

Research Article

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Genotype X Environment Interaction for Yield of Pickling Cucumber in 24 U.S. Environments

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Abstract: Reliable yield performance is important in cucumber because seed companies prefer to market cultivars adapted to multiple rather than single regions of the U.S. Also, growers benefit by using a cultivar that performs well in many environments. Future performance of cultivars is also important. The objectives of the study were to (i) evaluate the yield of cucumber genotypes over successive years and in different locations, and (ii) identify cucumber genotypes with high stability for yield. A diverse set of 22 pickling genotypes was evaluated over 3 years (1986, 1987 and 1988) and in 7 locations across the United States. Yield traits were evaluated using once-over harvest and counting the number of fruit that were marketable, culled or oversize. Total yield, marketable yield (total minus culled fruit), early yield (number of oversize fruit), percent culls and fruit per plant were calculated. Data were analyzed with SASGxE and RGxE programs using SAS and R programming languages, respectively. There were strong effects of environment(E) as well as genotype(G) xE interaction for all traits. Genotypes ‘Regal F1’, ‘Calypso F1’, ‘Carolina F1’, ‘Gy 3’, ‘Gy 14’ and ‘Fremont F1’ had high

marketable yield and medium to high stability for all traits. There was an advantage of hybrids over inbreds for trait performance. Hybrids fell into a single cluster with large prediction intervals. Based on the stability statistics and divisive clusters, it appears possible to breed stable cucumber genotypes with high yield. The genotype with highest performance for marketable yield, greatest stability for yield, lowest 1-R² ratio value (diverse and representative) were ‘Marbel F1’ and Gy 14.

Keywords: *Cucumis sativus*, single-harvest trials, variety testing, vegetable breeding

Abbreviations

AEC	= Average environment coordinate
ANOVA	= Analysis of variance
BLUP	= Best linear unbiased predictor
FL	= Leesburg, FL
GGE	= Genotype main effects plus genotypic x environment interaction effect
GGL	= Genotype main effects plus genotypic x location interaction effect
GxE	= Genotype x environment interaction
GxY	= Genotype x year interaction
GxL	= Genotype x location interaction
G	= Genotype
G01	= Addis
G02	= Calypso F1
G03	= Carolina F1
G04	= Castlepik F1
G05	= Chipper
G06	= Clinton
G07	= Colet F1
G08	= Earlipik 14 F1
G09	= Fremont F1
G10	= Gy 14
G11	= Gy 3
G12	= Ark. Littleleaf

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G13	= M 21
G14	= Marbel F1
G15	= Pioneer F1
G16	= Regal F1
G17	= Wis. SMR 18
G18	= Sumter
G19	= WI 1983G
G20	= WI 2757
G21	= WI 5096
G22	= Wautoma
HYHS	= High yield and high stable
HYLS	= High yield and low stable
L	= Location
LYHS	= Low yield and high stable
LYLS	= Low yield and low stable
MET	= Multi-environment trial
MI	= East Lansing, MI
MYHS	= Medium yield and high stable
MYLS	= Medium yield and low stable
NC	= Clinton, NC
OH	= Napoleon, OH
OK	= Bixby, OK
OR	= Brooks, OR
PC	= Principal component
PI	= Prediction interval at 95%
RGxE	= R language program for the analysis of genotype stability and location value
REML	= Restricted maximum likelihood
SASGxE	= SAS program for the analysis of genotype stability and location value
SVP	= Singular value partitioning
WI	= Hancock, WI
Y	= Year

1 Introduction

Cucumber (*Cucumis sativus* L.) is one of the 22 major vegetable crops grown in the United States. Plant breeders involved in improving yield of cucumbers are interested in understanding genotype x environment interaction (GxE), and the measurement of yield stability. Genotype x environment interaction occurs when there is a scale shift or rank shift in genotype performance across environments. Genotypes respond differently to environmental factors such as soil fertility or the presence of disease pathogens (Dia 2005; Dia et al. 2009; Weindorf et al. 2008a; Weindorf et al. 2008b; Board and Kahlon 2011; Board and Kahlon 2012; Board and Kahlon, 2013; Kahlon 2010; Kahlon et al. 2011; Kahlon et al. 2012). If there is significant GxE, then it

is useful to measure both performance and stability for genotypes being evaluated in breeding programs (Magari and Kang 1993; Ebdon and Gauch 2002). GxE may result in low correlation between phenotypic and genotypic values, thereby reducing progress from selection. This leads to bias in the estimation of heritability and in the prediction of genetic advance (Comstock and Moll 1963; Alghamdi 2004). Therefore, the magnitude and nature of GxE determines the features of a selection and testing program.

Several statistical methods for evaluating stability have been proposed, reflecting different aspects of GxE. These include univariate models, such as regression slope, deviation from regression, environmental variance, and Kang's yield-stability, and multivariate models, such as genotype main effect plus genotype by environment interaction (GGE) biplot (Finlay and Wilkinson 1963; Eberhart and Russell 1966; Yan 2001; Kang 1993; Yan and Kang 2003).

Analysis of variance (ANOVA) can be used to identify the existence of GxE in multiple-environment trials. ANOVA measures the components of variance arising from different fixed and random factors (for example, genotype, location, year, and replication) and their interactions. However, ANOVA has limitations, including the assumption of homogeneity of variance among environments, in its ability to explore the response of genotypes for GxE (Zobel et al. 1998).

The most widely used approach for stability analysis is based on linear regression: the slope (bi) or deviation from regression ($S2d$) of genotype performance relative to an environmental index derived from the average performance of all genotypes in each environment (Finlay and Wilkinson 1963; Eberhart and Russell 1966; Freeman 1973; Chakroun et al. 1990). Some researchers have found deficiencies in the regression method for evaluation of GxE patterns (Zobel et al. 1988; Nachit et al. 1992; Annicchiarico 1997; Kandus et al. 2010; Vita et al. 2010). The deficiencies are of four types. First, the estimates of best fitted line have high error when only a few low- and high-yielding locations are included in the study (Crossa et al. 1990). Second, the average of all genotypes evaluated in each environment (environmental index) is not independent of each genotype for that environment (Freeman and Perkins 1971). Third, the errors associated with the slopes of genotypes are not statistically independent (Kandus et al. 2010). Fourth, there is a required assumption of a linear relationship between interaction and environmental means when the actual responses of the genotypes to the environments are intrinsically multivariate (Crossa et al., 1990).

Shukla (1972) proposed an unbiased estimate of the variance (σ^2) of GxE plus an error term associated with genotype, in which a genotype with low σ^2 is regarded as stable. Kang's stability statistic (YS_i) is nonparametric, using both trait mean (M) and σ^2 , with equal weight on each. Genotypes with YS_i greater than the mean YS_i are stable (Kang 1993; Mekbib 2003; Fan et al. 2007).

Multivariate analysis includes the genotype main effects plus genotypic x environment interaction effect (GGE) method with a graphical display (Casanoves et al. 2005; Dehghani et al. 2006). GGE biplot is based on principal component (PC) analysis and has the ability to reveal structure in the data. It is constructed from the first two principal components (PC1 and PC2) that explain maximum variability in the data, derived by singular value decomposition of a two-way (genotype-by-environment) data matrix (Yan et al. 2000). Recently, hierarchical Bayesian and mixed models were introduced to model heterogeneous variance among environments and different correlation structures among environments (Jarquín et al. 2016; Jat et al. 2016; Li et al. 2010; Malosetti et al. 2004; Mathews et al. 2008; Mohan et al. 2017). Mixed models allow more flexibility to model unbalanced data using restricted maximum likelihood estimates (REML). Each statistical method reflects different aspects of the GxE, and no single method adequately explains genotype performance across environments (Dia et al. 2016a). Stability statistics are best used in combination with trait performance (mean or BLUP: Best Linear Unbiased Predictor is an estimate of random effect) and have successfully been used in plant breeding.

