Industry Recommendations for Implementing EFSA Guidance Document for Conducting GM Crop Studies

EuropaBio Industry Working Group

Key Objectives and Messages

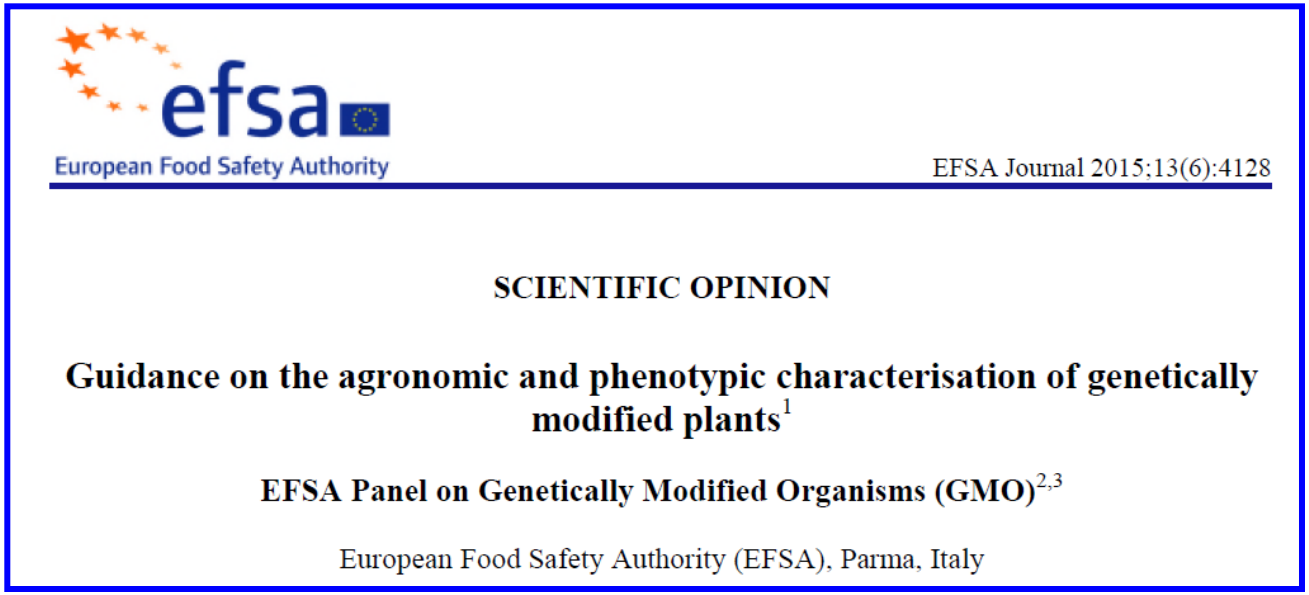
Objectives are to share with cooperators:

- Recent guidelines from EFSA (European Food Safety Agency)
- Industry effort associated with recommendations for interpreting EFSA guidelines

Key messages:

- Diversity of sites used for field trials
- More auxiliary data is required (soil characterization, cropping history, meteorological data)
- Less flexibility with missing data points
- This training provides general information. It is <u>not</u> a substitute for study specific protocols.

EFSA Guidance Document



European Food Safety Authority

EFSA Journal 2015;13(6):4128

SCIENTIFIC OPINION

Guidance on the agronomic and phenotypic characterisation of genetically modified plants[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

- EFSA Guidance Document was prepared to provide more specific information so applicants could meet their requirements

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- Text in red throughout this presentation signifies the recommendations by the EuropaBio Working Group.

2.3 Strategy for Site Selection

- Data from field trials should be from multiple sites that are representative of where the GM plants will be grown
- Representativeness of sites
- Commercial production region
- Soil characteristics
- Climatic conditions – average temperature and rainfall during the growing season
- Day length
- Soil moisture and fertility
- Biotic factors (presence/absence of pests)
- Crop maturity
- Some sites outside the most typical area for the GM line are desirable

3.2.2 Agrometeorological Data

3.2.2. Agrometeorological data

Agrometeorological data provide fundamental information needed for the description of the REs and of the occurrence of extreme weather. Applicants shall provide the necessary agrometeorological data to describe REs and identify potential differences in meteorological conditions across sites during critical growth stages of the GM plant (e.g. emergence, flowering, harvest).

Applicants are required to report descriptive statistics (such as mean and standard error on a weekly basis) throughout the cropping period for average air temperature and precipitation and to produce a combined thermopluviometric graph for each site, as this facilitates the comparison of trends across sites. Exceptional weather conditions (e.g. drought, frost, hail or wind storms), their time and duration of occurrence (day), and the crop growth stage (BBCH scale; see Meier, 2001) at which they occurred at each locality should also be reported.

- Descriptive Statistics (mean and SE) by week for average air temperature and precipitation
- Exceptional weather conditions: Time, Duration, Growth Stage

3.2.2 Agrometeorological Data

On a case-by-case basis (e.g. in the case of G × E interactions), the EFSA GMO Panel may request a more detailed analysis of data to further support the interpretation of the comparative assessment outcome. Applicants shall therefore record the following information in electronic format:

- daily maximum, minimum and average air temperature (°C);
- daily precipitation (mm);
- daily maximum, minimum and average air relative humidity taken at the same time as air temperature (% vol/vol);
- daily global radiation (MJ/m^2);
- daily maximum, minimum and average wind speed (m/s)
- historical thermopluviometric data (if available).

