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FEED THE FUTURE AREA PROJECT
MAIZE VARIETY TRIAL – SETUP AND PROCEDURES

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December 2020

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Maize Variety Trial – Setup and Procedures (December 2020)

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Feed the Future Haiti Appui à la Recherche et au Développement Agricole (AREA) | Support to Agricultural Research and Development (SARD)

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1 Maize Variety Trial Partnership

Introduction

Grain yield and quality alone do not provide sufficient information about the various strategies maize cultivars use to cope with diverse environments or how crop cultivars respond during the growing season to specific farming practices as well as biotic (pests, diseases, competition against weeds and ”neighbors”, etc.) and abiotic (hot, dry, wet growing conditions, lack of nutrients, etc.) stresses. Understanding the cultivar’s potential to respond to inputs and stresses is the first step to improving the corn crop’s productivity efficiently within Haiti’s diverse farming systems.

Farmers, breeders, and research coordinators taking part in the on-farm testing network will communicate regularly. Farmers will share information about field management. At the end of the growing season, the Variety Testing Coordinator will provide all farmers with a report about all varieties’ performance statistics. Each farmer will be informed about the performance data of the varieties grown on his or her farm and the average performance of all cultivars across all participating farms. Average on-farm and on-station results will be supplied separately.

Goals

- Farmers, breeders, and researchers work together in a Variety Trial Network to evaluate maize cultivars in on-station and on-farm experiments.
- Distribute information among farmers about new maize cultivars and their performance characteristics (e.g., yield, resistances, and issues).
- Provide breeders with direct feedback from farmers who test new cultivars under real-world farming conditions. Testing new germplasm in on-farm experiments will challenge the usefulness of breeding objectives.
- The on-farm testing network will be an integral part of the maize production system in Haiti.

2 Cultivar Supply and Selection

Three months before planting the Variety Trial Coordinator (VTC) will contact farmers to confirm their participation in the Variety Trial. Farmers who confirm participation will plant a total of N_{total} cultivars. N_{total} is composed out of the Core Set of cultivars and an Optional Set of cultivars.

- Core Set - Cultivars belonging to the Core Set will be evaluated by all farmers taking part in the Variety Trial. The use of a core set of cultivars will allow determining cultivar ("Genotype") by environment (GXE) interactions. If necessary, the Core Set will be tailored for areas based on cultivar maturity, cultivar seed availability, and farmer interest. Check varieties supplement the Core Set.
- Optional Set - This is a set of added cultivars that are of regional importance or interest to a specific group of farmers. Farmers will select optional cultivars from a list of maize cultivars available for the following main growing season.

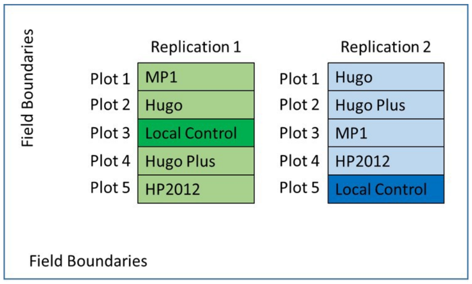
Information about cultivar maturity, quality, and agronomic characteristics will be provided to the farmers by the VTC during the recruitment process. Farmer's feedback about cultivars will be used to name OPVs/hybrids that will be increased in the following autumn season and then made available to farmers in the next spring season.

Table 1: Example set of cultivars offered to farmers. The list indicates which varieties are part of the Core or Optional Sets. The Source column provides information about when, where, and by what organization the seed was produced.

Cultivar	Maturity	Characteristics	Source	Set
Hugo		OPV		Core
Hugo Plus		OPV		Core
MP1		OPV		Core
HP2012		Population Hybrid		Core
Common Check		OPV		Core
Chicken Corn		OPV		Core
Local variety 1		OPV		Optional
Local variety 2		Hybrid		optional
...	...	...	...	...

