Claims

1. A method for creating a new gene in an organism, characterized by comprising the following steps:

simultaneously generating DNA breaks at two or more different specific sites in the organism's genome, wherein the specific sites are genomic sites capable of separating different genetic elements or different protein domains, and the DNA breaks are ligated to each other by a non-homologous end joining (NHEJ) or homologous repair, generating a new combination of the different genetic elements or different protein domains different from the original genomic sequence, thereby creating the new gene.

2. The method according to claim 1, characterized in that said two or more different specific sites locate on the same chromosome or on different chromosomes, or may be specific sites on at least two different genes, or may be at least two different specific sites on the same gene, wherein said at least two different genes may have the same or different transcription directions.

3. The method according to claim 1 or 2, characterized in that said DNA breaks are achieved by delivering a nuclease with targeting property into a cell of the organism to contact with the specific sites of the genomic DNA.

4. The method according to claim 3, characterized in that said nuclease with targeting property is selected from the group consisting of Meganuclease, Zinc finger nuclease, TALEN, and CRISPR/Cas system.

5. The method according to claim 3 or 4, characterized in that the nuclease with targeting property" is in the form of DNA, or exists in the form of mRNA or protein, but not DNA.

6. The method according to any one of claims 3-5, characterized in that the nucleases with targeting property are delivered into the cell by: 1) a PEG-mediated cell transfection method; 2) a liposome-mediated cell transfection method; 3) an electric shock transformation method; 4) a microinjection; 5) a gene gun bombardment; or 6) an *Agrobacterium*-mediated transformation method.

7. The method according to any one of claims 1-6, characterized in that said gene elements are selected from the group consisting of a promoter, a 5' untranslated region, a coding region or non-coding RNA region, a 3' untranslated region, a terminator of the gene, or any combination thereof.

8. The method according to any one of claims 1-7, characterized in that the combination of different gene elements is a combination of the promoter of one of the two genes with different

expression patterns and the coding region or the non-coding RNA region of the other gene,

a combination of the region from the promoter to 5'UTR of one of the two genes with different expression patterns and the CDS or non-coding RNA region of the other gene, or

a combination of adjacent gene elements of the same gene.

9. The method of claim 8, characterized in that, in the combination of different gene elements, one element is a strong endogenous promoter of the organism, and the other is the coding region of HPPD, EPSPS, PPO or GH1 gene.

10. The method according to claim 8 or 9, characterized in that the different expression patterns are different levels of gene expression, different tissue-specificities of gene expression, or different developmental stage-specificities of gene expression.

11. The method according to any one of claims 1-10, characterized in that the protein domain is a DNA fragment corresponding to a specific functional domain of a protein; preferably including but not being limited to a nuclear localization signal, a chloroplast leading peptide, a mitochondrial leading peptide, a phosphorylation site, a methylation site, a transmembrane domain, a DNA binding domain, a transcription activation domain, a receptor activation domain, or an enzyme catalytic center.

12. The method according to any one of claims 1-11, characterized in that the combination of different protein domains is a combination of the localization signal region of one of two proteins with different subcellular localizations and the mature protein coding region of the other gene,

a combination of two protein domains with different biological functions, or

a combination of adjacent protein domains of the same gene.

13. The method of claim 12, characterized in that the different subcellular locations are selected from the group consisting of nuclear location, cytoplasmic location, cell membrane location, chloroplast location, mitochondrial location, and endoplasmic reticulum membrane location, or the different biological functions are selected from the group consisting of recognition of specific DNA or RNA conserved sequence, activation of gene expression, binding to a protein ligand, binding to small molecular signal, binding to an ion, specific enzymatic reaction, and any combination thereof.

14. The method according to any one of claims 1-7 and 11, characterized in that the combination of gene elements and protein domains are a combination of protein domains and adjacent promoters, 5'UTR, 3'UTR or terminators of the same gene.

15. A new gene obtainable by the method according to any one of claims 1-14.

16. The new gene according to claim 15, characterized in that compared with the original gene, the new gene either has a different promoter and therefore is expressed with a different spatial-temporal characteristics or a different intensity characteristics or a different developmental stage characteristics, or has a new amino acid sequence; preferably, the "new amino acid sequence" may either be an integral fusion of two or more gene coding regions, or a partial fusion of coding regions or a doubling of a part of protein coding region of the same gene .

17. The new gene according to claim 15 or 16, characterized in that the new gene is a highly-expressed endogenous HPPD, EPSPS, PPO or GH1 gene in the organism.

18. A DNA containing the gene according to any one of claims 15-17.

19. A protein encoded by the gene according to any one of claims 15-17, or a biologically active fragment thereof.

20. A recombinant expression vector, which comprises the gene according to any one of claims 15-17 and a promoter operably linked thereto.

21. An expression cassette comprising the gene according to any one of claims 15-17.

22. A host cell, which comprises the expression cassette according to claim 21.

23. An organism regenerated from the host cell according to claim 22.

24. Use of the gene according to any one of claims 15-17 in conferring or improving a resistance/tolerance trait or a growth advantage trait in an organism.