- Daily Max, Min, and average temperatures, relative humidity, wind speed
- Daily precipitation, global radiation,
- Historical Data

3.2.2 Agrometeorological Data

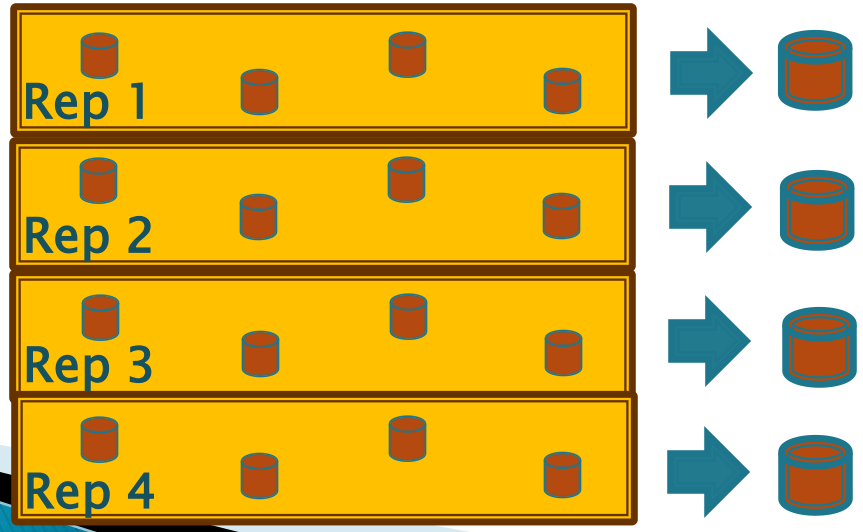
All the recorded meteorological parameters are to be collected at each site throughout the cropping period, i.e. from sowing to harvest, and can be annexed to the GM plant application at the time of submission.

If one or more of the selected sites are not equipped with an on-site weather station, then data generated from a nearby weather station can be used, provided that these are representative. In that case, the geographical coordinates of this station (see Section 3.2.1) and its distance (kilometres) from the field trial site should be given.

- Weather data collected from planting through harvest
- Nearby weather stations can be used, but <u>geographical coordinates of this station</u> and distance from the field trial site are required

3.2.3 Soil Characteristics

- Soil sampling must take place <u>before</u> planting or application of fertilizers or chemicals.
- Soil data must be provided from <u>each RCBD block</u> (*e.g.*, if 4 replications, then 4 soil samples will be sent for soil analysis).
- <u>EuropaBio:</u> Collect 4-6 soil sub-samples (6" deep) from <u>each</u> <u>block.</u> Mix sub-samples to create one soil sample per each block to be sent for analysis.



3.2.4 Cropping History

3.2.4. Cropping history

Applicants should provide information documenting the cropping history of the selected sites during the previous three years and the cultivation management practices applied (e.g. tillage). On a case-by-case basis, any additional information that may be useful to support the representativeness of sites and the interpretation of the comparative analysis, as well as information on the incidence and severity of plant disease epidemics and pest outbreaks at each site, should be supplied.

- Cropping history is required for previous three years.
- Includes cultivation management practices.
- EuropaBio: Recommended vocabulary for cultivation management practices: conventional tillage, minimum tillage, or no tillage.

3.3.1 Crop Management

3.3.1. Crop management

The following information for each field trial site should be supplied:

- Main form of tillage: (i) type, (ii) depth and (iii) date.
- Seedbed preparation: (i) type, (ii) depth and (iii) dates.
- Sowing or planting: (i) date, (ii) method, (iii) number of seeds or vegetative plant parts per square metre, (iv) number of rows per plot, (v) inter-row distance (cm), (vi) sowing depth and (vii) details of thinning (if any).

- EuropaBio: Recommended vocabulary
  - **Crop management types:**
    - Plow (chisel, inversion, no-till)
    - Seedbed preparation
    - In-season cultivation
    - Crop destruct
  - **Planting methods:**
    - Hand or mechanical

3.3.1 Maintenance Agrichemical Information

- EuropaBio:
  - **Agrichemical type:** herbicide, insecticide, fungicide, nematicide and plant growth regulator
  - **Agrichemical target for:**
    - Herbicides: broadleaf weeds, grass weeds and broadleaf/grass weeds
    - Insecticides: chewing insects, piercing/sucking insects, broad-spectrum insects, root-feeding insects
    - Fungicides: foliar disease, stem disease, fruit disease and broad-spectrum disease
    - Nematicide: nematodes
    - Plant growth regulator: plant growth, other (please specify)
  - **Application method**: foliar, side dress, broadcast on soil, soil incorporated
  - **Adjuvant type**: surfactant, buffering agent, conditioning agent, defoaming agent, drift control agent, oil concentrate

3.3.1 Crop Management

- Irrigation: (i) date(s), (ii) method, (iii) amount (mm).
- Harvest: (i) type, (ii) moisture (%) of harvested plant part, (iii) date(s), and (iv) growth stage of crop (BBCH scale).
- Any other crop-specific management interventions (post-emergence hoeing or cultivation, ridging/hilling, etc.): (i) type, (ii) date and (iii) growth stage of crop (BBCH scale).

