Regional Selection of Hybrid Nacional Cocoa Genotypes in Coastal Ecuador

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Abstract

Recent international demand for "nacional" flavour cocoa has increased the need for local cocoa producers in Ecuador to use high-yielding "nacional" hybrid genotypes. The relative potential of cocoa genotypes over various environments needs to be assessed prior to final selection of potential candidates. Extensive evaluation of germplasm with Nacional genetic background has been performed allowing for selection of high-yielding, disease-resistant clones. We investigated the productivity of selected high-yielding clones in 5 sites within the Ecuadorian Coastal region that vary in climate, soil and temperature. Occurrence of disease, number of diseased/healthy/underdeveloped pods, and yield (expressed as dry cocoa in kg/ha) were collected to determine performance of these clones. Though genotype is a major factor in evaluating overall productivity, variability in climate, soil type, management practices and genotype x environment interactions also impact on productivity. GLM and Mixed Model analysis were used to assess data over 4-5 years. Chongon was the highest yielding site, followed by Naranjal, Calceta, Valle de Patere and finally Valencia. Chongon had the lowest level of % diseased pods (3.2%) while Valle de Patere (82%) and Valencia (71%) had the highest due to high rainfall. The best producing hybrid Nacional clones were EET 575 (878.9 kg/ha/yr), EET 576 (808.4 kg/ha/yr), EET 544 (756.3 kg/ha/yr) and EET 558 (751 kg/ha/yr). CCN 51 (a non-Nacional genotype) and EET 103 (a Nacional genotype) were included as commercially used control clones, yielding 1301 kg/ha/yr and 918 kg/ha/yr, respectively. At two of the five sites studied (Chongon and Calceta), a few of the Nacional hybrid clones yielded as well as the CCN 51 clone. This suggests that there is a genotype × environment (site × clone) interaction. However, there were confounding factors that could partially account for this. This report allows for early conclusions on productivity and disease susceptibility of the 12 genotypes grown at the 5 sites, with the final aim of recommending the release of the best genotypes with Nacional genetics for use by producers in the coastal regions of Ecuador.

Introduction

For variety trials, it is of great importance to choose locations that are representative of the environments where a given crop is going to be grown. Genotype by environment interactions are also important components when selecting genotypes to reflect the relative potential of genotypes over a range of environments (Fan *et al*. 2001). Cocoa production, while influenced by the genetic potential of the variety, is also largely affected by soil and climatic characteristics. Deep, well-drained, loamy soils with a slightly acidic pH are best.

Cocoa grows naturally in moist, tropical forests with evenly distributed rain and undefined seasons (Wood 1985). Irrigated systems are used to supplement water availability in areas in Ecuador with less than 1500 mm rainfall (Amores 1992). Soils should be deep, without an impervious layer, clayey sand within 12 cm of surface and

sandy clay below 25 cm (Purdy and Schmidt 1996). Cocoa growing areas are influenced by moisture availability (the balance between rainfall and evaporation) (Alvim 1977). Many cocoa pathogens, especially the witches' broom pathosystem, are primarily driven and constrained by atmospheric moisture (Purdy and Schmidt 1996). In fact, 70% of the variation in annual cocoa seed yield is due to levels of annual radiation and rainfall (Almeida and Valle 2007).

The cultivation of cocoa in Ecuador is increasing annually, with 433,978 hectares currently in production (INEN 2002). The demand for Nacional cocoa in the international marketplace has also been increasing; resulting in incentives for producers in Ecuador to increase production and exportation of "arriba" flavoured cocoa. Locally, producers use seeds from pods from their own or from neighbouring farms, which are very variable in productivity and disease resistance. At INIAP in Pichilingue in the Los Rios Province, extensive evaluation of germplasm with Nacional genetic background has been performed allowing for selection of high-yielding, disease-resistant clones. However, no data exists about the productivity of these clones across the Ecuadorian regions where cocoa is cultivated.

This study investigates the productivity of selected high-yielding clones in various sites within the Ecuadorian Coastal region. Data were collected from each of five sites that were selected for variations in climate, soil and temperature. The number of mature diseased/healthy pods, disease incidence (% diseased pods), the number of cherelles and wet-bean weight per plot were collected to determine performance of these clones at the five sites being evaluated. We hypothesize that while the genotype of the clones is a major factor in evaluating their overall productivity, the influence of variability in climate, soil type and management practices also influence productivity. Therefore, this study also intends to investigate genotype x environment interactions. These data would allow the recommendation of genotypes selected to maximize productivity and adaptability to the intended region of cultivation.

