

3割～5割を占めることも明らかになったが、大豆やトウモロコシといった実用作物への利用も進んでおり、これらのフードチェーンへの混入に、今後一層注視する必要があると考えられる。

*SPK*遺伝子および*FLO2*遺伝子を標的としたTALEN遺伝子の構築においては、SSAアッセイによりこれらのTALEN遺伝子が部位特異的なDNA切断を行う能力を有することが確認された。この結果から、われわれが開発したEmerald Gateway TALENシステムおよびSSAアッセイシステムが期待通りに機能することがわかった。これらの成果は今後のゲノム編集技術の普及及び標準化に寄与するものと考えられる。

作製したTALEN遺伝子を用いてイネの形質転換を行いTALENを導入した形質転換体を得た。このうち、*SPK*遺伝子を標的とするTALENを導入した形質転換体では、導入したTALEN遺伝子の検出に成功したが、これらの形質転換個体ではゲノム編集を検出することができなかった。このことはゲノム編集が起こる効率があまり高くないことを示唆するものと考えられる。また、形質転換体に導入されたTALENは継続的に働くものと考えられるため、植物体を育成していく過程でゲノム編集が起こる可能性も考えられる。今後はこれらの再分化植物の中でのゲノム編集の事象について継続的に調べていく必要があると思われる。また、TALEN遺伝子を導入した形質転換体を継続的に作製することでゲノム編集効率が高まるものと考えられる。

## E. 結論

今年度は、昨年度に引き続き、TALEN

やCRISPR/Cas9といったNBTの植物分野への応用の状況について論文の調査を行い、NBT、特にTALENとCRISPR/Cas9がモデル植物だけでなく、穀類等の実用作物へも盛んに応用されている実態がより鮮明になった。とくに、CRISPR/Cas9の報告数が前年の約2倍となり、本技術の植物に対する利用が加速している実態が明らかになった。世界的には、米国と中国が二大NBT実施国となっているが、中国におけるTALEN及びCRISPR/Cas9の植物全般への適用状況について引き続き注視する必要があるといえる。

また、TALEN技術の確立と検知モデルの作成のため、イネの*SPK*遺伝子および*FLO2*遺伝子を標的としたTALEN遺伝子の構築を行い、これを用いた形質転換を行った。*SPK*遺伝子を標的とするTALENを導入した形質転換体が63個体得られたが、これらのうちでゲノム編集が検出された個体は見出されていない。このことはゲノム編集の効率がそれほど高いものではないことを示唆する。また、作製したTALENの機能検定を行い、SSAアッセイにより、これらが標的配列を認識して切断する活性を有することを示した。これらの技術および作出された形質転換体はTALENを用いたNBT技術の解析に供することができると考えている。

さらに、NBT適用植物の作成過程において「中間体」として構築されると想定される、TALEN遺伝子を導入した遺伝子組換えイネを、組換え体検知対象モデルとして使用し、その検知法を開発、評価した。その結果、一般的と考えられるTALEN遺伝子を標的とした組換え体のPCR法による

検知が可能であることが示された。

#### 参考文献

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- 2) Sakuma, T., Ochiai, H., Kaneko, T., Tomoji, M., Tokumaru, D., Sakane, Y., Suzuki, K., Miyamoto, T., Sakamoto, N., Matsuura, S., Yamamoto, T., *Sci. Rep.*, **3**:b 3379, 1-8 (2013)
- 3) Asano, T., Kunieda, N., Omura, Y., Ibe, H., Kawasaki, T., Takano, M., Sato, M., Furuhashi, H., Mujina, T., Takaiwa, F., Wu, C.Y., Tada, Y., Satozawa, T., Sakamoto, M. and Shimada, H. *The Plant Cell*, **14**(3), 619-628 (2002)
- 4) 「安全性未審査の中国産米加工品の検知法について」食安監発第 0220002 号 (平成 19 年 2 月 20 日)
- 5) Sakuma, T., Hosoi, S., Woltjen, K., Suzuki, K., Kashiwagi, K., Wada, H., Ochiai, H., Miyamoto, T., Kawai, N., Sasakuma, Y., Matuura, S., Okada, Y., Kawahara, A., Hayashi, S., Yamamoto, T., *Gene Cells*, **18**, 315-326 (2013)

