American Journal of Experimental Agriculture
3(2): 262-276, 2013

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Genotype by Environment (G x E) Modeling of the Variable Initiation of Parthenocarpy *sensu stricto* in *Musa*: Elucidation of the Environmental Components of Variable Expressivity of Parthenocarpy in a Facultative Apomictic *Musa acuminata* Subspecies Microcarpa Model System

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Authors' contributions

This work was carried out in collaboration between all authors. Author AAS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors PO, GU, BAO, II, AT, MNO, FM and CD manage the literature searches, read through and approved the final manuscript. All authors read and approved the final manuscript.

Research Article

Received 3rd July 2012
Accepted 17th January 2013
Published 9th March 2013

ABSTRACT

To better understand the genome by environment (G x E) interactions that need to be accommodated in order to better predict hybrid performance for a high breeding value

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vegetative parthenocarpy trait sensu stricto. An analysis of the possible environmental signals contributing to the variability of a vegetative parthenocarpy trait sensu stricto via the genome x environment initiation of a genetic lesion that temporally, developmentally and systematically results in abortion of a parthenocarpic developmental regime was performed utilizing Musa acuminata accession Borneo as a model plant. We examined the effect of the variable and potentially modulating environmental signals, and performed a dissection of the genetic components of expressivity and penetrance in the vegetative parthenocarpy in Borneo, utilizing 180 apomictic progeny planted at different developmental ages in duplicate at each of two ecoregional zones. A total of 2,160 floral rachis from 720 mats of Borneo were measured for their subsequent expressivity and penetrance for vegetative parthenocarpy across individual flowers produced from a single vegetative mat, across local duplicate mats, and across ecoregional zones. The results of our study have produced a predictive G x E Model for expressivity of vegetative parthenocarpy in Musa, with validation of this model by a variety of statistical and probabilistic methods. Since expressivity of vegetative parthenocarpy to similar environmental signals have been identified across the monocot to dicot plants such as tomato, the generalized use of models such as presented in our study may have broader applicability to a wider range of crop plants.

Keywords: Expressivity; genotype x environment; model; parthenocarpy; penetrance.

1. INTRODUCTION

Penetrance genetically defines a percentage of individuals with a given genotype who exhibit the phenotype associated with that genotype, while expressivity is a measure of the extent to which a given genotype is expressed at the phenotypic level within an individual [1]. Across many crop species, and understanding of the variable expression of traits due to incomplete penetrance or variable expressivity, is an essential component for both traditional and nontraditional approaches to better fruit production. For example, a *DefH9-iaaM* auxin biosynthesis enzyme transgene has been shown to broadly confer parthenocarpy to tomato and other species [2,3,4,5,6,7,8], however before now, all *DefH9-iaaM* transgenic tomato plants analyzed lack commercial viability, primarily due to the production of a predominance of malformations that occur in their resultant fruit structure. These malformations resemble what is often referred to as the "Pickelhauben" phenotype, and resembles the types of abnormalities that can develop in the nontransgenic fruit that are treated with exogenous auxin [3,9,11,12]. In *DefH9-iaaM* transgenic tomato plants, expressivity of a “Pickelhauben” fruit phenotype may in part be affected by growth temperature fluctuations, suggesting that alterations of auxin effects in relation to cooler temperatures at flowering, and current transgenic approaches are focusing on the alteration of the promoter of a DefH9-*iaaM* transgene toward a down regulated range that can work within a range of expressivity of parthenocarpy versus “Pickelhauben” phenotype and G x E temperature dependence, to effect a balance in fruit production in terms of parthenocarpic seedless fruit and other preferred fruit qualities [3,10]. Several approaches have been developed by researchers, for example a method for producing a lycopersicum plant that is homozygous for at least one of the genes have been developed by backcrossing the F1 generation and further generations for at least two generation with the first lycopersicum plant as recurrent parent [11,12]. In normal fruit development, the initiation of fruit set depends on the successful completion of pollination and fertilization. However, these processes depend on narrow environmental constrains [13]. Good pollen production is permitted by night a temperature ranging between 15 and 21ºC., and air circulation is necessary to ensure pollen shedding. In tomato, failure to