- EuropaBio:
  - Irrigation methods: drip, furrow, overhead
  - Harvest types: hand, mechanical
- Crop Specific Management Interventions
- Important to specify growth stage at every activity.

3.3.1.2 Post-Harvest Conditions

3.3.1.2. Post-harvest conditions

Applicants should describe the conditions under which the harvested/sampled materials have been stored. If materials have been transported for analysis to facilities distant from the place of storage, then the conditions during transport should also be described (see EFSA GMO Panel, 2011a, for further details on sample storage).

- Nothing new here.
- Important to provide details on storage of materials (if applicable).
- Details on conditions during transport required as well.

4. Agronomic Endpoints

For all endpoints and performed measurements, applicants shall supply documentation of the sampling representativeness, and of the recording methods.

The use of internationally agreed units of measurement (Thompson and Taylor, 2008) and of growth scales (Meier, 2001) is advocated to ensure a more comprehensive interpretation of agronomic and phenotypic data and agricultural management practices.

- It is critical to record the growth stage when agronomic endpoints are observed.
- EuropaBio:
- Recommended Growth stage conversions from V/R to BBCH developmental stages for soybean and corn.

Soybean Growth Stage Conversions

Proposed conversions from V/R to BBCH developmental stages of soybean

Developmental Stage	BBCH	BBCH DESCRIPTION
Soybean VE	10	Emergence / Cotyledon visible
Soybean VC	11	Unifoliate leaves unfolded
Soybean V1	12	1st trifoliate leaf unfolded
Soybean V2	13	2nd trifoliate leaf unfolded
Soybean V3	14	3rd trifoliate leaf unfolded
Soybean V4	15	4th trifoliate leaf unfolded
Soybean V5	16	5th trifoliate leaf unfolded
Soybean V6	17	6th trifoliate leaf unfolded
Soybean V7	18	7th trifoliate leaf unfolded
Soybean V8 -V18	19	8th - 18th trifoliate leaf unfolded
Soybean R1	61	Beginning bloom
Soybean between R1-R2	65	50% bloom
Soybean R2	65-69	Full bloom
Soybean R3	71	Fruit/propagule 10% final size
Soybean R4	75	Fruit/propagule 50% final size
Soybean R5	76	Fruit/propagule 70% final size
Soybean R6	77	Fruit/propagule final size
Soybean R7	79	Full ripeness/coloration
Soybean R8	89	Seed hardened
Soybean – several days after R8	99	Harvested seed

Pedersen, P. 2004. Soybean Growth and Development. Iowa State University Extension, PM 1945.

Corn Growth Stage Conversions

Conversions from V/R to BBCH developmental stages of corn

Developmental Stage	BBCH	BBCH DESCRIPTION
Corn VE	09	Emergence
Corn V1	11	First leaf unfolded
Corn V2	12	2 leaves unfolded
Corn V3	13	3 leaves unfolded
Corn V4	14	4 leaves unfolded
Corn V5	15	5 leaves unfolded
Corn V6	16	6 leaves unfolded
Corn V7	17	7 leaves unfolded
Corn V8	18	8 leaves unfolded
Corn V9-Vn	19	9 or more leaves unfolded
Corn VT	55	Middle of tassel emergence: middle of tassel begins to separate
Corn R1	63	Male: beginning of pollen shed Female: tips of stigmata visible
Corn R1	65	Male: upper and lower parts of tassel in flower; Female: stigmata fully emerged
Corn R2	71	Kernels at blister stage
Corn R3	75	Kernels in middle of cob yellowish-white, content milky
Corn R4	85	Dough stage: kernels yellowish to yellow
Corn R5	No equivalent BBCH code in reference	
Corn R6	87	Physiological maturity: black dot/layer visible at base of kernels
	89	Fully ripe: kernels hard and shiny
Corn - several days after R6	99	Harvested seed

Abendroth L.J., Elmore R.W., Boyer M.J., Marlay S.K. 2011. Corn Growth and Development. PMR 1009. Ames, IA: Iowa State University Extension. 49 pp.

Meier U. (ed.), 2001. Growth stages of mono-and dicotyledonous plants, 2nd edition. BBCH Monograph. Federal Biological Research Centre for Agriculture and Forestry, Bonn, Germany.

Lancashire P.D., Bleiholder H., Langeluddecke P., Stauss R., van den Boom T., Weber E., Witzen-Berger A. 1991. A uniform decimal code for growth stages of crops and weeds. *Ann. Appl. Biol.* **119** (3): 561–601. doi:10.1111/j.1744-7348.1991.tb04895.x.

