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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte JUSTIN M. LIRA, TERRY R. WRIGHT, SEAN M. RUSSELL, DONALD J. MERLO, STEVEN ROBERT WEBB, NICOLE L. ARNOLD, ANDREW E. ROBINSON, and KELLEY A. SMITH [1]

Appeal 2018-003927
Application 12/517,906
Technology Center 1600

Before ERIC B. GRIMES, JOHN G. NEW, and JAMIE T. WISZ, *Administrative Patent Judges.*

GRIMES, *Administrative Patent Judge.*

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134(a) involving claims related to phosphinothricin-resistant transgenic plant cells, which have been rejected as obvious. We have jurisdiction under 35 U.S.C. § 6(b).

We REVERSE.

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[1] Appellant identifies the real party in interest as Dow AgroSciences, LLC. Appeal Br. 2. We use the word Appellant to refer to "applicant" as defined in 37 C.F.R. § 1.42(a).

STATEMENT OF THE CASE

The invention relates to a gene referred to in the Specification as *DSM-2* and "plant-optimized genes, encoding DSM-2 proteins." Spec. 5. "The DSM-2 protein is distantly related to PAT and BAR" (*id.* at 3), which are both phosphinothricin acetyltransferase enzymes (*id.* at 2). "*DSM-2* can be useful as a transgenic trait to impart tolerance to plants to the herbicides glufosinate, phosphinothricin, and bialaphos." *Id.*

Claims 1, 5, 7–11, 31, 48, 49, 58, 59, 68, and 73–75 are on appeal. Claims 1 and 5, reproduced below, are illustrative:

> 1. A phosphinothricin-resistant transgenic plant cell comprising between 1 and 3 copies of the polynucleotide of SEQ ID NO:3 integrated in the genomic DNA of the plant cell, wherein the plant cell is resistant to glufosinate when applied at a rate of 560 g ai/ha.
>
> 5. A method of selecting for a transgenic plant cell, the method comprising:
>
> transforming a plurality of plant cells with a low copy number vector comprising a polynucleotide operably linked to a promoter operable in the plant cells, wherein the polynucleotide is SEQ ID NO:3, thereby producing transgenic plant cells comprising a low copy number of the polynucleotide; and
>
> growing the plurality of plant cells in a concentration of bialaphos or glufosinate that permits cells that express the polynucleotide to grow, while killing or inhibiting the growth of cells that do not express the polynucleotide,
>
> thereby selecting a transgenic plant cell that is resistant to glufosinate when applied at a rate of 560 g ai/ha.

OPINION

Obviousness

Claims 1, 5, 7–11, 31, 48, 49, 58, 59, 68, and 73–75 stand rejected under 35 U.S.C. § 103(a) as obvious based on Leemans I,[2] Leemans II,[3] UniProt,[4] GenBank,[5] Bedford,[6] Wright,[7] Kohli,[8] and Dudits.[9] Ans. 3. The Examiner finds that Leemans I discloses transgenic plant cells comprising DNA encoding a phosphinothricin acetyltransferase enzyme and "a method of producing a plant cell tolerant to phosphinothricin and other glutamine synthetase inhibitors." *Id.* at 4. The Examiner finds that Leemans II teaches similar transgenic plant cells comprising phosphinothricin acetyltransferase-encoding DNA stably integrated into the genome. *Id.* at 5.

The Examiner finds that the Leemans references do not teach transgenic plant cells comprising 1–3 copies of the polynucleotide of SEQ ID NO: 3, as recited in the claims. *Id.* However, the Examiner finds that Bedford discloses a "gene from *Streptomyces coelicolor* A3(2) conferring bialaphos resistance," which is shown by GenBank to be the same as SEQ ID NO: 1 of Appellant's Specification. *Id.* at 7. The Examiner finds that "[t]he instant specification teaches that SEQ ID NO: 3 represents a plant-optimized version of SEQ ID NO: 1 and that both nucleic acid sequences

