Eszett (ß; ß)-Glucuronidase, a False Positive of *Beta* (β)-Glucuronidase: Focus on Plant Genetic Transformation

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Research Article

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Abstract

The Escherichia coli beta (β)-glucuronidase gene (GUS), coded for by the uidA gene, is a popular reporter gene in plant genetic transformation experiments. As a result of a typographic-type error, leading to confusion between Eszett (uppercase ẞ; lowercase ß), a German special character, and Greek lowercase beta (β), some published papers claimed to have used Eszett (ẞ/ß)-glucuronidase, which does not exist. Attention was paid to the 114 false positive entries, i.e., ẞ/ß-glucuronidase, that were detected on PubMed on July 6, 2021. From the 114 entries, 81 (71.1%) were in papers in the field of plant science. After screening 79 of the full texts, the error was quantified in the article's location. The error was detected in 100% of abstracts on PubMed and also in 100% of the abstracts on the original journal/publisher websites, while 62.0% of papers had this error in the text (once or multiple times). The origin of these errors is unclear. Given that there are approximately 4000, 1100 and 10,600 hits for this false positive on sciencedirect.com, Springer Link, and Google Scholar, respectively, the quantification of this error based on PubMed suggests that a large and thorough quantitative post-publication analysis of papers claiming erroneously to have used non-existent Eszett (ẞ/ß)-glucuronidase is needed. Importantly, where possible, those errors should be corrected.

Gus In Plant Genetic Transformation

In plant genetic transformation, one of the historically most useful – due to its versatility and simplicity – and widely employed reporter genes is the *Escherichia coli* beta (β)-glucuronidase gene (*senso lato*, GUS; EC 3.2.1.31) (Jefferson, 1987; Jefferson et al., 1987). To appreciate how widely GUS has been used as a reporter gene, a search at Google Scholar revealed that the Jefferson et al. (1987) and Jefferson (1987) papers had been cited 11,455 and 6169 times, respectively (July 6, 2021). As one random and fairly recent example, GUS was used to confirm the tissue-specific expression of a small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RbcS) in transgenic sweet potato (Tanabe et al., 2015). 4-Methylumbelliferyl β-D-glucuronide (MUG) is a fluorescent substrate of the GUS fluorometric assay that is used to measure and quantify GUS activity, typically using the Jefferson (1987) or Jefferson et al. (1987) protocol. It is also common to observe the use of 5-bromo-4-chloro-3-indoxyl-β-D-glucuronic acid (X-gluc) in a histochemical assay to detect GUS activity in plant tissues (e.g., Dang et al., 2014). On occasion, X-gluc was abbreviated incorrectly, as 5-bromo-4-chloro-3-indolyl glucuronide (i.e., without beta) (e.g., Nyman and Wallin, 1992; Zeng et al., 2016), but this error was not quantified in the analyses conducted later on in this paper.

There are only two forms of glucuronidase, alpha (α-glucuronidase, or AGUS), and beta (BGUS). In humans and bacteria, BGUS is coded for by the *BGUS* (Oshima et al., 1987) and *uidA* (Martins et al., 1993) genes, respectively. *BGUS* is also used as a reporter gene in mammalian cells to explore the metabolism of drugs and other substances, such as the catalytic deconjugation of β-D-glucuronides, and serves as "a viable molecular target for several therapeutic applications" (Awolade et al., 2020). As demonstrated in AGUS and BGUS, Greek letters are used to differentiate the two forms.

Recently, it was discovered that the biomedical literature may be riddled with a unique typographic or linguistic error involving confusion between the Greek letter *beta* (β) and a German special character, *Eszett* (uppercase or capital ẞ; lowercase ß, or *ß* when written in italics), leading to the existence of an unknown number of undisclosed or unintentional errors (Teixeira da Silva, 2021a). As one example, a false positive of β-carotene was found, *Eszett*-carotene or ẞ/ß-carotene, which does not exist, but which may impact the claims made in food science and nutrition studies (Teixeira da Silva, 2021b). Similarly, in this paper, select cases of a false positive of *beta* (β)-glucuronidase, namely *Eszett* (ẞ/ß)-glucuronidase, are highlighted.