3 Field Layout

The basic variety trial unit is a plot of six rows (furrows) wide and 5 m long. This allows for the evaluation of each variety using the center four rows where the influence of neighboring cultivars is minimized. The plot length does not include the alleys between ranges. In general alleys have a width of 1 m. So, the total plot length (”center to center”) is 6 m, this includes a ”planting length” of 5 m and 0.5 m for the alleys on both ends. The distance between rows and between plants within rows follows local standards. To allow for a meaningful statistical analysis, each variety will be planted at least twice at each location using a randomized complete block design (see Figure 1).



	Replication 1		Replication 2
Plot 1	MP1	Plot 1	Hugo
Plot 2	Hugo	Plot 2	Hugo Plus
Plot 3	Local Control	Plot 3	MP1
Plot 4	Hugo Plus	Plot 4	HP2012
Plot 5	HP2012	Plot 5	Local Control

Figure 1: General variety trial design. As an example, varieties in Table 1 are used.

If the seed is limited, it is possible to use augmented designs. For an excellent introduction to augmented designs, see `https://plant-breeding-genomics.extension.org/introduction-to-augmented-experimental-design/#part1`. If space is limited, it is possible to use smaller plot sizes, but row number per plot should not be smaller than four. Again, using the center two rows for evaluation will reduce biases caused by competition effects, e.g., a tall variety is planted next to a shorter variety.

Individual farmers will complete the design of their variety trial when they meet with the Variety Trial Coordinator. The example below illustrates what a variety trial may look like (Figure 1). The contents of farm action plans, which will vary from farm to farm because of seed availability, climatic conditions, interests, and infrastructure of the farmer, and perceived opportunities, will be documented.

4 Phenotyping

4.1 Pollen Date, Anthesis

Description and Procedure

The Pollen Date is the date when 50 percent of the plants in a plot show anther on more than half of the central tassel spike (see Figure 2). Figure 3 shows the tassel one day after the pollen date was recorded.

Table 2: Male Flowering

Code	MF
Time of Evaluation	at flowering
Number of measures per plot	1
Unit	Date [MM/DD/YY]

Figure 2: Tassel with central spike 50% flowering.

Figure 3: Tassel completely flowering.

4.2 Silk Date

Description and Procedure

The Silk Date is the date when 50 percent of the plants in a plot show silk emergence (see Figure 4. Figure 5 shows the silk on an ear one day after the silk date was recorded.

Table 3: Female Flowering

Code	FF
Time of Evaluation	at flowering
Number of measures per plot	1
Unit	Date [MM/DD/YY]

Figure 4: Plant starts showing silks, i.e., female flowering begins.

Figure 5: Silk on ear one day after female flowering started.

4.3 Plant Height

Description and Procedure

Placing the measuring stick on ground next to the root crown, “plant height” is measured at the ligule of the flag leaf (see Figure 6, the green bar on the ruler in dicates the place of the ligule and the height of the plant). The ligule is a tissue that separates the leaf sheath from the leaf blade (Figure 7).

Table 4: Planth height

Code	PHT
Time of Evaluation	after flowering
Number of measures per plot	1 representative plant per plot
Unit	centimeter (cm)

Figure 6: Plant with measuring stick. PHT is measures at green bar on measuring stick

Figure 7: The flag leaf is the top leaf and the reference for measuring PHT.

4.4 Ear Height

Description and Procedure

Placing the measuring stick on the ground next to the root crown, “ear height” is measured at the primary ear bearing node (see Figures 8 and 9. If a plant carries multiple ears, the top ear is the primary ear and used for measuring ear height. It makes sense to evaluate plant height and ear height at the same time using the one representative plant per plot.

Table 5: Ear height

Code	EHT
Time of Evaluation	after flowering
Number of measures per plot	1 representative plant per plot
Unit	Date

Figure 8: Maize plant with measuring stick.

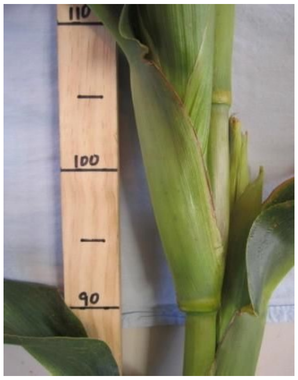


Figure 9: Node carrying the primary ear.