fruit set is therefore a common phenomenon under certain field conditions (high or low humidity combined with low or high temperatures) and in unheated greenhouses or tunnels during winter or early spring cultivation [13]. Parthenocarpic fruit development, which is the growth of the ovary into a seedless fruit in absence of pollination and/or fertilization, offers an opportunity to overcome this problem of poor fruit set under harsh conditions. In tomato three sources of natural parthenocarpy have been widely studied because of their perspectives for practical application to produce seedless fruits [12]. To date, mapped gene for parthenocarpy in tomato is localized on the long arm of Chromosome 3 [11]. The previous invention relates to tomato plants carrying parthenocarpy genes the presence of which may be assessed by their genetic linkage to molecular markers which relates to seed of the plants and to seedless tomatoes obtainable from the plants, as well as to methods for obtaining the plants using the molecular markers linked to parthenocarpy genes for assistance in breeding [14]. Further, they [15,16] have recently developed a set of introgression lines. The present research have now characterized and mapped two novel parthenocarpy genes (one major and one minor) that are responsible for the seedless fruit development. In addition the position of the style exertion locus was also identified. Furthermore two further parthenocarpy genes combined were identified and mapped, using an F_2 segregating population. DNA isolation, for the selection of relevant BC_5S_2 progenies, was performed by a rapid alkaline (NaOH) based extraction method [17]. This method was up-scaled to a 96-well format as described by [18]. Genotypes were determined using PCR-based markers. Primers and enzymes of CAPS and SCAR markers as described by [19, 20]. Other CAPS and SCAR markers were generated based on RFLP and COS marker sequences previously mapped by [21,22]. Several genomic works has been done on tomato [23].

Although a variety of traits have economic impact in *Musa*, all edible cultivar banana and plantain fruit cultivar breeding requires an ability to produce progeny with two essential traits, to be considered: vegetative parthenocarpy and seedlessness [24,25,26,27,28,29,30,31,32]. A variable form of vegetative parthenocarpy *sensu stricto* has been described by [33], which appeared to be produced as a result of a genome x environment initiation of a genetic lesion that temporally, developmentally and systematically results in abortion of a parthenocarpic developmental regime. To better understand the genome by environment (G x E) interactions that might need to be accommodated in order to better predict *Musa* hybrid performance for a high breeding value vegetative parthenocarpy trait, an analysis was performed with a Borneo accession of *Musa acuminata* subspecies Microcarpa exhibiting this variably expressed form of vegetative parthenocarpy *sensu stricto* and the possible environmental signals contributing to the genome by environment (G x E) dependence exhibited by this trait, as identified by [33]. We examined the effect of the variable and potentially modulating environmental signals, and performed a dissection of the genetic components of expressivity and penetrance in this *sensu stricto* defined trait of vegetative parthenocarpy in Borneo, utilizing 180 apomictic progeny planted at different developmental ages in duplicate at each of two ecoregional zones. A total of 2, 160 floral rachis from 720 mats of Borneo were measured for their subsequent expressivity and penetrance for vegetative parthenocarpy across individual flowers produced from a single vegetative mat, across local duplicate mats, and across ecoregional zones.

2. MATERIALS AND METHODS

2.1 Plant Materials and Ecoregional Field Sites

The experiments were performed with 180 *Musa* apomictic progenies vegetatively propagated as described by Vuylsteke et al., 1990a, and established in duplication in a randomized complete block experimental design across two locations in Nigeria as described by [33] an Onne site (Coastal High Rainfall Humid Forest ecoregional zone); and b) an Ibadan site (Inland Derived Savannah Forest Transition ecoregional zone). The plant genotypes grown were produced from apomictic seed and somatically propagated clones of a *Musa acuminata* subspecies Microcarpa accession Borneo described by [33]. For clonal propagation, apomictic seed were produced by pollination barrier bagging, and 384 embryos and plants were recovered in high frequency by embryo culture methods, out of which 180 plants were selected for subsequent vegetative propagation of suckers in a shade house, and were designated as the TMB2x 28383 population of apomicts. A description of Borneo and developmental genetic characterization of the apparent genetic lesion producing the variably expressed form of vegetative parthenocarpy exhibited in Borneo, as it relates to other known forms of parthenocarpic expression in *Musa*, is further described. Somatic propagation of individuals of TMB2x 28383 apomictic population was performed by growing plants in the shade house, and random desuckering of suckers from mother plant mats, producing daughter sucker plants of different developmental ages. Each sucker planted in a 6 square meter planting area. When suckers of a clone failed to be transplanted, they were replaced with clones of same TMB2x 28383 designate that were maintained in reservoir in either field or shade house.