The contribution of GxE on yield performance of field and row crops has been widely reported (Bednarz et al. 2000; Mekbib 2003; Riday and Brummer 2006; Fan et al. 2007; Mulema et al. 2008; Miranda et al. 2009; Vitta et al. 2010; Panthee et al. 2012; Dia et al. 2012a; Dia et al. 2012b; Dia et al. 2012c; Kumar et al. 2013). Cucumber yield is often measured in the final stage of cultivar testing using the value of the fruit obtained from multiple harvests after grading them by diameter, and removing culls (nubbins and crooked). However, yield can be measured more efficiently in the early stages of trialing by counting the number of fruit from a single harvest of small (6.4 to 10.3 m²) unbordered plots (Wehner 1989). Therefore, in this study we estimated GxE on cucumber yield components measured in thousand fruit per hectare (1000 ha⁻¹). The objectives of this study were to (i) evaluate the GxE of cucumber genotypes, (ii) predict yield performance and estimate dissimilarities among cucumber genotypes based on trait performance, and (iii) identify cucumber genotypes with high stability for yield.

2 Materials and methods

2.1 Germplasm and Location

Twenty two genotypes of pickling cucumbers were evaluated for 3 years (1986, 1987, and 1988) and in 7 locations across the United States. Locations were chosen to represent diverse and major cucumber production regions in the United States: Leesburg FL, Clinton NC, Bixby OK, Napoleon OH, East Lansing MI, Hancock WI, and Brooks OR (Figure 1). Twenty two genotypes were chosen to represent monoecious vs. gynoeceous, small vs. large fruit, anthracnose resistant vs. susceptible, and inbred vs. hybrid type (Table 1). Hereafter, the word 'genotype' will be used to indicate cultigen, cultivar, variety, line or genotype. Hybrids are identified with F1 after their name.

2.2 Cultural Practices

The experiment design was a split-plot treatment arrangement in a randomized complete block with 3 years, 7 locations, 4 blocks (replications), and 22 genotypes. Years and locations were whole plots, and genotypes were sub-plots. Sixty seeds of each genotype were planted in 3.3-m-long plots on raised, shaped beds 1.5 m apart (center to center) with 1.5 m alleys between plots. Plots were thinned to 30 plants approximately 5 weeks after planting. All research was conducted using standard cultural practices for cucumber production in North Carolina (Hughes et al. 1983; Schultheis 1990). Plots were harvested when 10% of the fruit had reached oversize (>51 mm diameter) using paraquat to defoliate plants and to simulate once-over harvest.

2.3 Data Collection and Traits

At each location, the 22 cucumber genotypes were evaluated for traits including total yield, marketable yield, early yield, percent culls (100 x cull fruit number/total fruit number) and fruit per plant. Marketable yield was total minus cull fruit number. Early yield was the number of fruit that were oversize (>51 mm diameter). Total, marketable and early yield were measured in thousands of fruit per hectare (1000 ha⁻¹).

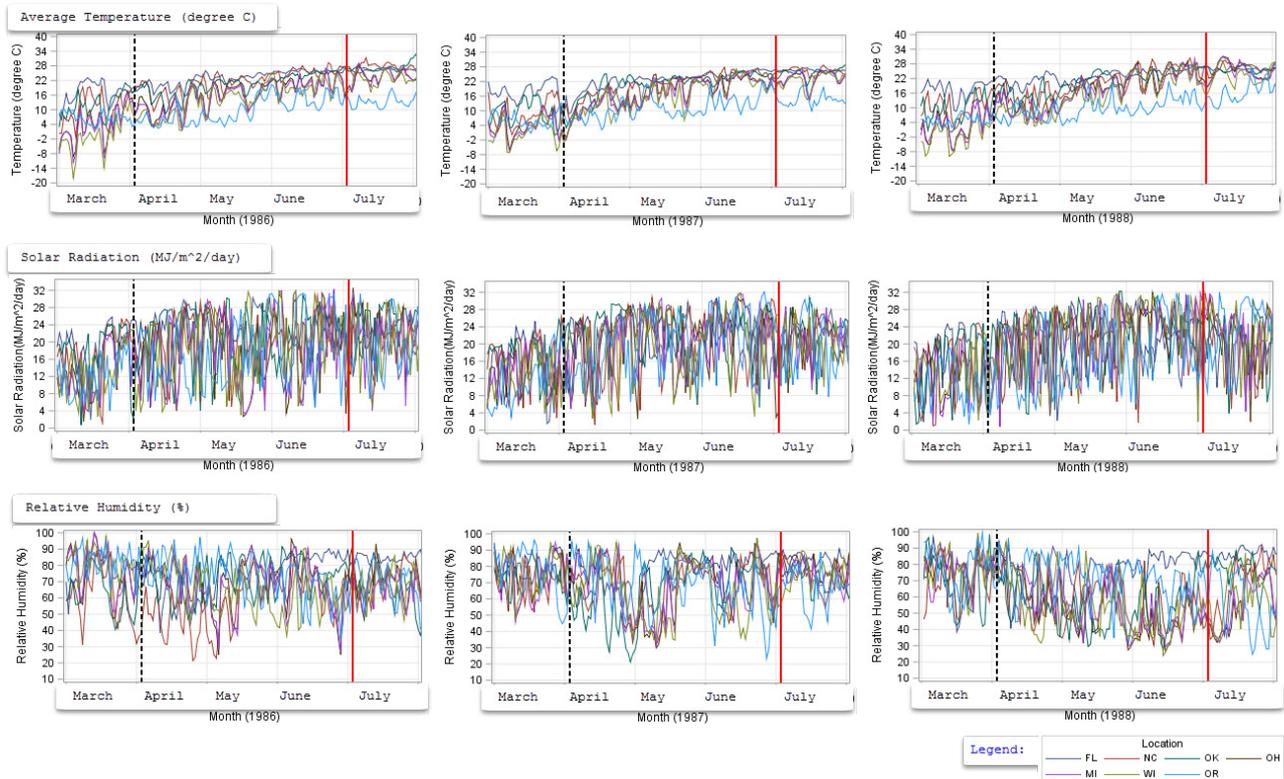


Figure 1: Daily average temperature (°C), solar radiation (MJ m⁻² day⁻¹) and relative humidity (%) for location FL, NC, OK, OH, MI, WI and OR for the year 1986 to 1988. Normal growing season of cucumber across tested location is from April to June. This duration is represented between the vertical dashed black and solid red lines

Table 1: The 22 cucumber genotypes tested with pedigree and general characteristics.

ID	Genotype	Parentage	Sex expression	Heterozygosity	Anthracnose resistance
G01	Addis	SC 19B x Pixie x NCARS lines	Monoecious	Inbred	Resistant
G02	Calypso F1	Gy 14 x Addis	Gynoecious	Hybrid	Resistant
G03	Carolina F1	Gy 14 x SC 38A	Gynoecious	Hybrid	Resistant
G04	Castlepick F1	N/A†	Gynoecious	Hybrid	Resistant
G05	Chipper	Asgrow lines, PI 197087 and PI 196289	Monoecious	Inbred	Resistant
G06	Clinton	Pixie x NCARS lines x Clemson lines	Monoecious	Inbred	Resistant
G07	Colet F1	N/A	Gynoecious	Hybrid	
G08	Earlipik 14 F1	Gy 3 x NK male	Gynoecious	Hybrid	Susceptible
G09	Fremont F1	WI 1983G x Clinton	Gynoecious	Hybrid	Susceptible
G10	Gy 14	PI 197087 lines	Gynoecious	Inbred	Resistant
G11	Gy 3	N/A	Gynoecious	Inbred	Resistant
G12	Ark. Littleleaf	N/A	Monoecious	Inbred	Resistant
G13	M 21	(Poinsett x Pixie) x (SC 19B x NH Tiny Dill)	Monoecious	Inbred	Resistant
G14	Marbel F1	N/A	Gynoecious	Hybrid	Resistant
G15	Pioneer F1	Gy 3 x Wisconsin Wis. SMR 18	Gynoecious	Hybrid	Resistant
G16	Regal F1	Gy 14 x M 21	Gynoecious	Hybrid	Resistant
G17	Wis. SMR 18	Wis. SMR 12 x Ohio MR 17	Monoecious	Inbred	Susceptible
G18	Sumter	Involves PIs 197087, 196289, Asgrow lines, and Wisconsin Wis. SMR 18	Monoecious	Inbred	Resistant
G19	WI 1983G	N/A	Gynoecious	Inbred	Resistant
G20	WI 2757	WI 1589 x Expo S4	Gynoecious	Inbred	Resistant
G21	WI 5096	N/A	-	-	-
G22	Wautoma	Gy 14 x WI 409M	Monoecious	Inbred	Resistant

† Not available

2.4 Data Analysis

Data were analyzed for genotype, environment, and genotype x environment interactions with the SASGxE (Dia et al. 2016a; Dia et al. 2016b; Dia et al. 2016c) and RGxE (Dia et al. 2016d; Dia et al. 2017) programs using SAS and R programming language, respectively.