Methods

Description of Sites

The field trials began in 2002 and the present study includes data up to May 2008. Trials were established to compare hybrid cocoa clones with a Nacional genetic background to CCN 51 in five farms representing different climatic zones in the coastal regions of Ecuador (Figure 1):

- "La Roma" (2° 40' 5" L. S and 79° 36' L. W) in the Naranjal region, Guayas Province;
- AGROTRASVASE Farm (2° 14' L. S and 80° 04' L. W) in the Chongón region, Santa Elena Province;
- ESPAM farm, (0° 50' L. S and 80° 09' L. W) in the Calceta region, Manabí Province;
- El Chollo farm, (0° 59' L. S and 79° 21' L. W) in Valencia, Los Ríos Province and Miguez farm in Valle de Patere in the Esmeraldes Province.

The start of the clonal trials varied with time of first plantings as follows: Naranjal- July 2001, Chongon- February 2002, Calceta- August 2002, Valencia- December 2002 and Valle de Patere- April 2003.

Climate and Soils

The sites in the Coastal region of Ecuador were chosen for their variation in climate and soil type (Table 1). Chongon has the lowest and Valencia the highest annual rainfall. However, the distribution of rainfall over the sites is similar with the first five months of the year receiving 90% of total annual rainfall. The climate for all five sites can be categorized as typical of the Ecuadorian coastal region. The soil chemical composition is quite different for the sites, resulting in varying levels of fertility based on differences in physical and chemical properties. Naranjal has a loamy-sandy soil type while Calceta has a clayey soil type (Table 1). The Sum of Bases (Staff 2003), which predicts the ability of the soil to hold nutrients is also quite variable between sites. In addition, the pH is quite variable between sites ranging from Chongon with the most basic soils (pH = 7.3) to Valencia, which has the most acidic soil (pH = 5.7).

Irrigation was performed in Chongon due to the low annual rainfall (500mm), and during the dry season in Naranjal and Calceta. In Valencia and Valle de Patere, the average rainfall was sufficient so that irrigation was not performed at any time.

Figure 1. Topographical Map of Ecuador showing farm location. **A.** Valle de Patere, Esmeraldes Province; **B.** Calceta (ESPAM), Manabí Province; **C.** Valencia (Granja Nestle), Los Rios Province; **D.** Chongon (Granja experimental de Agrotrasvere), Santa Elena Province; **E.** Naranjal (Hacienda Las Romas), Guayas Province.