2.2 Data Collection and Analyses

From each mat, 3 successively rachis of flowers were analyzed, for a total of 2,160 rachis of flowers and bunches of fingers of fruit, produced from 720 clonal mats of *Musa* plants. To verify an expression of parthenocarpy, all flowers upon emergence were bagged with pollination barrier bags, to prevent pollination, such that only fruit development in the absence of fertilization would be scored. The phenotyping assay for the developmental genetic characterization of the variably expressed form of vegetative parthenocarpy exhibited by Borneo, is further described in Okoro et.al. [33]. Measurement of penetrance was done by presence or absence of parthenocarpic expression of an individual after 3 flowering cycles. Measurement of expressivity was performed by the number of fingers in a bunch that persisted and developed as fruits. When non parthenocarpic expression occurred in ovaries, such ovaries typically dried out and dehisced from the rachis. Data analyses for determining G x E interactions involved in the variable expressivity of vegetative parthenocarpy (VPE) was performed with a variety of ANOVA models, with statistical computations and estimation for correlation and regression analysis pertinent to G x E interactions, carried out using the SAS Computer Package, utilizing both standard and stepwise regression procedures [34,35]. Trait variables were encoded and scored as follows: VPP= vegetative parthenocarpy % penetrance as the number of individual TMB 28383 population individuals which expressed at least two parthenocarpic fingers in one of their clonal mats distributed across locations. VPE= vegetative parthenocarpy expressivity in parthenocarpic hands/bunch; and DTRE= days to rachis and inflorescence emergence from a planting date of a TMB 28383 individual sucker. For environmental variables, a (0) encoding designation indicates a measured variable on the date of a rachis inflorescence's emergence from a pseudostem of a mat, and a (-10) designation represents the same value

measures at ten days prior to emergence. International Institute of Tropical Agriculture (IITA), maintained weather stations built and maintained at the Ibadan and Onne sites, provided the specific daily environmental data that was collected for data analyses. Environmental variables comprised daily measurements, with the exception of DL= daylength, which constitutes a daylength average for the week of rachis and flower emergence. Data variables were extracted from weather station data as follows: Rnf= rainfall in millimeters; Evap= pan evaporation in millimeters: Mintemp= minimum temperature in degrees Celsius; Maxtemp= maximum temperature in degrees Celsius; Minrelh= minimum relative humidity measured in atmospheric % water saturation at ground level; and Maxrelh= maximum relative humidity measured in % atmospheric water saturation at ground level.

2.3 Theory

2.3.1 G X E model data collection experimental design

Tests of significance of components of two to multi-location trials were used for estimating relative contribution of the various components to observed variation [34, 35]. The design of the experiment included the use of a daylength neutral plant, which was accommodated by the *Musa* accession utilized. Randomization to provide variation in flowering dates at different times of the year and under different environmental requirements, with a sufficient sampling size are requirements of the G x E modeling, as well as continuous collection of environmental data, by locally established commercially purchased weather stations.

2.3.2 The development of a G x E model for expressivity of vegetative parthenocarpy *sensu stricto* in *Musa*

Across all of TMB2x 28383 population plants at all sites, variation assessment was performed for all measured environmental and trait data (Table 1). These results as shown in Table 1 indicated that a wide range of environmental conditions were collected during those daily periods and dates at which flower emergence, and are evidence of validation of the precept in our experimental design, that the developmental age of the suckers planted as well as the locations utilized, would provide substantive variation in flowering dates and environmental conditions.

Utilizing procedures described in part by Hussein et al. [34], and SAS [35], we analyzed for trends in the variability of VPE as a dependent variable, with all available environmental and phenotypic data variables as independent variables. Our analysis included the use of both standard and stepwise regression procedures. From a regression analysis of all variable data, all significantly identified output variables were specifically reanalyzed with a “stepwise regression analysis" which functioned to eliminate the least significant variables one at a time to lead to output of those variables which provide the greatest probability of contribution within a specific model. In summary, the results of stepwise regression analysis produced essentially the same outputs and probabilities for the Minrelh (-10), and Mintemp (0) environmental variables, however an initial Minrelh (0) environmental variable was output as a less significant variable, in relation to the VPE variable under the initial regression analysis; but was output with greater significance and a greater probability, as an independent variable contributor to a dependent VPE variable.

The results of the stepwise regression, whose outputs are shown in Table 2, indicate that two environmental variables (Minrelh and Mintemp) and one developmental phenotypic variable (DTRE) comprising temporal components could be identified with predictive

significance. Results of stepwise regression also indicated that small incremental changes in the downward temperature trend of a Mintemp (0) variable correlated to greater increases in VPE (parameter estimate of –1.524), in comparison to increases in VPE correlated to increases in % relative humidity that were drawn to each of the Minrelh (-10) or Minrelh (0) variables (parameter estimates of 0.0758 and 0.0974 respectively). Further examination of the weather data indicated that the daily minimum temperature measurements always occurred at night, while the min relative humidity measurements had less temporal daily certainty, fluctuating perhaps by impact of weather patterns of rainfall (day or night) as well as relative humidity.

