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DETERMINING GRAIN SEED MICRONUTRIENT CONTENTS (IRON AND ZINC) AND COOKING TIME FOR SELECTED DRY BEAN CULTIVARS

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ABSTRACT

Micronutrient deficiencies caused by lack of Iron (Fe), Zinc (Zn) and Vitamin A in the human body have negative effect with regard to health issues worldwide. Imbalances of these nutrients in the human body create a significant risk of illness and mortality among children under five years of age, pregnant women and lactating mothers. Some of the food crops that thought to reduce micronutrients include rice, sweet potato, dry bean, sorghum, corn, barley, and finger millet have been biofortified through ways of agronomic practices, conventional breeding, or modern biotechnology. Despite the fact that dry beans address Fe and Zn deficiencies based on recommended dietary allowances (RDA), they often take a long time to cook. Many communities have limited cooking resources which make them rely on burning wood, charcoal or other biofuels that require more time and money. This study identified dry bean cultivars with enhanced Fe and Zn concentration levels and fast cooking time from 200 cultivars. Experiments were conducted under a complete randomized block design with two replications, 200 cultivars, and two different agro-ecologies. Iron and Zinc concentration levels were determined from the harvested seed grains of each bean cultivar using X-Ray Fluorescence while cooking time of similar cultivars were determined using the Mattson cooker. The variation of Fe and Zn concentration was significantly different at ($p<0.001$) among the treatments and environments. The range was between 46.76 mg/kg to 107.25 mg/kg for Fe and 21.70 mg/kg to 42.35 mg/kg for Zn concentration. From two testing sites, the concentration of Fe was higher at Lyamungo than SARI and Zn showed inversely proportional to these tested sites which means that the environment and soils were not homogenous in terms of soil health. The highest water uptake was 61.54% and the lowest was 3.70% and the higher the water uptake the lower the cooking time. Fast cooking time was ~15 min and the longest was ~76 min. Small seed-sized cultivars showed fast cooking time than large-seeded. These two traits in identified cultivars will enhance dry bean consumption, which will contribute to alleviating micronutrient deficiencies in this global growing population.

Key words: Micronutrient deficiencies, Biofortification, Cultivars, Cooking time, Vulnerable group, Variation

INTRODUCTION

Iron and Zinc are essential micronutrients for body growth, development, reproduction and other physiological functions in the human body [1]. The available data on zinc level in serum and seminal plasma suggests that zinc has a significant role in spermatogenesis and maintaining the total number of sperms as well as sperm motility and DNA integrity [1, 2, 3]. Also, Iron deficiency in the human body is one of the most common micronutrient deficiencies affecting women, children, and infants most severely and it is especially prevalent in resource-poor individuals [4]. Among the functional consequences of iron deficiency include decreased physical performance and physical activity, decreased cognitive performance, depression, and fatigue [5]. The nutritional strategies to reduce this burden of micronutrient deficiency in the growing global population include dietary diversity, supplementation, fortification, and biofortification of food crops [6, 7]. This study addresses one of these strategies to enhance Fe and Zn in staple foods by selecting crops that have the potential to become a sustainable, inexpensive, and effective solution of addressing Fe and Zn deficiencies in people. One of the crops identified for biofortification is dry beans (*Phaseolus vulgaris* L.). This dry bean is a leguminous grain, which plays an important role in human nutrition being one of the main foods in the standard diet of low-income people in developing countries [8]. Dry bean is an important source of nutrients for more than 300 million people in parts of Eastern Africa and Latin America providing Fe, Zn, thiamin and folic acid [9]. It represents 65% of the total protein consumed [10, 11]. Despite the fact that dry beans are a rich source of Fe and Zn, some cultivars often take a long time to cook. This causes people not to add beans to their meals. Moreover, it requires the use of either gas, charcoal or firewood depending on the type of fuel source used [12,13]. In developed countries, consumers do not have time to invest in cooking [14] and hence the need for fast cooking dry beans to reduce the time of staying in the kitchen. For example, in the United States of America (USA). The term convenience is highly significant because the average household spends only 60 minutes per day on meal preparation [15]. Some studies showed that the iron bioavailability is also higher in the quicker cooking beans in different tested bean market classes [16]. Fast cooking with better micronutrient bioavailability has an impact on bean consumption. Therefore, in this study, our objective was to screen the dry bean germplasm for the identification of cultivars with low cooking time and enhanced micronutrient content through genotype and environment interactions.