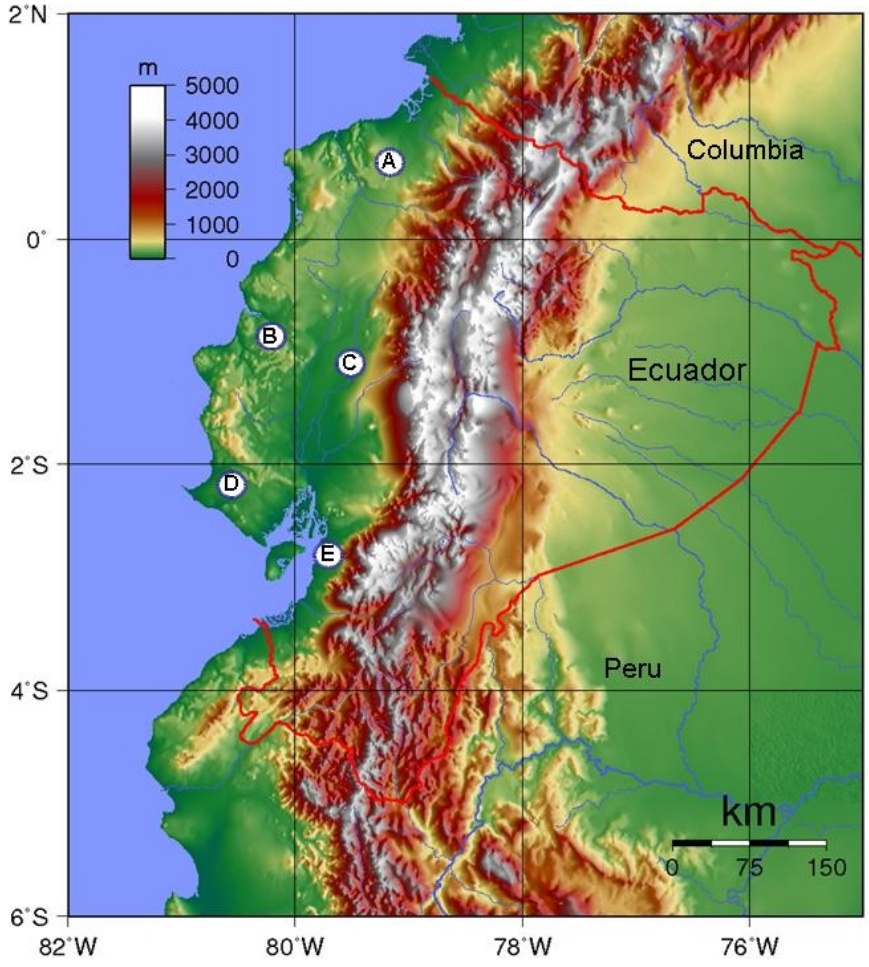

Table 1: Characteristics of the climate and soil type in the zones where the five experimental sites are located

Site	Temperature			Annual rainfall (mm)	Relative humidity (%)	Climate type	Soil Characteristics			
	Mean	High	Low				Texture	pH	Sum of bases meq/100g	OM (%)
Chongon	28	28.4	24.9	500	80	Tropical sub-desert	Loamy-clayey	7.3	22.05	2.3
Naranjal	26.5	28.4	21.2	1200	84	Tropical dry forest	Loamy-sandy	6.3	12.2	1.7
Calceta	26.8	27.8	24.5	838	68.4	Tropical very dry	Clayey	6.9	29.43	1.9
Valle de Patere	25.8	n/a	n/a	2097.5	84	Tropical rainy forest	Loamy	5.7	25.34	5
Valencia	24.3	26	23.67	2370	75	Tropical rainy	Loamy	5.9	15.05	4.3

Genotypes Selected and Experimental Design

In this regional trial, twelve (12) genotypes were selected based on potential yield as described in Table 2. Each was planted using a randomized complete-block design with four replications called “plots”. Each plot consisted of 25 trees in a 5 x 5 plant block design, with the 9 central plants evaluated out of the 25 for each of the four replications. The plot design minimizes border effects. The trees were planted at a distance of 3 x 3 m. The total planted area in each site was approximately 10,800 m^2 with each plot contained within 225m^2. Fertilization was performed twice per year, and weeds were controlled chemically during the rainy season and also supplemented by manual weeding.

Several of the clones included originated from the “Centro de Cacao de Aroma” Tenguel (CCAT) in Ecuador. Other clones were selected from traditional cocoa farms in different zones in Ecuador. The clone, EET 454, was selected in a cross between and Upper Amazon clone (SCA 6) with Nacional (EET19) in the 1960’s and was obtained from the H1 germplasm Collection at INIAP-Pichilingue. All other clones, except EET 454 were selected from the collection of Nacional genotypes (CGN) established in 1995 at INIAP-Pichilingue. The initial selection of hybrid clones was based on production and resistance to diseases.

The main objective of the regional trials was to determine any variability if these high-producing, high-flavour hybrids were grown in regions that geographically differ from Estacion Pichilingue, Quevedo. CCN 51 (Trinitario/Nacional X Upper Amazon) was included as a control in the trials since it is a high yielding non-Nacional clone commonly used. EET 103 was also included as it is a Nacional-type genotype that is also used in commercial cocoa production.

Table 2: Description of genotypes used at the five experimental sites

Clone number	Name	Genotype	Origin	Pod index	Seed index	Self-compatibility
1	EET 576	Nacional	Ecuador-Tenguel	18.83	1.36	Yes
2	EET 525	Nacional	Ecuador-Zapotal	25.10	1.08	No
3	EET 558	Nacional	Ecuador-Tenguel	24.43	1.12	Yes
4	EET 559	Nacional	Ecuador-Tenguel	31.85	0.84	Yes
5	EET 544	Nacional	Ecuador-Tenguel	22.05	1.02	Yes
6	EET 510	Nacional	Ecuador Chontillal	27.36	1.09	Yes
7	EET 575	Nacional	Ecuador - Tenguel	22.63	1.10	Yes
8	EET 522	Nacional	Ecuador -Vinces	28.09	0.97	Yes
9	EB 27-02	Nacional	Nestlé			Yes
10	EET 454	SCA 6 X EET 19	Ecuador - Pichilingue	19.15	1.24	Yes
11	EET103	N x VA	Ecuador - Tenguel	20.29	1.10	Yes
12	CCN 51	(ICS-95 X IMC-67) x Unknown	Ecuador - Naranjal	16.00	1.53	Yes

