

January 17, 2023

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Re: Proposed Exemptions: Movement of Organisms Modified or Produced through Genetic Engineering

Dear Dr. Hoffman,

The American Society of Plant Biologists (ASPB) is pleased to offer this comment in response to APHIS's proposed exemptions of organisms modified or produced through genetic engineering.

ASPB was established to promote the growth and development of plant biology, to encourage and publish research in plant biology, and to promote the interests and professional advancement of plant scientists in general. ASPB members educate, mentor, advise, and nurture future generations of plant biologists; they advocate in support of plant biology research; they work to convey the relevance and importance of plant biology; and they provide expertise in policy decisions world-wide.

Thank you for your foresight in proposing a rule that would allow for additional exemptions as science advances and for your leadership in expanding these exemptions in accordance with your policy and with plant pest risk.

ASPB is supportive of the five proposed exemptions. They each have a sound scientific basis and will serve to open research, teaching, and product development opportunities in the plant sciences that have long been on hold. However, there are a handful of opportunities for improvement. Here we provide examples of what we like, additional concerns with the proposed policy, and suggestions for improvement.

As part of the First Proposed Exemption, BRS will allow diploid or autopolyploid plants with loss of function modifications at a single genetic locus on up to four homologous chromosomes. While this is a scientifically sound exemption that, indeed, can be achieved through conventional breeding, it fails to include allopolyploid crops like wheat, cotton, canola, and peanuts. In most cases, but not always, differences in the parental genomes make it impossible to achieve homozygosity through conventional breeding if only one chromosome of an allopolyploid is mutated. However, non-transgenic approaches such as TILLING (targeted induced local lesions in genomes) and doubled haploids have been used to produce complete homozygosity across six chromosomes in hexaploid crops such as wheat<sup>1,2</sup>.

We laud BRS in its approach in regulating the end result rather than the methods that were used to make gene knockouts. However, the paragraph starting with "*Modifications resulting from insertions of exogenous DNA do not currently qualify for exemption...*" in the First Proposed Exemption would benefit from clarification. An approach to targeted mutagenesis often involves transforming plants using CRISPR/Cas9 constructs, screening for the desired mutation(s), and then segregating away the Cas9 transgenes and guide RNA using genetic crosses. Thus, these modifications resulted from insertions of exogenous DNA, even if that DNA is no longer present in the final product. We urge BRS to clarify that insertions of exogenous DNA can be used as an intermediate step to create knockout mutations, and that these mutations are considered exempt if the transgenes are no longer present.

An additional clarification that would be helpful in the proposed exemptions is the modification of previously BRS-approved transgenic crops, *e.g.*, maize expressing *Bt* toxin or Roundup Ready soybeans. Will such plants be considered exempt if they are targeted with loss-of-function mutations, as described in the proposed exemptions?

The Fourth Proposed Exemption would limit the allowed number of modifications to four in any individual plant. Although this rather small number of permitted modifications can be increased after further evaluations, as described in the Fifth Proposed Exemption, this additional step will impede both basic research and crop development. Indeed, natural gene knockouts, classical genetic crosses, and marker-assisted breeding<sup>3</sup>, which are not subject to BRS regulation, are commonly used to combine larger numbers of mutations in plant species such as maize<sup>4</sup>, soybean<sup>5</sup>, tomato<sup>6</sup>, rice<sup>7</sup>, and Arabidopsis<sup>8</sup>. As there is no overriding scientific rationale for limiting the exemption to four mutations, we urge BRS to modify the Fourth Proposed Exemption to include a larger number of allowed mutations. This would be in line with what is currently permitted in Canada and rules that have been proposed, though not yet approved in the European Union.

Thank you for your consideration of this important issue. We encourage APHIS to implement these exemptions as soon as possible and look forward to working with you in the future.

## References

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