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RESEARCH

Consistent Risk Assessment Outcomes from Agronomic Characterization of GE Maize in Diverse Regions and as Single-Event and Stacked Products

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ABSTRACT

In commercializing a genetically engineered (GE) crop, agronomic characterization studies that contribute to environmental risk assessment (ERA) may be repeated in different global regions. Likewise, these studies may be done both for single-event GE products and for traditional breeding crosses that combine GE events (breeding stacks). The objectives of this research were to assess the need for de novo agronomic characterization if previously done in another region or for each event in a breeding stack. Data were obtained for the GE maize (*Zea mays* L.) products MON 89034 (insect protected), NK603 (herbicide tolerant), and the breeding stack MON 89034 × NK603. The field trials were done from 2004 to 2014 in Argentina, Brazil, Mexico, Pakistan, and/or the United States. Sources of environmental diversity among the regions (i.e., countries) included differences in the prevalent climate classes of their sites. Although values for the agronomic characteristics varied among regions, event × region interactions caused <1% of the total variability for each GE product. Within each region, comparisons of GE products and near-isogenic conventional controls were largely nonsignificant. When considering agronomic characteristics, a consistent risk assessment outcome—no evidence of increased potential to become a plant pest—was found in each region and for the single-event products and the breeding stack. The results support ERA policies that provide for (i) acceptance of agronomic characterization data from other regions (data transportability) and (ii) exemption of breeding stacks from agronomic characterization, based on case-by-case assessments of plausible risks.

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Abbreviations: *Bt*, *Bacillus thuringiensis;* CP4 EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain *CP4*; ERA, environmental risk assessment; GE, genetically engineered.

Genetically engineered (GE) crops (also known as genetically modified or GM crops) were grown on 189.8 million ha globally in 2017 (ISAAA, 2017). Crops with multiple GE traits (stacks) accounted for 41% of this area (ISAAA, 2017). The use of GE crops has been associated with economic benefits to farmers (Klümper and Qaim, 2014; Smyth et al., 2015) and consumers (Smyth et al., 2015), reductions in pesticide use (Klümper and Qaim, 2014; Brookes and Barfoot, 2017), and changes to farming practices leading to lower greenhouse gas emissions (Brookes and Barfoot, 2017).

Environmental risk assessment (ERA) is conducted before GE crop commercialization. Trials that contribute to ERA include agronomic characterization (e.g., Horak et al., 2007, 2015; Sammons et al., 2014), in which a GE variety and a near-isogenic conventional control are compared across multiple field sites for agronomic characteristics such as plant population, flowering timing, lodging, and yield. References (typically conventional

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commercial varieties) may be included to provide a quantitative measure of the variability already common to each characteristic. The potential for effects from event-related differences in pest pressure is minimized by conventional pest management practices applied uniformly across each site as needed. Risks are assessed relative to comparators (e.g., the control and references) that, as conventional and/or commercial varieties, are assumed to be acceptable for environmental release. Observed differences between the GE variety and the control are assessed for risk implications or needs for further study (EFSA, 2015). The risks considered typically include the potential for the GE crop to become a plant pest through persistence or invasiveness in agricultural or natural ecosystems (Raybould et al., 2012; EFSA, 2015).

Field trials for GE crop ERA, including agronomic characterization studies, are commonly located within the intended cultivation region (e.g., the United States, Argentina, or Brazil) (Garcia-Alonso et al., 2014). However, requirements for this are not universal. The USDA has considered foreign site agronomic characterization data when submitted along with data from US sites (e.g., USDA APHIS, 2011, 2013), and Canadian Food Inspection Agency regulations allow field trial data from foreign sites with environments similar to those in Canada (CFIA, 2017). Furthermore, needs for specificity in testing environments may depend on the nature of the GE event. If not related to differences among environments in GE event effects, environmental biases to risk assessment outcomes are minimized by comparisons of the GE variety with a near-isogenic conventional control at each site. Agronomic characterization data that support similar ERA conclusions despite originating in geographically diverse regions have been reported by Horak et al. (2015) and Nakai et al. (2015).

Some regulatory authorities require agronomic characterization for traditional breeding crosses that combine multiple GE events (breeding stacks) even if previously completed for each event individually. For example, the European Food Safety Authority requires agronomic characterization for many breeding stacks (EFSA, 2011; European Commission, 2013). In contrast, breeding stacks are regulated in the United States only if they contain a novel combination of events that produce pesticidal substances. Risk assessments for such stacks typically do not rely on agronomic characterization (e.g., USEPA, 2009). Traditional breeding has an extensive history of safety that may be applicable to breeding stacks (Pilacinski et al., 2011; Weber et al., 2012; Steiner et al., 2013), particularly when risks from interactions of stacked events are unlikely (Pilacinski et al., 2011; Steiner et al., 2013). Similar values for agronomic characteristics for breeding stacks and conventional controls have been reported for MON 89034 × TC1507 × NK603 × DAS-40278–9 and MON 89034 × TC1507 × NK603 (Rezende de Cerqueira et al., 2017), and for MON 89034 × MON 88017 and MON 89034 × NK603 (Heredia Díaz et al., 2017). Kok et al. (2014) reviewed European Food Safety Authority scientific opinions for >20 breeding stacks, noting that "in all cases, the conclusion was that the crossing of the single GM events did not result in interactions that cause compositional, agronomic, or phenotypic changes that would raise safety concerns."