Data Collection and Analysis

Phenotypic data were recorded on a single tree basis starting in year 3 after planting. All sites included 4 years of data except Calceta, which included 5 years of data. Each pod was scored as either healthy or diseased or under-developed (cherelles). The following data were collected per plant per plot per year for each clone at each site: number of healthy pods (no sign of disease), number of diseased pods (any evidence of witches' broom and frosty pod), "cherelles" or under-developed fruit (assessed as younger than two months old), number of witches' brooms (assessed once in 2006 and not collected in Chongon or Valle de Patere) and yield in wet weight per plot (includes healthy pods and beans from partially diseased pods). The dry weight bean yield (kg/ha) was calculated from the wet weight using the following conversion: dry weight kg/ha = [(wet weight (g) x 0.4) / 1000g] x 10000 m^2 /plot area m^2. Natural logarithmic transformations were performed on yield (dry bean weight kg/ha) to achieve a sufficiently normal distribution. GLM and Mixed Model analyses were performed on data to produce least square means (LSMeans) for all traits, and were each used to analyze data by clone and by site, respectively. All calculations were performed with SAS (version 9.1 for Windows; SAS Institute, Cary, N.C.). The descriptive analysis was performed with the UNIVARIATE procedure.

Results and Discussion

Overall, there was significant variation in yield, number of healthy pods, number of diseased pods, and number of cherelles between the clones studied at each site ($p<0.001$). Furthermore, significant differences were observed in analysis of variance for site, clone, site x clone, year, clone x year and month (year) ($p<0.001$).

Table 3 describes the significant differences for traits at all sites per clone, annually. Of the hybrid Nacional genotypes, when yields were adjusted and ranked over all sites per year, EET 103 had the highest dry-bean yield, followed by EET 575, EET 576, EET 544 and EET 558.

Figure 2 illustrates the dry-bean yield (kg/ha) of each clone for each year of data

collection at each site (A-E) and overall at all five sites (F). Once corrected LS means were used minimizing sampling error in the Mixed Model analysis, Chongon was the highest yielding site, followed by Naranjal, Calceta, Valle de Patere and finally Valencia. At Chongon, EET 558 produced the highest yield, followed by EET 544 then CCN 51. At Calceta, EET 575 was the highest producing genotype, followed by CCN 51 then EET 576. CCN 51 was the highest producing clone in all other sites. However, at Naranjal, EET 559 and EET 103 were the next highest producers, while at Valle de Patere and Valencia, EET 103 was the second most productive genotype after CCN 51.

Table 3: Production and disease data for hybrid clones over all sites. Averages are for all sites over all years of data collection with clones ranked by average yield (kg/ha)

CLONE	Annual Yield (kg/ha) [1]	Average Healthy Pods per plot[2,3]	Average Diseased Pods per plot[2,4]	Average Cherelles per plot[2,5]	Diseased Pods per plot %[6]	Rank by Yield (kg/ha)
CCN 51	1301.1 [a]	142.4 [a]	175.1 [c]	332.7 [a]	52.2	1
EET 103	918.3 [a]	145.3 [a]	254.6 [ab]	413.6 [a]	60.9	2
EET 575	878.9 [ab]	133.4 [a]	215.5 [bc]	220.3 [b]	58.9	3
EET 576	808.4 [ab]	129.0 [a]	215.4 [bc]	216.7 [b]	59.8	4
EET 544	756.3 [b]	128.2 [a]	255.4 [a]	332.6 [a]	63.9	5
EET 558	751.3 [b]	131.4 [a]	264.7 [a]	168.2 [bc]	64.2	6
EET 559	721.9 [b]	140.3 [a]	260.2 [a]	213.1 [b]	62.2	7
EET 454	616.9 [b]	104.5 [b]	106.7 [d]	61.9 [d]	47.6	8
EET 525	325.9 [c]	66.3 [c]	80.4 [de]	45.2 [d]	51.9	9
EET 510	325.9 [c]	63.4 [dc]	46.5 [ef]	91.7 [de]	39.5	10
EB27 02	221.2 [cd]	40.2 [de]	36.2 [f]	39.3 [e]	44.4	11
EB 1928	185.7 [d]	38.6 [e]	33.2 [f]	32.6 [d]	43.8	12
Average	656.4	105.2	162.0	180.8		
CV (%)	123.1	130.4	143.9	202.8		