Incidence of Eszett **(ẞ/ß)-glucuronidase in plant science using PubMed as the litmus test**

Using a similar method as Teixeira da Silva (2021b), a search (July 6, 2021) was made on Google Scholar, PubMed, Elsevier's sciencedirect.com and Springer Nature's Springer Link for the false positive, ẞ/ß-glucuronidase, revealing 10,600, 114, 4083, and 1084 results, respectively (Table 1). Similarly, approx. 83,000, 21,681, 22,506 and 11,124 results for the corresponding control, β-glucuronidase, were found. These likely represent crude searches that themselves may include false positives (i.e., results not related to either β-glucuronidase or ẞ/ß-glucuronidase). Of note, whereas PubMed and sciencedirect.com did not differentiate the use of the alphabetized form of *beta* and the Greek letter itself (β), vastly different results were observed for Springer Link (6001 vs. 11,124, respectively) and Google Scholar (55,900 vs. 83,000, respectively). This is important because academics searching for literature on these two platforms/databases might find very different results, thereby potentially biasing their selection of literature to cite. Scientists are thus cautioned about their choice of search word, and it is suggested that they search using both the form that includes the Greek letter and also the alphabetized form, to ensure a maximum array of results. Curiously, none of the four platforms and/or databases that were searched recognized the term "*Eszett*". Finally, all four platforms or databases recognized the uppercase and lowercase *Eszett* as equal terms, giving identical outputs when searching for ẞ/ß-glucuronidase, sometimes representing this as "ss" in the alphabetized form of the German *Eszett*.

Table 1

Incidence[1] of false positive entry (□/ß-glucuronidase) and control entry (β-glucuronidase) in four search engines or indexes/databases

	PubMed	sciencedirect.com	Springer Link	Google Scholar
beta glucuronidase	21,681	22,506	6,001	Approx. 55,900
β-glucuronidase	21,681	22,506	11,124	Approx. 83,000
Eszett glucuronidase	0	0	0	0
□-glucuronidase[2]	114	4,083	1,084	Approx. 10,600
ß-glucuronidase[3,4]	114	4,083	1,084	Approx. 10,600

[1] These are raw searches which themselves may contain false positive search results, so the numbers only offer a crude appreciation of the volume of possible papers in each category. A search was conducted on June 17, 2021 (and verified for PubMed, which was analyzed in more detail, on July 6, 2021)

[2] Upper-case *Eszett*

[3] Lower-case *Eszett*

[4] The appearance changes, resembling *beta* (β), when written in italics: *ß* (in contrast, the visual appearance of the upper-case □ does not change in its italicized form)

Given its manageable sample size (n = 114), and a recent focus on the accuracy and reliability (or lack thereof) of PubMed in academic publishing (Teixeira da Silva, 2021c), a small analysis was conducted using the PubMed data set. Using PubMed's 114 apparent β-glucuronidase false positives (i.e., □/ß-glucuronidase), studies directly related to plant science were searched. Studies using plant-based products, for example, in medical studies, or applied studied, such as in microbiology, were not considered. A total of 81 papers on plant science carrying this error, were found (Suppl. Table 1). These represented errors in the title, abstract and keywords, which would be detected during a search on PubMed. The original texts, where available, were also analyzed, as were the source webpages, i.e., of the journal/publisher, to assess whether the error was exclusive to PubMed, or in common with the original publication and/or publisher website. Where possible, an automatic search of β and □/ß was conducted in PDF files, but for older files that could not read text automatically, a thorough search of the entire text was conducted manually.

Except for two papers (Chateau et al., 2000; Karimi et al., 2002), which had no errors in the original publication or journal website (i.e., PubMed-specific errors), the remaining 79 papers had errors in the abstract, text, keywords and/or abbreviations.

On PubMed, the error (false positive) was found in 100% of the 81 (and 79 in refined dataset) abstracts. In the original publications, including website, html and PDF versions of the papers, the false positive was

found in 100% of the abstracts and in 49/79 (62.0%) of the texts (any location excluding the abstract, references, abbreviations, and key words). In the 79 papers examined, 38 (48.1%) also carried this error in the abbreviations. To give readers some notion of the range of errors that exist in this sample set, some examples include: one error (abstract) in Kong et al. (2020); three errors in the abstract and four in the text and abbreviations (Kuai and Morris, 1996); a mixture of correct β-glucuronidase and the false positive ß/ß-glucuronidase (Markulin et al., 2019), with 6/19 mentions of β or ß/ß being attributed to ß/ß); 21 errors anywhere in the text (Obertello et al., 2005).

Moreover, a number of papers had errors in MUG and X-gluc (5.1% (4/79) and 24.1% (19/79) errors, respectively). PubMed also displayed another two categories of deficiencies that were not existent in the original publications, namely the lack of italics (91.1% (72/79) errors), or abbreviated authors' names (62.0% (49/79) errors).