#### F. 研究発表

##### 論文発表

Kusano, H., Onodera, H., Kihira, M., Aoki, H., Matsuzaki, H., Shimada, H. A simple Gateway-assisted construction system of TALEN genes for plant genome editing. (投稿中)

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- 2) 小野寺瞳、紀平望帆、草野博彰、島田浩章:TALEN のための 2 遺伝子発現ベクターの開発と植物ゲノム編集の簡略化、第 33 回日本植物細胞分子生物学会大会・シンポジウム、東京大学弥生キャンパス(2015. 8)
- 3) 草野博彰、小野寺瞳、紀平望帆、島田浩章:植物で TALEN 遺伝子を構築するためのエントリーベクター pPlat シリーズの開発、第 33 回日本植物細胞分子生物学会大会・シンポジウム、東京大学弥生キャンパス(2015. 8)
- 4) 紀平望帆、小野寺瞳、青木裕美、草野博彰、島田浩章:植物細胞内における TALEN 活性評価のためのプラスミド開発:第 33 回日本植物細胞分子生物学会大会・シンポジウム、東京大学弥生キャンパス(2015. 8)
- 5) 紀平望帆、青木裕美、小野寺瞳、板垣文子、堀江峻晃、松崎ひかる、草野博彰、島田浩章:植物ゲノム編集のための 2 遺伝子発現ベクター構築系と SSA アッセイプラスミドの開発。第 38 回日本分子生物学会年会・第 38 回日本生化学会大会合同大会(BMB2015)、神戸(2015. 11)
- 6) 小野寺瞳、紀平望帆、青木裕美、堀江峻晃、河野徳昭、吉松嘉代、近藤一成、

松崎ひかる、板垣文子、草野博彰、島田浩章 : Construction of the desired TALEN gene using the Emerald Gateway TALEN kit for the rice genome editing and its evaluation、第

57回日本植物生理学会年会、岩手大学  
上田キャンパス(2016. 3)

G. 知的所有権の出願・登録状況  
該当なし

query: ZFN, zinc finger nuclease, plant

Year	Applied plant species
ID	Affiliation, country
Identifiers	Title
Category	Description
	Details
2015 1 PMID:26687994 ZFN	Arabidopsis University of Minnesota, MN, USA. Histone H2AX and the small RNA pathway modulate both non-homologous end-joining and homologous recombination in plants. Qi Y, Zhang Y, Baller JA, Voytas DF. Mutat Res. 2016 Jan;783:9-14. doi: 10.1016/j.mrfmmm.2015.12.002. Epub 2015 Dec 4.
2015 2 PMID:26681515 ZFN	soybean University of Minnesota, MN, USA. MicroRNA Maturation and MicroRNA Target Gene Expression Regulation Are Severely Disrupted in Soybean dicer-like1 Double Mutants. Curtin SJ, Michno JM, Campbell BW, Gil-Humanes J, Mathioni SM, Hammond R, Gutierrez-Gonzalez JJ, Donohue RC, Kantar MB, Eamens AL, Meyers BC, Voytas DF, Stupar RM. G3 (Bethesda). 2015 Dec 17;6(2):423-33. doi: 10.1534/g3.115.022137.
2015 3 PMID:26452472 ZFN	rice University of Arkansas, AR, USA. Gene stacking in plant cell using recombinases for gene integration and nucleases for marker gene deletion. Nandy S, Zhao S, Pathak BP, Manoharan M, Srivastava V. BMC Biotechnol. 2015 Oct 9;15:93. doi: 10.1186/s12896-015-0212-2.
2015 4 PMID:26426390 ZFN	tobacco Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Germany Targeted gene exchange in plant cells mediated by a zinc finger nuclease double cut. Schneider K, Schiermeyer A, Dolls A, Koch N, Herwartz D, Kirchhoff J, Fischer R, Russell SM, Cao Z, Corbin DR, Sastry-Dent L, Ainley WM, Webb SR, Schinkel H, Schillberg S. Plant Biotechnol J. 2015 Oct 1. doi: 10.1111/pbi.12483. [Epub ahead of print]
2015 5 PMID:25913173 ZFN	corn Dow AgroSciences LLC, IN, USA A modular gene targeting system for sequential transgene stacking in plants. Kumar S, AlAbed D, Worden A, Novak S, Wu H, Ausmus C, Beck M, Robinson H, Minnicks T, Hemingway D, Lee R, Skaggs N, Wang L, Marri P, Gupta M. J Biotechnol. 2015 Aug 10;207:12-20. doi: 10.1016/j.jbiotec.2015.04.006. Epub 2015 Apr 23.
2014 6 PMID:25528147 ZFN	apple, fig Agricultural Research Organization, Bet-Dagan, Israel Targeted mutagenesis using zinc-finger nucleases in perennial fruit trees. Peer R, Rivlin G, Golobovitch S, Lapidot M, Gal-On A, Vainstein A, Tzfira T, Flaishman MA. Planta. 2014 Dec 21. [Epub ahead of print]
2014 7 PMID:25018764 ZFN	rice International Rice Research Institute Metro Manila, Philippines Identification of safe harbor" loci in indica rice genome by harnessing the property of zinc-finger nucleases to induce DNA damage and repair." Cantos C, Francisco P, Trijatmiko KR, Slamet-Loedin I, Chadha-Mohanty PK. Front Plant Sci. 2014;5:302. doi: 10.3389/fpls.2014.00302.
2014 8 PMID:24443519 ZFN	tobacco University of Minnesota, MN, USA. DNA replicons for plant genome engineering. Baltes NJ, Gil-Humanes J, Cermak T, Atkins PA, Voytas DF. Plant Cell. 2014 Jan;26(1):151-63. doi: 10.1105/tpc.113.119792. Epub 2014 Jan 17.
2014 9 PMID:24057367 ZFN	Arabidopsis University of Minnesota, MN, USA. Tailor-made mutations in Arabidopsis using zinc finger nucleases. Qi Y, Starker CG, Zhang F, Baltes NJ, Voytas DF. Methods Mol Biol. 2014;1062:193-209. doi: 10.1007/978-1-62703-580-4_10.