Oilseed Rape Growth Stages

BBCH developmental stages of oilseed rape

BBCH	BBCH DESCRIPTION
11	1 true leaf unfolded (not including cotyledons)
12	2 true leaves unfolded (not including cotyledons).
13	3 true leaves unfolded (not including cotyledons).
15	5 true leaves unfolded (not including cotyledons).
33	3 visibly extended internodes.
50	Flower buds present; still enclosed by leaves.
59	First petals visible; flowers still closed; yellow bud.
60	First flowers open.
61	10% of flowers on main raceme open, main raceme elongating
65	Full flowering; 50% of flowers open on main raceme open, older petals falling.
69	End of flowering.
79	Nearly all pods have reached full size.
83	30% of pods ripe; seeds dark and hard.
85	50% of pods ripe; seeds dark and hard.
86	60% of pods ripe; seeds dark and hard.
87	70% of pods ripe; seeds dark and hard
89	Fully ripe; nearly all pods ripe, seeds dark and hard.
90	Senescence; plant drying down.
97	Plant dead and dry

Lancashire P.D., Bleiholder H., Langeluddecke P., Stauss R., van den Boom T., Weber E., Witzen-Berger A. 1991. A uniform decimal code for growth stages of crops and weeds. *Ann. Appl. Biol.* **119** (3): 561–601. doi:10.1111/j.1744-7348.1991.tb04895.x.

Cotton Growth Stages

Appendix 4. BBCH developmental stages of cotton

BBCH	BBCH DESCRIPTION
09	Emergence: Hypocotyl with cotyledons breaking through soil surface ("crook stage")
10	Cotyledons completely unfolded
11	First true leaf unfolded [1]
12	2nd true leaf unfolded [1]
13	3rd true leaf unfolded [1] Stages continuous til . . .
19	9 or more true leaves unfolded, no side shoots visible [2]
21	First vegetative side shoot (2nd order) visible [3]
22	2 vegetative side shoots (2nd order) visible Stages continuous til . . .
29	9 or more vegetative side shoots (2nd order) visible
31	Beginning of crop cover: 10% of plants meet between rows
32	20% of plants meet between rows
33	30% of plants meet between rows Stages continuous til . . .
39	Canopy closure: 90% of plants meet between rows
51	First floral buds detectable ("pin-head square") [4]
52	First floral buds visible ("match-head square") [4]
55	Floral buds distinctly enlarged
59	Petals visible: floral buds still closed
60	First flowers opened (sporadically within the population)
61	Beginning of flowering ("Early bloom"): 5-6 blooms/25 ft (7.5 m) of row
65	Full flowering: ("Mid-bloom"): 11 or more blooms/25 ft (7.5 m) of row
67	Flowering finishing: majority of flowers faded ("Late bloom")
69	End of flowering
71	About 10% of bolls have attained their final size
72	About 20% of bolls have attained their final size Stages continuous til . . .
79	About 90% of bolls have attained their final size

Cotton Growth Stages

Appendix 4. BBCH developmental stages of cotton (Continued)

BBCH	BBCH DESCRIPTION
80	First open bolls on the first fruiting branches
81	Beginning of boll opening: about 10% of bolls open. Nodes Above White Flower (NAWF)
82	About 20% of bolls open
83	About 20% of bolls open. Nodes Above Cracked Boll (NACB)
8..	Stages continuous til . . .
89	About 90% of bolls open
91	About 10% of leaves discolored or fallen
92	About 20% of leaves discolored or fallen Stages continuous til . . .
9..	Stages continuous til . . .
96	About 60% of leaves discolored or fallen
97	Above ground parts of plant dead; plant dormant
99	Harvested product (bolls and seeds)

[1] Leaves are counted from the cotyledon node (= node 0).
[2] Side shoot development may occur earlier. If there is a vegetative side shoot, continue with principal growth stage 2. If there is a reproductive side shoot (fruiting branch), continue with the principal growth stage 5.
[3] Vegetative side shoots are counted from the cotyledon node.
[4] "pin-head square" or "match-head square" is the first square which forms at the first fruiting position of the first fruiting branch.

Munger, P., H. Bleiholder, H. Hack, M. Hess, R. Staub, T. van den Boom and E. Weber: Phenological Growth Stages of the Cotton plant (Gossypium hirsutum L.) Codification and Description according to the BBCH Scale. Journal of Agronomy and Crop Science (1998).

Generic Endpoints

(Soybean, Corn, Cotton, and Oilseed rape)

Phase	Endpoint	Application for		Growth Stages (BBCH)
		Import	Cultivation	
Vegetative	Early Stand Count	☑	☑	11-14
	Crop Development		☑	31-39
Reproductive	Days to Flowering	☑	☑	61-69
	Flowering Duration		☑	61-69
Maturity	Lodging	☑	☑	80-89
	Final Stand Count	☑	☑	31-99
	Plant Height	☑	☑	71-89
	Days to Maturity	☑	☑	81-89
	Seed Loss		☑	86-89
	Fruit/Ear Count	☑	☑	81-89
Harvest	Seed Moisture	☑	☑	99
	Seed Weight	☑	☑	99
	Yield	☑	☑	99

4.1.1 Stand Count

Method

The number of plants within a given area or distance (e.g. a given length of row or a quadrat of stated area) should be reported. If there are large numbers of plants per plot, then the quantification should be conducted by counting sub-sections of the entire plot (e.g. transects of 1 m length in two or more rows, 1-m^2 quadrats, etc.). Where possible, the same part of the plot should be used for measuring stand count at each different growth stage, in order to be in a position to make suitable comparisons.

- EuropaBio: This endpoint should be expressed as the number of plants per m^2.
  - **Cotton** and **Corn**: Count the number of plants per plot (or per row), then calculate the number of plants per m^2.
  - **Oilseed rape** and **Soybean**: Count the number of plants from 3 representative 1-linear-meter sections of the plot. (Alternatively, count the number of plants from 3 representative 1m^2 quadrats.)

