Strategy for RA with Stacked Events

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with inputs from

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Stacked transformation events are defined as new products with more than one transformation event (OECD, 2002) through

* Crossing between GM plants carrying separate individual events
* Transformation with multiple novel genes either simultaneously or consecutively

A cassette carrying multiple genes in one gene construct will remain linked within one event and therefore will not be categorised as “stacked events”

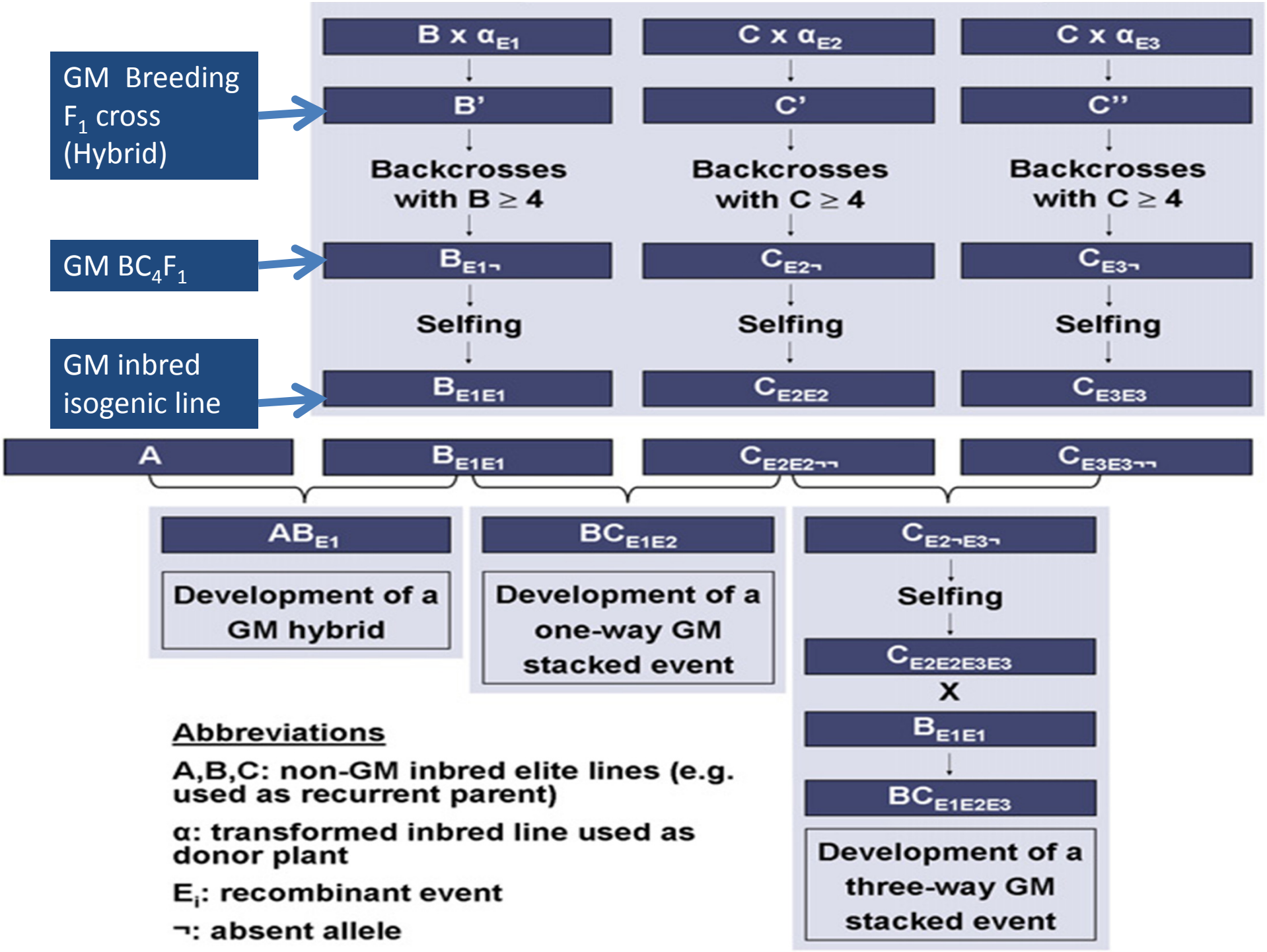
Stacked “Event and Product”