表 1. NBT 応用植物に関する文献調査結果(ZFN) (その 1)

query: ZFN, zinc finger nuclease, plant

Year	Applied plant species
ID	Affiliation, country
Identifiers	Title
Category	Description
	Details
2015 1	Arabidopsis University of Minnesota, MN, USA. Histone H2AX and the small RNA pathway modulate both non-homologous end-joining and homologous recombination in plants. PMID:26687994 ZFN Qi Y, Zhang Y, Baller JA, Voytas DF. Mutat Res. 2016 Jan;783:9-14. doi: 10.1016/j.mrfmmm.2015.12.002. Epub 2015 Dec 4.
2015 2	soybean University of Minnesota, MN, USA. MicroRNA Maturation and MicroRNA Target Gene Expression Regulation Are Severely Disrupted in Soybean dicer-like1 Double Mutants. PMID:26681515 ZFN Curtin SJ, Michno JM, Campbell BW, Gil-Humanes J, Mathioni SM, Hammond R, Gutierrez-Gonzalez JJ, Donohue RC, Kantar MB, Eamens AL, Meyers BC, Voytas DF, Stupar RM. G3 (Bethesda). 2015 Dec 17;6(2):423-33. doi: 10.1534/g3.115.022137.
2015 3	rice University of Arkansas, AR, USA. Gene stacking in plant cell using recombinases for gene integration and nucleases for marker gene deletion. PMID:26452472 ZFN Nandy S, Zhao S, Pathak BP, Manoharan M, Srivastava V. BMC Biotechnol. 2015 Oct 9;15:93. doi: 10.1186/s12896-015-0212-2.
2015 4	tobacco Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Germany Targeted gene exchange in plant cells mediated by a zinc finger nuclease double cut. PMID:26426390 ZFN Schneider K, Schiermeyer A, Dolls A, Koch N, Herwartz D, Kirchhoff J, Fischer R, Russell SM, Cao Z, Corbin DR, Sastry-Dent L, Ainley WM, Webb SR, Schinkel H, Schillberg S. Plant Biotechnol J. 2015 Oct 1. doi: 10.1111/pbi.12483. [Epub ahead of print]
2015 5	corn Dow AgroSciences LLC, IN, USA A modular gene targeting system for sequential transgene stacking in plants. PMID:25913173 ZFN Kumar S, AlAbed D, Worden A, Novak S, Wu H, Ausmus C, Beck M, Robinson H, Minnick T, Hemingway D, Lee R, Skaggs N, Wang L, Marri P, Gupta M. J Biotechnol. 2015 Aug 10;207:12-20. doi: 10.1016/j.jbiotec.2015.04.006. Epub 2015 Apr 23.
2014 6	apple, fig Agricultural Research Organization, Bet-Dagan, Israel Targeted mutagenesis using zinc-finger nucleases in perennial fruit trees. PMID:25528147 ZFN Peer R, Rivlin G, Golobovitch S, Lapidot M, Gal-On A, Vainstein A, Tzfira T, Flaishman MA. Planta. 2014 Dec 21. [Epub ahead of print]
2014 7	rice International Rice Research Institute Metro Manila, Philippines Identification of safe harbor" loci in indica rice genome by harnessing the property of zinc-finger nucleases to induce DNA damage and repair." PMID:25018764 ZFN Cantos C, Francisco P, Trijatmiko KR, Slamet-Loedin I, Chadha-Mohanty PK. Front Plant Sci. 2014;5:302. doi: 10.3389/fpls.2014.00302.
2014 8	tobacco University of Minnesota, MN, USA. DNA replicons for plant genome engineering. PMID:24443519 ZFN Baltes NJ, Gil-Humanes J, Cermak T, Atkins PA, Voytas DF. Plant Cell. 2014 Jan;26(1):151-63. doi: 10.1105/tpc.113.119792. Epub 2014 Jan 17.
2014 9	Arabidopsis University of Minnesota, MN, USA. Tailor-made mutations in Arabidopsis using zinc finger nucleases. PMID:24057367 ZFN Qi Y, Starker CG, Zhang F, Baltes NJ, Voytas DF. Methods Mol Biol. 2014;1062:193-209. doi: 10.1007/978-1-62703-580-4_10.