[abcdef] Means with the same letter indicate no significant difference in accordance to a Tukey test (p<=0.01)
[1] Annual Yield (kg/ha): Average production in dry weight (kg/ha) (converted from Wet weight) per clone per year for all sites.
[2] Per Plot corresponds to 9 plants of each clone free of border effect
[3] Average Healthy Pods: Number Healthy pods per plot over 4 years of data at each site, except Calceta, which has 5 years of data.
[4] Average Diseased Pods: Number of Diseased pods (all diseases) per plot over 4 years of data at each site, except Calceta which has 5 years of data.
[5] Average Number of accumulated "cherelles" or under-developed fruits for all sites (July-November 2005 and May-August 2006) excluding Valle de Patere
[6] Diseased Pods %: total diseased pods as % of total accumulated healthy and diseased pods per year during 4 years of data collection except Calceta where there are 5 years of data included.

The two top producing Nacional hybrid genotypes EET 103 and EET 575 produced almost a metric tonne of dry beans per ha annually in this study, which is quite comparable to the non-Nacional based high-yielding clone, CCN 51. There is also no significant difference in the average annual yield for the top three producing hybrid clones (EET 103, EET 576, EET 575). In addition, it is important to note that there was no significant difference observed in the top seven producing hybrid clones and CCN 51 in terms of number of healthy pods produced annually averaged over all 5 sites. This indicates that pod index and percentage of recovered beans in partially "diseased" pods played a major role in the ranking of the clones in terms of kg/ha.

The sites selected in the Coastal region of Ecuador possess favourable characteristics of climate, soil and precipitation for producing cocoa. The selected genotypes had a variable productivity dependent on site. In addition, the high annual rainfall in Valencia (2370mm) and Valle de Patere (2097.5mm) could account for the high degree of % diseased pods (>70%) over all years since many cocoa pathogens, especially the witches' broom and monilia pathosystems, are primarily driven and constrained by atmospheric moisture (Purdy and Schmidt 1996). The best yielding site for all clones was Chongon, which had an annual precipitation of 500 mm, producing only 3.2% diseased pods total over all the years of collection. Of note is the variation in soil pH as Chongon has the most basic pH (7.3) and Valencia has the most acidic pH (5.7), which may also have a factor in fertility. It is possible that rainfall and soil type were factors reducing production at Valencia.

One confounding factor is that the start of planting varied between sites (Figure 3) therefore although the plants included in this study were at the third year of growth, precocity would influence productivity causing variation in productivity among genotypes. Since our data were averaged for all clones and years of data collection regardless of start time, we hoped to minimize this effect in estimating yield per genotype per site. This variability introduced by more precocious genotypes giving higher yields earlier than others would eventually lessen over time as the trials are continued. The less precocious hybrids would eventually be evaluated on an equal basis as all the trees included in the regional trials attain maturity.

There are several reasons while these multi-locational trials would also have other confounding effects. Since all trials were conducted at farms that were large distances apart, farm management and collection of data were contracted to the local farms. Data collection was adversely affected by improper management issues, larceny and other confounding factors contributing to overall error in the analysis. There was also a high degree of disease at Valencia and Valle de Patere that would affect the overall outcome and estimation of yield. In addition, irrigation during the dry season was insufficient at Naranjal and Calceta, resulting in plants suffering from serious periods of water stress. Management problems at Chongon also resulted in the experiment ending prematurely. Ideally, there would be the same start and end dates for all clones at all sites, but this particular design and distance between sites did not allow for this.

These sources of variation would account for the strong effect of genotype x environment interaction observed in this analysis of the multi-locational trials to date, with site x clone and clone x year interactions being highly significant ($p<0.001$). Further analysis of the genotype x environment interactions will be performed to examine the choice of locality when selecting cocoa for high yield and general adaptability.