The output of this data from stepwise regression analysis, in its most logical interpretation, led to an initial design of a predictive model for an increased expressivity of vegetative parthenocarpy, based on precepts that the following variables contributed to an increased expressivity of vegetative parthenocarpy, if they exhibited the following characteristics: 1) a minimum temperature on the date of flowering trending toward a lower temperature range (i.e. Cooler nights); and 2) a determinative persistent relative humidity trend occurring for a period extending from 10 days prior to flowering until the flowering, exhibiting a higher minimum value (ie. The overall relative humidity is persistently higher). Because of the temporal relationship of the environmental variables themselves, specifically that of environmental variables Mintemp and Minrelh measured at the DTRE date (0), or ten days prior in relation (-10), the DTRE output in Table 2 can be assessed as simply a validation of the experimental design precept that signaling events occurring at (time 0) or around (time –10) would most likely impact the variable expressivity of vegetative parthenocarpy (VPE).

Table 1. Trait and environmental variable r-square, mean, and coefficient of variation values, with an estimation of the various levels of significance (sig.) across all mats of the TMB 28383 population, and across locations. Environmental and trait variable encodings are as described in materials and methods

Source of variation	R^2	Means	Coeff. of variation	Sig. across TMB 28383	Sig. across location
Vegetative Parthenocarpy Expressivity (VPE)	0.234	7.372	256.57	0.0062	0.0001
Rnf (-10)	0.219	3.302	257.17	0.0013	0.0001
Evap (-10)	0.264	3.270	34.70	0.0001	0.0001
Mintemp (-10)	0.322	22.026	6.35	0.0001	0.0001
Maxtemp (-10)	0.435	31.300	6.05	0.0001	0.0001
Minrelh (-10)	0.384	49.681	28.22	0.0001	0.0001
Maxrelh1 (-10)	0.495	94.930	2.50	0.0001	0.0001
DL	0.680	11.925	1.66	0.0005	0.0001
Rnf (0)	0.199	3.100	262.97	0.0001	0.0001
Evap (0)	0.283	3.389	34.44	0.0007	0.0001
Mintemp (0)	0.360	22.103	6.12	0.0001	0.0001
Maxtemp (0)	0.345	31.097	7.52	0.0001	0.0001
Minrelh (0)	0.375	50.298	26.53	0.0001	0.0001
Maxrelh (0)	0.524	95.062	2.16	0.0001	0.0001
DTRE	0.615	222.150	22.75	0.0001	0.0001

Table 2. Step-wise regression outputs of significant variable trends of high probability (Pr) for predictability for a trend of an increased expressivity of vegetative parthenocarpy

Variable	DF	Parameter estimate	Standard error	t-value	Pr > |t|
Intercept	1	22.118	9.125	2.42	0.0155
Minrelh (-10)	1	0.0758	0.0368	2.06	0.0398
Mintemp (0)	1	-1.524	0.374	-4.08	<0.0001
Minrelh (0)	1	0.0973	0.0371	2.62	0.0089
Dfl	1	0.0496	0.010	4.94	<0.0001

3. RESULTS AND DISCUSSION

3.1 Variable Expression of Vegetative Parthenocarpy in Borneo Is Due To Variable Expressivity Rather Than Incomplete Penetrance

All clonal reps of each of the TMB 28383 individuals, across both locations were analyzed for individual expression of at least one parthenocarpic fruit, to determine the penetrance of vegetative parthenocarpy, over the course of emergence of three successive floral rachis, from independent pseudostems of the same mat. As describe by Okoro et al. [33], Borneo is monecious and facultative apomictic, with the female flowers of its rachis are developmentally isolated from the male flowers; and as such is an outcrossing genotype. Furthermore, Borneo exhibits a form of variable expression of parthenocarpy *sensu stricto*, given its facultative apomictic biology. Fig. 1. Illustrates some of the typical results in parthenocarpic expression observed from assays of the TMB2x 28383 population and its clonal replicates. For example in different inflorescences produced from the same clonal mat at diff times of year in Ibadan, as shown for the TMB 28383-34 plant in Fig. 1 A and C, the expression of parthenocarpy *sensu stricto* can appear to be completely or incompletely penetrant. However, all clonal plants of individual apomicts of the population, when examined in summary, often exhibited an intermediate form parthenocarpic *sensu stricto* expression as shown in Fig. 1B. Results from the scoring of every clone of each original individual 180 apomictic members of TMB2x 28383 population, indicate that the expression of the penetrance of vegetative parthenocarpy (VPP) was 100% across all individual TMB 28383 individuals, because at least one clone of each of the TMB28383 population exhibited vegetative parthenocarpy. These results indicate that the variation of expression of vegetative parthenocarpy was not due to incomplete penetrance (VPP), because each individual could be scored as comprising the trait; but rather that the variation in parthenocarpic expression was the result of the variable expressivity of vegetative parthenocarpy (VPE) [1].