表1. NBT応用植物に関する文献調査結果(ZFN) (その2)



Year	Applied plant species
ID	Affiliation, country
Identifiers	Title
Category	Description
	Details
2013 10 PMID:23996306 ZFN	soybean University of Minnesota, MN, USA. Targeted mutagenesis for functional analysis of gene duplication in legumes. Curtin SJ, Anderson JE, Starker CG, Baltes NJ, Mani D, Voytas DF, Stupar RM. Methods Mol Biol. 2013;1069:25-42. doi: 10.1007/978-1-62703-613-9_3.
2013 11 PMID:23979943 ZFN	Arabidopsis University of Minnesota, MN, USA. Targeted deletion and inversion of tandemly arrayed genes in Arabidopsis thaliana using zinc finger nucleases. Qi Y, Li X, Zhang Y, Starker CG, Baltes NJ, Zhang F, Sander JD, Reyon D, Joung JK, Voytas DF. G3 (Bethesda). 2013 Oct 3;3(10):1707-15. doi: 10.1534/g3.113.006270.
2013 12 PMID:23953646 ZFN	corn Dow AgroSciences LLC, IN, USA Trait stacking via targeted genome editing. Ainley WM, Sastry-Dent L, Welter ME, Murray MG, Zeitler B, Amora R, Corbin DR, Miles RR, Arnold NL, Strange TL, Simpson MA, Cao Z, Carroll C, Pawelczak KS, Blue R, West K, Rowland LM, Perkins D, Samuel P, Dewes CM, Shen L, Sriram S, et al. Plant Biotechnol J. 2013 Dec;11(9):1126-34. doi: 10.1111/pbi.12107. Epub 2013 Aug 19.
2013 13 PMID:23625357 ZFN/TALEN	Nicotiana benthamiana, Arabidopsis the Weizmann Institute of Science, Rehovot, Israel A rapid assay to quantify the cleavage efficiency of custom-designed nucleases in planta. Johnson RA, Gurevich V, Levy AA. Plant Mol Biol. 2013 Jun;82(3):207-21. doi: 10.1007/s11103-013-0052-1. Epub 2013 Apr 28.
2013 14 PMID:23509176 ZFN	tobacco, Arabidopsis University of Michigan, Michigan, USA Nonhomologous end joining-mediated gene replacement in plant cells. Weinthal DM, Taylor RA, Tzfira T. Plant Physiol. 2013 May;162(1):390-400. doi: 10.1104/pp.112.212910. Epub 2013 Mar 18.
2013 15 PMID:23282329 ZFN	Arabidopsis University of Minnesota, MN, USA. Increasing frequencies of site-specific mutagenesis and gene targeting in Arabidopsis by manipulating DNA repair pathways. Qi Y, Zhang Y, Zhang F, Baller JA, Cleland SC, Ryu Y, Starker CG, Voytas DF. Genome Res. 2013 Mar;23(3):547-54. doi: 10.1101/gr.145557.112. Epub 2013 Jan 2.
2013 16 PMID:23279135 ZFN	Arabidopsis Leiden University, Leiden, The Netherlands ZFN-mediated gene targeting of the Arabidopsis protoporphyrinogen oxidase gene through Agrobacterium-mediated floral dip transformation. de Pater S, Pinas JE, Hooykaas PJ, van der Zaal BJ. Plant Biotechnol J. 2013 May;11(4):510-5. doi: 10.1111/pbi.12040. Epub 2012 Dec 28.
2012 17 PMID:22082504 ZFN	Arabidopsis University of Michigan, Michigan, USA Zinc finger nuclease and homing endonuclease-mediated assembly of multigene plant transformation vectors. Zeevi V, Liang Z, Arieli U, Tzfira T. Plant Physiol. 2012 Jan;158(1):132-44. doi: 10.1104/pp.111.184374. Epub 2011 Nov 14.
2011 18 PMID:21848915 ZFN	Arabidopsis the Weizmann Institute of Science, Rehovot, Israel Localized egg-cell expression of effector proteins for targeted modification of the Arabidopsis genome. Even-Faitelson L, Samach A, Melamed-Bessudo C, Avivi-Ragolsky N, Levy AA. Plant J. 2011 Dec;68(5):929-37. doi: 10.1111/j.1365-313X.2011.04741.x. Epub 2011 Oct 4.