Conclusions

This report describes in brief, the initial analysis of our data allowing for preliminary conclusions on productivity and disease susceptibility of the 12 genotypes used in the 5 sites. It is interesting to note that at two of the dryer sites, Chongon and Calceta, several Nacional clones seem to be able to yield as well as CCN 51. At Chongon, EET 558 produced the highest yield, followed by EET 544 then CCN 51. At Calceta, EET 575 was the highest producing genotype, followed by CCN 51 then EET 576. We are in the process of performing quality tests to evaluate the hybrid clones selected as the top producers to confirm Nacional flavour. The final aim of this study is to recommend the release of the top four high-yielding genotypes with Nacional genetics from this study for use by producers in the coastal regions of Ecuador.

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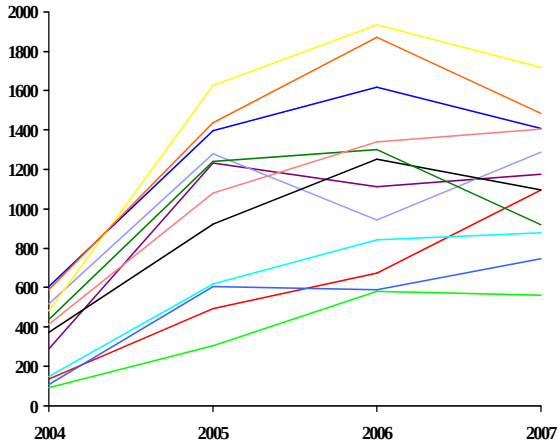
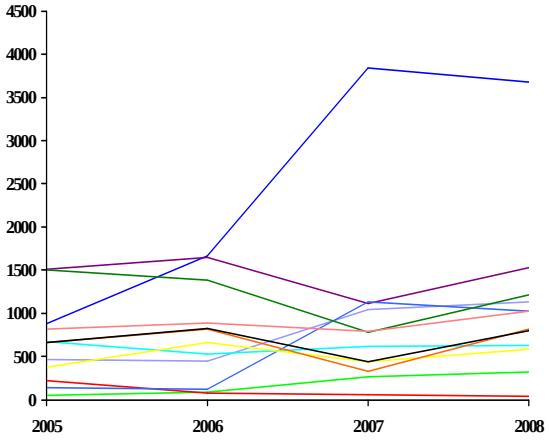
Figure 2. Production yield (Kg/ha) per genotype.

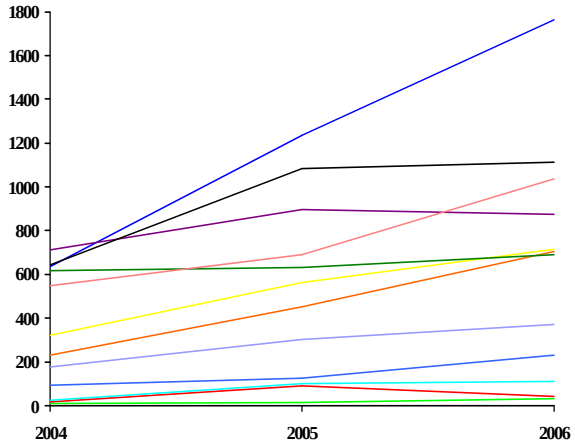
A. Chongon Yield (Dry Bean Wt. kg/ha)



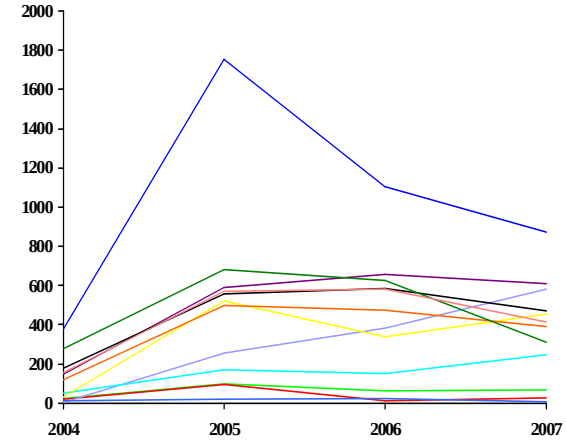
D. Valle de Patere Yield (Dry Bean Wt. kg/ha)