The conduct of agronomic characterization studies in multiple regions and on breeding stacks is time and resource intensive. The impacts may include delays in GE crop availability for commercial use, with potential for significant opportunity costs such as those noted by Biden et al. (2018). These additional assessments also contribute to current barriers to GE crop commercialization that greatly affect small organizations or those in the public sector (Garcia-Alonso et al., 2014; Conko et al., 2016) and reduce the likelihood of commercialization of beneficial GE events in crops with limited market value (Conko et al., 2016). Given these concerns, the objectives of this study were to assess the need for de novo agronomic characterization when (i) data are available from another region, or (ii) data are available for each of the individual events in a breeding stack.

MATERIALS AND METHODS

Data were obtained from 25 agronomic characterization field studies conducted for ERA from 2004 to 2014 across a total of 104 sites in Argentina, Brazil, Mexico, Pakistan, or the United States. Data from Mexico were included as part of a prior publication (Heredia-Díaz et al., 2017), as were a small portion of the data from the United States (Nakai et al., 2015). Inclusion of these data allowed more robust assessments in the current study.

The GE products assessed were insect-protected maize (*Zea mays* L.) MON 89034 (YieldGard VT PRO), glyphosate-tolerant maize NK603 (Roundup Ready Corn 2), and the associated breeding stack MON 89034 × NK603 (VT Double PRO) developed by Monsanto (St. Louis, MO, USA). MON 89034 is a single event that produces two insecticidal proteins that protect against feeding damage caused by lepidopteran insect pests: Cry1A.105, a modified *Bacillus thuringiensis* (*Bt*) Cry1A protein, and Cry2Ab2, a *Bt* (subsp. *kurstaki*) protein. The NK603 event produces a 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain *CP4* (CP4 EPSPS) that confers tolerance to the herbicide glyphosate.

The MON 89034 and NK603 events were selected for commercialization based on agronomic testing in many environments and extensive assessment of molecular characteristics. Heck et al. (2005) documented NK603 event selection with a focus on molecular testing. Extensive agronomic and molecular testing is typical of commercial GE events and facilitates selection of events that are unlikely to have significant unintended effects (Prado et al., 2014; Glenn et al., 2017). Furthermore, the functions of the proteins produced by these events do not suggest hypotheses for risks that would be evident via agronomic characterization. The *Bt* proteins (such as those encoded by MON 89034) lack known metabolic activity in plants (Steiner et al., 2013). As reviewed by CERA (2010), CP4 EPSPS and endogenous plant EPSPS proteins are functionally equivalent except in affinity for glyphosate. In a breeding stack, *Bt* proteins are unlikely to interact with herbicide tolerance events such as NK603 (Steiner et al., 2013).

The GE events were tested in 22 different genetic backgrounds, including temperate, subtropical, and tropical hybrids (Table 1). One GE hybrid had an originally transformed inbred line as a parent. The others had parental lines with GE events introgressed via backcrossing, a traditional breeding technique that recovers most of a recurrent parent genome but includes a targeted trait from a donor genome (Hallauer and Miranda, 1988). Agronomic comparisons of the GE hybrids with near-isogenic conventional hybrids may have occurred before their use in the current study, potentially in the same region for which data are currently reported. Hybrids tested in this way are representative of those available in the marketplace, as testing of this or a similar nature is standard when integrating GE events into new hybrids for commercial use (Stojšin and Behr, 2004; Prado et al., 2014).

The MON 89034, NK603, and MON 89034 × NK603 products were evaluated at 81, 59, and 24 of the sites, respectively (Table 2). For 21 of 25 studies, each included GE product was tested in the same hybrid(s) across all study sites. Although hybrids may have differed among studies or sites, at each site, each GE product and corresponding near-isogenic conventional control were in the same hybrid background with all parental lines in common. Furthermore, each site included three to seven commercial maize hybrids as references. There were 100 sites with exclusively conventional reference hybrids and four sites that included one or two commercial GE reference hybrids that contained a different event than the GE test material.

The 104 sites represented a diverse range of geography and climate classes (Fig. 1, Table 2). Additional sources of environmental diversity included variation in planting year, growing season, and date; differences in other production practices; and a wide range of soil properties (data not shown). A site was defined as a location within a study. Within each study, all sites were within a single region (i.e., country). Within all studies but one, all sites were planted within a single growing season.

Crop management practices were implemented uniformly across all plots at each site, including those of the GE products

Table 1. Hybrid backgrounds of genetically engineered (GE) maize products and conventional controls for 2004 to 2014 agronomic characterization studies in Argentina, Brazil, Mexico, Pakistan, and the United States.