Stacked gene plants are produced through the following methods:

- (1) Insertion of an additional transgene by transformation of an existing biotech plant (New Stacked Event)
- (2) Insertion of multiple genes into a non-transgenic plant with a vector containing two or more genes (New Stacked Event)
- (3) Traditional breeding approach that combines genes previously introduced by plant biotechnology (**New Stacked Product**)

CBD Secretariat (Cartagena Protocol)

- **GUIDANCE ON RISK ASSESSMENT OF LIVING MODIFIED ORGANISMS**
- *(REVISED ON 15 SEPTEMBER 2011)*
- A separate section on GMOs with stacked event produced through conventional hybridization with two different single event GMOs


GM Breeding F_1 cross (Hybrid)
GM BC_4F_1
GM inbred isogenic line
B x α_{E1}
C x α_{E2}
C x α_{E3}
B'
C'
C''
Backcrosses with B ≥ 4
Backcrosses with C ≥ 4
Backcrosses with C ≥ 4
$B_{E1\neg}$
$C_{E2\neg}$
$C_{E3\neg}$
Selfing
Selfing
Selfing
B_{E1E1}
C_{E2E2}
C_{E3E3}
A
B_{E1E1}
$C_{E2E2\neg\neg}$
$C_{E3E3\neg\neg}$
AB_{E1}
Development of a GM hybrid
BC_{E1E2}
Development of a one-way GM stacked event
$C_{E2\neg E3\neg}$
Selfing
$C_{E2E2E3E3}$
X
B_{E1E1}
BC_{E1E2E3}
Development of a three-way GM stacked event
Abbreviations
A,B,C: non-GM inbred elite lines (e.g. used as recurrent parent)
α: transformed inbred line used as donor plant
E_i: recombinant event
¬: absent allele

Stacked genes can segregate

- The cassettes containing the individual event' transgenes that were inserted in the original transformation events will be physically unlinked (i.e. located separately in the genome) and can segregate independently from a stacked product

Kinds of Stacked Products

- **Category 1:** Unrelated traits (e.g., Insect protection and herbicide tolerance; male sterility and virus resistance; insect protection and food quality improvement, etc.).
- **Category 2:** Related traits, but involving different pathways or distinct modes-of-action (e.g., glyphosate herbicide tolerance and glufosinate herbicide tolerance; multiple proteins with different modes-of-action that provide insect protection, etc.).
- **Category3:** Related traits functioning in the same metabolic or biosynthesis pathway (*e.g.*, two enzymes involved in starch biosynthesis or lipid biosynthesis).

Approved Events : Good Comparators

- “The choice of comparators” section of the Roadmap, the GMOs that were involved in the cross-breeding process leading to the stacked GM plant under consideration may also be used as comparators, as appropriate and according to national regulations.

Comparators

- *Points to consider:*
- Level of heterozygosity between the non-modified recipient organisms used to produce the parental GMOs;
- Phenotypic variability between non-modified hybrids produced through crosses between the non-modified recipient organisms;
- Number of crossings and the use of intermediate stacked GMOs as additional comparators.

Will the stacked gene products interact?

- **Potential interactions between combined genes and their resulting phenotypic changes and effects on the environment**
- *Points to consider:*
- Effects of the parental GMOs on the environment;
- Information on transcriptional and post-transcriptional regulation of genes and their products that may be predictive of interactions between the novel and endogenous genes and/or DNA elements in the stacked GM plant;
- Whether transgenes of similar functions or belonging to the same metabolic pathways were stacked.
- Levels of expression of the transgenes compared to the parental GMOs and to the non-modified recipient organisms.