表1. NBT応用植物に関する文献調査結果(ZFN) (その3)

Year	Applied plant species
ID	Affiliation, country
Identifiers	Title
Category	Description
	Details
2011 19 PMID:21464476 ZFN	soybean University of Minnesota, MN, USA. Targeted mutagenesis of duplicated genes in soybean with zinc-finger nucleases. Curtin SJ, Zhang F, Sander JD, Haun WJ, Starker C, Baltes NJ, Reyon D, Dahlborg EJ, Goodwin MJ, Coffman AP, Dobbs D, Joung JK, Voytas DF, Stupar RM. Plant Physiol. 2011 Jun;156(2):466-73. doi: 10.1104/pp.111.172981. Epub 2011 Apr 4.
2011 20 PMID:21181530 ZFN	Arabidopsis University of Minnesota, MN, USA. Targeted mutagenesis in Arabidopsis using zinc-finger nucleases. Zhang F, Voytas DF. Methods Mol Biol. 2011;701:167-77. doi: 10.1007/978-1-61737-957-4_9.
2011 21 PMID:21151135 ZFN	Arabidopsis, soybean Massachusetts General Hospital, Massachusetts, USA Selection-free zinc-finger-nuclease engineering by context-dependent assembly (CoDA). Sander JD, Dahlborg EJ, Goodwin MJ, Cade L, Zhang F, Cifuentes D, Curtin SJ, Blackburn JS, Thibodeau-Beganny S, Qi Y, Pierick CJ, Hoffman E, Maeder ML, Khayter C, Reyon D, Dobbs D, Langenau DM, Stupar RM, Giraldez AJ, Voytas DF, Peterson RT, Yeh JR, et al. Nat Methods. 2011 Jan;8(1):67-9. doi: 10.1038/nmeth.1542. Epub 2010 Dec 12.
2010 22 PMID:20876340 ZFN	tobacco, petunia Danziger Innovations Ltd., Beit Dagan, Israel Nontransgenic genome modification in plant cells. Marton I, Zuker A, Shklarman E, Zeevi V, Tovkach A, Roffe S, Ovadis M, Tzfira T, Vainstein A. Plant Physiol. 2010 Nov;154(3):1079-87. doi: 10.1104/pp.110.164806. Epub 2010 Sep 27.
2010 23 PMID:20508152 ZFN	Arabidopsis University of Minnesota, MN, USA. High frequency targeted mutagenesis in Arabidopsis thaliana using zinc finger nucleases. Zhang F, Maeder ML, Unger-Wallace E, Hoshaw JP, Reyon D, Christian M, Li X, Pierick CJ, Dobbs D, Peterson T, Joung JK, Voytas DF. Proc Natl Acad Sci U S A. 2010 Jun 29;107(26):12028-33. doi: 10.1073/pnas.0914991107. Epub 2010 May 27.
2010 24 PMID:20508151 ZFN	Arabidopsis NIAS, Tsukuba, Japan Site-directed mutagenesis in Arabidopsis using custom-designed zinc finger nucleases. Osakabe K, Osakabe Y, Toki S. Proc Natl Acad Sci U S A. 2010 Jun 29;107(26):12034-9. doi: 10.1073/pnas.1000234107. Epub 2010 May 27. Erratum in: Proc Natl Acad Sci U S A. 2011 Jan 4;108(1):433.
2010 25 PMID:20454835 ZFN	tobacco Dow AgroSciences LLC, IN, USA Zinc finger nuclease-mediated transgene deletion. Petolino JF, Worden A, Curlee K, Connell J, Strange Moynahan TL, Larsen C, Russell S. Plant Mol Biol. 2010 Aug;73(6):617-28. doi: 10.1007/s11103-010-9641-4. Epub 2010 May 8.
2009 26 PMID:19754840 ZFN	Arabidopsis Leiden University, Leiden, The Netherlands ZFN-induced mutagenesis and gene-targeting in Arabidopsis through Agrobacterium-mediated floral dip transformation. de Pater S, Neuteboom LW, Pinas JE, Hooykaas PJ, van der Zaal BJ. Plant Biotechnol J. 2009 Oct;7(8):821-35. doi: 10.1111/j.1467-7652.2009.00446.x.
2009 27 PMID:19404259 ZFN	corn Dow AgroSciences LLC, IN, USA Precise genome modification in the crop species Zea mays using zinc-finger nucleases. Shukla VK, Doyon Y, Miller JC, DeKever RC, Moehle EA, Worden SE, Mitchell JC, Arnold NL, Gopalan S, Meng X, Choi VM, Rock JM, Wu YY, Katibah GE, Zhifang G, McCaskill D, Simpson MA, Blakeslee B, Greenwalt SA, Butler HJ, Hinkley SJ, Zhang L, et al. Nature. 2009 May 21;459(7245):437-41. doi: 10.1038/nature07992. Epub 2009 Apr 29.