Years, locations and genotypes were analyzed as random effects. Estimates and significance of random effects were computed using RGxE. The random effect model was fitted using the `lmer()` function of **lme4** (linear mixed effects models) package (Bates et al. 2015). The significance of random effects was computed using a likelihood ratio test to attain *p*-values. Likelihood is the probability of the data given a model. The logic of the likelihood ratio test is to compare the likelihood of two models with each other using restricted maximum likelihood (REML) methodology. The model without the factor of interest (the null model) is compared with the model with the factor of interest (the full model) using the `anova()` function. It gives a chi-squared value, the associated degrees of freedom and *p*-value. According to Wilk's theorem, the negative two times the log likelihood ratio of two models approaches a chi-squared distribution with *k* degrees of freedom, where *k* is number of random effects tested (Winter 2013).

Univariate stability statistics [regression slope (*bi*), deviation from regression (*S2d*), Shukla's stability variance (σ^2), and Kang's yield-stability statistics (*YSi*)], and BLUP for genotypes were computed using RGxE. Regression slope (*bi*) and deviation from regression (*S2d*); Shukla's stability variance (σ^2) and Kang's yield-stability statistics (*YSi*); and best linear unbiased predictor (BLUP) for genotypes were computed using `lm()` function of R (R Core Team 2016); `stability.par()` function of the **agricolae** package (Mendiburu, 2015); and `ranef()` function of **lme4** package (Bates et al. 2015), respectively. Tests for significance were derived using a *t*-test for each *bi* and an *F* test for each *S2d* for statistical differences from one and zero, respectively, at 0.05, 0.01 and 0.001 levels of probability.

SASGxE provided R code that is ready to use in R statistical software (R Core Team 2016) for the analysis of multivariate stability statistics (GGE biplot) (Dia et al. 2016c). GGE biplot analysis was computed using the '**GGEbiplotGUI**' package (Frutos et al. 2014), in the helper application 'RStudio' (RStudio 2014) in R statistical software. GGE biplot analysis was used to visually assess the presence of genotype x environment interaction and to rank genotype based on stability and mean in each management practice (Yan et al. 2000; Yan and Kang 2003).

Identical performing genotypes across locations and years were clustered using PROC VARCLUS of SAS v9.4 (SAS 2016). The VARCLUS procedure has a user-defined second eigenvalue cutoff and underlying algorithm called divisive clustering to split a given set of genotypes into two groups. Eigenvalues are the coefficients of principal component analysis. The value 1 of the second eigenvalue is a common choice for cutoff because it represents the average size of the eigenvalues. However, we have used the smaller value of the second eigenvalue as 0.7 to account for sampling variability (Jackson 1991). PROC VARCLUS identified clusters and computed 1-R² ratio ($(1-R^2_{\text{own cluster}}) / (1-R^2_{\text{next closest}})$), which identifies a cluster of genotypes that are highly correlated among themselves and not highly correlated with genotypes in other clusters. The graphical representation of divisive clustering, 1-R² ratio, forest plot of BLUP along with prediction interval, and mean were computed using SAS PROC TEMPLATE in conjugation with PROC SGRENDER while utilizing the graphical template language (GTL) of SAS v9.4. The bullet graphs were generated for graphical summary of stability statistics, mean and BLUP of each genotype under different traits studied using SAS PROC GPLOT in conjugation with PROC GREPLAY of SAS v9.4.

Ethical approval: The conducted research is not related to either human or animals use.

3 Results

The pooled analysis revealed statistically significant environment (E), genotype (G), genotype x location (GxL) and GxE effects for total yield, marketable yield, early yield, percent culls and fruit per plant (Table 2).

3.1 Polygon View of GGE Biplot

The 'polygon' (which-won-where) view of the GGE biplot divides the biplot into sector via perpendicular lines (rays) passing from the polygon sides (Figure 2). The polygon is drawn by joining extreme genotypes of the biplot. If environments fall into different sectors, then different genotypes won in different sectors, and a crossover GxE pattern exists. The winning genotype for an environment or set of environments in a sector is the vertex genotype. Conversely, if all environments fall into a single sector, a single genotype had the highest yield in all environments. The vertex genotype in a sector where no environment is present is considered to be a poor performer in all

Table 2: Variance analysis of total, marketable and early yield (1000 ha⁻¹), percent culls and fruit per plant of 22 cucumber genotypes tested in 3 years and 24 environments

Source	Estimate	Standard Deviation	χ^2 probability†
Total yield			
Location (L)	1608.28	40.10	**
Year (Y)	0	0	NS
Environment (LxY)	673.57	25.95	***
Replication within E	83.78	9.15	***
Genotype (G)	1461.98	38.24	***
GxL	217.24	14.74	***
GxY	4.41	2.10	NS
GxLxY (GxE)	654.15	25.58	***
Pooled Error	938.64	30.64	
Marketable yield			
Location (L)	1591.95	39.90	***
Year (Y)	4.21	0.002	NS
Environment (LxY)	567.98	23.83	***
Replication within E	76.22	8.73	***
Genotype (G)	775.62	27.85	***
GxL	231.33	15.21	***
GxY	9.99	3.16	NS
GxLxY (GxE)	566.88	23.81	***
Pooled Error	767.65	27.71	
Early yield			
Location (L)	507.66	22.53	**
Year (Y)	69.20	8.32	NS
Environment (LxY)	218.02	14.77	***
Replication within E	14.11	3.76	***
Genotype (G)	120.88	10.99	***
GxL	129.80	11.39	***
GxY	12.82	3.58	*
GxLxY (GxE)	113.68	10.66	***
Pooled Error	146.89	12.12	
Percent culls			
Location (L)	61.59	7.85	*
Year (Y)	16.65	4.08	NS
Environment (LxY)	50.61	7.11	***
Replication within E	3.95	1.99	**
Genotype (G)	51.36	7.17	***
GxL	20.56	4.53	***
GxY	2.86	1.69	NS
GxLxY (GxE)	57.19	7.56	***
Pooled Error	139.34	11.80	
Fruit plant ⁻¹			
Location (L)	0.18	0.42	NS
Year (Y)	0.02	0.13	NS
Environment (LxY)	0.12	0.35	***
Replication within E	0.01	0.11	***
Genotype (G)	0.23	0.48	***
GxL	0.02	0.15	**
GxY	0.001	0.03	NS
GxLxY (GxE)	0.09	0.30	***
Pooled Error	0.17	0.41	

†*, **, and *** = significant at 0.05, 0.01, and 0.001 levels of probability, respectively; NS =non-significant

test environments. Genotypes within the polygon were less responsive to location than the vertex genotypes. A polygon view of the GGE biplot explained 92%, 88%, 95%, 87% and 92% of the genotype and genotype x environment variation for the total yield, marketable yield, early yield, percent culls and fruit per plant, respectively (Figure 2: Panel A, B, C, D and E). Other than percent cull, yield traits had environments in two sectors with different wining genotypes (vertex genotype) in each (Figure 2: Panel A, B, C, D and E). This confirms the existence of GxE for total yield, marketable yield, early yield and fruit per plant. (Figure 2: Panel A, B, C and E). Genotype main effects plus genotype x location interaction effect (GGL) biplots for individual year were constructed and showed that location grouping did not vary across years. Results of GGL biplots are not presented here.