	Hybrid (type)				
GE product	Argentina	Brazil	Mexico	Pakistan	United States
MON 89034	MPA618, NF6066 (temperate)	AG7000, AG9020, BF9424, DKB199, EXP9707 (tropical)			DK622, DKC51-43, MPA618, MPA636B, MPA640B, NH6212 (temperate)
NK603	MPA618, NF6066, NA5051 (temperate)	DKB390 (tropical)	CANGURO, CEBU, TIGRE (subtropical), MI6313 (temperate)	DKC61-42, DKC6876, ND6628 (temperate), 919 (tropical)	MPA636B, MPA640B, NH6212 (temperate)
MON 89034 × NK603	MPA618 (temperate)	AG9020, BF9424, DKB199, EXP9707 (tropical)	CANGURO, CEBU (subtropical), MI6313 (temperate)	DKC61-42, DKC6876, ND6628 (temperate)	

Table 2. Site climatic characteristics by region for 2004 to 2014 agronomic characterization studies of genetically engineered (GE) maize in Argentina, Brazil, Mexico, Pakistan, and the United States.

GE product	Characteristic	Argentina	Brazil	Mexico	Pakistan	United States
MON 89034	Median site latitude†	−33.8	−23.3			40.7
	Köppen-Geiger climate class, number of sites‡					
	Aw: Equatorial savannah with dry winter		4			
	Cfa: Warm temperate climate, fully humid, hot summer	8	5			33
	Dfa: Snow climate, fully humid, hot summer					29
	Dfb: Snow climate, fully humid, warm summer					2
NK603	Median site latitude	−33.6	−25.1	26.0	30.9	40.2
	Köppen-Geiger climate class, number of sites					
	Aw: Equatorial savannah with dry winter		1			
	BSh: Steppe climate, hot steppe/desert			1	3	
	BWh: Desert climate, hot steppe/desert			6	3	
	Cfa: Warm temperate climate, fully humid, hot summer	11	1	2		18
	Dfa: Snow climate, fully humid, hot summer					13
MON 89034 × NK603	Median site latitude	−34.0	−23.3	26.0	30.7	
	Köppen-Geiger climate class, number of sites					
	Aw: Equatorial savannah with dry winter		3			
	BSh: Steppe climate, hot steppe/desert			1	1	
	BWh: Desert climate, hot steppe/desert			6	2	
	Cfa: Warm temperate climate, fully humid, hot summer	5	4	2		

† A site is defined as a location within a study.

‡ Sources: http://koeppen-geiger.vu-wien.ac.at (1986–2010 climate data), Kottek et al. (2006), Rubel et al. (2017).

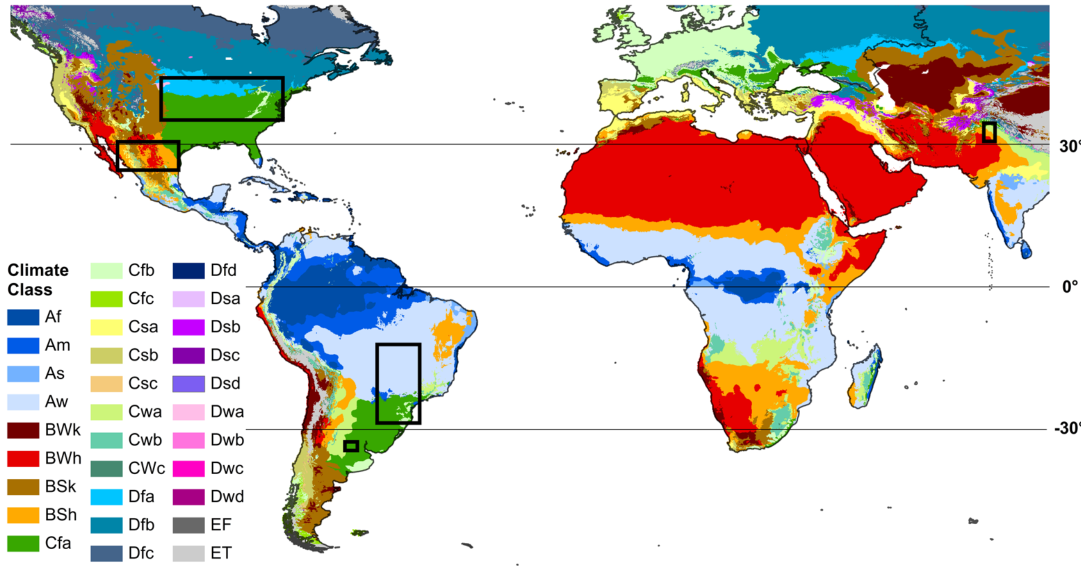

Fig. 1. Köppen–Geiger climate classification map with outlines encompassing the experimental sites for 2004 to 2014 agronomic characterization studies of genetically engineered (GE) maize in Argentina, Brazil, Mexico, Pakistan, and the United States, respectively (from south to north). All sites fell within the following main climate classes: arid (BSh, BWh), equatorial (Aw), snow (Dfa, Dfb), and warm temperate (Cfa). Sources: http://koeppen-geiger.vu-wien.ac.at (1986–2010 climate data), Kottek et al. (2006), Esri (2015), Rubel et al. (2017). Color scheme similar to Peel et al. (2007).

and controls. The potential for event-related differences in pest pressure among plots was reduced by targeting agronomically acceptable levels of insect and weed control. The insect control achieved varied with local insect pressure and treatment decisions. Planting dates were within or near ranges typical of the local area and growing season. The majority of sites were thinned at an early growth stage, after observations of early stand, to promote uniform plant population among plots. Other sites were not thinned, and two sites were thinned before observations of early stand. These sites were hand planted with two seeds per hill and thinned to one-half of the planting rate to promote retention of a single plant per hill. Postemergence glyphosate applications (independent of weed control practices, which were applied uniformly across all plots) were made exclusively to the NK603 plots at 26 of the 59 NK603 sites.