3.2 Validation and Predictiveness Testing of a G X E Model for the Variable Expressivity of Vegetative Parthenocarpy in *Musa*

Additional analyses were performed to examine the predictiveness and validation of this model. We analyzed performance and predictiveness of this model in relation to expression at locations and to expression after successive rachis generation from a clonal mat. Since the coastal and inland zone sites were well known to comprise different temperature and humidity ranging zones, as well as radically different soil types, an analysis of variability was performed across locations, to examine if the outputs across and within ecoregional zones were consistent with the outputs of Table 2. The results of an analysis of variability across

locations, supported evidence to indicate a high reliability and predictiveness of the developed G x E model for VPE. All of the environmental and trait variables examined exhibited significant differences across both ecoregional zone locations (Table 3). Utilizing the G x E model of VPE, the VPE LSMeans measurements identified in Table 3, include values that would be predicted by the model, and as such, a variety of variables including the radically different soil types at the two locations, could be excluded as significant variables in our G x E dependent modeling of VPE to a major extent.

Table 3. The Standard Error (S.E.) associated with the Least Square Means (LSMeans) for each of the Inland (Ibadan) and Coastal (Onne) ecoregional site locations for environmental and trait variables.

Variables	Inland Ibadan site		Coastal onne site	
	LSMeans	S.E.	LSMeans	S.E.
VPE	13.97016	0.861655	2.999064	0.905333
Rnf (-10)	5.789579	0.325913	1.246274	0.367556
Evap (-10)	3.017004	0.044167	3.539868	0.049386
Mintemp (-10)	21.58121	0.053692	22.41001	0.060553
Maxtemp (-10)	30.12703	0.072711	32.3804	0.082001
Minrelh (-10)	56.53732	0.53839	42.89453	0.606844
Maxrelh (-10)	96.80869	0.090958	93.26445	0.10258
DL	12.14356	0.007583	11.66348	0.008552
Rnf (0)	4.055292	0.312976	2.173531	0.354044
Evap (0)	2.95302	0.045646	3.819101	0.050938
Mintemp (0)	21.5026	0.051938	22.63872	0.058754
Maxtemp (0)	30.06936	0.0898	32.03955	0.101583
Minrelh (0)	56.69222	0.512954	44.07873	0.579808
Maxrelh (0)	96.83294	0.07875	93.46837	0.089084
DTRE	276.9113	1.941886	175.0175	2.187684

For example, as shown in Table 3, the overall Least Square Means (LSMeans) of the expressivity of the parthenocarpy was approximately 14 (i.e. 13.97016) fingers at the inland Ibadan site, while the LSMeans expressivity was significantly different at appr. 3 (i.e. 2.999064) fingers at the coastal Onne site. The LSMeans of Mintemp (0) at the Ibadan site was 21.5 (i.e. 21.5026) degrees Celsius (C) and the LSMeans of Mintemp (0) at the Onne site was 22.6 (i.e. 22.63872) C, suggesting that an increase in expression to appr. 14 parthenocarpic fingers from appr. 3 fingers could be predicted in part by a moderating drop in night time temperature of appr. 1.1 degree Celsius under certain conditions of relative humidity, with such combined environmental signals more likely to occur at an inland temperature fluctuating Ibadan location, than a temperature moderating coastal Onne location. This small increment of 1.1 degrees was thus statistically significant and highly predictive for VPE, consistent with the comparatively large negative Mintemp (0) parameter value associated with the analysis of all samples, as indicated in Table 2. Furthermore, both Minrelh (-10) and Minrelh (0) variables exhibited relatively low but positive parameter values compared to Mintemp (0) with the total sample size, as shown in Table 2, with parameter values of Minrelh (-10) exhibiting a lower correlated change to VPE compared to Minrelh (0) (values of 0.758 compared to 0.0973). This suggested that a persistence in a lower value of relative humidity, with a signal from this variable increasing in genetically determinative impact of expressivity, as rachis emergence approached (over a 10 day period). These results also indicated, that to perform more detailed model validation, analysis of Minrelh (0) and Maxrelh (0) as well Minrelh (-10) and Maxrelh (-10) needed to be examined across