3.2 Mean vs. Stability views of GGE biplot

The ‘average environment coordinate’ (AEC) view based on genotype-focused singular value partitioning (SVP = 1) can be referred as the ‘mean vs. stability’ view of GGE

biplot (Yan et al. 2007). That view facilitates genotype comparisons based on mean performance and stability across environments within a mega-environment. The ‘mean vs. stability’ view of GGE biplot explained 92%, 88%, 95%, 87% and 92% of the genotype and genotype x environment variation for the total yield, marketable yield, early yield, percent cull and fruit plant¹, respectively (Figure 3: Panel A, B, C, D and E). The arrow shown on the AEC abscissa points in the direction of higher trait performance of genotypes and ranks the genotypes with respect to trait performance. Thus, genotype ‘Colet F1’ (G07) had the highest total yield and ‘Ark. Littleleaf’ (G12) had the lowest (Figure 3: Panel A). Similarly, WI 5096 (G21), ‘Regal F1’ (G16), and ‘Colet F1’ (G07) had the highest marketable yield, early yield, and percent cull and fruit per plant, respectively. ‘Ark. Littleleaf’ (G12), WI 2757 (G20), ‘Wautoma F1’ (G22), ‘Clinton’ (G06), and WI 2757 (G20) and ‘Ark. Littleleaf’ (G12) had the lowest total yield, marketable yield, early yield, percent cull and fruit per plant, respectively (Figure 3: Panel A, B, C, D and E). The stability of each genotype was explored by its projection onto the AEC vertical axis. The most stable genotype was located almost on the AEC abscissa (horizontal axis) and

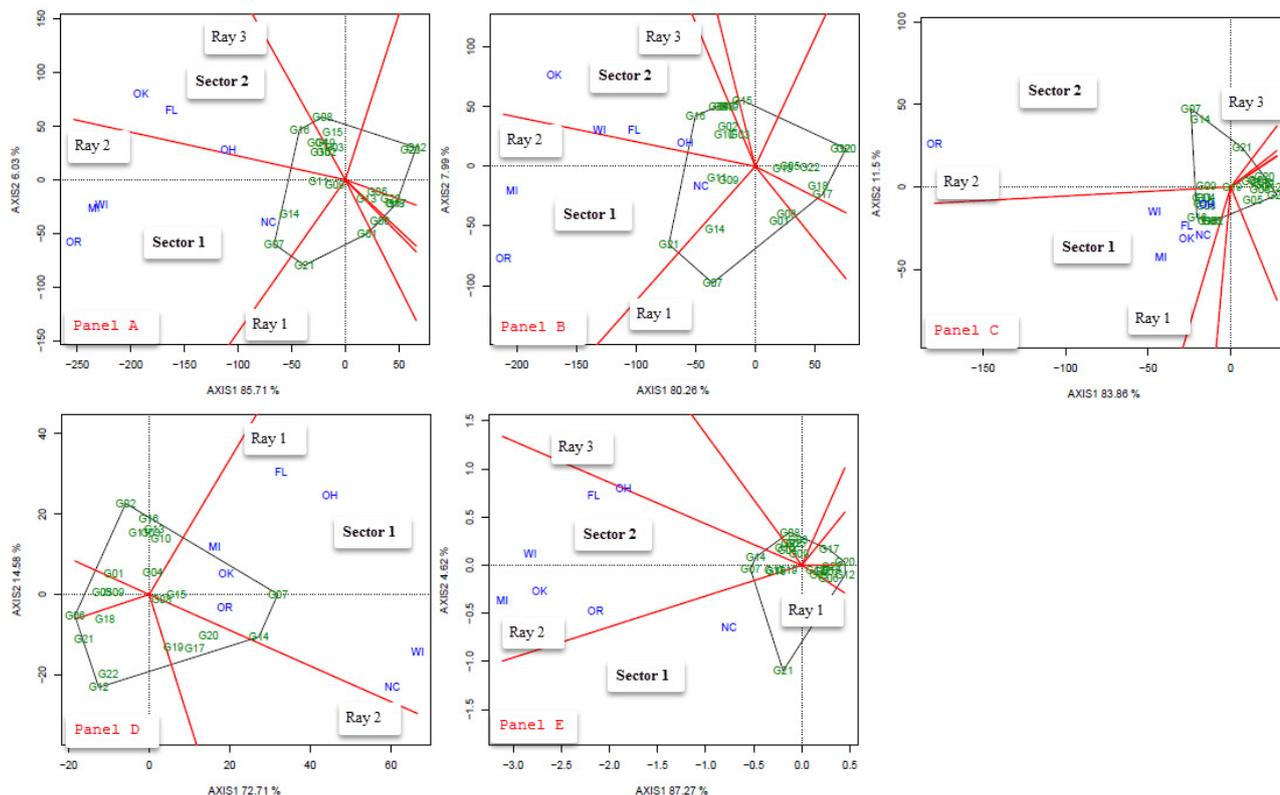


Figure 2: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 22 cucumber genotypes tested in 3 years and 7 locations for total yield (Panel A), marketable yield (Panel B), early yield (Panel C), percent cull (Panel D) and fruit plant¹ (Panel E). The biplots were based on ‘Scaling = 0’, ‘Centering = 2’ and ‘SVP = 2’. Key to the labels of genotype and management practices is presented in abbreviation section

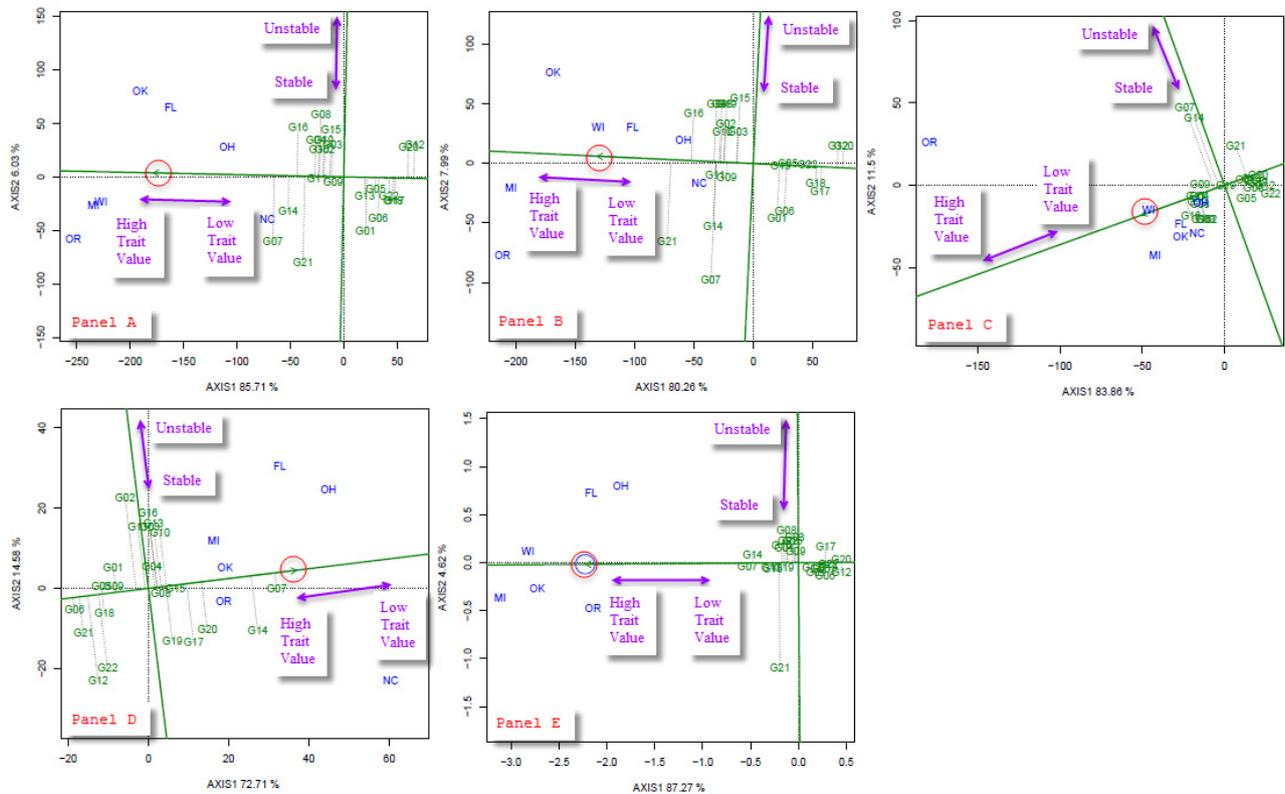


Figure 3: The mean vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 22 cucumber genotypes tested in 3 years and 7 locations for total yield (Panel A), marketable yield (Panel B), early yield (Panel C), percent cull (Panel D) and fruit plant⁻¹ (Panel E). The biplots were based on ‘Scaling = 0’, ‘Centering = 2’ and ‘SVP = 1’. The ‘ideal’ genotype is represented by a circle on average environment coordinate (AEC)-abscissa which passed through biplot origin. Key to the labels of genotype and management practices is presented in abbreviation section

had a near-zero projection onto the AEC (vertical axis). Thus, Gy 3 (G11) and ‘Fremont F1’ (G09), and ‘Colet F1’ (G07), WI 5096 (G21) were the most and least stable for total yield, respectively (Figure 3: Panel A). Similarly, M 21 (G13), ‘Chipper’ (G05) and ‘Wautoma’ (G22), and ‘Colet F1’ (G07), WI 5096 (G21), ‘Pioneer F1’ (G15) and ‘Regal F1’ (G16) were the most and least stable for marketable yield, respectively (Figure 3: Panel B). Likewise, ‘Colet F1’ (G07), ‘Calypso F1’ (G02) and WI 5096 (G21) were the least stable for early yield, percent cull and fruit per plant, respectively (Figure 3: Panel C, D and E).