Combinatorial and cumulative effects

- *Points to consider:*
- Effects of the use of pesticides, other chemicals or agricultural practices commonly used in the cultivation of the parental GMOs;
- Phenotypic characteristics compared to the parent GMOs and to the non-modified recipient organisms;
- Interactions between the stacked transgenes or their products, or interactions between the physiological pathways in which the transgenes are involved. Considerations on whether these could result in potentially harmful substances (e.g. anti-nutritional factors)and the possibility of persistence and accumulation of these substances in the environment, such as in the food chain;
- Combinatorial and cumulative effects arising from the presence of two or more modified traits in the environment that could result in a broadened target range or increased toxicity.

RCGM : Considerations in RA of GMOs with stacked events

- Key question :

  Is the stack (comprising approved or unapproved events) likely to create any new or additional risk to biosafety?

Crossing and segregation of transgenes

- *Points to consider:*
- Presence of sexually-compatible non-modified relatives and their ecological function;
- Presence of other single-event and stacked GMOs of the same species;
- Possible new combinations of transgenes and/or DNA fragments should the stacked event under consideration cross, intentionally or unintentionally, with other GM plants, stacked or not, or with non-modified relatives;
- Possible impacts of the new stacked events on non-target organisms or a change in the range of non-target organisms;
- Scientifically plausible risk scenarios or risk hypotheses involving the stacked events with different combinations of transgenes and DNA fragments.

RA data needs for stacks : RCGM

- When all concerned events are individually approved
  - Not likely to undergo complete RA for food, feed and environmental safety
  - Data to be focused on possible interaction products

RA data needs for stacks : RCGM

- When all concerned events are individually approved
  - **Molecular characterization** (Southern blot evidence for presence) for stability and integrity of the stacked events (visualized by Southern for size and trait expression vis a vis parental event line expression)
  - **Phenotypic, agronomic and compositional characterization** (Visualized by careful assessment of performance in CFT, additional feeding or environmental studies may required)

RA data needs for stacks : RCGM

- When all concerned events are individually approved
  - **Food/Feed toxicity and allergenicity tests** :
    - tests required only when the expression of one or both traits exceed the parental line expression
    - An overall allergenicity potential to be assessed due to possible interaction between the events/genes
  - Compositional analysis :
    - Required for the stack to identify any adverse effects due to change in composition by two or more event introgression

RA data needs for stacks : RCGM

- When one or more events are not individually approved
  - **The stack would be considered a 'new' event and therefore a complete RA is required of the stack**
  - If the biosafety RA is carried out only on stacked line, the parental event line (unapproved event donor) will not be considered as approved. If the latter is to be used for commercial seed production, a separate approval application needs to be filed
  - The above apply to a stack where none of the events is approved

Status in Australia, Canada, US, New Zealand

- A stacked event will be licensed after analyzing risk to identified through RA as different from the single events
- A new application will have to be filed only if new risk/s are identified through RA

Status in Japan

- **Category 1 traits**: traits that do not alter the metabolic pathway of host plants
- **Category 2 traits**: traits that alter (promote or inhibit) the metabolic pathway of host plants
- **Category 3 traits**: traits that introduce new metabolites that have previously not been present in the host plant.
- **No need for safety clearance** in stacked products developed by **Category 1 X Category 1** traits if individual traits have been previously approved.
- **A separate safety clearance is required** for combined trait products comprised of **Category 2 or Category 3** traits

Category 1 clearance in Japan

- Cotton tolerant to glyphosate herbicide and glufosinate herbicide and resistant to Lepidoptera (*2mepsps, modified bar, modified cry1Ac, modified cry2Ab, Gossypium hirsutum L.) (GHB614×LLCotton25×15985, OECD UI:* BCS-GHØØ2-5×ACS-GHØØ1-3×MON-15985-7) (including the progeny lines isolated from the cotton lines, GHB614, LLCotton25 and 15985, that contain a combination of any of the transferred genes in the individual cotton lines
- Maize resistant to Lepidoptera and Coleoptera and tolerant to glufosinate and glyphosate herbicides (modified *cry1F, cry1Ab,cry34Ab1, cry35Ab1, pat, modified cp4 epsps, Zea mays subsp. mays (L.) Iltis)*