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[2] US 5,646,024; July 8, 1997.
[3] US 5,561,236; Oct. 1, 1996.
[4] UniProt Accession Number JH0246 (submitted on September 10, 1999).
[5] GenBank Accession Number M62753 (submitted on April 26, 1993).
[6] Bedford et al., Characterization of a gene conferring bialaphos resistance in *Streptomyces coelicolor* A3(2), *Gene* 104:39–45 (1991).
[7] US 7,838,733 B2; Nov. 23, 2010.
[8] Kohli et al., Transgene integration, organization and interaction in plants, *Plant Mol. Biol.* 52:247–258 (2003).
[9] US 6,284,945 B1; Sept. 4, 2001.

encod[e] the same protein, SEQ ID NO: 2." *Id.* at 8. (The Examiner cites UniProt as evidence that Bedford's gene encodes SEQ ID NO: 2. *Id.* at 5–6.)

The Examiner finds that Wright teaches "codon optimizing bacterial genes for expression in plants," and specifically teaches transgenic plants encoding an enzyme making them tolerant to 2,4-D and a method of controlling weeds. *Id.* at 8. The Examiner finds that Wright also teaches "screening out transgenic lines that had high copy number of the transgene." *Id.* The Examiner finds that Kohli teaches that "low copy number transgene loci result in improved stability of expression" and that "*Agrobacterium*-mediated transformation gives rise to lower transgene copy numbers compared to direct transformation." *Id.* at 8–9. Finally, the Examiner finds that Dudits teaches "expression of a pat [phosphinothricin acetyltransferase] gene in maize plants conferred resistance to 2.0 kg of active ingredient per hectare of glufosinate." *Id.* at 9.

The Examiner concludes that it would have been obvious "to modify the teachings of Leemans-I and II using the polynucleotide sequence of . . . Bedford . . . , to obtain the plants, plant cells, and seeds comprising a polynucleotide encoding a protein with phosphinothricin acetyltransferase activity." *Id.* The Examiner also concludes that modifying the sequence

> to codon-optimize it for expression in plants would have been *prima facie* obvious given the teachings of Wright et al. The sequence thus obtained and the instant SEQ ID NO: 3 would be considered obvious variant[s] of each other, given that they would encode the same protein (SEQ ID NO: 2).

Id.

The Examiner also concludes that

> [s]electing the transgenic plants, cells and seeds for low copy number of the transgene, as taught by Wright et al, would have been obvious given the direct suggestion of Wright et al. Given

> the advantages of having a transgene integrated at low copy number, as taught by Kohli et al . . . , one would have been motivated to screen for transgenic plants and cells with 1–3 copies of the transgene . . . and one would have reasonably expected to obtain such low copy number transformants using the art-standard *Agrobacterium*-based transformation.

Id. at 9–10. The Examiner reasons that "[o]ne would have been motivated to combine the above teachings because of the known agronomic advantages of transgenic plants resistant to glutamine synthase inhibitors, such as phosphinothricin or bialaphos." *Id.* at 11.

We agree with the Examiner that the cited references support a prima facie case of obviousness. Leemans I discloses a gene from *Streptomyces hygroscopicus* that inactivates bialaphos. Leemans I at 2:36–39.[10] Leemans I discloses "DNA fragments capable, when introduced into plant cells and plants, to confer on them protection against Bialaphos or PPT [phosphinothricin]." *Id.* at 5:56–58. Leemans I also discloses recombinant DNAs comprising its DNA fragments and heterologous DNA; "[p]referred recombinant DNA contains heterologous DNA compatible with plant cells, particularly Ti-plasmid DNA." *Id.* at 6:63–65, 7:26–27.

