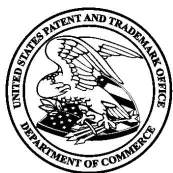

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/913,614	02/22/2016	Andrew Cigan	BB2284-US-PCT	2600

27310 7590 09/14/2020
PIONEER HI-BRED INTERNATIONAL, INC.
7250 NW 62ND AVENUE
PO BOX 552
JOHNSTON, IA 50131-0552

EXAMINER	
COLLINS, CYNTHIA E	

ART UNIT	PAPER NUMBER
1662	

NOTIFICATION DATE	DELIVERY MODE
09/14/2020	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

IPsupport@corteva.com
sue.smith@corteva.com

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte ANDREW MARK CIGAN, SAVERIO CARL FALCO,
HUIRONG GAO, ZHONGSEN LI, ZHAN-BIN LIU,
L. ALEKSANDER LYZNIK, JINRUI SHI, SERGEI SVITASHEV, and
JOSHUA K. YOUNG

Appeal 2020-001230
Application 14/913,614
Technology Center 1600

Before ERIC B. GRIMES, TIMOTHY G. MAJORS, and
MICHAEL A. VALEK, *Administrative Patent Judges*.

VALEK, *Administrative Patent Judge.*

DECISION ON APPEAL

Appellant[1] submits this appeal[2] under 35 U.S.C. § 134(a) involving claims to a method for editing a nucleotide sequence in the genome of a

---

[1] We use the word "Appellant" to refer to "applicant" as defined in 37 C.F.R. § 1.42(a). Appellant identifies Pioneer Hi-Bred International, Inc. and E.I. DuPont de Nemours & Company as the real parties in interest. Appeal Br. 4. Herein, we refer to the Final Office Action mailed October 4, 2018 ("Final Act."); Appellant's Response after the Final Action filed January 4, 2019 ("Response"); Examiner's Advisory Action mailed February 7, 2019 ("Adv. Act."); Appellant's Appeal Brief filed April 1, 2019 ("Appeal Br."); Examiner's Answer mailed October 4, 2019 ("Ans."); and Appellant's Reply Brief filed December 3, 2019 ("Reply Br.").
[2] This Appeal is related to Appeal 2019-003067 (Application 14/463,687),

plant cell that have been rejected for anticipation under 35 U.S.C. § 102 and obviousness under 35 U.S.C. § 103. We have jurisdiction under 35 U.S.C. § 6(b).

We AFFIRM.

STATEMENT OF THE CASE

"Recombinant DNA technology has made it possible to insert foreign DNA sequences into the genome of an organism, thus, altering the organism's phenotype." Spec. 1. According to the Specification, "[a]lthough several approaches have been developed to target a specific site for modification in the genome of a plant, there still remains a need for more efficient and effective methods for producing a fertile plant, having an altered genome comprising specific modifications in a defined region of the genome of the plant." *Id.* at 2.

Appellant's Specification describes "compositions and methods for genome modification of a target sequence in the genome of a plant or plant cell" that "employ a guide RNA/Cas endonuclease system, wherein the Cas endonuclease is guided by the guide RNA to recognize and optionally introduce a double strand break at a specific target site into the genome of a cell." Spec. 27. A Cas endonuclease is a protein encoded by a "CRISPR-associated (Cas)" gene such as Cas9. *Id.* at 28–29.

"Once a genomic target site is identified, a variety of methods can be employed to further modify the target sites such that they contain a variety of polynucleotides." Spec. 27. According to the Specification, "[s]uch methods can employ homologous recombination to provide integration of

---

Decision affirming the rejections of record entered June 24, 2020.

the polynucleotide of Interest at the target site" through the use of "a donor DNA construct," i.e., "a DNA construct that comprises a polynucleotide of Interest to be inserted into the target site of a Cas endonuclease." *Id.* at 39. Such constructs have "region[s] of homology that flank the polynucleotide of Interest" and "share homology to a first and a second genomic region, respectively, present in or flanking the target site of the plant genome" so as "to promote homologous recombination at the cleaved target site." *Id.*

Claims 29, 31, 32, and 38 are on appeal and can be found in the Claims Appendix of the Appeal Brief. Claim 29 is illustrative of the claims on appeal. It reads as follows:

> 29. A method for editing a nucleotide sequence in the genome of a plant cell, the method comprising:
>
> (a) introducing at least one guide RNA and at least one polynucleotide modification template into a plant cell comprising at least one Cas endonuclease, wherein the Cas endonuclease introduces a double-strand break at a target site in the genome of said cell, wherein said polynucleotide modification template comprises at least one nucleotide substitution, insertion, deletion, or a combination thereof, as compared to the sequence of the target site; and
>
> (b) obtaining a whole plant from the plant cell, wherein the plant comprises the nucleotide sequence edit introduced at the target site in the genome of at least one cell of the plant.