4.5 Root Lodging

Description and Procedure

The number of plants that show root lodging per plot, i.e., those stems that lean substantially to one side (> 15% from vertical) (Figure 10). The count includes “goosenecked” plants that have “straightened up” after becoming lodged earlier in the season (Figure 10).

Note - Emphasis is on the number of plants, not on a % estimate oflodged plants. Accurate stand counts and lodging counts are essential and will be used to calculate a % lodging in the later analyses.

Table 6: Root Lodging

Code	RLD
Time of Evaluation	before harvest
Number of measures per plot	1 **count** per plot
Unit	number of plants with RLD

Figure 10: Plants leaning substantially to one side.

Figure 11: Plants straightening after root lodging (”goosenecking”).

4.6 Stalk Lodging

Description and Procedure

Number of plants broken between the ground level and the top ear node (Figure 12).

Note - Emphasis is on the number of plants, not on a % estimate of broken plants. Accurate stand counts and stalk lodging counts are essential and will be used to calculate a % stalk lodging in the later analyses.

Table 7: Stalk Lodging

Code	SLD
Time of Evaluation	before harvest
Number of measures per plot	1 **count** per plot
Unit	number of plants with SLD

Figure 12: Plant showing stalk breakage below the ear.

4.7 Green Snap

Description and Procedure

Number of plants broken between the ground level and the top ear node **before flowering** (Figure 13.

Notes - Collaborators may choose to take counts of green snap following a weather event occurring before flowering that causes substantial numbers of stalks to snap. Please also record date of event.

Emphasis is on the number of plants, not the %, which does not tell us much. Accurate stand counts and lodging counts are essential and will be used to calculate a % snapped plants in later analyses.

Table 8: Green Snap

Code	GSP
Time of Evaluation	before flowering
Number of measures per plot	1 count per plot
Unit	number of plants with GSP and date of triggering event [MM/DD/YY]

Figure 13: Corn plants with snapped stalk before flowering.

4.8 Stand Count

Description and Procedure

The number of plants per plot at harvest.

Notes - Main consideration is how many plants were in the plot at harvest time. Accurate stand counts are important for calculating the percentage of lodged or broken plants per plot. Counting can occur earlier but if a plot damage occurs before harvest they will need to be recounted.

Table 9: Stand Count

Code	STC
Time of Evaluation	at harvest
Number of measures per plot	1 count per plot
Unit	count

4.9 Plot Weight

Description and Procedure

The shelled grain weight per plot.

Table 10: Plot Weight

Code	PLW
Time of Evaluation	at harvest
Number of measures per plot	1
Unit	kilogram (kg)

4.10 Grain Moisture

Description and Procedure

The water content of the grain harvested from one plot.
moisture meter oven drying weighing method

Table 11: Grain Moisture

Code	MST
Time of Evaluation	at harvest
Number of measures per plot	1
Unit	percent

5 Derived Traits

5.1 Grain Yield

Grain yield will be calculated using the ”Plot Weight” and ”Grain Moisture” measurements (see Chapters 9 and 10) applying Equation 1:

$$Y\,LD = PWT \times \left(1 - \frac{(15.5 - MST\,)}{100}\right) \times \frac{10,000}{PlotSize} \tag{1}$$

The ”Plot Size” is provided in square meters (m^2).

Table 12: Grain Yield

Code	YLD
Time of Evaluation	at harvest
Number of measures per plot	1
Unit	kilogram (kg) per hectar (ha) adjusted to MST = 15.5%

6 Statistics

6.1 Software

The randomization of field experiments and the subsequent data analysis will be conducted using program packages available in the R software. RStudio will be used as the integrated development environment that allows for an easy and efficient access to R packages, data management, programming, and program debugging. The R software, RStudio, and all R packages used here are publicly available and can be downloaded without cost for Windows, Apple, or Linux environments.