表1. NBT応用植物に関する文献調査結果(ZFN) (その4)

Year	Applied plant species
ID	Affiliation, country
Identifiers	Title
Category	Description
	Details
2009 28 PMID:19404258 ZFN	tobacco University of Minnesota, MN, USA. High-frequency modification of plant genes using engineered zinc-finger nucleases. Townsend JA, Wright DA, Winfrey RJ, Fu F, Maeder ML, Joung JK, Voytas DF. Nature. 2009 May 21;459(7245):442-5. doi: 10.1038/nature07845. Epub 2009 Apr 29.
2009 29 PMID:19112554 ZFN	tobacco Dow AgroSciences LLC, IN, USA Targeted transgene integration in plant cells using designed zinc finger nucleases. Cai CQ, Doyon Y, Ainley WM, Miller JC, Dekelver RC, Moehle EA, Rock JM, Lee YL, Garrison R, Schulenberg L, Blue R, Worden A, Baker L, Faraji F, Zhang L, Holmes MC, Rebar EJ, Collingwood TN, Rubin-Wilson B, Gregory PD, Urnov FD, Petolino JF. Plant Mol Biol. 2009 Apr;69(6):699-709. doi: 10.1007/s11103-008-9449-7. Epub 2008 Dec 27.
2008 30 PMID:18657511 ZFN	tobacco Massachusetts General Hospital, Massachusetts, USA Rapid open-source" engineering of customized zinc-finger nucleases for highly efficient gene modification." Maeder ML, Thibodeau-Beganny S, Osiak A, Wright DA, Anthony RM, Eichinger M, Jiang T, Foley JE, Winfrey RJ, Townsend JA, Unger-Wallace E, Sander JD, Miller-Lerch F, Fu F, Pearlberg J, Gabel C, Dassie JP, Pruett-Miller SM, Porteus MH, Sgroi DC, Lafrate AJ, Dobbs D, et al. Mol Cell. 2008 Jul 25;31(2):294-301. doi: 10.1016/j.molcel.2008.06.016.
2005 31 PMID:16262717 ZFN	tobacco University of Minnesota, MN, USA. High-frequency homologous recombination in plants mediated by zinc-finger nucleases. Wright DA, Townsend JA, Winfrey RJ Jr, Irwin PA, Rajagopal J, Lonosky PM, Hall BD, Jondle MD, Voytas DF. Plant J. 2005 Nov;44(4):693-705.
2005 32 PMID:15677315 ZFN	Arabidopsis University of Utah, UT, USA Targeted mutagenesis using zinc-finger nucleases in Arabidopsis. Lloyd A, Plaisier CL, Carroll D, Drews GN. Proc Natl Acad Sci U S A. 2005 Feb 8;102(6):2232-7. Epub 2005 Jan 26.