locations. As Shown in Table 3, the Minrelh (-10) and Minrelh (0) LSMeans were not statistically significantly different from each other, within each of the two locations. The Maxrelh (-10) and Maxrelh (0) LSMeans measurements for Ibadan were ~97% relative humidity (i.e. 96.80869 and 96.83294 % respectively), and for Onne were ~93 % relative humidity (i.e. 93.26445 and 93.46837 % respectively). The Maxrelh variables failed to output as significant independent variables contributing to a trend of a dependent VPE, although the Minrelh variable output as such (Table 2). While these variables indicated daily periods of very high water saturation of the air at ground level, reflective of the tropical environs, these high values did not appear to be associated with rainy versus dry seasons, since the rainfall variables themselves failed to exhibit any statistically significant correlative trend with a dependent VPE variable (the Rnf variable of Table 3). Furthermore, the measurements at the Ibadan site were 56.53732 [S.E. 0.53839] and 56.69222 [S.E. 0.512954] % relative humidity, and at Onne site for 42.89453 [S.E. 0.606844] % and 44.07873 [0.579808] % relative humidity for Minrelh (-10) and Minrelh (0) LSMeans variables respectively. Thus, a G x E Model for VPE that a higher persistent relative humidity provided by a higher persistent minimum relative humidity value would produce a dependent increase in expressivity of parthenocarpy developed for all data was predictive within these different locations regardless of their differences in soil types and other ecologies, the expectation would be that under a Minrelh LSMeans of 43-44 % minimum relative humidity at Onne ten days prior and at rachis emergence, one would predict a lower VPE LSMeans value in relation to the same rachis emerging at the same developmental time periods, but under a 56.5-56.7 % relative humidity. Furthermore, for the 43-44% LSMeans of Minrelh at the Onne site, and the 56.5-56.7 % LSMeans of Minrelh at the Ibadan site, the VPE LSMeans is indeed relatively higher at the Ibadan versus Onne sites at a significant difference of 14 to 3 LSMeans parthenocarpic fingers respectively, providing evidence of validation of the proposed G x E model for VPE, across locations.

For further G x E Model validation testing, a closer examination of the DTRE variable was performed, to determine if the model derived from the total dataset was consistent in VPE prediction across the three successive flowers produced across a mat, regardless of flowering location. As shown in Table 4, the LSMeans number of days after planting of the mother plant that gave rise to each successive rachis and inflorescence from that mother plant derived mat were: 196.6357 days for the first rachis (rachis 1) from the mother; 236.6203 days for the second rachis (rachis 2) from the most apically dominant sucker of the mother mat; and 244.6372 days for the third rachis (rachis 3) of the second most apically dominant sucker from the mother mat. For rachis 1, rachis 2, and rachis 3 samplings, the LSMeans Mintemp (0) were 22.2036, 21.8528, and 21.93044 degrees Celsius, with comparisons of rachis combinations 1 to 2 and 1 to 3 exhibiting significant differences, but not rachis 2 to 3 combinations. The LSMeans measurements of the expressability of parthenocarpy in bunches on rachises 1, 2, and 3 were 5.872002, 9.147032, and 10.4348 fingers respectively, consistent with a trend toward a downward Mintemp (0) value correlating at high probability to an increased expressability of parthenocarpy, as entailed in our G x E model. Furthermore, although the LSMeans Minrelh (-10) measurements were insignificantly different at measurements of 50.336, 50.67061, and 50.14982 % relative humidity, as shown in Table 3, a between location LSMeans Minrelh (-10) value range of 56 % downward to 42 % relative humidity would be predicted by our G x E model to produce a trend of a decrease in expressivity of parthenocarpy from appr. 14 to 3 fingers respectively. The LSMeans calculated values of the variable expressivity of parthenocarpy consisted of values between 10 to 6 fingers, associated with an LSMeans Minrelh (-10) value of appr. 50 degrees Celsius, was thus consistent with a variable expressivity model in which a higher daily relative humidity before flower emergence and a minimum temperature at night during

flower emergence contributes to an increased expressivity of parthenocarpy, providing further supporting evidence of prediction performance of the G x E Model for VPE.