The experimental design at each site was a randomized complete block with three or four replications. A total of 11 characteristics commonly assessed in agronomic research and plant breeding were considered in this study (Table 3). In most cases, plants from two inner rows were used for data collection in each plot. Across sites, the area harvested for yield ranged from 4.0 to 14.4 m^2, averaging 9.6 m^2, with harvested row length ranging from 5 to 10 m. Many of the characteristics, such as timing of flowering, ear and plant height, and yield, reflect a cumulative response to the environment over time and are therefore robust assessments for unintended effects. Some, such as root and stalk lodging, are strongly influenced by environmental stresses.

The dataset was reviewed to ensure that included data were of high quality. In addition, to minimize potential for plant population differences to confound event effects, plots with final stand <80% of the intended plant population (defined as the 75th quantile of all final stands at the site) were excluded from analyses of yield, grain moisture, dropped ears, root lodging, and stalk lodging. Plots with final stand <60% of the intended plant population were excluded from analyses of all characteristics other than early and final stand.

Table 3. Plant characteristics measured in 2004 to 2014 agronomic characterization studies of genetically engineered (GE) maize in Argentina, Brazil, Mexico, Pakistan, and the United States.

Characteristic	Timing	Description (units)
Early stand	Early vegetative	Plant population early in the season (plants ha^{-1})
Final stand	Pre-harvest	Plant population late in the season (plants ha^{-1})
50% pollen shed	Pollen shed	Timing of 50% anthesis (DAP)†
50% silking	Silking	Timing of 50% silking (DAP)
Ear height	After flowering	Distance from ground to primary ear attachment node (m)
Plant height	After flowering	Distance from ground to flag leaf collar (m)
Dropped ears	Pre-harvest	Number of ears completely detached from the plant (no. per 100 plants)
Root lodging	Pre-harvest	Number of plants leaning more than 30° from vertical (percentage of final stand)
Stalk lodging	Pre-harvest	Number of plants broken below the ear (percentage of final stand)
Grain moisture	Harvest	Moisture of harvested grain (%)
Yield	Harvest	Grain yield, standardized to 15.5% moisture (Mg ha^{-1})

† DAP, days after planting.

Variance Components Analyses

The following model (Eq. [1]) was fit to the data for each of the three test and associated control combinations (excluding reference materials) to assess the different components of variance, by characteristic:

$$y_{ijklm} = \mu + r_i + t_j + rt_{ij} + s\left(r\right)_{ik} + ts\left(r\right)_{ijk} + z\left(sr\right)_{ikl} + tz\left(sr\right)_{ijkl} + \varepsilon_{ijklm} \quad [1]$$

where y_{ijklm} is the observed characteristic response for the jth event in the mth replication at the lth site for the kth study in the ith region; μ represents the overall mean response; r_i represents the random effect of the ith region; t_j represents the random effect of the jth event; rt_{ij} represents the random effect of the ith region crossed with the jth event; $s(r)_{ik}$ represents the random effect of the kth study nested within the ith region; $ts(r)_{ijk}$ represents the random effect of the jth event crossed with the kth study nested within the ith region; $z(sr)_{ikl}$ represents the random effect of the lth site nested within the kth study and the ith region; $tz(sr)_{ijkl}$ represents the random effect of the jth event crossed with the lth site nested within the kth study and the ith region; and ε_{ijklm} represents the residual error.

Equation [1] was fit to the data, by characteristic, using the MIXED procedure in SAS (SAS, 2012). Variance was estimated for each of the random model components. Variance components for the main effects of region, event, study within region (hereafter referred to as "study"), and site within study and region (hereafter referred to as "site") all represent the amount of variability among the responses due to those effects. The other variance components are interactions with event and show the amount of variability among the responses due to unique combinations of the events and the other main effects.

Dropped ears, root lodging, and stalk lodging were not a part of the variance components analyses, as the distributions of these data do not satisfy the statistical assumptions for this analysis.

Comparisons of Means

The following model (Eq. [2]) was fit to the data (including test, control, and reference materials) to compare the average response of the test and control materials by region for each characteristic:

$$y_{ijklm} = \mu + \tau_i + s\left(t\right)_{jk} + g\tau s\left(t\right)_{ijkl} + r\left(st\right)_{jkm} + \varepsilon_{ijklm} \quad [2]$$

where y_{ijklm} is the observed characteristic response for the lth genetic background of the ith material in the mth replication nested within the kth site and jth study; μ represents the overall mean response; τ_i represents the fixed effect of the ith material; $s(t)_{jk}$ represents the random effect of the kth site nested within the jth study; $g\tau s(t)_{ijkl}$ represents the random effect of the lth genetic background, ith material, and kth site nested within the jth study; $r(st)_{jkm}$ represents the random effect of the mth replication nested within the kth site and the jth study; and ε_{ijklmn} represents the residual error.