Bedford discloses the *bar* gene from *Streptomyces coelicolor* A3(2). Bedford 39, abstract. Bedford states that "[a]lthough *S. coelicolor* is sensitive to low levels of Bp [bialaphos] (2 µg/ml), *bar* confers high-level resistance to Bp (500 µg/ml) when cloned on a high-copy-number plasmid in this species." *Id.* at 43, bridging sentence. Bedford discloses that "[t]he K_m for PPT [phosphinothricin] of the *S. coelicolor bar* gene product is approx. l mM, a concentration nearly 20-fold higher than the K_m of the *S.*

[10] Leemans I is a divisional of Leemans II, so their disclosures are substantively identical. We therefore discuss only Leemans I.

hygroscopicus enzyme (0.06 mM . . .)." *Id.* at 43, right col. Bedford states that

> the *S. coelicolor* chromosomal copy of *bar* does not confer appreciable resistance to Bp. Moreover, high levels of identity (> 70%) are usually found between the products of genes encoding resistance to the same antibiotic in different *Streptomyces* species. . . . In contrast, the product of the *S. coelicolor bar* gene shows only approx. 30% similarity to products of the *bar*/*pat* genes associated with the Bp biosynthetic clusters.

Id. Bedford states that "[t]hese considerations may imply that the physiological substrate of the *bar* gene product is not PPT, but a structurally related compound." *Id.*

Wright discloses transgenic plants producing an enzyme (AAD-1) that makes them tolerant to the herbicide 2,4-D. Wright 3:58 to 4:9, 4:33–35. Wright states that its "invention also relates to 'plant optimized' genes that encode proteins of the subject invention." *Id.* at 20:16–18. The "microbial gene has been redesigned such that the protein is encoded by codons having a bias toward both monocot and dicot plant usage (hemicot)." *Id.* at 20:10–12. Wright states that "the exemplified rebuilt gene was more efficacious in conveying herbicide resistance to the plant, as compared to the bacterial gene." *Id.* at 20:19–21. Wright provides an example of a "plant-optimized" AAD-1 gene comprising specific codons at specific frequencies. *Id.* at col. 57–58 (Table 12).

The Examiner finds that Genbank shows that Appellant's SEQ ID NO: 1 is identical to the *S. coelicolor* gene disclosed by Bedford, and that UniProt shows that Appellant's SEQ ID NO: 2 is the amino acid sequence encoded by both Bedford's gene and SEQ ID NO: 3. Ans. 5–7. Appellant does not dispute this finding. *See, e.g.*, Appeal Br. 10 ("[T]he DSM-2 (SEQ

ID NO:2) polypeptide [is] encoded by the claimed SEQ ID NO:3 and the bacterial gene of Bedford."). Nor does Appellant dispute that SEQ ID NO: 3, recited in the claims, would be an obvious plant-optimized variant of Bedford's gene. *Id.* at 14–16.

Kohli states that one of the two alternative strategies for producing transgenic plants "exploits the natural ability of *Agrobacterium tumefaciens* to transfer DNA from a resident plasmid into the plant genome." Kohli 247, left col. More specifically, "*Agrobacterium tumefaciens* can transfer a small segment of DNA (known as T-DNA) from a resident Ti plasmid into the plant genome." *Id.* at 252, right col. "Generally *Agrobacterium*-mediated transformation gives rise to lower transgene copy numbers compared to direct transformation methods." *Id.* at 253, left col. Kohli states that, in one experiment, "more than one third contained a single T-DNA insert, half contained 2–3 copies and the remainder contained 4–5 copies." *Id.*

Based on the above disclosures, it would have been obvious to a skilled artisan to make transgenic plants expressing the enzyme encoded by Bedford's *bar* gene, because Bedford discloses that expression of the gene (albeit on a high-copy-number vector) conferred resistance to phosphinothricin (PPT) on PPT-sensitive bacteria. PPT resistance is a desirable trait in transgenic crop plants because Leemans I discloses that PPT and bialaphos are nonselective herbicides that inhibit growth of all (PPT-sensitive) plant species present, causing their total destruction. Leemans I at 1: 62–67.

In addition, it would have been obvious to modify the gene disclosed by Bedford to use codons optimized for plant expression, as taught by Wright, because Wright teaches that such a rebuilt gene is more efficacious in conveying herbicide resistance to plants, as compared to the bacterial

gene. Finally, Kohli teaches that using *Agrobacterium*-mediated transformation is one of two alternative strategies for making transgenic plants, and therefore it would have been an obvious choice as a method for making transgenic plants comprising the modified *bar* gene. *Agrobacterium*-mediated transformation transfers the transgenic DNA into the plant genome (Kohli 252, right col.) and results in a high percentage of low copy-number transformants (1–3 copies of the transgene) (*id.* at 253, left col.), as recited in claims 1 and 5.