3.3 In What Context Might this Type of G X E Model Be Used, and How Broadly Applicable is it?

The objective of this study was to better understand the environmental components comprising a set of the G x E interactions that need to be accommodated in order to better predict hybrid performance of vegetative parthenocarpy using Musa as a model plant. The stature of *Musa* spp. as a gigantic herbs, typically grown in a 6 square planting area over the course of up to a 3 year planting and multiple flowering evaluation cycle make it simply difficult to manage for growth chamber based experimental cycles [27, 28, 32]. That might otherwise be possible for in other fruit crops that might be utilized for genetic models of gene expression of parthenocarpy, such as tomato [36, 37, 38, 3, 4, 5, 6, 7, 10, 8]. The accession Borneo is a *Musa acuminata* subspecies Microcarpa classified plant, with a low degree of apical dominance and a high production of suckers from a mat, by comparison to many *Musa* germplasm utilized in cultivation and breeding. This relatively low apical dominance and high vegetative sucker production from an initial mother corm, inherently produces an acceleration of the flowering of such plants, within the first two years, that is often not observed in other *Musa* cultivars and accessions. This temporal developmental production of inflorescences from a single mat of Borneo, is in part represented in Table 4, which shows that across all zones and plantings that: the first inflorescence emerged with an LSMeans of appr. 237 days after planting; the second with an LSMeans 40 days after the first inflorescence; and the third with an LSMeans appr. 8 days after the second inflorescence. The reliability of the model presented in this study, thus might to some extent be less predictive in genotypes that exhibit greater apical dominance, and further study in this regard is suggested.

Table 4. The Least Square Means (LSMeans) and Standard Error (S.E.) of environmental and trait variables for the first three successive rachises that Emerged from independent pseudostems produced from the same mat

Variables	Levels of	*Rachis 1*		*Rachis 2*		*Rachis 3*	
	probability	*LSMeans*	S.E.	*LSMeans*	S.E.	LSMeans	S.E.
VPE	0.0016	5.872002	0.864934	9.147032	1.032758	10.4348	1.1538276
Rnf (-10)	0.0162	2.756736	0.368964	3.41835	0.404041	4.378694	0.44005821
Evap (-10)	0.749	3.308128	0.049873	3.255206	0.054408	3.271975	0.05937374
Mintemp (-10)	0.0001	22.2036	0.060785	21.8528	0.066563	21.93044	0.0724969
Maxtemp (-10)	0.0001	31.53144	0.082315	31.02413	0.090141	31.20557	0.0981763
Minrelh (-10)	0.187	48.92819	0.609131	50.53167	0.667444	49.68792	0.7264484
Maxrelh (-10)	0.0001	94.27248	0.102973	95.31432	0.112762	95.52291	0.1228143
DL	0.0001	11.99221	0.008585	11.88085	0.009401	11.83749	0.0102392
Rnf (0)	0.6093	2.909014	0.354763	3.004127	0.388718	3.430094	0.4239236
Evap (0)	0.0693	3.475836	0.051387	3.307043	0.056222	3.375301	0.061264
Mintemp (0)	0.0152	22.19852	0.058873	21.9548	0.064508	22.05866	0.0703501
Maxtemp (0)	0.1285	31.20801	0.10179	30.91296	0.111532	31.04239	0.1216335
Minrelh (0)	0.8414	50.336	0.580888	50.67061	0.637832	50.14982	0.6940993
Maxrelh (0)	0.0001	94.5198	0.089265	95.42102	0.097808	95.51114	0.1066667
DTRE	0.0001	196.6357	2.196041	236.6203	2.404706	244.6372	2.62234

Data presented was derived from data from all duplicates and mats at both planting locations of Ibadan and Onne.

The results of this study reliably demonstrate that in the accession Borneo of *Musa acuminata* subspecies Microcarpa, plasticity in expression of a trait of vegetative parthenocarpy is not due to incomplete penetrance, but rather due to a G x E interactions resulting in variable expressivity. Our results also provide strong evidence to demonstrate that the most probable G x E model is one by which the environmental signals of a persistently higher daily relative humidity ten days before flower emergence, and a cooler night time minimum temperature at flower emergence function as environmental signals that produce a trend toward an increase in expressivity of vegetative parthenocarpy in Borneo. In terms of how broadly applicable this G x E model might be to other plants, or in general to a view of variable expressivity of a trait, is difficult to ascertain, but the methodology in developing such a model can be considered as extensible. In terms of parthenocarpic expression in plants in general, an effect on variable expressivity of parthenocarpy by external signals is not unique to Musa, and is typically found much less across the range of plants to dicots. However, in tomato, much as shown for *Musa* in this study, parthenocarpic fruit production in certain mutant PAT and wild type tomato cultivar genotypes occurs not by incomplete penetrance, but by variable expressivity in which increased expression of parthenocarpy is correlated to cooler temperatures of the plants at flowering [38, 10], and this methodology should be extensible at minimum to a crop such as tomato. Given the concurrence of cooler temperatures at flowering as common signals for tomato and Musa, a general statement that a temperature-modulating signal for a variable expressivity of parthenocarpy occurs across both monocots and dicots is a reasonable interpretation.