The purpose of the model was to account for the general structure of the designed experiments from which the data were collected. Genetic background was considered within the model to account for one study in which test materials for differing GE products had differing genetic backgrounds. The respective genetic backgrounds were distinguished in the analyzed dataset for this study only. As a result, the model ensured that comparisons between test and control paired each test material with its associated near-isogenic conventional control for this study. In each of two additional studies, there were three test materials of differing genetic backgrounds (with corresponding near-isogenic conventional controls) for the same GE product. In these situations, the model pooled the variability across the three genetic backgrounds within each replication.

Equation [2] was fit to the data, by region for each characteristic, using the MIXED procedure in SAS (SAS, 2012). Model assumptions were checked. Dropped ears, root lodging, and stalk lodging, analyzed as proportions, did not satisfy model assumptions for normality and equal variance per qualitative assessments of residual and quantile plots.

The least squares means from Eq. [2] were provided for each characteristic for the test and control materials in each region. Pairwise differences between the test and control materials were tested at the 5% level of significance.

Distributions of Reference Hybrid Means

Arithmetic means were calculated for each commercial reference hybrid within each site. The minimum, fifth percentile, 25th percentile, 50th percentile (i.e., median), 75th percentile, 95th percentile, and maximum were calculated on those means within each region to demonstrate the statistical distribution of the reference means. Distributions are not shown for early or final stand due to potential for influences by planting rate and/or thinning. Likewise, they are not shown for grain moisture due to potential for influences by harvest timing.

RESULTS AND DISCUSSION

In agronomic characterization data for MON 89034, NK603, and MON 89034 × NK603 across five global regions, factors of region, study, and site each accounted for >25% of the total variability in agronomic characteristics for each GE product (Fig. 2). In contrast, little variability (≤1.2% of the total) was observed due to main effects of event or interactions of event with region, study, or site. These findings are consistent with reports of greater contributions to GE soybean [*Glycine max* (L.) Merr.] compositional variability from region, growing season, and/or genetic background than from GE events (Harrigan et al., 2010; Berman et al., 2011).

The minimal nature of the event effects and event interactions was further evident in comparisons of means (Tables 4–6). Within each region tested, the GE products MON 89034, NK603, and MON 89034 × NK603 were phenotypically similar to conventional controls. A total of 120 statistical comparisons of GE products and conventional controls were conducted for agronomic characteristics. For the great majority of the 120 comparisons (92%), significant differences (α = 0.05) were not detected. Across the 120 comparisons, some detected differences may have been spurious, as 5% of comparisons are expected to show

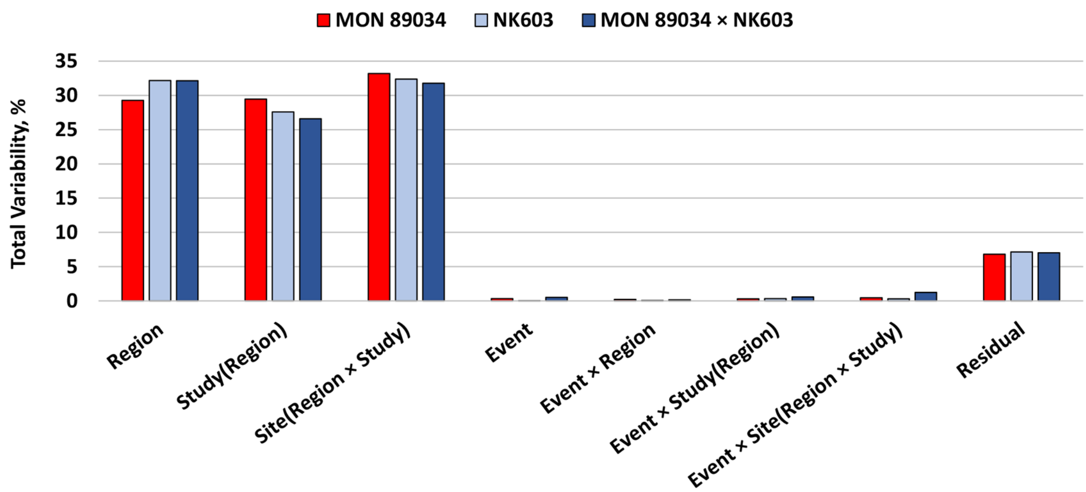

Fig. 2. Percentages of total variability attributable to variance components for 2004 to 2014 agronomic characterization studies of genetically engineered (GE) maize in Argentina, Brazil, Mexico, Pakistan, and the United States. Percentages were averaged over all characteristics except dropped ears, root lodging, and stalk lodging, which did not meet model assumptions. Reference hybrid data were excluded. Event effects arise from differences between the specified GE product and near-isogenic conventional controls. For brevity, text references to this figure use the terms "study" and "site" for "study(region)" and "site(region × study)," respectively.