In summary, these examples all employed the erroneous false positive *Eszett* (ß/ß)-glucuronidase to some extent (title, abstract, and/or main text), on PubMed and/or the publisher's website.

Discussion

The number of errors at or on PubMed or PubMed Commons are increasing (Teixeira da Silva, 2021c), all of which should be corrected, if possible (Teixeira da Silva, 2016a). The errors found in PubMed are often limited to the Abstract only, or to the title if the error is also in the title, unless the paper's open access version is archived in PubMed Commons, in which case, "text" was also registered (Table 2). Errors on PubMed are then also reflected in the "Cite" function, i.e., the citation that academics might use then allows the errors to be carried forward to downstream literature. For example, in the case of Gao et al. (2019), where the false positive ß-glucuronidase exists in the title, the erroneous title also appears on the AMA, APA, MLA and NLM citation formats offered by PubMed. In most other cases, there are stylistic errors, such as the lack of italics for plants' Latin botanical names or genes, e.g., Yu et al. (2019). In one serious case (Ni et al., 2011), the Chinese authors' names are misrepresented on PubMed, with the citation citing the first names (Jun, Gaohang, Zhenxing, Huanhuan, Yunrong, and Ping) rather than the family names (Ni, Wang, Zhu, Zhang, Wu, and Wu). The Ni et al. (2011) paper has been cited 92 times according to Google Scholar (July 8, 2021), although a thorough and careful post-publication analysis would be needed to appreciate if any of the 92 "citations" are false positives, erroneous citations or duplications, and then to assess whether the authors that cited this paper conscientiously corrected the authors' names, or if they merely propagated the error by citing the paper as "Jun et al., 2011".

It can thus be argued that PubMed, by not correcting these errors, is allowing academics who cite such papers to propagate these errors to the downstream academic literature (Teixeira da Silva, 2015). The failure to sufficiently correct errors in the literature, or erroneous papers, may result in unfair citations to those papers (Teixeira da Silva and Dobránszki, 2018). In the worst-case scenario, journals and publishers benefit unfairly (e.g., article processing charges for open access papers, subscriptions, citations, journal impact factor, CiteScore, etc.) from erroneous literature (Teixeira da Silva and Vuong,

2021). The elimination by PubMed of italics in titles and abstracts might be perceived by some as unscholarly because of the importance of italics in some academic aspects, such as gene names, Latin botanical names (Teixeira da Silva, 2016b), etc. Academics are thus advised to not rely blindly on PubMed information and citation data but to also verify information based on the original journal or publisher website and the actual publication itself (PDF and/or HMTL versions).

It is unclear which party (e.g., authors, editors, proof setters, publishers, indexing agency, etc.) introduced such errors into these papers. Given that most academics, including editors, might consider this to be a "minor" error that does not "affect the conclusions" of a paper, it is likely that none of these errors will be corrected, but it is important to formally document them, nonetheless, if not only as a historical record. This error (β-glucuronidase ≠ ß-glucuronidase) in the biomedical literature still needs to be quantified based on the large numbers detected in Google Scholar, PubMed, sciencedirect.com and SpringerLink indicated above (Table 1). Such a future study would also benefit from a more comprehensive search and analysis using Scopus and Web of Science.

Conclusion, Limitations, And Cautious Note

The values indicated as false positives of β-glucuronidase (i.e., ß/ß-glucuronidase) are likely an under-representation of the existence of this error since full texts, in which the error may be found, are not always indexed on these platforms or databases.

Readers should be careful about making erroneous or excessive extrapolations from this dataset. For example, even though most of the errors (62/81 papers, or 76.5%) were found in Springer Nature journals, this does not imply that in reality, Springer Nature journals are the most frequent bearers of this error because, among other reasons, not all publishers' journals might be indexed in PubMed, not all papers published within the same journal might be indexed in PubMed, etc. Thus, even though the journal displaying the error most frequently was *Plant Cell Reports* (55/81 papers, or 67.9%), a Springer Nature title, that scientists must be careful about extrapolating what might be a simple error to claiming that the journal publishes, for example, erroneous science. Any accurate or meaningful journal- or publisher-based conclusions will require a thorough and detailed post-publication peer review analysis, as has been suggested in this paper.

Declarations

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Conflicts of interest

The author declares no conflicts of interest relevant to this topic.

Author contributions

The author contributed exclusively to the intellectual discussion underlying this paper, literature exploration, writing, reviews and editing, and accepts responsibility for the content and interpretation.

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