4.1.1 Thinning

Recommendations

Unless thinning is the normal agronomic practice, it is strongly discouraged[19]. If carried out, thinning should be described in detail, explaining the time of execution, the method of removal of the plants and the target plant density. The decision to perform thinning must be based only on plant density and not on the phenotypic characteristics of the plants. Correct thinning is required to ensure a reliable comparative assessment of the agronomic and phenotypic endpoints to be measured later during the vegetative and reproductive phases of growth, including yield and yield components.

- EuropaBio: If thinning is performed, provide:
  - Date
  - Growth stage
  - Method (cut at soil level or pulled out)

4.1.3 Days to Flowering

Method

Time to flowering can be visually estimated at the plot level between BBCH 61 and 69. In case of GM plant applications for import/processing for food and feed uses, time to flowering should be measured at a specific growth stage (e.g. BBCH 65) and be provided as days from planting to when flowers occur on a specific percentage of the plants in the plot.

- **EuropaBio**:
  - **Cotton, Oilseed rape, Soybean**: Determine the number of days from planting to 50% flowering
  - **Corn**: Determine the number of days from planting to 50% anthesis

4.1.5 Lodging

Method

Lodging should be measured at plant maturity, before harvest, by visually estimating the proportion of lodged plants per plot (to the nearest 10 %) inclined more than 45 ° from the vertical.

If brackling occurs, then this can be measured following the same method described for lodging and measured along with lodged plants.

- EuropaBio: Across crops, lodging should be recorded to the nearest 10%.
  - **Cotton**, **Oilseed rape**, and **Soybean**: Determine the estimated % lodging
  - **Corn**: Determine separate % lodging estimates for stalk lodging and root lodging, but add together and report as one number.

4.1.6 Plant Height

Method

Measurements should be performed at completion of stem elongation, which is usually during or at the end of flowering (BBCH 69). A representative number of plants per plot should be measured for length (cm) from the base (soil level) of the plant to the uppermost terminal meristem, or in maize to the base of the tassel.

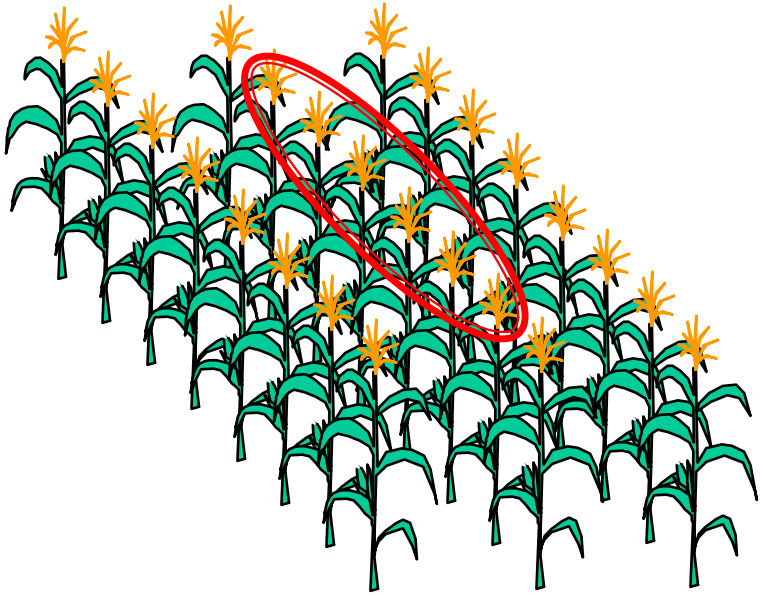
Recommendations

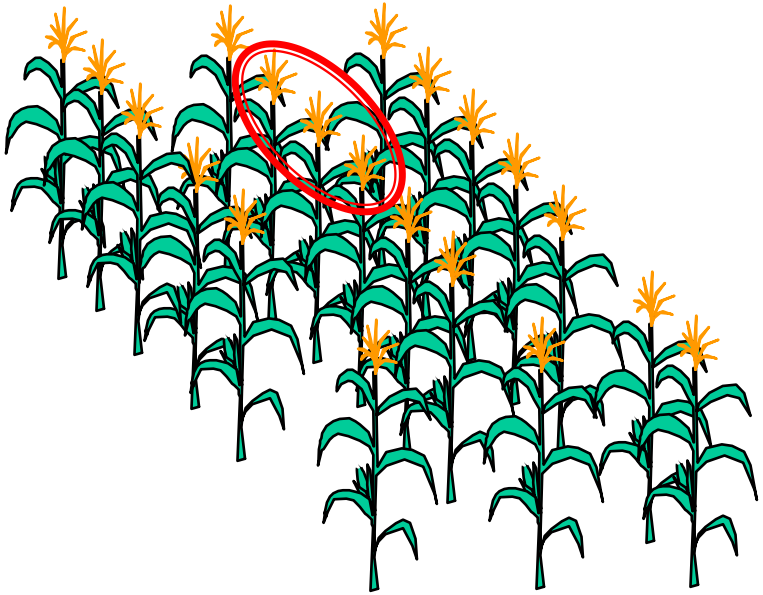
The total number of sampled plants per plot is to be justified and reported, as well as the individual values measured.