表2. NBT応用植物に関する文献調査結果(TALEN) (その1)



query: TALEN(s), TAL effector, plant (as of 2016/2/19)

Year	Applied plant species
ID	Affiliation, country
Identifiers	Title
Category	Description
	Details
2016 1	Arabidopsis, (Linum usitatissimum, CRISPR/Cas9) Cibus, CA, USA
PMID:26864017	Oligonucleotide-mediated genome editing provides precision and function to engineered nucleases and antibiotics in plants.
TALEN/CRISPR	Sauer NJ, Narváez-Vásquez J, Mozoruk J, Miller RB, Warburg ZJ, Woodward MJ, Mihiret YA, Lincoln TA, Segami RE, Sanders SL, Walker KA, Beetham PR, Schöpke CR, Gocal GF Plant Physiol. 2016 Feb 10. doi:pii: pp.01696.2015. [Epub ahead of print]
2015 2	rice National Institute of Agrobiological Sciences, Japan
PMID:26668331	A Defect in DNA Ligase4 Enhances the Frequency of TALEN-Mediated Targeted Mutagenesis in Rice.
TALEN	Nishizawa-Yokoi A, Cermak T, Hoshino T, Sugimoto K, Saika H, Mori A, Osakabe K, Hamada M, Katayose Y, Starker C, Voytas DF, Toki S. Plant Physiol. 2016 Feb;170(2):653-66. doi: 10.1104/pp.15.01542. Epub 2015 Dec 14.
2015 3	rice Chinese Academy of Sciences (CAS), Beijing, China
PMID:26641666	TALEN-Based Mutagenesis of Lipoxxygenase LOX3 Enhances the Storage Tolerance of Rice (Oryza sativa) Seeds.
TALEN	Ma L, Zhu F, Li Z, Zhang J, Li X, Dong J, Wang T. PLoS One. 2015;10(12):e0143877. doi: 10.1371/journal.pone.0143877.
2015 4	soybean Nanjing Agricultural University, Nanjing, China
PMID:26603121	Efficient targeted mutagenesis in soybean by TALENs and CRISPR/Cas9.
TALEN/CRISPR	Du H, Zeng X, Zhao M, Cui X, Wang Q, Yang H, Cheng H, Yu D. J Biotechnol. 2015 Nov 18. doi:pii: S0168-1656(15)30178-4. 10.1016/j.jbiotec.2015.11.005. [Epub ahead of print]
2015 5	barley Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany
PMID:26153077	Targeted Modification of Gene Function Exploiting Homology-Directed Repair of TALEN-Mediated Double-Strand Breaks in Barley.
TALEN	Budhagatapalli N, Rutten T, Gurushidze M, Kumlehn J, Hensel G. G3 (Bethesda). 2015 Jul 6;5(9):1857-63. doi: 10.1534/g3.115.018762.
2015 6	Nicotiana benthamiana Cellestis Plant Sciences Inc., MN, USA.
PMID:26011187	Multiplexed, targeted gene editing in Nicotiana benthamiana for glyco-engineering and monoclonal antibody production.
TALEN	Li J, Stoddard TJ, Demorest ZL, Lavoie PO, Luo S, Clasen BM, Cedrone F, Ray EE, Coffman AP, Daulhac A, Yabandith A, Retterath AJ, Mathis L, Voytas DF, D'Aoust MA, Zhang F. Plant Biotechnol J. 2015 May 25. doi: 10.1111/pbi.12403. [Epub ahead of print]
2015 7	rice Chinese Academy of Sciences, Shanghai, China.
PMID:25867543	TALEN-mediated targeted mutagenesis produces a large variety of heritable mutations in rice.
TALEN	Zhang H, Gou F, Zhang J, Liu W, Li Q, Mao Y, Botella JR, Zhu JK. Plant Biotechnol J. 2015 Apr 13. doi: 10.1111/pbi.12372. [Epub ahead of print]
2015 8	rice Zhejiang University, Hangzhou, China.
PMID:25856577	Gene editing by co-transformation of TALEN and chimeric RNA/DNA oligonucleotides on the rice OsEPSPS gene and the inheritance of mutations.
TALEN	Wang M, Liu Y, Zhang C, Liu J, Liu X, Wang L, Wang W, Chen H, Wei C, Ye X, Li X, Tu J. PLoS One. 2015;10(4):e0122755. doi: 10.1371/journal.pone.0122755.