3.3 Univariate Stability Statistics

According to Eberhart and Russell (1966), a regression coefficient (bi) approximating unity, along with deviation from regression ($S2d$) near zero, indicates stability. For total yield and marketable yield, the bi value for all the genotypes was close to unity ($P > 0.01$), except for WI 2757 and ‘Addis’, respectively (Table 3). Similarly, except

‘Chipper’ and WI 2757 the bi value for all the genotypes was close to unity ($P > 0.01$) for percent culls and fruit per plant. Genotypes ‘Wautoma’ and WI 2757 had negative bi value for percent culls and fruit per plant, respectively. Conversely, for early yield almost half of the genotypes had significantly different bi value from unity. Except for ‘Calypso F1’, ‘Carolina F1’ and ‘Fremont F1’; M 21; ‘Clinton’ and ‘Sumter’; and ‘Carolina F1’, ‘Marbel F1’ and WI 1983G all genotypes evaluated for total yield and marketable yield; early yield; percent culls; and fruit per plant had significant $S2d$.

According to Shukla (1972), a genotype with low σ_i^2 is regarded as stable. Most of the genotypes evaluated in this study had non-significant σ_i^2 for all the evaluated traits. The exceptions were ‘Colet F1’, ‘Earlipik 14 F1’, ‘Marbel F1’, WI 5096, WI 2757, and ‘Ark. Littleleaf’ (Table 3). These genotypes had high σ_i^2 value. Similarly, according to YS_i , genotypes with YS_i higher than the mean YS_i are stable (represented with symbol ‘v’ in Table 3). The mean YS_i for total yield, marketable yield, early yield, percent culls, and fruit per plant was 105 (1000 ha⁻¹), 90 (1000 ha⁻¹),

Table 3: Significance value of regression coefficient (*bi*), deviation from regression (*S2d*), Shukla's stability variance (*σi2*), and Kang stability statistics (*YSi*) for total, marketable and early yield (1000 ha⁻¹), percent cull and fruit plant⁻¹ of 22 cucumber genotypes tested in 3 years and 24 environments

Genotype	Total yield			Marketable yield			Early yield			Percent culls			Fruit plant ⁻¹			
	<i>bi</i>	<i>S2d</i>	<i>σi2</i>	<i>YSi</i>	<i>bi</i>	<i>S2d</i>	<i>σi2</i>	<i>YSi</i>	<i>bi</i>	<i>S2d</i>	<i>σi2</i>	<i>YSi</i>	<i>bi</i>	<i>S2d</i>	<i>σi2</i>	<i>YSi</i>
Addis	0.65	2477***	1903	0.58*	2028***	1513	0.28***	380***	51	1.12	184***	25	0.72	0.35***	0.29	.
Calyso F1	1.13	933	149	0.99	1014	286	1.39	468**	59	1.00	381***	254	1.31	0.22	0.05	✓
Carolina F1	0.63	1264	919	0.65	1166	498	1.16	373**	121	0.90	250***	111	0.40	0.21*	0.11	✓
Castlepick F1	1.03	1849**	866	1.15	1668***	1019	1.52	293**	154	1.32	158***	85	0.95	0.33***	0.06	✓
Chipper	1.05	1054**	587	0.96	871**	499	0.67	513***	285	0.34*	135*	21	0.51*	0.16**	0.11	.
Clinton	0.61	2317***	1227	0.53	1976***	1210	0.39**	374***	125	0.51	69	42	0.98	0.35***	0.21	.
Colet F1	1.67	7586***	5435**	1.51	6087***	4335**	0.84	1194***	650**	0.79	529***	377*	1.93	1.03***	0.51**	✓
Earlipik 14 F1	0.81	3228***	2049*	1.17	2552***	1440	1.33	660**	184	1.65	187**	60	0.52	0.47***	0.24	✓
Fremont F1	1.28	1256	630	1.40	1130	598	0.97	261**	97	0.55	103*	52	1.03	0.21**	0.12	✓
Gy 14	1.15	2129***	331	1.01	1907***	247	1.84**	380***	67	0.92	191*	118	1.27	0.33**	0.12	✓
Gy 3	1.20	1767*	63	1.42	1740**	95	2.34***	442***	148	0.68	266***	111	1.40	0.71**	0.33	✓
Ark. Littleleaf	0.63	10099***	1693	0.61	9184***	1314	0.21*	653***	164	0.05	807***	201	0.52	1.68***	0.48**	.
M 21	1.00	2355***	837	0.88	1532***	520	0.38	342	103	0.99	274*	142	0.68	0.34***	0.13	.
Marbel F1	1.34	8619***	4268**	1.52	8560***	4607**	1.58*	829***	424**	1.16	573***	482**	2.18	0.69	0.31	✓
Pioneer F1	0.99	1944**	1126	1.32	1914***	1264	1.78*	752***	240	1.50	168***	54	1.12	0.28***	0.13	✓
Regal F1	1.50	3056**	1281	1.26	2714**	1050	1.84	462*	166	0.89	213***	189	0.91	0.46**	0.02	✓
Wis. SMR 18	0.66	1601***	1287	0.65	1499***	1349	0.98	112***	15	0.14	830***	257	0.91	0.26***	0.06	.
Sumter	0.72	1174***	435	0.76	734**	348	0.36***	205***	36	1.16	151	16	0.98	0.27***	0.11	.
WI 1983G	1.22	2132**	1454	1.30	1970***	1462	1.26	307**	70	0.98	252***	166	1.33	0.26	0.16	✓
WI 2757	0.22**	4678***	1740	0.03*	2724***	701	0.18***	236***	71	4.79***	1803**	512**	-0.15***†	0.81***	0.34	.
WI 5096	1.48	9056***	4068**	1.41	7914***	4189**	0.18*	449***	150	0.67	116*	68	1.03	1.14***	0.57**	✓
Wautoma	0.67	2390***	1147	0.57	1926***	867	0.42**	286***	68	-0.04***†	313***	215	1.01	0.44***	0.12	.

*, **, *** indicate significantly different from unity for the regression coefficients or slope (*bi*) and from zero for the deviation from regression (*S2d*) at 0.05, 0.01 and 0.001 levels of probability, respectively.

† indicates negative slope.

‡ indicate stable according to Kang stability statistic (*YSi*).

23 (1000 ha⁻¹), 19, and 1.3, respectively. According to YSi the top five genotypes for marketable yield and stability were ‘Regal F1’, WI 5096, ‘Marbel F1’, Gy 3 and ‘Colet F1’ (Table 3).

3.4 Genotype BLUPs

BLUPs are the estimates of random effects. The estimates of cucumber genotype (random effect) for total yield, marketable yield, and early yield ranged from 56.66 to 171.13 (1000 ha⁻¹), 39.09 to 122.32 (1000 ha⁻¹), and 8.99 to 37.66 (1000 ha⁻¹) (Figure 4: Panel A and Panel B, and Figure 5: Panel A). The highest total yield and marketable yield was estimated for genotypes ‘Colet F1’, ‘Marbel F1’, ‘Regal F1’ and WI 5096. Similarly, the highest early yield was estimated for genotypes ‘Regal F1’, ‘Pioneer F1’, Gy 14 and Gy 3. The estimates of percent cull per plant ranged from 9.05 to 35.70 and fruit per plant from 0.60 to 2.22, respectively (Figure 5: Panel B and Figure 6). High total and marketable yield was correlated with high percent culls and fruit per plant. Genotype ‘Colet F1’ and ‘Marbel F1’ had high estimated yield and the highest estimated percent culls and fruit per plant. Other genotypes with high estimated percent culls and fruit per plant were ‘Regal F1’, Gy 3 WI 5096, ‘Wis. SMR 18’ and WI 2757, respectively. The lowest estimated total yield, marketable yield, early yield, percent culls and fruit per plant were recorded for ‘Ark. Littleleaf’, WI 2757, ‘Wautoma’, ‘Clinton’ and WI 5096, respectively.