- R software: `https://cran.r-project.org/`
- RStudio: `https://rstudio.com/products/rstudio/`
- For a detailed description of how to download and install R and RStudio see: `https://rstudio-education.github.io/hopr/starting.html`

The R package ”Agricolae” will be applied to conduct the randomization and analysis of all on-station and on-farm variety trials. A detailed Agricolae tutorial is available online at `https://cran.r-project.org/web/packages/agricolae/vignettes/tutorial.pdf`. All features Agricolae offers are described in the following document `https://cran.r-project.org/web/packages/agricolae/agricolae.pdf`.

6.2 Randomization

All R packages needed for experiment randomization and data analysis are installed and loaded for use using the following R commands.

```
# ###########Design Randomization##################

install.packages(" agricolae")
install.packages("predictmeans")
install.packages("lme4")

library ( agricolae)
library ( predictmeans )
library ( lme 4 )
```

With *Agricolae* a large number of experimental designs can be randomized. These include ”Complete Randomized Designs (CRD)”, ”Randomized Complete Block Designs (RCBD)”, incomplete block designs, alpha designs, and split plot designs. In addition, the program outputs field books that can be exported to Excel for subsequent note taking in the field. In the following example an experiment is randomized using an RCBD and Split-Plot layout.

```
#  ########################################################
#RCBD
trt=c("A","B","C","D")
rep = 5
out.design=design.rcbd(trt,r=rep,seed=123456,serie=2)

book.rcbd= out.design$book
book.rcbd #Field Book

# 2 ways to see field layout
out. design $ sketch
matrix(book.rcbd[,1],nrow=rep,byrow=T)

# Output -----------------------------------------------
> book.rcbd #Field Book
   plots block  trt
1      101     1    D
2      102     1    A
3      103     1    C
4      104     1    B
5      201     2    B
6      202     2    C
7      203     2    A
8      204     2    D
9      301     3    B
10     302     3    C
11     303     3    D
12     304     3    A
13     401     4    D
14     402     4    C
15     403     4    A
16     404     4    B
17     501     5    C
18     502     5    B
19     503     5    A
20     504     5    D

> out.design$sketch
     [,1 ] [,2 ] [,3 ] [,4 ]
[ 1 ,] "D"   "A"   "C"   "B"
[ 2 ,] "B"   "C"   "A"   "D"
[ 3 ,] "B"   "C"   "D"   "A"
[ 4 ,] "D"   "C"   "A"   "B"
[ 5 ,] "C"   "B"   "A"   "D"

> matrix(book.rcbd[,1],nrow=rep,byrow=T)
     [,1 ] [,2 ] [,3 ] [,4 ]
[1 ,]   101   102   103   104
[2 ,]   201   202   203   204
```

```
[3 ,]  301   302   303   304
[4 ,]  401   402   403   404
[5 ,]  501   502   503   504


# Serpentine Layout
# Accommodates easier phenotyping in the field
book .rcbd .serpentine= zigzag ( out. design )
book .rcbd .serpentine
matrix(book.rcbd.serpentine[,1],nrow=rep,byrow=T)

> book.rcbd.serpentine=zigzag(out.design)
> book.rcbd.serpentine
   plots block trt
1    101     1   D
2    102     1   A
3    103     1   C
4    104     1   B
5    204     2   B
6    203     2   C
7    202     2   A
8    201     2   D
9    301     3   B
10   302     3   C
11   303     3   D
12   304     3   A
13   404     4   D
14   403     4   C
15   402     4   A
16   401     4   B
17   501     5   C
18   502     5   B
19   503     5   A
20   504     5   D
> matrix(book.rcbd.serpentine[,1],nrow=rep,byrow=T)
     [,1 ] [,2 ] [,3 ] [,4 ]
[1 ,]  101   102   103   104
[2 ,]  204   203   202   201
[3 ,]  301   302   303   304
[4 ,]  404   403   402   401
[5 ,]  501   502   503   504

write. csv ( book . rcbd . serpentine ,  " book . rcbd . serpentine . csv ") #

##########################################################
#Split-Plot in an RCBD
trt1=c("A","B","C","D")
trt2=c("X","Y","Z")
rep = 5
split.design=design.split(trt1,trt2,r=rep,serie=2,seed=123456)
```