Appeal Br. 22.

Appellant seeks review of the following rejections:[3]

I. Claims 29, 31, 32, and 38 under 35 U.S.C. § 102(a)(2) as anticipated by Yang;[4] and

---

[3] Appellant does not appeal Examiner's rejections of claim 43. Appeal Br. 5, 7.

[4] US 2015/0067922 A1, published March 5, 2015 ("Yang"). Yang claims

II. Claims 29, 31, and 38 under 35 U.S.C. § 103 as unpatentable over Zhang[5] in view of Li.[6]

Appeal Br. 7.

I. ANTICIPATION REJECTION OVER YANG

Issue

Appellant does not argue the rejection of dependent claims 31, 32, and 38 separately from the rejection of independent claim 29. Accordingly, we focus our analysis on claim 29, and the other claims stand or fall with claim 29. *See* 37 C.F.R. § 41.37(c)(iv).

The issue for this rejection is whether a preponderance of the evidence supports Examiner's conclusion that Yang anticipates the method recited in claim 29.

Findings of Fact

FF1. Yang discloses "compositions and methods for specific gene targeting and precise editing of DNA sequences in plant genomes using the CRISPR (cluster regulatory interspaced short palindromic repeats) associated nuclease." Yang, Abstr.; '737 Provisional 79.[7] Yang teaches that

---

priority to provisional application no. 61/828,737, filed on May 30, 2013 ("'737 Provisional").

[5] US 9,840,713 B2, issued Dec. 12, 2017 ("Zhang"). Zhang claims priority to a number of applications, including provisional application no. 61/835,931, filed on June 17, 2013 ("'931 Provisional").

[6] Ting Li et al., *High-efficiency TALEN-based Gene Editing Produces Disease-resistant Rice*, 30 Nature Biotechnology 390–92 (2012) ("Li").

[7] We include parallel citations to supporting disclosure in the '737 Provisional. The '737 Provisional was filed prior to the earliest possible effective filing date of Appellant's present application. Appellant does not dispute that the '737 Provisional provides written description support for one or more claims in Yang.

"[n]on-transgenic, genetically modified crops can be produced using these compositions and methods." Yang, Abstr.

FF2. Yang discloses:

> a series of plant-specific RNA-guided Genome Editing vectors (pRGE plasmids) . . . provided for expression of the CRISPR/Cas9 system in plants. The plasmids may be optimized for transient expression of the CRISPR/Cas9 system in plant protoplasts, or for stable integration and expression in intact plants via *Agrobacterium*-mediated transformation. In one aspect, the plasmid vector constructs include a nucleotide sequence comprising a DNA-dependent RNA polymerase III promoter, wherein said promoter operably linked to a gRNA molecule and a Pol III terminator sequence, wherein said gRNA molecule includes a DNA target sequence; and a nucleotide sequence comprising a DNA-dependent RNA polymerase II promoter operably linked to a nucleic acid sequence encoding a type II CRISPR-associated nuclease.

Yang ¶ 17; '737 Provisional 3, 37–38 (describing plasmid vectors).

FF3. Yang teaches "gene editing may be obtained . . . via deletion or insertion" by co-introducing a polynucleotide modification template, i.e., a "donor DNA fragment," with the CRISPR/Cas system. Yang ¶ 23; '737 Provisional 4. Specifically, Yang discloses that "a donor DNA fragment with positive (e.g., herbicide or antibiotic resistance) and/or negative (e.g., toxin genes) selection markers could be co-introduced with the CRISPR/Cas system into plant cells for targeted gene repair/correction and knock-in (gene insertion and replacement) via homologous recombination." *Id.*; *see also* Yang ¶ 69; '737 Provisional 10–11 (further describing use of "a 'donor' molecule to template repair of a 'target' molecule (i.e., the one that experienced the double-strand break)").

