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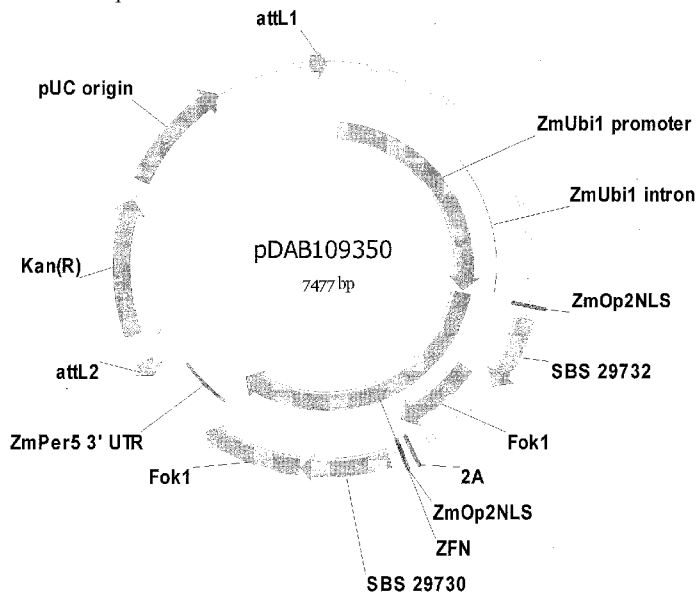
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[Continued on next page]

(54) Title: METHODS AND COMPOSITIONS FOR INTEGRATION OF AN EXOGENOUS SEQUENCE WITHIN THE GENOME OF PLANTS

FIGURE 1: pDAB109350



(57) Abstract: Disclosed herein are methods and compositions for parallel or sequential transgene stacking in plants to produce plants with selected phenotypes. The present disclosure provides methods and compositions for precision transformation, gene targeting, targeted genomic modification and protein expression in plants. In particular, the present disclosure describes a novel, transgenic marker-free strategy for integrating an exogenous sequence and to stack traits that exploit differential selection at an endogenous locus, e.g., the acetohydroxyacid synthase (AHAS) locus) in plant genomes.





MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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**Published:**

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

**(88) Date of publication of the international search report:**

15 January 2015

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2014/032706

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(8) - A01H 1/00 (2014.01) CPC - C12N 15/8274 (2014.09) According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC(8) - A01H 1/00, 5/00, 5/10; C12N 5/04, 9/88, 15/60, 15/82 (2014.01) CPC - C12N 9/88, C12N 9/1022, C12N 15/821, C12N 15/8274, C12N 15/8278 (2014.09)		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC - 435/419, 468; 800/278, 320.3 CPC - C12N 9/88, C12N 9/1022, C12N 15/821, C12N 15/8274, C12N 15/8278 (2014.09) (keyword delimited)		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PatBase, Google Patents, PubMed Search terms used: plant herbicide resistance zinc finger nuclease homologous recombination exogenous target TALES TALEN		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/0029860 A1 (MOFFATT et al) 29 January 2009 (29.01.2009) entire document	1-8, 10, 24-26
A	US 7,705,139 B2 (JAMIESON et al) 27 April 2010 (27.04.2010) entire document	1-8, 10, 22-26
A	TZFIRA et al. "Genome modifications in plant cells by custom-made restriction enzymes," Plant Biotechnology Journal, 03 January 2012 (03.01.2012), Vol. 10, Iss. 4, Pgs. 373-389. entire document	1-8, 10, 22-26
A	DURAI et al. "Zinc finger nucleases: custom-designed molecular scissors for genome engineering of plant and mammalian cells," Nucleic Acids Research, 26 October 2005 (26.10.2005), Vol. 33, No. 18, Pgs. 5978-5990. entire document	1-8, 10, 22-26
A	US 2007/0033670 A1 (KONZAK et al) 08 February 2007 (08.02.2007) entire document	1-8, 10, 22-26
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/>		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 23 October 2014		Date of mailing of the international search report 20 NOV 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201		Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/032706

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- 1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
- 2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
- 3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

See Extra Sheet.

- 1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
- 2.  As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
- 3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:  
all part of claims 1-8, 10, and 24-26; and claims 22 and 23 restricted to zinc finger protein that binds to a target site, wherein the target site is selected to be SEQ ID NO:35, and wherein the zinc finger protein comprises recognition helix regions, wherein the recognition helix regions are selected to be SEQ ID NOs:181-185.
- 4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I: claims 1-8, 10, and 24-26 are drawn to a plant cell.

Group II+: claims 22 and 23 are drawn to a zinc finger protein that binds to a target site.

The first invention of Group II+ is restricted to a zinc finger protein that binds to a target site, wherein the target site is selected to be SEQ ID NO:35, and wherein the zinc finger protein comprises recognition helix regions, wherein the recognition helix regions are selected to be SEQ ID NOs:181-185 (first row of Table 2).

Applicant is invited to elect additional target sites with specified SEQ ID NO and/or recognition helix regions with specified SEQ ID NO to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be a zinc finger protein that binds to a target site, wherein the target site is selected to be SEQ ID NO:36, and wherein the zinc finger protein comprises recognition helix regions, wherein the recognition helix regions are selected to be SEQ ID NOs:186-191. Additional target sites and/or recognition helix regions will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I and II+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I, a plant cell, are not found in Groups II+; the special technical features of Groups II+, a zinc finger protein, are not found in Group I.

Groups I and II+ share the technical features of a target site. However, these shared technical features do not represent a contribution over the prior art. Specifically, US 7,705,139 B2 to Jamieson et al. discloses a target site (a modified plant zinc finger protein (ZFP) that binds to a target sequence, Col. 3, Lns. 16 and 17; ZFP having a target site specificity, Col. 2, Ln. 29).

The Groups II+ formulas do not share a significant structural element, requiring the selection of alternatives for the target site "target site selected from the group consisting of SEQ ID NOs:35-56 and 263-278" and the recognition helix regions "the recognition helix regions shown in a single row of Table 2 or Table 12".

Groups II+ share the technical features of a zinc finger protein that binds to a target site. However, these shared technical features do not represent a contribution over the prior art. Specifically, US 7,705,139 B2 to Jamieson et al. discloses a zinc finger protein that binds to a target site (a modified plant zinc finger protein (ZFP) that binds to a target sequence, Col. 3, Lns. 16 and 17).

The inventions listed in Groups I and II+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

INTERNATIONAL SEARCH REPORT

International application No.

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**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

on paper

in electronic form

b. (time)

in the international application as filed

together with the international application in electronic form

subsequently to this Authority for the purposes of search

2.  In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 35, 181-185 were searched.