25. A composition, which comprises:

(a) a promoter of one of two genes with different expression patterns and a coding region or non-coding RNA region of the other gene;

(b) a promoter to a 5' untranslated region of one of two genes with different expression patterns and a coding region or non-coding RNA region of the other gene;

(c) adjacent gene elements within the same gene;

(d) a localization signal region of one of the two protein coding genes with different subcellular localizations and a mature protein coding region of the other gene;

(e) two protein domains with different biological functions;

(f) adjacent protein domains in the same gene; or,

(g) a protein domain and an adjacent promoter, 5' untranslated region, 3' non-coding region or terminator in the same gene.

26. An editing method for increasing the gene expression level of a target endogenous gene in an organism, which is independent of an exogenous DNA donor fragment, which comprises the following steps:

simultaneously generating DNA breaks separately at selected sites between the promoter and the coding region of each of the target endogenous gene and an optional endogenous highly-expressing gene; ligating the DNA breaks to each other by means of non-homologous end joining (NHEJ) or homologous repair, thereby generating an in vivo fusion of the coding region of the target endogenous gene and the optional strong endogenous promoter to form a new highly-expressing endogenous gene, wherein the target endogenous gene and the optional endogenous highly-expressing gene are located on the same chromosome or different chromosomes.

27. An editing method for knocking up the expression of the endogenous HPPD, EPSPS or PPO gene in a plant, characterized in that it comprises fusing the coding region of the HPPD, EPSPS or PPO gene with a strong endogenous promoter of a plant in vivo to form a new highly-expressing plant endogenous HPPD, ESSPS or PPO gene, respectively.

28. The editing method according to claim 27, characterized in that it comprises the following steps: simultaneously generating DNA breaks respectively in selected specific sites between the promoter and the coding region of each of the HPPD, EPSPS or PPO gene and an optional endogenous highly-expressing gene , ligating the DNA breaks to each other through an intracellular repair pathway, generating in vivo a fusion of the coding region of the HPPD, EPSPS or PPO gene and the optional strong endogenous promoter to form a new highly-expressing HPPD, EPSPS or PPO gene, respecitively.

29. The editing method according to claim 27 or 28, characterized in that it comprises fusing the coding region of the HPPD gene with a strong endogenous promotor of a rice, wherein the strong promoter is a promoter of ubiquitin2 gene,

it comprises fusing the coding region of the EPSPS gene with a strong endogenous promotor of a rice, wherein the strong promotor is a promotor of TKT gene, or

it comprises fusing the coding region of the PPO gene with a strong endogenous promotor of a rice or an *Arabidopsis*, wherein in rice, the strong promotor is a promotor of CP12 gene, and in *Arabidopsis*, the strong promotor is a promotor of ubiquitin10 gene.

30. A highly-expressing plant endogenous HPPD, EPSES or PPO gene obtainable by the

editing method according to any one of claims 27-29.

31. A highly-expressing rice endogenous HPPD gene, which has a sequence selected from the group consisting of:

(1) the nucleic acid sequence as shown in SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 18 or SEQ ID NO: 19 or a portion thereof or a complementary sequence thereof;

(2) a sequence having an identity of at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% to any one of the sequences as defined in (1); or

(3) a nucleic acid sequence capable of hybridizing to the sequence as shown in (1) or (2) under a stringent condition.

32. A highly-expressing rice endogenous EPSPS gene, which has a sequence selected from the group consisting of:

(1) the nucleic acid sequence as shown in SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 or SEQ ID NO: 14 or a portion thereof or a complementary sequence thereof;

(2) a sequence having an identity of at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% to any one of the sequences as defined in (1); or

(3) a nucleic acid sequence capable of hybridizing to the sequence as shown in (1) or (2) under a stringent condition.

33. A highly-expressing rice endogenous PPO gene, which has a sequence selected from the following:

(1) the nucleic acid sequence as shown in SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, or SEQ ID NO: 26 or a portion thereof or a complementary sequence thereof;

(2) a sequence having an identity of at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% to any one of the sequences as defined in (1); or

(3) a nucleic acid sequence capable of hybridizing to the sequence as shown in (1) or (2) under a stringent condition.

34. A DNA comprising the gene according to any one of claims 30-33.

35. A protein or a biologically active fragment thereof encoded by the gene according to any one of claims 30-33.

36. A recombinant expression vector, which comprises the gene according to any one of

claims 30-33, and a promoter operably linked thereto.

37. An expression cassette comprising the gene according to any one of claims 30-33.

38. A plant host cell, which comprises the expression cassette according to claim 37.

39. A plant regenerated from the plant host cell according to claim 38.

40. A method for producing a plant with an increased resistance or tolerance to an herbicide, which comprises regenerating the plant host cell according to claim 38 into a plant and a progeny derived therefrom.

41. A rice resistant to a herbicide, which comprises one or a combination of two or more of the highly-expressing rice endogenous HPPD gene according to claim 31, the highly-expressing rice endogenous EPSPS gene according to claim 32, and the highly-expressing rice endogenous PPO gene according to claim 33.

42. A method for controlling a weed in a cultivation site of a plant, wherein the plant is selected from the group consisting of the plant according to claim 39, a plant prepared by the method according to claim 40, or the rice according to claim 41, wherein the method comprises applying to the cultivation site one or more of HPPD, EPSPS or PPO inhibitory herbicides in an amount for effectively controlling the weed.