FF4. Yang discloses that such "DNA constructs may be introduced into the genome of a desired plant host by a variety of conventional techniques." Yang ¶ 105; '737 Provisional 33–34 (listing techniques and supporting references). For instance, Yang discloses:

> the gRNA-Cas construct can be introduced together with a donor DNA construct into plant cells (via protoplast transformation or the *Agrobacterium*-mediated transformation) to create precise nucleotide alterations (substitution, deletion and insertion) and sequence insertion. In one embodiment, herbicide-tolerant crops can be generated by substitutions of specific nucleotides in plant genes such as those encoding acetolactate synthase (ALS) and protoporphyrinogen oxidase (PPO). In addition to targeted mutation of single genes, gRNA-Cas constructs can be designed to allow targeted mutation of multiple genes, deletion of chromosomal fragment, site-specific integration of transgene, site-directed mutagenesis in vivo, and precise gene replacement or allele swapping in plants. Therefore, the invention has . . . broad applications in gene discovery and validation, mutational and cisgenic breeding, and hybrid breeding. These applications should facilitate the production of a new generation of genetically modified crops with various improved agronomic traits such as herbicide resistance, disease resistance, abiotic stress tolerance, high yield, and superior quality.

Yang ¶ 122; '737 Provisional 39.

FF5. Yang discloses that "[t]ransformed plant cells which are produced by any of the above transformation techniques can be cultured to regenerate a whole plant which possess the transformed genotype and thus the desired phenotype." Yang ¶ 108; '737 Provisional 35. Yang cites references describing techniques for both "[p]lant regeneration from cultured protoplasts" and "regeneration . . . from plant callus, explants, organs, pollens, embryos or parts thereof." *Id.*

FF6. In addition, Yang discloses a working example demonstrating the use of a CRISPR-Cas9 system in rice protoplasts "for precise cleavage at the desired sites [in the rice genome] and [to] introduce mutation (insertion or deletion) by error prone non-homologous end joining DNA repairing." Yang ¶¶ 123–156; '737 Provisional 40–44 (Ex. I); *see also* '737 Provisional 54 ("These results demonstrate that the engineered gRNA-Cas9 can precisely generate [double-strand breaks] at specific sites of the plant genome, leading to targeted gene mutations introduced by the [non-homologous end joining] DNA repairing machinery."). According to Yang, "[u]sing rice (a model plant and important crop) as an example, we demonstrated that Cas9 could be guided by engineered gRNA for precise cleavage and editing of the plant genome" and "[t]herefore, the CRISPR-Cas system can be exploited as a powerful genome editing and gene targeting tool for functional characterization of plant genes and genetic modification of agricultural crops." Yang ¶ 134; '737 Provisional 56.

FF7. At least claim 1 of Yang is supported by the written description in the '737 Provisional. *See*, *e.g.*, '737 Provisional 3–4, 10–11, 37–44, and 56.

Analysis

Examiner finds that Yang discloses "a method comprising introducing at least one guide RNA into a plant cell comprising at least one Cas9 Type-II CRISPR-associated nuclease" along with "a donor DNA fragment with positive (e.g., herbicide or antibiotic resistance) and/or negative (e.g., toxin genes) selection markers when co-introduced with the CRISPR/Cas system into plant cells for targeted gene repair/correction and knock-in (gene insertion and replacement) via homologous recombination." Final Act 4. Examiner further determines that Yang discloses its "transformed plant cells

can be cultured to regenerate a whole plant . . . using techniques that were known in the art." *Id.* at 5. Examiner further determines these disclosures are supported in the '737 Provisional and, therefore, Yang is entitled to the filing date of the '737 Provisional for purposes of assessing it as prior art. *See Id.* at 6. For these reasons, Examiner concludes that claim 29 is anticipated by Yang. *Id.* at 5.

We agree with, and adopt, Examiner's findings of fact and reasoning supporting the determination that Appellant's claims are anticipated by Yang. *See* Final Act. 3–5; Ans. 9–31; FF1–FF7. We are not persuaded by Appellant's arguments, as explained below.

Appellant notes the Examiner's findings regarding Yang's disclosure of co-introducing a donor DNA fragment along with a CRISPR/Cas system into plant cells to affect gene editing through homologous recombination, but points out that the "corresponding paragraph" in the '737 Provisional refers to a "Fig. 13" that cannot be found in the "publicly available file history of the '737 provisional application." Appeal Br. 9. Appellant urges that because "Fig. 13" is missing, "[n]o specific guidance was given by Yang for the use of a polynucleotide template for plant genome modification before the priority date of the instant application" and, therefore, Yang does not anticipate claim 29. *Id.*

We disagree. Yang discloses a genome editing method in which a donor DNA template is "co-introduced" with a CRISPR/Cas system, i.e., a guide RNA and Cas endonuclease, into a plant cell. FF2–FF3. Yang explains the purpose of doing so is to create "precise nucleotide alterations (substitution, deletion and insertion)" via homologous recombination. FF4. These disclosures provide specific guidance to perform step (a) of claim 29.