MATERIALS AND METHODS

Acquisition of dry bean cultivars: A collection of 200 cultivars with diverse sources and market classes was used in this study. Twelve cultivars were collected from Ethiopia, 173 from International Center for Tropical Agriculture (CIAT)– Kawanda, 10 from Kenya, 3 from Tanzania and 2 from Rwanda based on the previous season under Tropical Legume three project. These cultivars were morphologically different from market classes to seed size classifications (Fig. 1).

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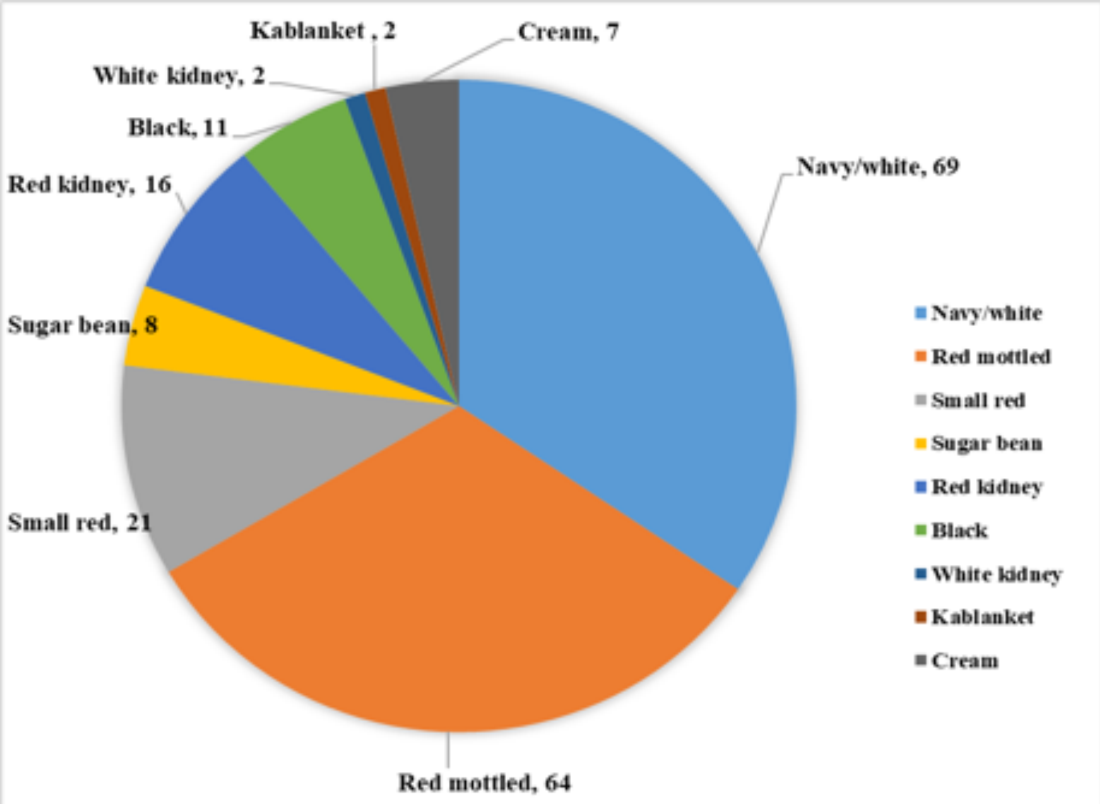

Figure 1: Dry bean market classes used in this research

Field experimental design. The experiment was conducted at low to high altitudes with 1407 m above sea level (a.s.l) of S03°21.690' and E36°37.879' Selian Agricultural Research Institute (SARI) and Tanzania Coffee Research Institute commonly called Lyamungo with 992 m a.s.l of S03°19.905'and E037°14.067', respectively. The soil characteristics of these areas were Eutrophic Brown Soils on volcanic and Alluvial sediments - medium texture (loamy soils), range of Fe is 29.85mg/kg to 39.24mg/kg and Zn is 0.33mg/kg to 0.60mg/kg [17]. Trials were laid out in Complete Randomized Block Design (CRBD) with two replications for 200 common bean cultivars in two agro-ecologies. The experimental plot size was 4 rows, 3.2 m long and 50 cm apart and 20 cm within a row and was under rainfed.