3.5 Divisive Clusters and 1-R² ratio

For total yield, marketable yield, early yield, percent culls and fruit per plant, similarly performing genotypes were grouped into 3, 4, 3, 7 and 3 clusters, respectively (Figure 4, Figure 5 and Figure 6). The most representative and distinct genotype within the cluster has high correlation with its own cluster and low correlation with other clusters (SAS, 2017). Thus, an ideal representative genotype has a low 1-R² ratio $([1-R^2_{\text{own cluster}}] / ([1-R^2_{\text{next closest}}]))$ value. For total yield, ‘Earlipik 14 F1’, WI 5096 and ‘Ark. Littleleaf’ were the representative genotype of cluster 1, cluster 2 and cluster 3, respectively (Figure 4: Panel A). Similarly, for marketable yield ‘Castlepik F1’, ‘Clinton’, ‘Ark. Littleleaf’; and ‘Colet F1’ and ‘Marbel F1’ were the representative genotype of cluster 1, cluster 2, cluster 3 and cluster 4, respectively (Figure 4: Panel B). Genotypes ‘Colet F1’ and ‘Marbel F1’ had same 1-R² ratio value (0.19) and are equally representative for distinctiveness for marketable

yield. Thus, these genotypes can be used interchangeably. Likewise, Gy 14, ‘Clinton’ and ‘Ark. Littleleaf’ were the representative genotypes of cluster 1, cluster 2 and cluster 3, respectively for early yield (Figure 5: Panel A).

For percent culls, ‘Pioneer F1’, ‘Castlepik F1’ and M 21; ‘Ark. Littleleaf’, ‘Clinton’, Gy 3; WI 2757 and ‘Marbel F1’ were the representative genotypes of cluster 1, cluster 2, cluster 3, cluster 4, cluster 5, cluster 6 and cluster 7, respectively (Figure 5: Panel B). Cluster 3 and cluster 6 consisted of single distinct genotypes with zero value for 1-R² ratio. The zero value for the 1-R² ratio was due to the presence of a single entity in the cluster and, thus, correlation within its own cluster was 1 $([1-1] / ([1-R^2_{\text{next closest}}] = 0 / ([1-R^2_{\text{next closest}}] = 0))$. In cluster 2, genotypes ‘Castlepik F1’ and M 21 had equal 1-R² ratio value (0.19). Thus, ‘Castlepik F1’ and M 21 were equally representative for distinctiveness and could be used interchangeably (Figure 5: Panel B). Likewise, ‘Chipper’ and WI 5096, and ‘Ark. Littleleaf’ and WI 2757 had the same 1-R² ratio value (0.45 and 0.22, respectively) and the most representative genotypes in cluster 2 and cluster 3, respectively, for fruit per plant (Figure 6). The other representative genotypes for cluster 1 and cluster 4 for fruit per plant were ‘Castlepik F1’ and WI 1983G, respectively (Figure 6).

4 Discussion

For all the yield traits evaluated in this study, estimates of location (L), environment (E), genotype (G) and GxE explained most of the variation (Table 2). The large estimates of L, LxY and G suggested that the agro-ecological conditions of the test locations and the germplasm studied were extremely diverse, and accounted for most of the yield variation. Except for fruit per plant, significant location effect suggests that plant breeders can either develop specialist genotypes for selected environments or generalist genotypes adapted to a wide range of environments. Since location x year was significant for all traits evaluated, plant breeders should develop stable genotypes that perform well in different environments. The ideal genotype would have both high mean and high stability.

The number of divisive clusters varied for the traits evaluated in this study. However, the pattern of grouping of inbreds vs. hybrids remained consistent, except for percent culls. For total yield, marketable yield, early yield and fruit per plant, hybrids recorded high predicted performance with relatively large prediction interval and tend to be grouped into a single cluster. These high trait performance hybrids

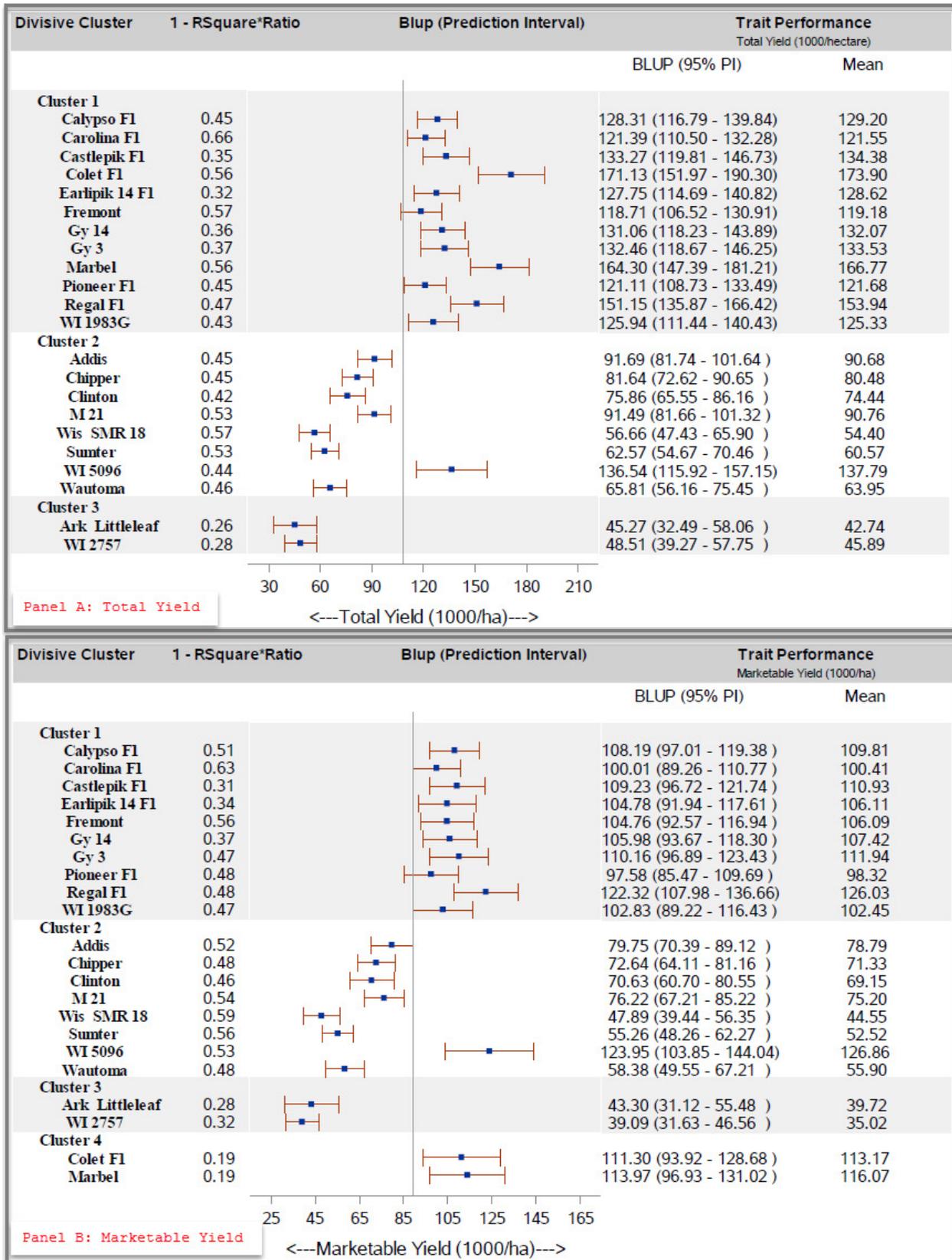


Figure 4: Divisive cluster, 1-R² ratio, forest plot of BLUP along with 95% prediction interval, mean, BLUP and 95% prediction interval of 22 cucumber genotypes tested in 3 years and 24 environment for total yield (Panel A) and marketable yield (Panel B). Vertical line on x-axis (horizontal scale) represent trait mean across genotypes