Finally, Dudits teaches transgenic maize plants expressing phosphinothricin acetyltransferase (PAT) that are resistant to phosphinothricin at a concentration corresponding to 2.0 kg/ha (Dudits 19:10–42), which is more than the 560 g/ha glufosinate required by claims 1 and 5. Thus, the claimed references would have made obvious a transgenic plant cell, and a method of selecting for transgenic plant cells, meeting all of the limitations of claim 1 and 5.

Appellant argues, however, that the claimed invention provides unexpectedly superior results. Appeal Br. 16–20.[11] Appellant argues that

> the person of ordinary skill could never have expected that DSM-2 would be a ***superior*** selectable marker to PAT, given the fact that Bedford reports its affinity to be 20x lower for the common substrate, PPT. In contrast, . . . Appellants have found that this protein actually is a very favorable PPT resistance marker.

Id. at 16–17 (citing Wright Decl.[12] ¶ 23).

[11] Appellant also argues that the cited references do not support a prima facie case of obviousness but, for the reasons discussed below, we need not address those arguments.
[12] Declaration under 37 C.F.R. § 1.132 of Terry Wright, filed Oct. 21, 2016.

We agree with Appellant that the Wright Declaration provides persuasive evidence of unexpected results for the claimed invention. The evidence of record supports Appellant's position that a person of ordinary skill in the art would have had relatively low expectations for the performance of DSM-2 (encoded by SEQ ID NO: 3) as a transgene for conferring phosphinothricin (PPT) resistance in plants.

Those expectations would have been informed by Bedford, which states that the chromosomal copy of the gene in *S. coelicolor* does not confer resistance, instead requiring expression on a high copy-number plasmid to do so. Bedford 43, right col. Bedford also states that the K_m for the enzyme is twenty-fold higher than the K_m for the comparable *S. hygroscopicus* enzyme, and that the *S. coelicolor* enzyme has a significantly lower degree of amino acid sequence similarity to the *S. hygroscopicus* and *S. viridochromogenes* enzymes (BAR and PAT, respectively) than is "usually found between the products of genes encoding resistance to the same antibiotic in different *Streptomyces* species." *Id.* In view of these considerations, Bedford suggests that the physiological substrate of its enzyme may not even be PPT, but a related compound. *Id.*

Thus, Bedford would have led a skilled artisan to expect that DSM-2 could provide resistance to PPT when expressed at a high level, but a skilled artisan would not reasonably have expected that DSM-2 would provide PPT resistance equivalent to PAT when both were expressed in low copy number. The Wright Declaration supports this conclusion. Dr. Wright states that,

> [p]rior to the disclosure of the present application, PAT was considered the best available PPT selectable marker in plant cells. No proteins having such a low level of sequence identity

> as DSM2 to PAT . . . had been shown prior to the present application to provide useful activity in plants.

Wright Decl. ¶ 23.

Dr. Wright also states that "[w]hen tested in the model plant system, *Arabidopsis*, it was found that each of PAT . . . and DSM2 provided some PPT resistance with low copy number (relative ranking PAT>DSM2 . . .)." *Id.* "From this result, it was expected that a greater copy number of *DSM2* would be required to obtain equivalent activity to that observed with *PAT*." *Id.*

The Wright Declaration, however, provides evidence that "even though DSM2 was worse than PAT at conferring PPT resistance in bacteria, it was equivalent to PAT at providing PPT tolerance in whole plants in the greenhouse. Appendix 1, at pp. 14–15." *Id.*[13] Page 14 of Appendix 1 provides results for a "[s]ide by side comparison" of DSM-2 and PAT in canola. At 840 g/ha glufosinate,[14] the average injury for PAT is 5.0%, while the lowest comparable result for DSM-2 is 7.3%; at 1680 g/ha glufosinate, the average injury for PAT is 17.5%, and the best result for DSM-2 is 11.0%. Appendix 1, page 14. The results are said to show that "[t]olerance is surprisingly equivalent to PAT." *Id.*