- EuropaBio: Individual plant height values need to be provided in electronic form.
  - **Cotton**, **Oilseed rape**, and **Soybean**: Measure the height of 5 representative and competitive plants per plot.
  - **Corn**: Measure the height (from the soil level to the collar of the flag leaf as a good visual indicator of the base of the tassel) of 5 representative and competitive plants per plot.

Competitive plants

- Competitive plants are those that are surrounded by other plants on all sides (not plants at the end of the plots or those flanked by stand gaps).





4.1.7 Maturity

Method

Visual estimation of fruit/kernel/seed maturity is to be measured when the plants reach full physiological maturity (BBCH 89) by recording days from planting to the date when mature fruits/kernels/seeds occur on 90 % of the plants.

Recommendations

If it is not possible to assess the physiological maturity of the crop (for instance, owing to the use of desiccants, swathing), then applicants should characterise the level of maturity (BBCH growth stage) at the time of the intervention.

- **Europa Bio**: Number of days from planting to maturity.
  - **Cotton**: Maturity data should be recorded prior to applying defoliant or boll opener (10%–70% open bolls).
  - **Corn**: When approximately 90% of plants have reached black layer stage.
  - **Oilseed rape**: When 60–70% of the pods are ripe and the seed color changes on the main stem.
  - **Soybean**: when approximately 90% of the plants have pods of physiologically mature color (R8).

4.1.9 Fruit Count

Method

Record the number of fruits per plant from a representative sample of plants in the plot at maturity (BBCH 89).

- EuropaBio: The number of fruits per plant.
  - **Cotton**: Count bolls on 5 representative and competitive cotton plants per plot
  - **Corn**: Count ears on 5 representative and competitive corn plants per plot.
  - **Oilseed rape** and **Soybean**: Count pods on 5 representative and competitive plants per plot.

4.1.10 Seed Moisture at Harvest

Method

Seed moisture content analysis should be based on representative samples taken from the entire harvest. Samples should be tested immediately after harvest (BBCH 99) and before any processing (e.g. natural or artificial air drying, storage). "Weight loss on drying", moisture meters or other suitable devices can be used to determine moisture content. Specification of the methodology applied should be always provided.

Moisture content should be recorded as percentage water of the fresh harvested weight. The remainder is the dry matter.

- EuropaBio: This is a standard measurement recorded at harvest for grains to normalize yield data.
  - **Corn**, **Oilseed rape**, and **Soybean**: No changes
  - **Cotton**: The seed moisture values are determined during composition analysis instead of at harvest.

4.1.11 Seed Weight

Method

A representative sample of harvested (BBCH 99) seeds per plot is weighed and counted in order to determine the mass (g) of 100 or 1 000 seeds. The value obtained is then corrected to a standard moisture content using the moisture content assessment (see Section 4.1.10).

EuropaBio:

- **Cotton:** Determine the weight of 100 seeds. Cannot be corrected for moisture, since moisture is not determined until composition analysis.
- **Corn** and **Soybean**: Determine the weight of 100 seeds and correct for moisture.
- **Oilseed rape**: Determine the weight of 1,000 seeds and correct for moisture.

4.1.12 Yield

Method

Harvest a representative area or the whole plot and weigh the harvested plant material. Yield can be expressed as mass (g or kg) of the harvested material per sampled plot area (m^2) corrected for moisture content. Alternatively, yield can also be converted to tonnes or kilograms per hectare.

Recommendations

Harvested plant material should be as free as possible of non-harvest fractions (e.g. straw, stems, leaves) and contaminants (e.g. soil for potato). Secondary cleaning can be done to remove these materials. In addition, it should be clearly stated whether the yield refers to grain, grain and cob, or grain plus attached husk. In the case of crops harvested before physiological maturity (e.g. sweetcorn or forage maize), the maturity and/or growth stage of the crop at harvest should be described.

- EuropaBio:
  - **Corn, Soybean** and **Oilseed rape**: Expressed as t/ha of grain.
  - **Cotton**: Expressed as kg/ha for seed cotton.

Additional endpoints

If EFSA application is done for cultivation of products then additional endpoints are needed:

Phase	Endpoint	Application for		Growth Stages (BBCH)
		Import	Cultivation	
Vegetative	Early Stand Count	☑	☑	11-14
	Crop Development		☑	31-39
Reproductive	Days to Flowering	☑	☑	61-69
	Flowering Duration		☑	61-69
Maturity	Lodging	☑	☑	80-89
	Final Stand Count	☑	☑	31-99
	Plant Height	☑	☑	71-89
	Days to Maturity	☑	☑	81-89
	Seed Loss		☑	86-89
	Fruit/Ear Count	☑	☑	81-89
Harvest	Seed Moisture	☑	☑	99
	Seed Weight	☑	☑	99
	Yield	☑	☑	99

4.1.2 Plant Development

For most crops, percentage ground cover[20], estimated visually, is a useful and practical method (Steven et al., 1986). Generally, a 0.5 × 0.5 m quadrat is placed over the crop, and the amount of ground covered by the crop is estimated visually. For tall crops or crops with wide row spacing such as maize, measurement of plant height[21] will give a good assessment of vegetative growth. In general, other measurements that can provide information on crop development are considered appropriate, such as non-destructive quantification of leaf surface or leaf area index (e.g. Liu and Pattey, 2010). A detailed description of the applied methodology for the endpoint measurements should be provided.