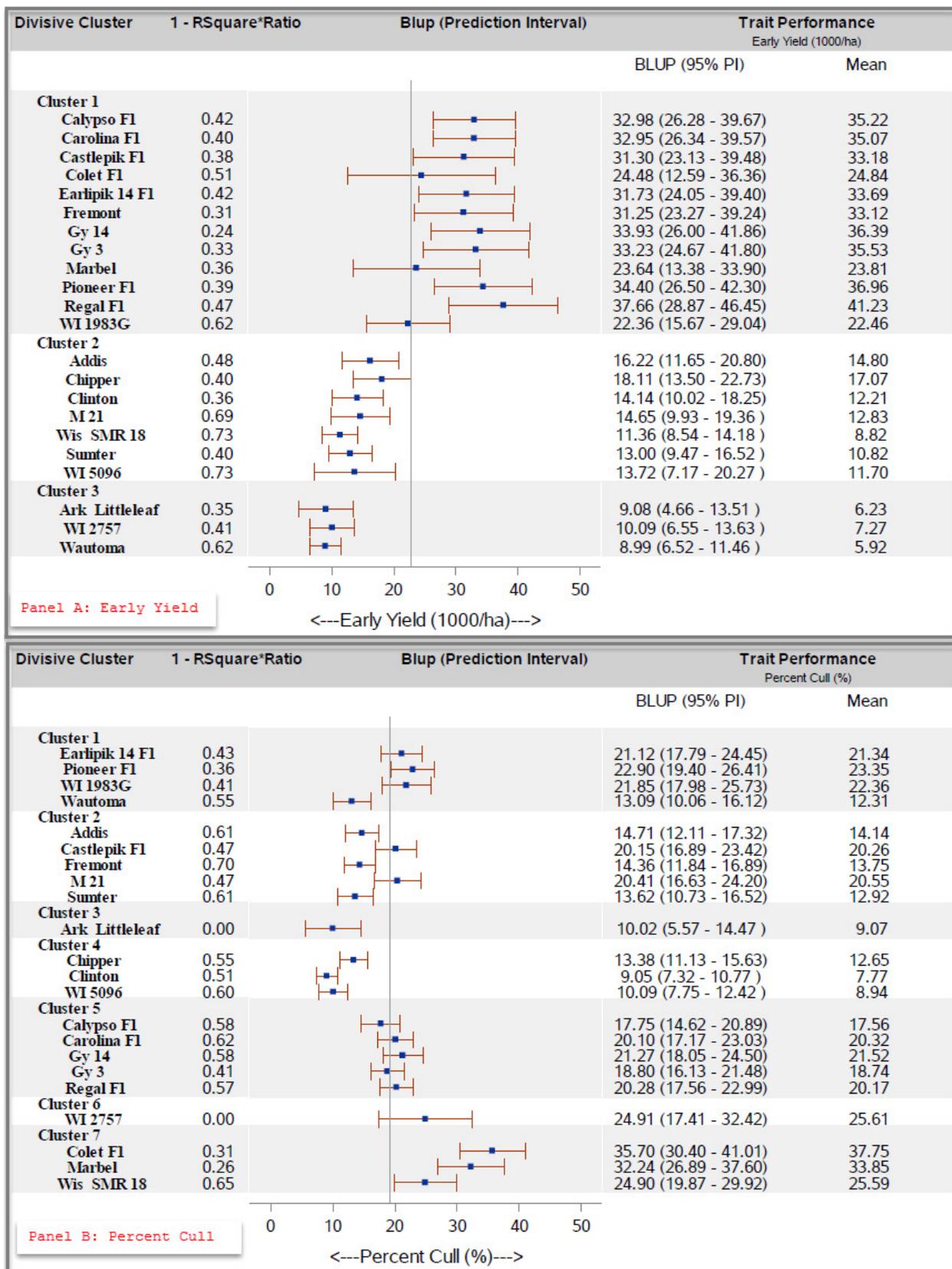


Figure 5: Divisive cluster, 1-R² ratio, forest plot of BLUP along with 95% prediction interval, mean, BLUP and 95% prediction interval of 22 cucumber genotypes tested in 3 years and 24 environment for early yield (Panel A) and percent cull (Panel B). Vertical line on x-axis (horizontal scale) represent trait mean across genotypes

include ‘Regal F1’, ‘Colet F1’, ‘Castlepick F1’, ‘Calypso F1’ and ‘Earlipik F1’ (Figure 4, Figure 5 and Figure 6). Conversely, inbreds had low to high trait performance with variable prediction interval and tended to group into multiple clusters. The distinct values for the 1-R² ratio of inbreds in each cluster indicated the existence of some dissimilarity among genotypes within the cluster. This finding is also supported by the unique genetic makeup and distinct parents being used in each genotype (Table 1). Among all the traits, marketable yield is most important, since the income of growers is based on it. Divisive cluster analysis grouped genotypes into 4 clusters based on marketable yield performance: high (cluster 4, average cluster yield - 112 thousand fruit ha⁻¹), mid-high (cluster 1, average cluster yield -106 thousand fruit ha⁻¹), mid-low (cluster 2, average cluster yield - 73 thousand fruit ha⁻¹) and low (cluster 3, average cluster yield - 41 thousand fruit ha⁻¹) yielding genotypes (Figure 4: Panel B). High and low yielding inbreds were WI 5096, ‘Marbel F1’, Gy 3, Gy 14 and ‘Fremont F1’; and WI 2757, ‘Ark Littleleaf’, ‘Wautoma’, ‘Wis SMR 18’ and ‘Sumter’, respectively. Inbreds ‘Marbel F1’, Gy 14,

‘Wautoma’ and WI 2757 had the lowest 1-R² ratio and, thus, were the most representative genotypes of their respective clusters. Cucumber breeders can use the most representative and distinct genotype (lowest 1-R² ratio) as a parent from a unique cluster for future breeding purposes to exploit that extra genetic variability for trait improvement.

Based on average ranking generated from multiple stability measures (BLUP, mean, *bi*, *S2d*, *oi2*, *YSi*, ‘mean vs. stability’ view of GGE biplot) we developed a bullet graph summary of the traits (Figure 7) and classified cucumber genotypes into three categories. Category 1 included genotypes having medium to high marketable yield and high stability. These genotypes are widely adapted across diverse environments. Those genotypes were ‘Regal F1’ (G16), ‘Calypso F1’ (G02), ‘Carolina F1’ (G03), Gy 3 (G11), Gy 14 (G10) and ‘Fremont F1’ (G09) (Figure 7). Hybrids ‘Regal F1’ (G16), ‘Calypso F1’ (G02) and ‘Carolina F1’ (G03) had high early yield, average percent culls and average to high fruit per plant. In contrast, genotypes Gy 3 (G11), Gy 14 (G10) and ‘Fremont F1’ (G09) had high early yield, average to low percent culls and high fruit per plant. These high