Yang further discloses that after the plant cells have been edited in this manner, the whole plant comprising those edits can be obtained using standard techniques. FF5. Thus, both Yang and its priority application disclose all of the steps of claim 29 "arranged or combined in the same way as in the claim." *In re Gleave*, 560 F.3d 1331, 1334 (Fed. Cir. 2009) (quotations omitted). The fact that the '737 Provisional additionally cites to Figure 13, a figure that is apparently missing from that application, does not change the fact that the other disclosure present in Yang and the '737 Provisional is itself sufficient to anticipate claim 29.

Appellant's argument that the "general molecular biology techniques" disclosed in Yang are insufficient to anticipate claim 29 is also unpersuasive. *See* Appeal Br. 11–12. Yang is a published patent application and its disclosure of techniques for, *inter alia,* introducing DNA constructs into plant cells and regenerating whole plants from edited plant cells "are presumptively enabling." *See In re Antor Media Corp.*, 689 F.3d 1282, 1287 (Fed. Cir. 2012). It is the patent applicant who bears the burden to "submit rebuttal evidence" to show these teachings are not enabled. *Id.* Appellant, however, has not persuasively explained, much less provided evidence demonstrating, that the anticipating disclosures in Yang (*see* FF1–FF5) are not enabling.

We are unpersuaded by Appellant's argument that Yang "did not teach template-directed repair of a double strand break" because "Yang's work in protoplasts was limited to the error-prone NHEJ [i.e., Non-Homologous End Joining] pathway." Appeal Br. 9–10. As Examiner points out, Yang provides a working example demonstrating the use of a guide RNA and Cas endonuclease to induce targeted double-strand breaks at

precise locations in the genome of rice protoplasts. *See* Ans. 25; FF6. In this example, Yang used NHEJ to introduce mutations designed to knock out genes at those locations. But Yang specifically discloses that "in addition to targeted mutation" through NHEJ, "the gRNA-Cas construct can be introduced together with a donor DNA construct . . . to create precise nucleotide alterations," such as the substitution of nucleotides encoding particular genes, at those sites. FF4. Thus, Yang is not limited to the specific repair pathway employed in Example I because it also discloses methods in which a polynucleotide modification template is introduced with the CRISPR/Cas system to introduce edits via homologous recombination. Moreover, while the method in Yang Example I does not itself involve a donor DNA template, it does demonstrate the successful use of a guide RNA and Cas endonuclease to induce a targeted double-strand break in rice protoplasts. FF6. In this regard, Example I provides additional disclosure to help enable the practice of Yang's methods of using CRISPR/Cas systems to edit plant cells, including those involving the co-introduction of a donor DNA template.

Appellant contends that Yang's work with protoplasts does "not teach Cas endonuclease-mediated editing of a polynucleotide in plant cells comprising a cell wall (intact plant cell), as claimed in Claim 29." Appeal Br. 12. We disagree for several reasons. First, claim 29 is not limited to plant cells comprising a cell wall, but rather recites a "plant cell" generally.[8]

[8] The amendment Appellant proposed after the Final Office Action was not entered. Advisory Act. 2; *see* Response 2 (seeking to amend claim 29 to recite "an intact plant cell").

Moreover, as Examiner points out, Appellant's Specification indicates that "protoplasts" are plant cells:

> The term "plant" refers to whole plants, plant organs, plant tissues, seeds, plant cells, seeds and progeny of the same. Plant cells include, without limitation, cells from seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen and microspores. Plant parts include differentiated and undifferentiated tissues including, but not limited to roots, stems, shoots, leaves, pollens, seeds, tumor tissue and various forms of cells and culture (e.g., single cells, protoplasts, embryos, and callus tissue).

Ans. 27–28 (quoting Spec. 85). Accordingly, the protoplasts described in Yang constitute a "plant cell," as recited in claim 29.

Second, the disclosure of plant cells in Yang is not limited to just protoplasts. Yang states that its gene editing methods are broadly applicable to editing plants and crops generally. *See* FF1, FF4, and FF5; *see also* Yang ¶¶ 101–103 (describing various monocot and dicot plants "the polynucleotides and vectors described herein can be used to transform"). Moreover, Examiner found and we agree, that Yang describes techniques for introducing DNA constructs into plant cells "that are understood in the art to be applicable to plant cells that retain their cell wall." Ans. 24 (citing Yang ¶ 105); FF4. Thus, Yang discloses that its methods may be used to edit the genome of protoplasts as well as plant cells comprising a cell wall. *Bristol-Myers Squibb Co. v. Ben Venue Labs., Inc.*, 246 F.3d 1368, 1379 (Fed. Cir. 2001) (holding that "anticipation does not require actual performance of suggestions in a disclosure").

