LETTER

RE: Use of regression analysis in plant cell, tissue and organ culture experiments (44(3): 229–232)

Jeffrey Adelberg

I am writing to express a differing opinion from the authors of a recent article "Use of regression analysis in plant cell, tissue and organ culture experiments" (*In Vitro Cellular Developmental Biology–Plant* 44(3): 229–232). The authors' problem was in "using trend analysis to compare means of related quantitative treatment." I will quote only from the abstract and final conclusion, being careful not to use the authors' words out of context. The authors assert, "one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD)" has "clearly supported the biological observations," whereas regression analysis "was not useful to describe our experimental results."

The authors were correct; the regression models drawn in Fig. 1c–f did not fit the data (p. 230). The lack of fit analysis involving replication would be a better indicator of this failure than R^2 presented. Like the authors, I also have had biological data that cannot be fit by the linear models that come loaded in my high-quality software. It is desirable to have a rational model based on biological principle that would indicate correct fit, as opposed to empirical line fitting. Unfortunately, we do not often have enough theoretical understanding for a rational model.

This does not justify considering the treatment levels (0, 100, 200, 300, and 400 mg l^{-1}) as ordinal data, so we may perform multiple comparison test following an ANOVA.

Choosing the highest point from ANOVA produces a similar answer as fitting a fourth degree polynomial, where the line changes direction between the five treatment levels four times. The problem with both approaches is that we lack any reason to believe that this many changes in the slope are relevant. However, the polynomial is still a more correct approach because functional activity does not abruptly change if treatment levels are not evenly divisible by 100, as shown in Fig. 1b. The worst manifestation of the incorrect assertion is immediately above and below 200 mg l^{-1}—"the most adequate concentration."

It is a difficult job to find the optimal point. The raw data in Fig. 1a has not been adequately described by any of the methods in this paper, including Fig. 1b—ANOVA and Tukey's HSD. Since the optimal range is obviously between 100 and 300, a better approach is to conduct a second experiment with more points within that range and hone in on the optimum. If time or resources would not allow a second experiment, a pragmatic strategy would be to re-analyze this data as a second-order polynomial, in the range of 100–300, by omitting 0 and 400. Lacking the original data, I will guess the optimum would be closer to 180. If this were true, less reagent would yield more product than simply choosing a treatment level of 200.

Quantitative treatments require quantitative methods, even when the data does not fit the simple empirical models. The authors incorrectly conclude "it is better to perform one-way ANOVA followed by Tukey's HSD, which will give clear biological meaning." Empirical data does not produce biological meaning, and correct methods gives better estimates of optima.

Thank you for publishing this alternative opinion.

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