Table 4. Comparisons of MON 89034 and conventional controls in 2004 to 2014 agronomic characterization studies of genetically engineered (GE) maize in Argentina, Brazil, and the United States. Values are least squares means.

	Argentina†		Brazil		United States	
Characteristic	MON 89034	Control	MON 89034	Control	MON 89034	Control
Early stand, plants ha^{-1}	81,800	83,000	60,600	61,000	83,600	84,100
Final stand, plants ha^{-1}	69,300	71,000	56,900	57,100	71,100	71,400
50% pollen shed, DAP‡	60.7	60.3	59.1	59.2	64.9	64.7
50% silking, DAP	61.4	60.7	57.7	57.5	64.6*	64.3
Ear height, m	0.80	0.82	1.31	1.27	1.03	1.04
Plant height, m	1.82	1.85	2.42	2.37	2.31	2.31
Dropped ears, no. per 100 plants	0.0	0.0	–	–	0.2	0.3
Root lodging, %	5.6	4.7	–	–	1.9	2.9
Stalk lodging, %	8.0	12.6	–	–	3.0	3.5
Grain moisture, %	20.8	20.1	18.5	17.3	18.8	18.6
Yield, Mg ha^{-1}	8.8*	7.8	11.1*	9.8	12.1	12.0

* Significant at the 0.05 probability level.

† Data from eight sites in Argentina, nine sites in Brazil, and 64 sites in the United States.

‡ DAP, days after planting.

Table 5. Comparisons of NK603 and conventional controls in 2004 to 2014 agronomic characterization studies of genetically engineered (GE) maize in Argentina, Brazil, Mexico, Pakistan, and the United States. Values are least squares means.

	Argentina†		Brazil		Mexico		Pakistan		United States	
Characteristic	NK603	Control	NK603	Control	NK603	Control	NK603	Control	NK603	Control
Early stand, plants ha^{-1}	84,300	83,000	59,000	61,000	91,900	90,600	92,200	89,300	84,500	84,100
Final stand, plants ha^{-1}	71,800	71,000	56,100	57,100	69,300	69,800	73,000	73,100	71,100	71,400
50% pollen shed, DAP‡	60.7	60.3	58.3	59.2	75.0	75.9	–	–	65.0	64.7
50% silking, DAP	61.3	60.7	56.5	57.5	76.4*	77.7	–	–	64.6*	64.3
Ear height, m	0.83	0.82	1.21	1.27	0.96	0.94	1.05	1.05	1.06	1.04
Plant height, m	1.87	1.85	2.27	2.37	1.98	1.96	2.25	2.27	2.33	2.31
Dropped ears, no. per 100 plants	0.1	0.0	0.0§	0.0	0.1	0.2	0.0§	0.0	0.2	0.3
Root lodging, %	4.5	4.7	0.7	0.0	0.7	0.9	4.8	3.7	1.6	2.9
Stalk lodging, %	11.8	12.6	1.9	2.2	1.2	0.8	4.3	5.6	3.8	3.5
Grain moisture, %	20.7	20.1	17.9	17.3	19.3	19.7	17.6	17.9	18.7	18.6
Yield, Mg ha^{-1}	7.8	7.8	10.5	9.8	9.5	9.5	9.7	9.9	12.0	12.0

* Significant at the 0.05 probability level.

† Data from 11 sites in Argentina, two sites in Brazil, nine sites in Mexico, six sites in Pakistan, and 31 sites in the United States.

‡ DAP, days after planting.

§ Statistical comparisons could not be made because all data were zero.

significant differences by chance when performing large numbers of comparisons at $\alpha = 0.05$.

Those differences that were detected were unlikely to represent increased potential for the GE products to become plant pests. As risks of GE products are assessed in a relative sense, differences that are small in the context of variation already occurring in maize may lack implications for ERA. Differences of 0.3 to 1.3 d in 50% silking and 0.04 m in ear height were far less than the ranges of values observed among the commercial references (Table 7). Differences of ≤3500 plants ha^{-1} in early or final stand were small compared with the ranges of >15,000 plants ha^{-1} observed among references at the MON 89034 × NK603 sites in Brazil (single-site means for each reference; uniform planting rate). These differences were also small in the context of experience with maize plant populations. For example, Fancelli and Dourado Neto (2004) noted a history of high maize yields in Brazil under populations from 55,000 to 72,000 plants ha^{-1} (irrigated) and 45,000 to 55,000 plants ha^{-1} (nonirrigated). Differences in yield were ≤1.3 Mg ha^{-1} and consistently represented greater yield for a GE product containing MON 89034. These differences may reflect the intended insect protection provided by the MON 89034 event. However, regardless of cause, they were less than the range of values among the reference hybrids. Additionally, greater yield is insufficient to cause increased invasiveness or persistence in maize. Although yields have increased substantially over many years of breeding and improved management (Duvick, 2005), maize still lacks the ability to establish self-sustaining populations as a weed or in natural ecosystems (Crawley et al., 2001; OECD, 2003; Raybould et al., 2012)

Thus, in each region tested, agronomic characterization of the GE maize products MON 89034, NK603, and MON 89034 × NK603 resulted in a consistent risk assessment outcome: no evidence of increased potential for the GE products to become plant pests. This outcome, which would be considered during the broader ERA for GE products, was consistent not only across regions but also between the single-event GE products and the breeding stack. These results are aligned with the conclusions reached for the GE products by regulatory agencies of multiple countries. They are also consistent with extensive commercial experience in diverse global regions, which has not resulted in evidence that these GE products are persistent or invasive in agricultural or natural ecosystems.