We are also unpersuaded by Appellant's argument that Yang does not anticipate because "Yang did not obtain a whole plant with an edited

genome because Yang's cells were limited to isolated protoplasts." Appeal Br. 14–15 (emphasis omitted). Yang discloses various techniques for obtaining a whole plant from edited plant cells and discloses that these techniques may be used to regenerate a whole plant containing the transformed genotype, i.e., the edited nucleotide sequence resulting from Yang's gene editing methods. FF5. Thus, Yang discloses a method comprising both steps of claim 29.

Appellant's arguments that Yang and/or the '737 Provisional do not "*demonstrate* generating a plant from a plant protoplast that was edited using CRISPR-Cas endonuclease" and a donor DNA template are not persuasive. *See* Appeal Br. 15 (emphasis added); Reply Br. 4–8 (asserting "Yang neither demonstrated nor provided the requisite enabling guidance to show that the instant claims are anticipated"). As Examiner correctly points out, Yang need not provide a working example comprising both steps of the claimed method to anticipate. Ans. 15. What is required is that the prior art reference disclose a method comprising all of the steps "arranged or combined in the same way as in the claim," which Yang does. *See Gleave*, 560 F.3d at 1334. Appellant's arguments that Yang does not *demonstrate* the full method of claim 29 in a working example does not overcome the presumption Yang's disclosure is presumptively enabled because Appellant has not explained, much less provided evidence to show, why Yang's detailed description of the claimed method, and supporting citations, is insufficient to enable a skilled artisan to practice that method.

For these reasons, we determine that Examiner's rejection is supported by the preponderance of the evidence and therefore affirm the

rejection of claim 29 as anticipated by Yang. We affirm the rejection of Appellant's other claims as anticipated for the same reasons.

II. OBVIOUSNESS REJECTION OVER ZHANG AND LI

Issue

Appellant does not argue the rejection of dependent claims 31 and 38 separately from the rejection of independent claim 29. Accordingly, we focus our analysis on claim 29, and the other claims stand or fall with claim 29. *See* 37 C.F.R. § 41.37(c)(iv).

The issue for this rejection is whether a preponderance of the evidence supports Examiner's conclusion that Zhang in combination with Li renders the method in claim 29 obvious.

Findings of Fact

FF8. Zhang teaches "[a] CRISPR-Cas complex-mediated method" for producing a "genetically modified plant" that involves introducing a "CRISPR-Cas vector system" comprising a guide RNA and a Cas endonuclease into a plant cell to induce targeted cleavage of a polynucleotide locus and teaches that this method may further include the introduction of a "template" into the cleaved polynucleotide locus. Zhang claims 1 and 14; '931 Provisional ¶¶ 13, 15, 136, 145.[9]

FF9. Zhang teaches that

> [t]he CRISPR/Cas or the CRISPR-Cas system (both terms are used interchangeably throughout this application) does not require the generation of customized proteins to target specific

---

[9] We include parallel citations to supporting disclosure in the '931 Provisional. The '931 Provisional was filed prior to the earliest possible effective filing date of Appellant's present application. Appellant does not dispute that the '931 Provisional provides written description support for one or more claims of Zhang.

> sequences but rather a single Cas enzyme can be programmed by a short RNA molecule to recognize a specific DNA target, in other words the Cas enzyme can be recruited to a specific DNA target using said short RNA molecule.

Zhang 2:39–46; ’931 Provisional ¶ 6. Zhang explains that in these CRISPR-Cas systems the Cas protein “directs cleavage of one or two strands at the location of the target sequence” of the guide RNA. Zhang 3:40–42, 6:17–19; ’931 Provisional ¶ 24.

FF10. Zhang teaches that “[i]n some embodiments, the method further comprises repairing said cleaved target polynucleotide by homologous recombination with an exogenous template polynucleotide, wherein said repair results in a mutation comprising an insertion, deletion, or substitution of one or more nucleotides of said target polynucleotide.” *Id.* at 7:38–44, 31:37–44; ’931 Provisional ¶¶ 15, 136.

FF11. Zhang teaches that its methods may be used to edit plant cells. Zhang 6:36–37, 35:17–19; ’931 Provisional ¶¶ 145, 148–150. For example, Zhang explains that “[w]ith recent advances in crop genomics, the ability to use CRISPR-Cas systems to perform efficient and cost effective gene editing and manipulation will allow the rapid selection and comparison of single and multiplexed genetic manipulations to transform such genomes for improved production and enhanced traits.” *Id.* at 35:47–52; ’931 Provisional ¶ 148. According to Zhang, the “present invention” provides “plant breeders . . . with a new tool to induce mutations.” *Id.* at 36:33; ’931 Provisional ¶ 150. “Accordingly, one skilled in the art can analyze the genome of sources of resistance genes, . . . with more precision than previous mutagenic agents and hence accelerate and improve plant breeding programs.” *Id.* at 36:33–39; ’931 Provisional ¶ 150.

