



SDI FINAL EVALUATION FORM 1.1

PART 1:

Journal Name:	American Journal of Experimental Agriculture
Manuscript Number:	2015_AJEA_17696
Title of the Manuscript:	Somatic embryo formation from the fused protoplasts of <i>Brassica rapa</i> & <i>B. juncea</i>
Type of the Article	Original Research Article

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	Authors' response to final evaluator's comments
REQUEST IN THE FIRST ROUND OF REVISION TITLE Since the authors used no means or methods to demonstrate that microcalli was produced only from fused protoplasts I suggest an adjustment for the title from “Somatic embryo formation from the fused protoplasts of <i>Brassica rapa</i> & <i>B. juncea</i>” to, for instance, Somatic embryo formation from co-cultivated protoplasts of <i>Brassica rapa</i> & <i>B. juncea</i> FEEDBACK OF AUTHORS: We fused the protoplast of two species with a fusogen and cultured the fused protoplasts. We did not co-cultivated isolated protoplasts of two species. The suggestion is not appropriate to us. However, we thank you for your thoughts and we agree that we did not study the ploidy level of produced somatic embryos. Can authors assure, by any means, that 100% fused protoplasts came from different species?? Can protoplasts coming from the same leaf/species fuse in the conditions used for experimentation? If it is possible to have “interspecific” fusions as well as “intraspecific” protoplasts fusions, as a request for the final revision, I suggest authors should include in MATERIAL AND METHODS that fused protoplasts were subjected to selection following co-cultivation and before cultivation to obtain embryogenic calli and explain what was the criterion/method used for selection, once the chromosome number fo protoplasts fused was not assessed. Otherwise I maintain my suggestion of modifications for the TITLE. REQUEST IN THE FIRST ROUND OF REVISION MATERIAL AND METHODS Authors should include the number of calli analyzed for each repetition and include the information about the number of replicas in this section of Methods in addition to the abstract. Authors included that three replicas were counted/evaluated but did not included the number, at least approximately, of calli or protoplasts measured or classified by color. It would be interesting to know and improve the quality of the manuscript.	100% hybrid protoplasts are generally not possible. We microscopically examined fused cells. Generally the protoplasts of Agrani were comparatively larger (significantly) in size compared to those of BINA Sharisha7 and BINA sharisha8 (see Fig. 4). In Fig. 3b upper cell is of Agrani (<i>B. rapa</i>) and the lower positioned cell is of BINA sharisha7 (<i>B. juncea</i>). Thus we were assured about interspecific fusion occurred. I fully agree that to some extent intraspecific fusion is also possible. As the hybrid cells were not selected therefore at this stage to avoid any confusion of the readers I am convinced to adopt the earlier suggestion of Reviewer 3. Many thanks. In abstract: The experiment was carried out in completely randomized design with three replications for each hormone treatment of each variety combination. See line 90: The size of five isolated protoplasts from each cultivar was measured. Line 129: All data related to calli and somatic embryos were recoded for three replicates of each hormone treatment from each fusion combination. Table 2: Each data point is the average of six observations. See line 110: There were three replicates for each hormone combination meaning that there were a total of six replicates for each cultivar combination (fusion



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	combination) x hormone combination Fig. 4: Each data point is the average of five observations. See Fig. 5 & 6 (micro-calli and somatic embryos): Each data point is the average of six observations. Many thanks to reviewer and editor.
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