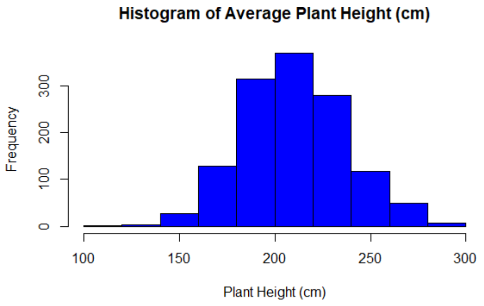
```
book.split=split.design$book
head(book.split)
View(book.split)

# Output -----------------------------------------------
> head(book.split)
  plots  splots  block  trt1  trt2
1   101       1      1     D     X
2   101       2      1     D     Z
3   101       3      1     D     Y
4   102       1      1     A     Y
5   102       2      1     A     X
6   102       3      1     A     Z
```

6.3 Data Analysis

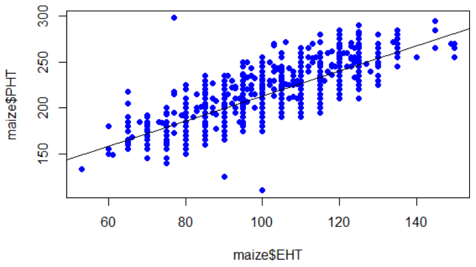
As an example, a ”real-world” maize data set is used. A total of 72 maize hybrids were evaluated in three years using a randomized complete block design with three replications per environment.

```
############Loading and Manipulating Data ################
# Remove all previous dataframes, etc.
rm(list=ls())
# Set the path to folder containing data sets and programs.
setwd("C:/Users/...")

# Import data set saved as a CSV file.
maize=read.csv("maize.csv",head=T)
View(maize)
names(maize)

# Creating new variables.
maize$sum=maize$PHT+maize$EHT
maize$Avg=maize$sum/2
maize #As opposed to View(maize)

#Calculating overall summary statistics
# This notation directs the program to the "GY" column in "maize".
summary(maize$GY)
# This notation directs the program to column 9 and 10 in "maize".
summary(maize[,c(9,10)])
var(maize[,c(9,10)])
var(maize$Avg)
sd(maize$PHT)

# Output -----------------------------------------------
> sd(maize$PHT)
[1] 26.98663
```

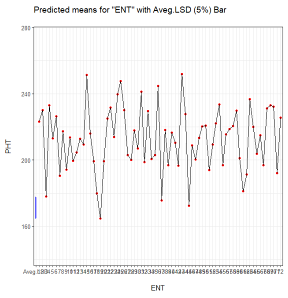
```
#Calculating Means, Medians, and Variances by Group
aggregate(maize[,c(10:13)],list(maize$TYPE),mean)
aggregate(maize[,c(10:13)],list(maize$TYPE),median)
aggregate(maize[,c(10:13)],list(maize$TYPE),var)

# Output ---------------------------------------------
> aggregate(maize[,c(10:13)],list(maize$TYPE),var)
    Group.1      PHT      EHT      DTS      DTP
1     INTER 778.8764 749.3992 33.21756 20.32546
2 INTRANSSS 681.4022 208.0904 30.42254 21.04506
3  INTRASSS 608.9595 189.1674 34.00796 20.01419

# ################### PLOTS ####################
#Making Histograms
hist(maize$PHT,main="HistogramuofuAverageuPlant
uuuuuHeightu(cm)",xlab="PlantuHeightu(cm)",col="Blue")
```

