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Dr. Larissa Jarzylo, Science Editor

*JoVE*

17 Sellers St.

Cambridge, MA 02139

Dear Dr. Jarzylo:

# Enclosed please find our revised manuscript “Assaying proteasomal degradation in a cell-free system in plants”. The revision addresses all comments of the Editor and three reviewers as detailed in our specific point-by-point response and in a marked copy of the revised paper.

I hope that our revisions will meet the high publication standards of *JoVE*.

Sincerely yours,



Elena García-Cano, Ph. D.

Marie Curie Scholar

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STATE UNIVERSITY OF NEW YORK

**Editorial comments**

1. Editorial Policies of PNAS in regard to Figure 1 adapted from our paper Zaltsman et al., *Proc. Natl. Acad. Sci. USA* **110**, 169-174 (2013):

“The right to permit others to use your original figures or tables published in PNAS for noncommercial and educational use (i.e., in a review article, in a book that is not for sale), provided that the original source is cited. Third parties need not ask PNAS for permission to use figures and tables for such use.”

<http://www.pnas.org/site/aboutpnas/authorfaq.xhtml>

“PNAS authors need not obtain permission for the following cases: (1) to use their original figures or tables in their future works”.

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**Reviewer 1**

The Figure was attributed correctly as requested and the relevant link to the PNAS editorial policies was included in our response to the Editor.

**Reviewer 2**

1. The light regime for agroinoculated plants, which is identical to the growth conditions of untreated plants, was detailed in the revised paper.

2. The sentence in Discussion was corrected and the Figure legend was also corrected as suggested.

**Reviewer 3**

1. The reviewer is correct that at least part of the degradation process may occur in planta, before the extracts are made. But the assay monitors degradation only within the extract, upon its incubation, with the time zero serving as the initial reference point. Thus, strictly speaking, we assay that part of the process which occurs in the extract during the incubation. Proteasomal inhibitors indeed act in the plant, but the do so by inhibiting the proteasome, which is then unable to act in the extract. This point was clarified in the revised paper.

2. We added an online reference for a bead-beating procedure protocol and gave example of the required equipment.

3. The protease inhibitor cocktail mainly affects serine, cysteine, aspartic, and metalloproteases, and does not interfere with the 26S protease. This point was clarified in the revised paper.

4. We indeed use the Bradford protein assay. Our extensive experience shows that, because protein concentration of the sample is known, precise quantification of RuBisCo content or serial dilutions are not required; these would only complicate the assay. Furthermore, in any given time-course experiment, all samples are derived from the same batch of extract which inherently assures equal loading. Potential pipetting variations, which normally are not dramatic, are verified by comparing intensities of the RuBisCo band on stained gels or membranes. These points were clarified in the revised paper.

5. Immunoblotting is indeed a standard procedure; however, it does have many different variations in terms of blocking conditions, etc. Thus, we feel that the specific variation used in our lab warrants description as the entire assay depends on the correct blotting protocol.

6. In our experience, the NIH ImageJ software reliably quantifies ECL band intensity. Again, there is no need for serial dilutions of the western reactions.