3.4 What other Traits Might Best be Analyzed with G X E Models of the Type as Presented in this Study?

Besides parthenocarpy, a variety of other plant crop traits of high economic value, exhibit G x E dependence in plasticity of expression, that can heavily impact a breeding process, or result in negative or positive heterotic trait expression in developed cultivars or landraces that might are translocated into new ecoregional planting zones [39, 40, 41, 42, 43, 44, 45]. For example in *Musa*, other traits that affect yield stability, disease resistance, ovule and pollen fertility, as well as embryo and seed production, often appear to exhibit G x E dependent variable expressivity [39, 46, 47, 48, 29, 27, 30, 31]. While often observed, this variable expression is often not identified as correlating to any specific set of environmental conditions. To date, only observations of correlations between environmental conditions of relative humidity to alterations in pollen viability, or to the degree of expression of meiotic restitution mechanisms that result in pollen ploidy have been reported for *Musa* [48].

Is there any other potential utility for a model of this type? To some extent, the ability to predict the temporal component of genetic determination of a trait that is the result of temporal concerted gene action, may have a very useful utility in gene discovery for some important genes expressed genetically downstream of environmental signals and their putative receptor components. For example, if the production of certain gene transcripts of Borneo at flowering are determinative, then to a certain extent, we can predict that the gene expression profile of plants with an increased determination for expressivity of parthenocarpy would have different gene expression profiles that could provide for gene discovery. With a model plant such as that of Borneo, if flowering occurs with a monitored 21 Celsius night time temperature and persistent relative humidity of 50%, then gene expression in flowers can be expected to have a high genetic determination of parthenocarpy, and a concurrent up or down regulation of transcription of genes accompanying such predicted genetic determination. Such an approach has been utilized extensively with a variety of chemical and other stress-induced paradigms, but rarely has been utilized with temporal

developmental genetically determinative paradigms, because a means of measure of genetic determination are often not available. The methodology presented here, could thus easily be extended across a variety of crop species and traits, for example with parthenocarpy in tomato, once a predictive G x E model for expressivity is reliably validated.

Variable expressivity of parthenocarpy in the form of complete expression of parthenocarpy (Fig. 1C) and complete lack of expression of parthenocarpy (Fig. 1A) are shown for different flowers of the same mat produced at different times of the year at an Ibadan site location, for plant 28383-26. Variable expressivity of parthenocarpy within the same rachis of a 28383-74 plant at an Ibadan site location is shown in Fig. 1B.

Fig. 1. Rachises and inflorescences demonstrating typical types of results from a phenotypic external pollination barrier assay for the variable expressivity of vegetative parthenocarpy sensu stricto in *Musa acuminata* subspecies

The Vegetative Parthenocarpy Expressivity (VPE) Variable trend is utilized as the dependent variable trend. Parameter estimates and t-value calculations are indicative of upward or downward trends. The main fact in this study is that “a higher daily relative humidity before flower emergence and minimum temperature at night during flower emergence contributes to an increased expressivity of parthenocarpy” in the species in the investigation. As earlier mentioned, parthenocarpy obtained via traditional genetics often shows a variable expressivity of the trait (e.g. some tomato parthenocarpic mutants). Temperature is certainly one of the major environmental factors affecting the expressivity of parthenocarpy.

Population and replicate sizes were identical across locations, comprising 360 clonal plant mats at each site over 3 flowering cycles, for a total sample size of 1080 rachises of flowers analyzed at each location. Environmental and variable trait encoding is as described in Materials and Methods.

4. CONCLUSION

The results of our study include the development of a predictive G X E model for the expressivity of vegetative parthenocarpy in *musa*, and validation of this model by a variety of statistical and probabilistic methodological methods. the results of this study also indicate, that while the apparent penetrance of vegetative parthenocarpic expression produced from a mat, or within any one individual plant provided an appearance of incomplete penetrance of parthenocarpy, that by increasing sample sizes and to duplicates across ecoregional zones demonstrated that the penetrance of vegetative parthenocarpy at the level of clonal individuals was in actuality complete, and that the variability in the trait expression observed was not the result of incomplete penetrance phenomenon, but rather the result of this variable expressivity phenomenon which could be modeled and reliably predicted, by the use G x E modeled trends that exhibited statistical and probabilistic dependence upon trends

of specific environmental variables. The study suggests that the reported findings might assist the discovery of novel parthenocarpic genes. Clearly transcript profiling has been used to identify novel parthenocarpic genes, and perhaps the information provided in this study might be helpful, but however, other approaches have been used in other species.

ACKNOWLEDGEMENTS

The authors wish to thank IITA for providing research environment and the tools for carrying out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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