FF12. Zhang further teaches that methods for producing transgenic plants are known in the art (Zhang 35:23–25; '931 Provisional ¶ 145) and incorporates by reference the disclosures in "U.S. Pat. No. 6,603,061—*Agrobacterium*-Mediated Plant Transformation Method; U.S. Pat. No. 7,868,149—Plant Genome Sequences and Uses Thereof and US 2009/0100536—Transgenic Plants with Enhanced Agronomic Traits" and "Morrell et al 'Crop genomics: advances and applications' Nat Rev Genet. 2011 Dec. 29; 13(2):85-96." *Id.* at 35:53–57; '931 Provisional ¶ 148.

FF13. Zhang also provides Example 15, which is titled "Engineering of Microalgae Using Cas9." Zhang, 129:7–9; '931 Provisional ¶¶ 475–491. In Example 15, Zhang discloses three methods for delivering Cas9 and a guide RNA into such plant cells and teaches that "[f]or Homologous recombination . . . an additional homology directed template" is provided. Zhang 129:12–130:13; '931 Provisional ¶¶ 476–479. Zhang also provides the nucleotide sequences for cassettes that include the Cas9 endonuclease as well as a sequence for a guide RNA that may be used in these methods. Zhang 129–132 (SEQ ID NOs: 275–277); '931 Provisional ¶¶ 480–485.

FF14. Zhang Example 15 further teaches a method of producing "a line of *Chlamydomonas reinhardtii*," i.e., alga, "that expresses Cas9 constitutively." *See* Zhang 132:60–67; '931 Provisional ¶¶ 487–488. Zhang teaches "[t]his can be done by using pChlamy1 (linearized using PvuI) and selecting for hygromycin resistant colonies" and provides the nucleotide sequence for "pChlamy1 containing Cas9." *Id.* According to Zhang, "to achieve gene knockout" in such Cas9-expressing alga "one simply needs to deliver RNA for the guide RNA" and "for homologous recombination" one "deliver[s]

guideRNA as well as a linearized homologous recombination template." *Id.* at 132:64–67; '931 Provisional ¶ 488.

FF15. At least claim 1 of Zhang is supported by the written description in the '931 Provisional. *See*, *e.g.*, '931 Provisional ¶¶ 7, 13–15, 24, 136, 145, 148, and 475–491.

FF16. Li describes the use of "TAL effector nucleases (TALENSs)" to create site-specific gene modifications in plant cells, e.g., rice. Li 390. Li further describes use of "TALEN technology to edit a specific S gene in rice to thwart the virulence strategy of *X. oryzae* and thereby engineer heritable genome modifications for resistance to bacterial blight." *Id.* Li teaches that after TALEN technology was used to edit the rice cells, "individual transformant cells were selected, propagated and regenerated into whole plants" that continued to exhibit the desired trait in subsequent generations. Li 390–91.

Analysis

Regarding step (a) of claim 29, Examiner finds "Zhang teaches a CRISPR-Cas complex-mediated method for the production of a multicellular genetically modified plant" comprising the introduction of vectors encoding a Cas9 protein and a guide RNA to "direct sequence-specific binding of the CRISPR complex to one or more target sequences" and "obtain[] cleavage of a polynucleotide loci [sic]" in a plant cell. Final Act. 8–9. In addition, Examiner finds Zhang teaches the method

> may further comprise repairing a cleaved target polynucleotide by homologous recombination with an exogenous template polynucleotide wherein the repair results in a mutation comprising an insertion, deletion, or substitution of one or more nucleotides . . . within or near a target sequence nicked or cleaved by a CRISPR enzyme as part of a CRISPR complex.