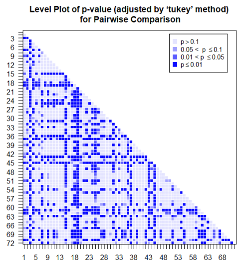


```
#Scatterplot
#Basic
plot(maize$PHT~maize$EHT,pch=16,col="Blue")
fit=lm(maize$PHT~maize$EHT)
abline(fit)
```



```
############# Data Analysis ####################

# Make sure that classification variables are recognized # by R
as factors.
maize$ENV=as.factor(maize$ENV)
maize$REP=as.factor(maize$REP)
maize$BLOCK=as.factor(maize$BLOCK)
maize$ENT=as.factor(maize$ENT)

#Conduct ANOVA with all factors regarded as fixed effects
fit.rcbd=aov(PHT~ REP:ENV + ENV + ENT + ENT:ENV,data=maize)
summary(fit.rcbd)

# Output ---------------------------------------------
> summary(fit.rcbd)
              Df  Sum Sq   Mean Sq  F value    Pr(>F)
ENV            2   25266     12633   33.299  9.32e-15  ***
ENT           71  458923      6464   17.037   < 2e-16  ***
REP :ENV       6   13788      2298    6.057  3.01e-06  ***
ENV :ENT     142   35579       251    0.660    0 .999
Residuals   1073  407085       379
---
Signif. codes:  0     ***     0.001     **     0 .01     *
                0.05     .     0.1            1

#How do I tell which differences between ENTs are different?
#install.packages("predictmeans")
library(predictmeans)
predictmeans(fit.rcbd,"ENT",,pairwise=T,adj="tukey")
```





```
#RCBD with Random Factors - install and load "lme4"
#install.packages("lme4")
library(lme4)
fit.rcbd=lmer(PHT~(1|REP:ENV)+(1|ENV)+ENT+(1|ENT:ENV) ,data=maize)
summary(fit.rcbd.2)
predictmeans(fit.rcbd.2,"ENT",pairwise=T,adj="tukey")
```

7 Checklists

The following check lists will provide guidelines regarding all the information that should be collected during the growing season by the VTC.

7.1 Field Metadata Collection

At Planting

☐ Planting dates [MM/DD/YY]

☐ Note latitude/longitude (GPS coordinates) of field location.

☐ Note row spacing and plot dimensions.

☐ Map of field layout.

☐ Note what local check cultivars were used.

☐ Note previous crop

☐ Note method of soil preparation

☐ Notes on planting errors, field anomalies, equipment, etc.

☐ If weather stations are used not their serial number.

☐ If soil analysis will be conducted, collect soil samples.

In Season

☐ Note pesticides and herbicides: type and amount applied.

☐ Note fertilizer: date, type, and amount applied.

☐ Note irrigation schedule: date and amount applied (if applicable).

☐ Notes on field anomalies, phenotyping errors and issues.

At Harvest

☐ Harvest dates [MM/DD/YY]

☐ Notes on field anomalies, whole-field issues, equipment and technical issues, or harvesting issues

7.2 Phenotype and Performance Data Collection

Evaluate cultivars for the following traits. See document ”Standard Operation Procedures - Phenotyping” for specific instructions.

In-Season

- Stand Count – may be taken as juveniles and at harvest
- Anthesis [MM/DD/YY]
- Silking [MM/DD/YY]
- Plant Height
- Ear Height
- If damaging winds occur, cooperators may choose to record green snap and date of event

At Harvest

- Stand Count
- Stalk Lodging – plant count (NOT percentage)
- Root Lodging – plant count (NOT percentage)
- Stand Count
- Plot Weight
- Grain Moisture

8 Image Credit

1. Figure 2: 2004, 2006; Purdue University, RL Nielsen.
2. Figure 3: 2004, 2006; Purdue University, RL Nielsen.
3. Figure 4: https://www.mississippi-crops.com/2018/07/05/ identifying-corn-reproductive-growth-stages-and-management-implications/
4. Figure 5: https://www.mississippi-crops.com/2018/07/05/identifying-corn-reproductive-growth-stages-and-management-implications/
5. Figure 6: Genome-to-Fields Standard Operation Procedure; https://www.genomes2fields.org/resources/.
6. Figure 7: Genome-to-Fields Standard Operation Procedure; https://www.genomes2fields.org/resources/.
7. Figure 12: UDel Extension, Gordon Johnson.
8. Figure 13: UGA Cooperative Extension.