Comparisons with near-isogenic controls were key to the consistent risk assessment outcomes across diverse testing conditions, including those that were present among the regions. The percentages of variability associated with factors of region, study, and site were high (>25% for each factor for each GE product, Fig. 2). However, both GE products and conventional controls were affected similarly, as reflected in low percentages of variability from event interactions and few significant differences in GE product vs. conventional control comparisons within regions (Tables 4–6).

Although the effect of environment was not examined independently of management practices and genetic background, the consistent results suggest that it did not influence risk assessment outcomes. It is noteworthy that risk assessment outcomes were the same across regions that were diverse in climate, even as defined by the broad classifications of the Köppen–Geiger system (Fig. 1, Table 2). Arid climates (desert or steppe) were represented only in Mexico and Pakistan, occurring at most or all of the sites, respectively. Equatorial climates were represented only in Brazil, occurring at >40% of the sites for each GE product.

Table 6. Comparisons of MON 89034 × NK603 and conventional controls in 2004 to 2011 agronomic characterization studies of genetically engineered (GE) maize in Argentina, Brazil, Mexico, and Pakistan. Values are least squares means.

	Argentina†		Brazil		Mexico		Pakistan	
Characteristic	MON 89034 × NK603	Control	MON 89034 × NK603	Control	MON 89034 × NK603	Control	MON 89034 × NK603	Control
Early stand, plants ha^{-1}	79,500	83,000	57,600*	61,000	85,300	90,600	–	–
Final stand, plants ha^{-1}	69,100	71,000	53,600*	57,100	69,800	69,800	72,100	73,100
50% pollen shed, DAP‡	61.0	60.3	59.3	59.2	74.8	75.9	71.8	71.8
50% silking, DAP	61.3	60.7	57.7	57.5	76.9	77.7	73.6	73.8
Ear height, m	0.83	0.82	1.25	1.27	0.98*	0.94	1.08	1.05
Plant height, m	1.85	1.85	2.31	2.37	1.99	1.96	2.24	2.27
Dropped ears, no. per 100 plants	0.0	0.0	–	–	0.0	0.2	0.0§	0.0
Root lodging, %	6.8	4.7	–	–	1.6	0.9	3.0	3.7
Stalk lodging, %	11.0	12.6	–	–	0.3	0.8	5.6	5.6
Grain moisture, %	20.1	20.1	17.6	17.3	20.0	19.7	17.7	17.9
Yield, Mg ha^{-1}	8.5	7.8	10.0	9.8	10.3*	9.5	10.9*	9.9

* Significant at the 0.05 probability level.

† Data from five sites in Argentina, seven sites in Brazil, nine sites in Mexico, and three sites in Pakistan.

‡ DAP, days after planting.

§ Statistical comparisons could not be made because all data were zero.

Snow climates were represented only in the United States, occurring at >40% of the sites for each GE product tested. Sites with a warm temperate climate were common in Argentina, Brazil, and the United States, but the sizable percentages of sites with differing climate classes in Brazil and the United States imply climatic diversity among the three regions. Consistency across climates is further seen in the minimal interactions of event with study and site despite differences in climate classification that occurred within regions.

Implications for Environmental Risk Assessment

Many ERA frameworks and recommendations incorporate principles of case-by-case determination of data requirements based on risk hypotheses (USEPA, 1998; SCBD, 2000; Raybould, 2006; EFSA, 2011; Wolt et al., 2010). The consistent risk assessment outcomes in the current study support a case-by-case approach to requirements for agronomic characterization in specific environments (including climates) or on breeding stacks.

Table 7. Distributions of single-site arithmetic means for individual commercial reference hybrids in 2004 to 2014 maize agronomic characterization studies in Argentina, Brazil, Mexico, Pakistan, and the United States.