Id. at 9. Examiner further finds that Zhang's claims and teachings are supported by and disclosed in the '931 Provisional. *Id.* However, Examiner acknowledges that Zhang does "not teach obtaining a whole plant from a plant cell," as recited in step (b) of claim 29. *Id.*

For step (b), Examiner relies on the teachings in Li. According to Examiner, Li "teach[es] methods for obtaining a whole plant from a plant cell comprising TALEN-generated mutations, wherein the plant comprises the mutation introduced at the target site in the genome of at least one cell of the plant." Final Act. 10. Examiner concludes that it would have been obvious to combine Zhang's teachings regarding the use of an "engineered, non-naturally occurring CRISPR-Cas vector system" and a repair template for introducing alterations in a plant cell with Li's teachings regarding "regenerating the transformed cell into a whole plant" because it "would have been a simple substitution of equivalent elements (plant cell transformation and subsequent regeneration for whole plant transformation) to obtain predictable results (a multicellular genetically modified plant)." *Id.*

We agree with, and adopt, Examiner's findings and reasoning as well as Examiner's conclusion of obviousness. *See* Final Act. 8–10; Ans. 31–45; FF8–FF16. We are not persuaded by Appellant's arguments, as explained below.

Appellant argues that "Zhang did not describe any method of editing a nucleotide sequence in the genome of a plant cell with a Cas endonuclease and a polynucleotide modification template" because, according to Appellant, Zhang:

> does not provide any method for the testing of CRISPR-Cas compositions in plants, or for the modification of a plant cell genome with a Cas endonuclease and guide RNA complex.

> Zhang does not disclose plant cells comprising a genome modification. Zhang does not provide any evidence demonstrating use of CRISPR-Cas compositions in plant cells or that it would work in plant cells. Zhang does not provide any data of any type of plant or plant cell comprising a Cas endonuclease mediated double strand break, nor does Zhang provide any disclosure of plants comprising modifications made by a Cas endonuclease. Each example of Zhang is specific to the usage of a Cas endonuclease in one specific type of human kidney cell (HEK293) or one specific type of mouse neuroblastoma cell (N2A). Zhang did not demonstrate any plant vectors, plant cells, or plant cell lines, plant genomic targets, or any indication that the alleged demonstration in animal cells would work in plant cells, as evidenced by the lack of any relevant data. Further Zhang does not mention any plant cell that comprises an edited nucleotide sequence created from a polynucleotide modification template and an RNA-guided Cas endonuclease.

Appeal Br. 16–17.

We are not persuaded. Zhang teaches the introduction of a CRISPR/Cas system, comprising a guide RNA and Cas endonuclease, into a plant cell along with a nucleotide template to introduce alterations via homologous repair at the site of the break induced by the CRISPR complex (i.e., the combination of the guide RNA and Cas enzyme). FF8–FF11. In addition, Zhang provides an example describing various methods for introducing a CRISPR/Cas system into an alga and teaches that plants, such as algae, may be transformed to express Cas9 "constitutively" so that subsequent alterations may be made simply by introducing an appropriate guide RNA and homologous recombination template. FF13–FF14. Moreover, Zhang teaches that these techniques represent a new tool to produce transgenic crops with improved traits and incorporates by reference

various disclosures relating to techniques for producing transgenic plants. FF11. As such, Zhang provides an express motivation to combine its teachings with those in Li in order to obtain whole plants with the desired phenotype from cells transformed using Zhang's methods.

Appellant's argument that Li cannot be combined with Zhang because "Li refers to an entirely different class of proteins (TALENs) than the Cas9 endonucleases" is also unpersuasive. Appeal Br. 17. As Examiner explains, the rejection is premised on Li's teaching of techniques for regenerating a whole plant expressing the desired phenotype from transformed plant cells. *See* Ans. 37; FF16. We agree with Examiner that a skilled artisan would reasonably expect that the same techniques could be successfully used to obtain a whole plant from cells transformed using a different technique, i.e., Zhang's method involving a Cas endonuclease, guide RNA and nucleotide repair template. We also agree with Examiner that Appellant has "provide[d] no explanation or evidence as to why the methods Li used to regenerate a whole plant from a plant cell that was edited by a TALEN could not be employed to regenerate a whole plant from a plant cell that was edited by a Cas endonuclease," as taught in Zhang.[10] *Id.*

---

[10] On the penultimate page of its Reply Brief, Appellant argues, citing its own Specification, "the probability of cellular toxicity" as a factor rendering "the production of a viable plant from an endonuclease-edited plant cell uncertain." Reply Br. 12–13. Because Appellant failed to present this argument in its Appeal Brief, the argument is untimely and Examiner did not have an opportunity to respond to it in the Answer. 37 C.F.R. § 41.41(b)(1) & (2). Even so, we are not persuaded that the toxicity Appellant mentions would have been significant enough to overcome Examiner's prima facie showing. In particular, Appellant has not offered sufficient evidence-backed argument to show that an ordinary artisan would have been discouraged from combining the art as proposed, or that such artisan would have lacked a

Therefore, the record provides more than adequate support for Examiner's prima facie showing of obviousness. Appellant's argument that Zhang does not provide "evidence demonstrating" or "data" to prove that CRISPR/Cas systems would work in plant cells and that whole plants could subsequently be obtained from those cells (*see* Appeal Br. 16) is not persuasive because "[t]he reasonable expectation of success requirement for obviousness does not necessitate an absolute certainty for success." *Par*, 773 F.3d at 1198.