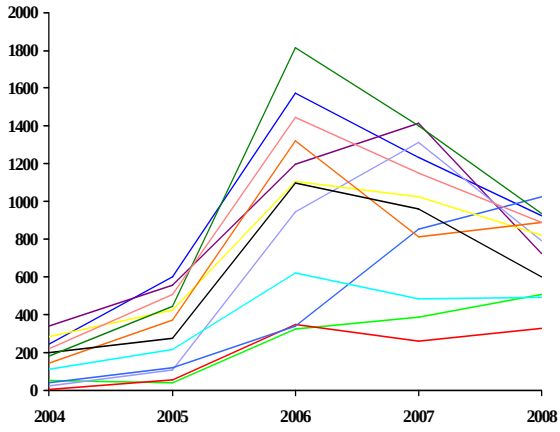
B. Naranjal Yield (Dry Bean Wt. kg/ha)



E. Valencia Yield (Dry Bean Wt. kg/ha)



C. Calceta Yield (Dry Bean Wt. kg/ha)



F. Average Yield All sites (kg/ha)

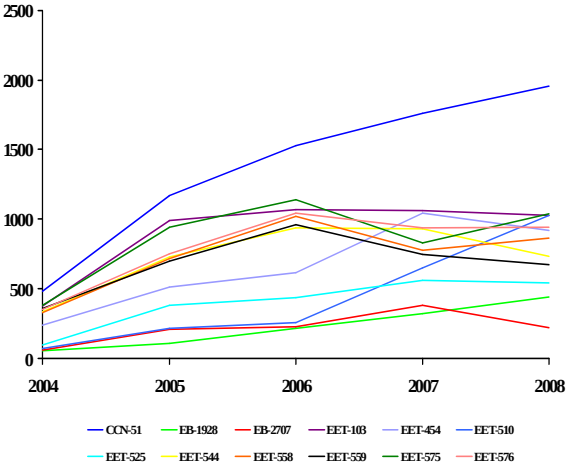
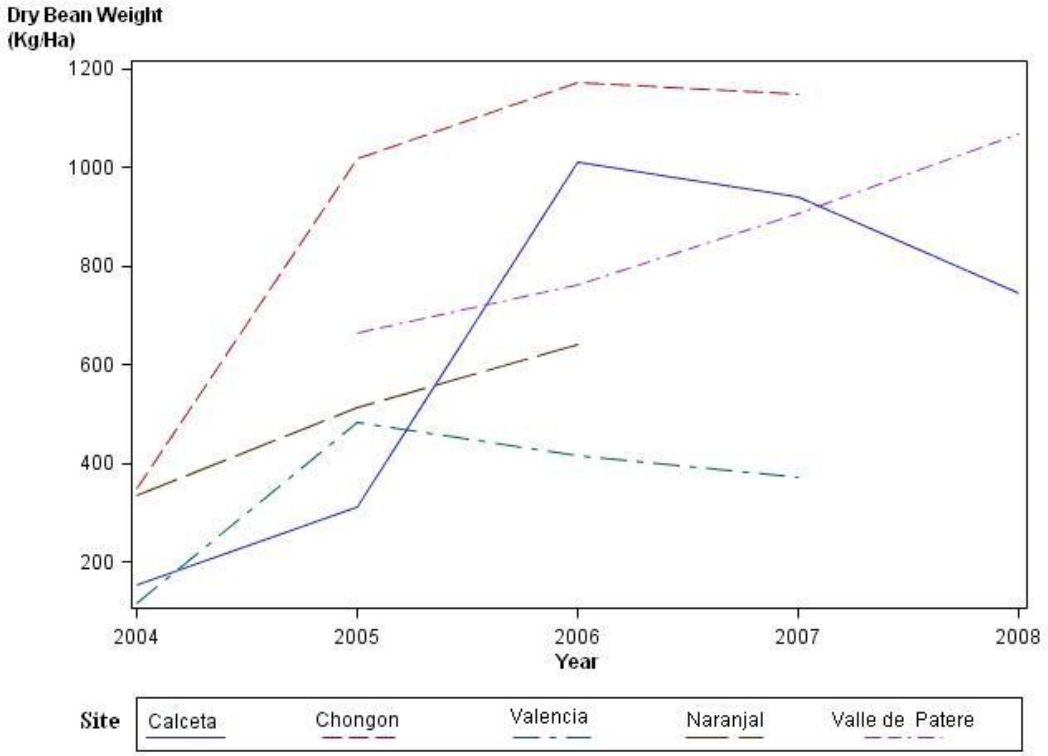


Figure 3: Production (kg/ha) overall sites for each year of data collection. Variation in start date is indicated by site and length of time of data collection varied between sites



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