表2. NBT応用植物に関する文献調査結果(TALEN) (その2)

Year	Applied plant species
ID	Affiliation, country
Identifiers	Title
Category	Description
	Details
2015 9 PMID:25848989 TALEN	potato Swedish University of Agricultural Sciences, Alnarp, Sweden. Targeted gene mutation in tetraploid potato through transient TALEN expression in protoplasts. Nicolia A, Proux-Wéra E, Åhman I, Onkokesung N, Andersson M, Andreasson E, Zhu LH. J Biotechnol. 2015 Jun 20;204:17-24. doi: 10.1016/j.jbiotec.2015.03.021. Epub 2015 Apr 4.
2015 10 PMID:25846201 TALEN	potato Cellecctis plant sciences Inc., MN, USA. Improving cold storage and processing traits in potato through targeted gene knockout. Clasen BM, Stoddard TJ, Luo S, Demorest ZL, Li J, Cedrone F, Tibebe R, Davison S, Ray EE, Daulhac A, Coffman A, Yabandith A, Retterath A, Haun W, Baltes NJ, Mathis L, Voytas DF, Zhang F. Plant Biotechnol J. 2015 Apr 7. doi: 10.1111/pbi.12370. [Epub ahead of print]
2015 11 PMID:25822541 TALEN	Arabidopsis Heidelberg University, Germany. Max Planck Institute, Tübingen, Germany. Germline-transmitted genome editing in Arabidopsis thaliana Using TAL-effector-nucleases. Forner J, Pfeiffer A, Langenecker T, Manavella PA, Lohmann JU. PLoS One. 2015;10(3):e0121056. doi: 10.1371/journal.pone.0121056.
2015 12 PMID:25644697 TALEN	corn Iowa State University, Ames, USA Heritable site-specific mutagenesis using TALENs in maize. Char SN, Unger-Wallace E, Frame B, Briggs SA, Main M, Spalding MH, Vollbrecht E, Wang K, Yang B. Plant Biotechnol J. 2015 Feb 3. doi: 10.1111/pbi.12344. [Epub ahead of print]
2015 13 PMID:25599829 TALEN	rice Chinese Academy of Sciences (CAS), Beijing, China Creation of fragrant rice by targeted knockout of the OsBADH2 gene using TALEN technology. Shan Q, Zhang Y, Chen K, Zhang K, Gao C. Plant Biotechnol J. 2015 Jan 20. doi: 10.1111/pbi.12312. [Epub ahead of print]
2014 14 PMID:25403732 TALEN/CRISPR	Nicotiana benthamiana Weizmann Institute of Science, Rehovot, Israel Comparative assessments of CRISPR-Cas nucleases' cleavage efficiency in planta. Johnson RA, Gurevich V, Filler S, Samach A, Levy AA. Plant Mol Biol. 2015 Jan;87(1-2):143-56. doi: 10.1007/s11103-014-0266-x. Epub 2014 Nov 18.
2014 15 PMID:25038773 TALEN/CRISPR	bread wheat Chinese Academy of Sciences (CAS), Beijing, China Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu JL. Nat Biotechnol. 2014 Sep;32(9):947-51. doi: 10.1038/nbt.2969. Epub 2014 Jul 20.
2014 16 PMID:24851712 TALEN	soybean Cellecctis plant sciences Inc., MN, USA Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. Haun W, Coffman A, Clasen BM, Demorest ZL, Lowy A, Ray E, Retterath A, Stoddard T, Juillerat A, Cedrone F, Mathis L, Voytas DF, Zhang F. Plant Biotechnol J. 2014 Sep;12(7):934-40. doi: 10.1111/pbi.12201. Epub 2014 May 23.
2014 17 PMID:24680698 TALEN	rice Iowa State University, IA, USA. TALEN utilization in rice genome modifications. Li T, Liu B, Chen CY, Yang B. Methods. 2014 Aug 15;69(1):9-16. doi: 10.1016/j.ymeth.2014.03.019. Epub 2014 Mar 27.

表2. NBT応用植物に関する文献調査結果(TALEN) (その3)