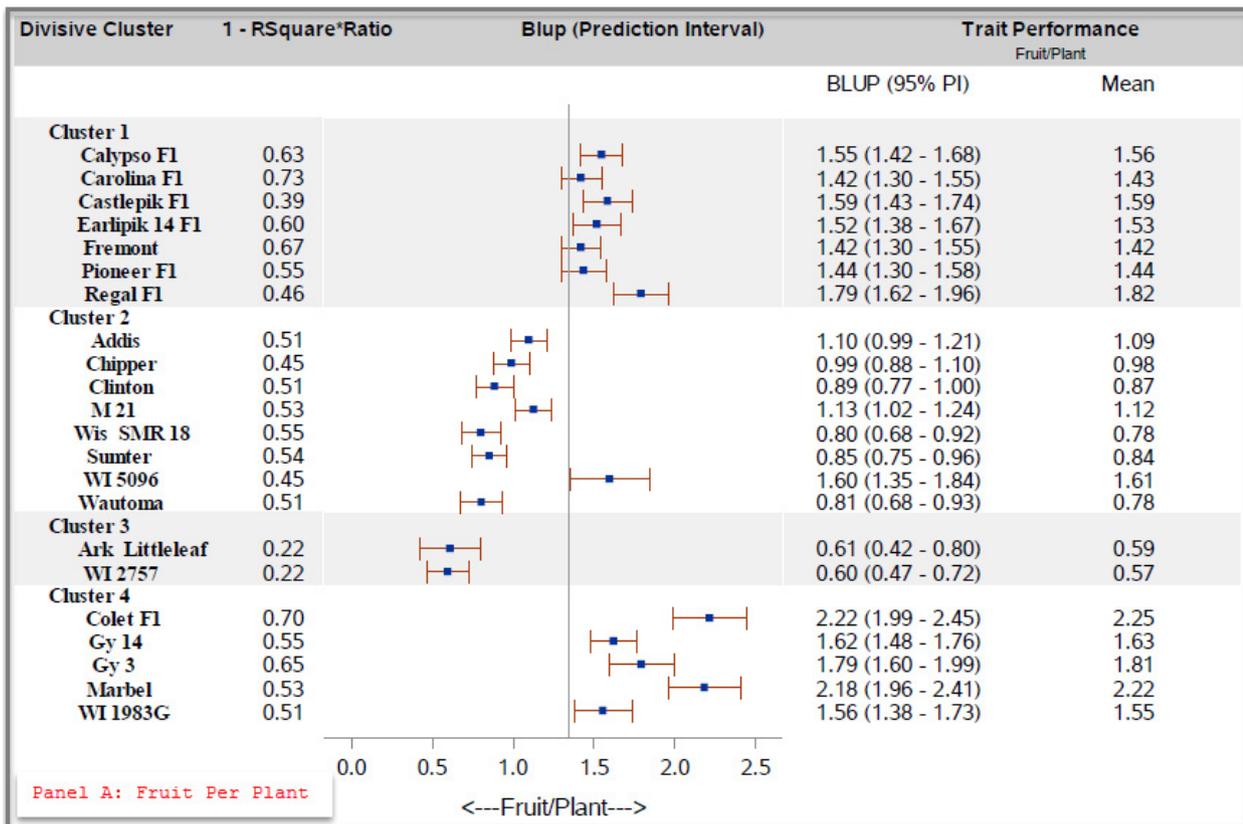


Figure 6: Divisive cluster, 1-R² ratio, forest plot of BLUP along with 95% prediction interval, mean, BLUP and 95% prediction interval of 22 cucumber genotypes tested in 3 years and 24 environment for fruit plant⁻¹ (Panel A). Vertical line on x-axis (horizontal scale) represent trait mean across genotypes

yielding hybrids and inbreds had high to medium stability for early yield and fruit per plant. Stability for percent culls was low for hybrids and low to high for inbreds.

Category 2 genotypes exhibited high marketable yield but low stability, so these genotypes were suited more for specific environments. This category includes genotypes ‘Castlepik F1’ (G04), ‘Colet F1’ (G07), WI 5096 (G21) and ‘Marbel F1’ (G14) (Figure 7). Hybrids ‘Castlepik F1’ (G04) and ‘Colet F1’ (G07) had average early yield, average to high percent culls, and high fruit per plant. Conversely, genotypes WI 5096 (G21) and ‘Marbel F1’ (G14) had average to low early yield, low to high percent culls, and high fruit per plant. Hybrids were highly stable, whereas inbreds were low to medium in stability for early yield, percent

culls and fruit per plant. Category 3 genotypes had low marketable yield and high stability. These genotypes would be useful in breeding traits other than yield, for example disease resistance or fruit quality. Category 3 genotype include ‘Ark Littleleaf’ (G12), ‘Wautoma’ (G22), and ‘Sumter’ (G18) (Figure 7). These genotypes had marketable yield significantly lower than the other genotypes. For other yield components, category 3 genotypes had percent culls and below average early yield and fruit per plant. Category 3 genotypes had low to high stability for early yield, percent culls and fruit per plant.

The highest performing inbred and hybrid genotypes for marketable yield and yield components (WI 5096, ‘Marbel F1’, ‘Regal F1’, ‘Colet F1’) were not the highest for yield

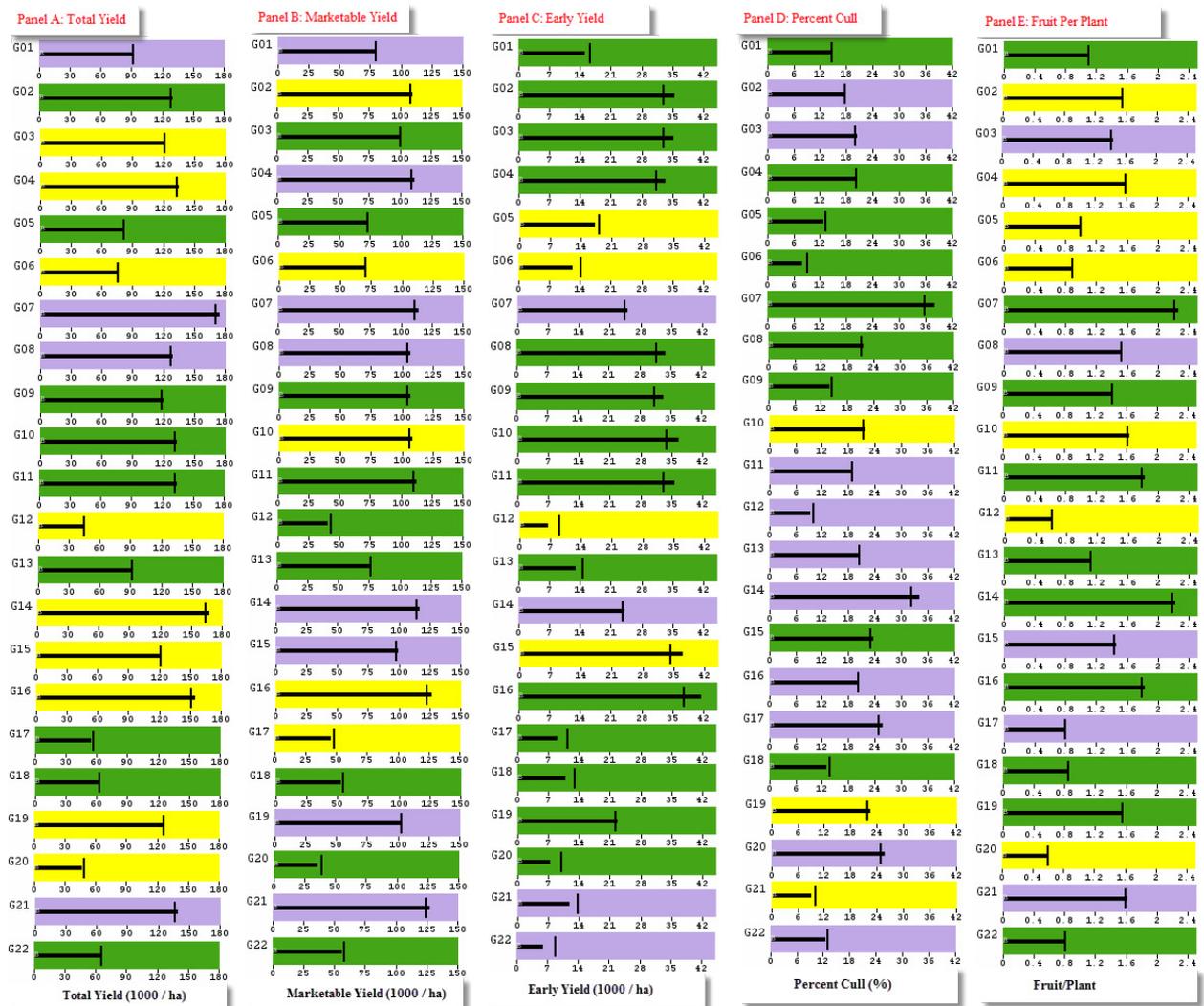


Figure 7: Bullet graph summary of stability statistics, mean and BLUP of 22 cucumber genotypes tested in 3 years and 24 environment for total yield (Panel A), marketable yield (Panel B), early yield (Panel C), percent cull (Panel D) and fruit plant⁻¹ (Panel E). The horizontal bars represent genotype (G01-G22). Back ground fill color of green, yellow and violet within each horizontal bar represent high, medium and low stability. The horizontal and vertical black line within each horizontal bar measure trait mean and BLUP, respectively, on quantitative scale (x-axis). Key to the labels of genotype is presented in abbreviation section

stability. Not all three genotypes were stable for all yield components. Therefore, there is room for improvement. Based on the bullet graph summary of stability statistics and forest plot of divisive clusters, it is evident that genetic diversity and stability exist among evaluated cucumber genotypes for yield and yield components.

Thus, it appears possible to breed stable cucumber genotypes with high yield and either high or low yield. The genotype with highest performance for marketable yield, greatest stability for yield component, lowest 1-R² ratio value (diverse and representative) were 'Marbel F1' and Gy 14.

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