The vegetative development should be measured during the elongation phase (BBCH 31–39). Vegetative development should be measured at least once during the season. Ideal timings to make the ground cover observations include during vegetative stem extension or prior to flowering. These phases can be identified between BBCH 16 and 39 but before BBCH 61.

EuropaBio:

- **Cotton**, **Oilseed rape**, and **Soybean**: Estimate percent ground cover based on 1x1m quadrat.
- **Corn**: Measure early plant height on 5 representative and competitive plants.

4.1.4 Flowering Duration

Method

Flowering is to be estimated visually at the plot level. The beginning and the end of the flowering period should be recorded as the dates when 10 % of the flowering is occurring and when 90 % of the plants have completed flowering, respectively. This will require visual observations to be carried from the onset to the completion of flowering (BBCH 61–69)[22].

EuropaBio:

- **Cotton, Oilseed rape, Soybean**: Determine the number of days from beginning of flowering (10% of plants flowering) to the end of flowering (90% of plants completed flowering).
- **Corn**: Determine the number of days from 10% to 90% anthesis.

4.1.8 Seed Loss

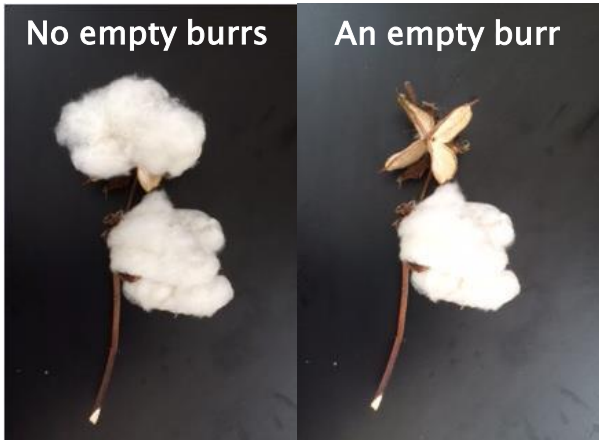
Method

Record the number of dropped ears/cobs/seeds per plot or shattered pods per specific area of plot. In some instances, "seed rain" trays may be positioned beneath the plots to collect falling or shattered seed (e.g. oilseed rape). Observations should ideally be made at plant maturity before harvest. Total number of mature dropped cobs or seeds per plot, or total number of shattered pods per specific length or area of the plot, should be recorded. Measurements should be converted to numbers per unit area (m^2).

For potatoes, standard areas of plots should be examined after harvest and the numbers of small tubers not harvested and fruit should be counted.

EuropaBio: Express as number of dropped fruits or shattered pods or empty burrs per m^2

- **Corn**: Number of dropped ears per plot.
- **Oilseed Rape** and **Soybean**: Number of shattered pods on 5 competitive and representative plants.
- **Cotton**: Number of empty burrs on 5 competitive and representative plants.



4.1.13 Plant Responses to Stressors

- Three types of stressors are considered: Arthropod, Disease, and Abiotic.
- Visual estimation per plot.
- EuropaBio: Evaluate stressors at each location four times during the growing season. For example:
  - ✓ Early vegetative stage
  - ✓ Late vegetative stage
  - ✓ Early reproductive stage
  - ✓ Late reproductive stage
- Additional observation times might be appropriate if a specific stressor is observed at a location.

4.1.13 Plant Responses to Stressors

EuropaBio: Observations should be collected from each plot using the categorical scale of increasing severity:

Category	Severity of plant damage
None	No symptoms observed
Slight	Symptoms not damaging to plant development (e.g. minor feeding, minor lesions, nutrient deficiency chlorosis); mitigation likely not required
Moderate	Intermediate between slight and severe; likely requires mitigation
Severe	Symptoms damaging to plant development (e.g. stunting or death); mitigation unlikely to be effective

4.1.13 Plant Responses to Arthropod Stressors

- Early in the season, at least 3 common pest species at each location need to be identified considering the following criteria:
  - Species need to feed on the crop of interest (avoid arthropods that use plants as shelter/reproduction site).
  - Select diverse feeding modes, if possible (*e.g.,* sucking, chewing, mining/boring, gall formation, root feeder, flower feeder, seed feeder…).
- Prioritize pests of biological and economic relevance.

4.1.13 Plant Responses to Disease Stressors

- At least 3 relevant plant diseases should be observed at each location considering the following criteria:
- Diverse categories (fungi, viruses, bacteria, *etc*)
- Different mechanisms of pathogenesis (biotrophic, necrotrophic)
- Consider diseases that affect different plant parts (*e.g.*, leaves, stem/stalk/trunk, roots, flowers, fruit, aboveground or subterranean plant parts…)
- Prioritize pests of biological and economic relevance.