Appellant argues that there is "no disclosure [in] any of Zhang's priority documents [that] mentions template-directed repair of a plant cell genome that has been edited with an RNA-guided Cas endonuclease." Appeal Br. 18–19. We disagree. The '931 Provisional discloses the same Example 15 in Zhang. FF13–FF14. That example provides methods and nucleotide sequences for incorporating a CRISPR/Cas systems into algae cells, i.e., a type of plant cell. FF13. Moreover, Zhang Example 15 describes embodiments in which a Cas9 endonuclease is constitutively expressed in the algal cell so that targeted editing can occur by the introduction of a guide RNA and polynucleotide repair template for homologous recombination, just as recited in claim 29 step (a). FF14.

Appellant asserts that by relying on Zhang's teachings regarding the "single-celled haploid flagellate alga" in Example 15, Examiner "unreasonably expands the interpretation of the term 'plant' as used in the

---

reasonable expectation of success in arriving at the claimed method. This is particularly so given that "absolute certainty" of success is not required for obviousness. *Par Pharm., Inc. v. TWI Pharms., Inc.*, 773 F.3d 1186, 1198 (Fed. Cir. 2014).

instant specification" because the Specification refers to monocot and dicot plants. Reply Br. 9–10. That argument is unpersuasive for several reasons. First, Appellant's claims do not recite, and are not limited to, methods for editing the genome of "monocot" and "dicot" plants. Indeed, the Specification confirms that the claims are not so limited, stating that "*[a]ny plant* can be used, including monocot and dicot plants." Spec. 98 (emphasis added); *see also* Spec. 85 (indicating that the term "plant" includes "various forms of cells and culture" including "single cells"). Accordingly, given its broadest reasonable interpretation, the term "plant cell" in claim 29 encompasses the alga of Zhang Example 15.

Second, even if Appellant's claims did exclude the particular plant cells in Zhang's example, Appellant has failed to persuasively explain why anything more than routine and obvious experimentation would have been required to use the methods described in Example 15 to edit other types of plant cells, particularly given Zhang's other disclosures and incorporation of references describing techniques for generating transgenic plants (*see* FF11). Appellant's unsupported assertions that Zhang's teachings of "general plant molecular biology methods and a prophetic example of microalgae" provide "***no*** guidance" (Reply Br. 10) do not overcome the legal presumption that such teachings are enabling. *See Antor*, 689 F.3d at 1287.

We are likewise unpersuaded by Appellant's argument that it is unreasonable to "extrapolat[e] Zhang's limited work in mammalian cells" to plant cells. Appeal Br. 20. While it is true that the bulk of Zhang's examples describe the use of CRISPR/Cas systems in animal cells, Zhang explains that its teachings regarding "animal cells may also apply, mutatis mutandis, to plant cells unless otherwise apparent." Zhang 35:65–67; '931

Provisional ¶ 148. Regardless, Appellant urges that a skilled artisan could not apply these teachings to plant cells given "the many differences between animal cells and plant cells." Appeal Br. 20. We are not persuaded. The differences Appellant identifies are not supported by, for example, expert testimony, or other persuasive evidence. *See In re De Blauwe*, 736 F.2d 699, 705 (Fed. Cir. 1984) (explaining that arguments and conclusions unsupported by factual evidence carry no evidentiary weight). Thus, while it is no doubt true that plant cells are different from animal cells, it is incumbent on Appellant to provide evidence-backed argument to explain how those differences relate to and allegedly overcome Examiner's prima facie showing, which relies, *inter alia*, on the presumptively enabled disclosures of the cited prior art. Appellant has not done that here.

For these reasons, we determine that Examiner's rejection is supported by the preponderance of the evidence and therefore affirm the rejection of claim 29 as obvious over Zhang and Li. We affirm the rejection of claims 31 and 38 for the same reasons.

CONCLUSION

In summary:

Claims Rejected	**35 U.S.C. §**	**Reference(s)/Basis**	**Affirmed**	**Reversed**
29, 31, 32, 38	102	Yang	29, 31, 32, 38	
29, 31, 38	103	Zhang, Li	29, 31, 38	
Overall Outcome			29, 31, 32, 38	

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a)(1)(iv).

AFFIRMED