Page 15 of Appendix 1 provides results for a "[s]ide by side comparison" of DSM-2 and PAT in soybeans. The results of plant growth at 0, 411, 822, and 1644 g ae/ha glufosinate are shown visually for DSM-2, PAT, and a control, and the results are said to show that "[t]olerance is

[13] Appendix 1 was entered into the record Oct. 21, 2016, separately from but concurrently with the Wright Declaration.
[14] "[T]he term 'glufosinate' is synonymous with 'phosphinothricin.'" Ans. 16.

surprisingly equivalent to PAT in greenhouse." *Id.*, page 15. The growth of the plants shown supports the statement that glufosinate tolerance is equivalent for DSM-2 and PAT.

Dr. Wright also states that the "equivalence has translated to whole plant tolerance in the field to high glufosinate rates. Appendix 1, at p. 16." Wright Decl. ¶ 23. Page 16 of Appendix 1 reproduces the greenhouse results shown on page 15, and beneath them shows field results for DSM-2 and PAT, in % injury, at 542 and 1084 g ae/ha. Appendix 1, page 16. The results are said to show that "[t]olerance is surprisingly equivalent to PAT in greenhouse" "[a]nd the field." *Id.*

In addition to the data regarding PPT resistance, the Wright Declaration and Appendix 1 present results for transformation frequency of DSM-2 and PAT. Wright Decl. ¶ 23; Appendix 1, page 13. Dr. Wright states that, "[w]hen compared in plant cells, it was surprisingly found that DSM2 was actually superior to PAT as a selectable marker in culture yielding 50–100% improvement in canola transformation efficiency and 125% improvement in soybean in side-by-side comparisons. Appendix 1, at p. 13" *Id.*

Dr. Wright declares that "[n]one of these experimental results would have been foreseen, let alone reasonably expected, by a person of ordinary skill in the art at the time of the present application." *Id.*

In summary, Bedford described the enzyme encoded by SEQ ID NO: 3 of the instant claims, noting its high K_m and low sequence similarity to enzymes with similar activity, and expressing doubt about whether the physiological substrate of the enzyme was phosphinothricin at all. The Wright Declaration, however, provides evidence that DSM-2 (the product of SEQ ID NO: 3) functions as well as PAT in providing resistance to

glufosinate in transgenic plants, both in the greenhouse and in the field, and also provides superior transformation frequency compared to PAT in canola and soybeans.

Dr. Wright has stated that the transformation results were surprising, and that neither the transformation frequency nor the PPT resistance in plants would have been expected. Wright Decl. ¶ 23. "Mere improvement in properties does not always suffice to show unexpected results. . . . [H]owever, when an applicant demonstrates *substantially* improved results . . . and *states* that the results were *unexpected*, this should suffice to establish unexpected results *in the absence of* evidence to the contrary." *In re Soni*, 54 F.3d 746, 751 (Fed. Cir. 1995) (emphasis in original).

Here, the Examiner addresses Appellant's data with respect to the comparison of DSM-2 with a phosphinothricin acetyltransferase identified in a search of annotated sequences (designated DSM-1, and not discussed above). Ans. 22. The Examiner, however, does not point to evidence or provide persuasive reasoning to show that a skilled artisan would have expected DSM-2, having the same amino acid sequence as Bedford's enzyme, to provide PPT resistance equivalent to that of PAT when expressed in plants. In view of Appellant's showing of unexpected results, we conclude that the rejection under 35 U.S.C. § 103(a) is not supported by a preponderance of the evidence of record.

DECISION SUMMARY

In summary:

Claims Rejected	35 U.S.C. §	Reference(s)/Basis	Affirmed	Reversed
1, 5, 7–11, 31, 48, 49, 58, 59, 68, 73–75	103(a)	Leemans I, Leemans II, UniProt, GenBank, Bedford, Wright, Kohli, Dudits		1, 5, 7–11, 31, 48, 49, 58, 59, 68, 73–75

REVERSED