4.1.14 Plant Responses to Abiotic Stressors

- At least 3 relevant abiotic stressor should be observed at each location considering the following criteria:
- A stressor that influence the environment beyond its typical range of variation (*e.g.*, cold temperatures of $\leq$ 40°F for 3 or more days)
- Consider abiotic stressor that show clear indication of symptoms on plant parts (*e.g.*, leaf rolling, short plant height, premature plant death)
- Prioritize stressor of biological and economic relevance.
- EuropaBio: Pesticide injury may be included as an abiotic stress if the exposure was uniform across plots. However, it should not be considered as an abiotic stress if only a few plots are affected.

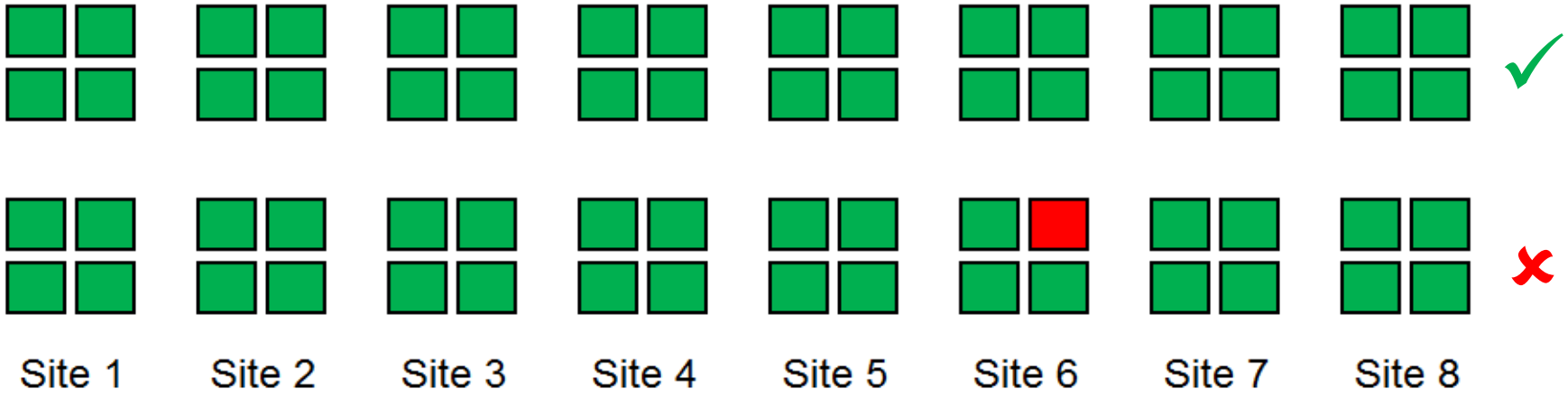
4.2 Case-specific Agronomic and Phenotypic Endpoints

- Other relevant endpoints may be included in the study on a case-by-case basis.
- Trait-specific
- Potential unintended effects
- Endpoints related to persistence and invasiveness

- Other relevant endpoints may be included in the study if required by Regulatory Authorities other than EFSA.

Missing Data

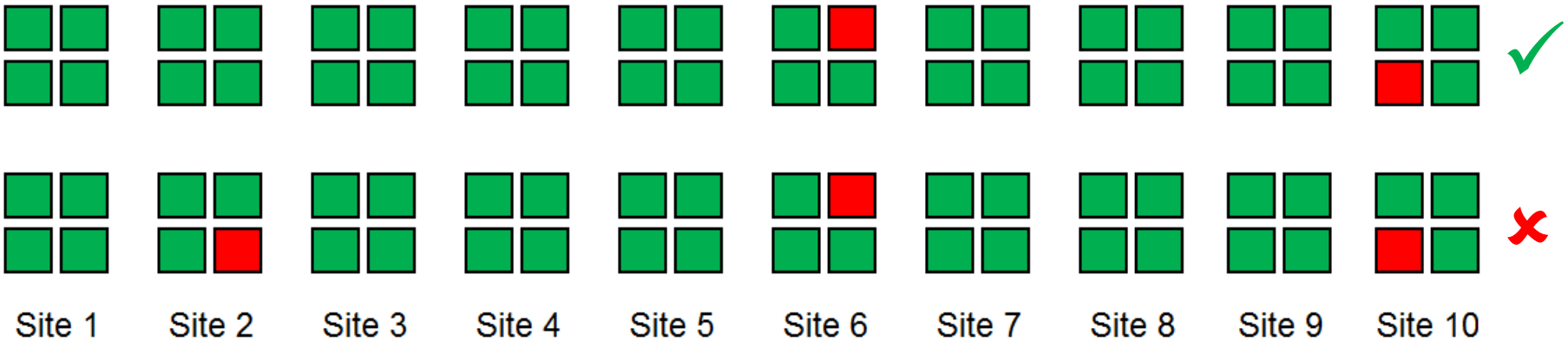
EFSA has strict requirements (minimum of 8 sites x 4 reps of data):



The second data set will not be considered in line with EFSA guidance documents: "The replication should never be <u>less than four</u> at any site".

Missing Data

If missing values are observed, then additional locations are needed to fulfil 8 sites x 4 reps requirement:



The second dataset will not be considered in line with EFSA guidance documents.

Summary

- Guidance has been provided by EFSA on agronomic and phenotypic characterization of GMO crops
- Site selection must be carefully thought out
- More auxiliary data is required (soil characterization, cropping history, meteorological data)
- Less flexibility with missing data points
- Study protocols provide study specific details

Thank you!