comprises" or "comprising" are used herein to denote the inclusion of a stated integer or stated integers but not to exclude any other integer or any other integers, unless in the context or usage an exclusive interpretation of the term is required.

Any reference to publications cited in this specification is not an admission that the disclosures constitute common general knowledge in Australia.

CLAIMS

1. A method for introducing heterologous DNA into at least one cell of a plant seed embryo comprising priming a plant seed ~~which has not undergone imbibitions~~ with a priming medium containing a ~~viral~~ virus-based DNA ~~expression~~ construct comprising the heterologous DNA~~, wherein said priming initiates seed germination but does not allow radicle emergence, wherein said priming allows uptake of the DNA by the plant seed,~~ under conditions enabling priming and uptake of the DNA by the plant seed, thereby obtaining the seed embryo comprising the heterologous DNA.

2. The method of claim 1, further comprising ~~subsequently~~ germinating and growing the ~~said~~ plant seed following the introducing so as to obtain a plant or part thereof comprising the heterologous DNA.

3. The method of claim 1, wherein the ~~viral~~virus-based DNA ~~expression~~ construct comprises viral genes or parts thereof enabling replication and symptomless spreading of the construct into adjacent plant cells ~~encoding a coat protein, a V2 protein and a C4 protein~~.

4. The method of claim 3, wherein the DNA ~~expression~~ construct is a Geminivirus ~~DNA expression~~ based construct.

5. The method of claim 4, wherein the DNA ~~expression~~ construct ~~further~~ comprises ~~a bacterial polynucleotide sequence~~ the heterologous DNA flanked by a non-contiguous nucleic acid sequences encoding Geminivirus replicase or replicase-associated proteins.

6. The method of ~~any one of~~ claims ~~4~~-5, wherein the DNA construct further comprises a polynucleotide sequence encoding a modified Geminivirus ~~is Tomato Yellow Leaf Curl Virus (TYLCV)~~ coat protein (CP).

7. The method of claim 6, wherein the ~~DNA expression construct comprises the nucleic acids sequence set forth in SEQ ID NO:1 or SEQ ID NO:2~~ modified Geminivirus coat protein comprises a mutation or deletion in nucleotides encoding an N-terminal 100 amino acids.

8. The method of claim ~~1~~5, wherein the DNA ~~expression~~ construct further

comprises ~~at least one regulatory element selected from the group consisting of an enhancer, a promoter, and a transcription termination sequence~~ a polynucleotide sequence encoding a modified Geminivirus V2 protein.

9. The method of ~~any one of~~ claims ~~1-2~~8, wherein the DNA ~~expression~~ construct further comprises a polynucleotide sequence~~marker for identifying the seed and plants comprising the heterologous DNA~~encoding a modified Geminivirus C4 protein.

10. The method of claim ~~1~~5, wherein the ~~heterologous~~ DNA construct further comprises a bacterial polynucleotide sequence~~encodes a product selected from the group consisting of a peptide, a polypeptide, a protein and an RNA molecule~~.

11. The method of any one of claims 4-10, wherein the Geminivirus is Tomato Yellow Leaf Curl Virus (TYLCV)~~RNA molecule is an inhibitory RNA selected from the group consisting of antisense RNA, dsRNA and siRNA~~.

12. The method of claim 1~~0~~, wherein the DNA construct is an expression construct thereby the heterologous DNA is expressed within the plant cell~~encoded product confers a desirable agronomic trait selected from the group consisting of resistance to biotic or abiotic stress, increased yield, increased yield quality and growth pattern~~.

13. The method of claim 12, wherein the ~~heterologous~~ DNA construct further comprises at least one regulatory element selected from the group consisting of an enhancer, a promoter, and a transcription termination sequence~~is transiently expressed in the at least one cell of the seed embryo and cells derived therefrom~~.

14. The method of claim 1 or claim 2, wherein the DNA construct further comprises a marker for identifying the seed and plants comprising the heterologous DNA~~priming medium is an aqueous solution~~.

15. The method of claim 1, wherein the ~~priming medium is a solid matrix~~ heterologous DNA encodes a product which confers a desirable agronomic trait selected from the group consisting of resistance to biotic or abiotic stress,

increased yield quality and preferred growth pattern.

16. The method of claim 1, wherein the ~~A dried, primed seed which comprises a Geminivirus-based DNA construct comprising~~ heterologous DNA~~, wherein saidheterologous DNA is expressed in the embryo of the seed~~ is transiently expressed in the at least one cell of the seed embryo and cells derived therefrom.

17. The method of claim 1, wherein ~~the seed is of a monocot origin~~ the heterologous DNA incorporated into the genome of the at least one cell of the seed embryo and cells derived therefrom.

18. The method of claim 1, wherein the ~~seed is of a dicot origin~~ priming medium is an aqueous solution.

19. The method of claim 1, wherein the ~~seed is of cone-bearing origin~~ priming medium is a solid matrix.

~~20. The method of claim 1, wherein said priming does not comprise microinjecting.~~

~~21~~20. A ~~The dried primed~~ seed produced ~~of claim 16, being generated according to~~ by the method of claim 1, wherein the seed comprises a virus-based DNA construct comprising heterologous DNA.