Characteristic	Region	No. of means	Min.	Percentiles 5th	25th	50th	75th	95th	Max.
50 pollen shed, DAP†	Argentina	39	54.0	55.0	59.7	62.0	64.0	71.5	74.0
	Brazil	48	51.5	52.0	54.5	59.6	63.5	71.3	73.8
	Mexico, Oct.–Feb. planting	27	74.8	77.8	82.0	93.0	114.7	118.5	118.5
	Mexico, July planting	15	49.0	49.0	53.3	57.0	60.0	61.3	61.3
	Pakistan	9	70.8	70.8	71.3	71.5	78.0	79.3	79.3
	USA	232	52.3	56.0	61.0	65.5	69.8	76.3	84.5
50 silking, DAP	Argentina	39	54.0	55.3	60.5	62.0	64.3	73.8	74.3
	Brazil	48	49.0	50.0	53.0	57.4	61.9	71.3	73.8
	Mexico, Oct.–Feb. planting	27	76.5	80.0	83.5	95.3	117.0	121.5	122.0
	Mexico, July planting	15	49.3	49.3	53.3	57.5	62.0	63.0	63.0
	Pakistan	9	72.5	72.5	72.5	73.0	79.0	80.3	80.3
	USA	232	52.0	55.5	60.5	64.9	68.8	75.0	83.5
Ear height, m	Argentina	39	0.46	0.46	0.76	0.91	0.98	1.18	1.29
	Brazil	48	0.91	0.94	1.07	1.31	1.43	1.61	1.70
	Mexico	48	0.66	0.68	0.78	1.02	1.21	1.34	1.44
	Pakistan	27	0.92	0.93	1.05	1.13	1.22	1.27	1.27
	USA	239	0.61	0.77	0.97	1.08	1.20	1.37	1.57
Plant height, m	Argentina	39	1.16	1.19	1.55	1.98	2.12	2.57	2.57
	Brazil	48	1.82	1.96	2.18	2.39	2.54	2.72	2.76
	Mexico	48	1.37	1.50	1.82	2.08	2.21	2.53	2.60
	Pakistan	27	1.83	2.01	2.10	2.24	2.42	2.66	2.97
	USA	239	1.60	1.85	2.14	2.33	2.49	2.69	2.97
Dropped ears, no. per 100 plants	Argentina	37	0.0	0.0	0.0	0.0	0.0	0.8	1.5
	Brazil	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mexico	48	0.0	0.0	0.0	0.0	0.2	0.6	1.5
	Pakistan	27	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	USA	232	0.0	0.0	0.0	0.0	0.0	1.1	3.7
Root lodging, %	Argentina	39	0.0	0.0	0.0	1.6	5.1	16.6	36.9
	Brazil	8	0.0	0.0	0.0	0.2	0.7	1.7	1.7
	Mexico	48	0.0	0.0	0.0	0.5	1.5	4.9	15.9
	Pakistan	27	0.0	0.0	0.0	1.9	10.1	24.8	26.0
	USA	236	0.0	0.0	0.0	0.0	0.5	6.3	40.9
Stalk lodging, %	Argentina	39	0.0	0.0	2.1	6.8	19.3	63.9	68.1
	Brazil	8	0.0	0.0	0.0	0.9	4.4	12.6	12.6
	Mexico	48	0.0	0.0	0.0	0.4	1.4	4.6	4.9
	Pakistan	27	0.0	0.0	0.0	1.0	3.3	23.8	31.3
	USA	236	0.0	0.0	0.0	1.0	2.6	11.7	92.9
Yield, Mg ha^{-1}	Argentina	37	3.2	3.9	6.3	7.7	8.3	11.8	12.0
	Brazil	42	7.1	7.6	9.8	11.0	12.0	14.3	15.2
	Mexico	48	5.4	5.7	8.8	9.9	11.6	13.0	13.6
	Pakistan	27	7.1	7.4	9.1	9.8	10.4	11.8	11.9
	USA	229	5.2	6.7	10.7	12.6	14.3	16.6	19.3

† DAP, days after planting.

Factors relevant to these decisions may include the rigor of the event selection process and any risk hypotheses suggested by event characteristics. For example, the MON 89034 and NK603 events were selected for commercialization based on agronomic testing in many environments and extensive assessment of molecular characteristics. These processes are powerful tools in selecting events that are unlikely to have significant unintended effects (Prado et al., 2014; Glenn et al., 2017). Furthermore, the functions of the proteins produced by the MON 89034 and NK603 events do not suggest hypotheses for risks that would be evident via agronomic characterization, regardless of environment or their combination in a breeding stack.

Implementation of the above approach may decrease the time required for regulatory approvals of affected GE products. Earlier availability of GE products may have significant benefits for farmers, consumers, and the environment, as documented by Biden et al. (2018) for countries differing in the timing of GE canola (*Brassica napus* L.) adoption. Fewer agronomic characterization studies could also play a role in reducing the current barriers to commercialization of GE products by smaller organizations (Garcia-Alonso et al., 2014; Conko et al., 2016) and for crops with limited market value (Conko et al., 2016), thereby encouraging needed agricultural innovations.

CONCLUSIONS

Risk assessment outcomes from agronomic characterization of the GE maize products MON 89034, NK603, and MON 89034 × NK603 were consistent across multiple global regions. Likewise, risk assessment outcomes were consistent between the breeding stack and the single-event products. The results support ERA policies that provide for (i) acceptance of agronomic characterization data from other regions (data transportability) and (ii) exemption of breeding stacks from agronomic characterization, based on case-by-case assessments of plausible risks for GE events or event combinations. These policies may benefit farmers, consumers, and the environment by facilitating regulatory approvals of GE crops.

Conflict of Interest

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