Review

Plants with genetically modified events combined by conventional breeding: An assessment of the need for additional regulatory data

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ARTICLE INFO

Article history:
Received 7 May 2010
Accepted 3 November 2010

Keywords:
Combined GM events
Combined events
GM event
Combined traits
GM trait
Stacked traits

ABSTRACT

Crop varieties with multiple GM events combined by conventional breeding have become important in global agriculture. The regulatory requirements in different countries for such products vary considerably, placing an additional burden on regulatory agencies in countries where the submission of additional data is required and delaying the introduction of innovative products to meet agricultural needs. The process of conventional plant breeding has predictably provided safe food and feed products both historically and in the modern era of plant breeding. Thus, previously approved GM events that have been combined by conventional plant breeding and contain GM traits that are not likely to interact in a manner affecting safety should be considered to be as safe as their conventional counterparts. Such combined GM event crop varieties should require little, if any, additional regulatory data to meet regulatory requirements.

Contents

Confirmatory Information Required

(Without additional bio & env. safety data)

Data that demonstrate the

- presence of the GM events and support lack of GM trait interactions affecting safety or efficacy of the product may be reasonable.
- These data may include greenhouse or field bioefficacy studies, gene or protein expression levels, and/or relevant composition analyses on the combined GM event product.
- Additional studies would be warranted if two or more of the traits present in the combined GM event product are likely to interact in a manner that would in some way change prior safety assessments. In this case, appropriate experiments should be designed to address the anticipated interaction

USA 2009: SMARTSTAX Maize

- EPA has conditionally registered events MON 89034 + TC1507 + MON 88017 + DAS-59122-7 called "***SmartStax,***", a new bioengineered corn seed product containing genes for two Bt plant incorporated protectants (PIPs) active against corn rootworm CRW and three Bt PIPs to control different corn borer pests.
- After reviewing all pertinent data, the Agency has concluded that a lower CRW refuge of 5% is scientifically justified for SmartStax corn and will further reduce the use of conventional insecticides.

USA 2009

- Monsanto and Dow have developed a new Bt corn product (SmartStax) with two Bt toxins (Cry34Ab1/Cry35Ab1 and Cry3Bb1) active against CRW. The use of multiple toxins against the same pest is termed a “pyramid.”
- SmartStax also contains three Bt PIPs to control different corn borer pests. (Corn borers have separate refuge requirements

Smartstax Maize

- ***Cry1A.105, Cry34Ab1, Cry35Ab1, Cry3Bb1, Cry2Ab2, Cry1F***
- ***CP4 EPSPS***
- ***PAT***
- Southern blot analysis confirmed in the combined trait corn product MON 89034 × TC1507 ×MON 88017 × DAS-59122-7 the presence of sequences identical to sequences derived from MON 89034 and MON 88017.
- Hybridization patterns for the combined trait product were identical to those of the parental lines with *cry1F, cry34Ab1, cry35Ab1, and the pat gene probes* indicating that the TC1507 and DAS-59122-7 insertions were unaffected by combining with MON 89034 and MON 88017 through conventional breeding.

Expression in Smartstax

- The levels of the Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS proteins in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn were comparable to those in the appropriate MON 88017 or MON 89034 positive control
- The test also included a conventional corn as a negative control and TC1507 and DAS-59122-7 parental event corn as positive controls
- The results indicate that the levels of all the cry proteins were comparable to the levels produced in the appropriate TC1507 or DAS-59122-7 control corn
- The level of PAT 88017 x DAS-59122-7 was higher in the combined trait products compared to TC1507 and DAS-59122-7, likely due to the presence of multiple copies of the *pat* gene in the stacks *(one from each of the DAS parent lines)*

acknowledgement

- Research material provided by Dr S. R. Rao, DBT and
- Vibha Ahuja of BCIL

is gratefully acknowledged

THANKS