Sampling and sample preparation for Fe and Zn determination: At full maturity, 30 well-filled pods were randomly harvested from each plot of two centered rows and placed in a new clean paper envelope. Total of 100 seeds per cultivar were sampled and dried in an Oven (Binder drying chamber, Model: ED 115, Tuttlingen, Germany) for 12 hrs. at 60℃ for <5°C, dried seeds moisture was measured by Grain Moisture Tester – Soybeans (Baton cooperation. Model: 8500, Michigan 48084) and ground by Retsch SK 100 (Retsch GmbH, Model: SK 1001C, 42781, Haan, Germany) to produce bean seed flour. About 5-10 g of flour were scanned using X-ray Fluorescence (XRF) (Bruker AXS GmbH, Model: Tracer 5i, Östliche Rheinbrückenstr, 76187 Karlsruhe Germany) and read as Fe and Zn concentration mg/kg for each genotype from two different environments.

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Sampling and sample preparation for cooking time determination: Harvested seeds from similar plots were sun-dried and mixed in a paper bag sized 6.5 cm ×14 cm and stored at room temperature. When seed moisture reached 10-14%, 30 seeds from each genotype were selected and measured (g) using electronic scale by (SONASH ®, Model: SKS-006, Frances) after that these seeds were soaked in 150 ml of water in a plastic jar overnight; before they were soaked, the seeds were weighed again to quantify water uptake (ml). Afterwards, 25 seeds were placed on top of each well of Matson Cooker (Customized Machining and Hydraulics Co., Winnipeg, Canada) which consisted of a plate with 25 wells for individual seeds and 25 metal pins [18]. Cooking time in minutes was recorded from 1 to 20 pins drop; that is, the time it took for 80% of the seeds to be completely pierced with an 85 g stainless steel rod with a 2 mm pin [19], after which average minutes were determined per cultivar. Proportional (%) of water uptake was determined by using below fequation;

$$\text{water uptake (\%)} = \frac{\text{seed weight after soaking (g)} - \text{seed weight before soaking (g)}}{\text{seed weight after soaking (g)}} \text{x } 100$$

Data analysis
The Analysis of Variance (ANOVA) from the factors were used to determine the significant effects ($p<0.05$) among the tested environments, while Fisher's protected least significant differences (LSD) was used to identify the populations whose means differed statistically [20]. A built-in formula in excel analysis software and Pearson correlation was used for plotting graph, correlation, regression, scatter plots and pie charts.

RESULTS AND DISCUSSION

Dry bean market classes
Nine different market classes (Fig. 1) were used in this study for the wider chance to cover demand needs for specific regions, even though every region has its specific market class for dry bean cultivars. For instance, farmers in East African countries prefer red mottled, yellow and kablanket. In the USA, consumers prefer mostly pinto and navy beans, while preference in Ethiopia is navy/white beans. In this study, 77.00 % of the collection was composed of three market classes, navy/white beans (34.50 %), red mottled (32.00 %), and small red (10.50 %).

Iron and Zinc concentration levels of dry bean seeds
The variation of Fe and Zn concentration levels was significantly different ($p<0.001$) for genotype by environmental interactions. Under combined analysis, SWP 09 (large and white kidney type) and ZABR 16575-24F22 (navy/white small seeded type) at Lyamungo showed a higher concentration of Fe with 128.00mg/kg and 109.00mg/kg, respectively. At SARI, IBC 2 (navy/white and small-seeded) showed higher concentration with 113.50mg/kg, while the lower concentration of Fe observed in this cultivar at a different location may be attributed to environmental effect as the variation stated for locations at the methodology part. Results revealed that more cultivars had higher concentration levels at Lyamungo site compared to the SARI site. The majority

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of cultivars had the concentration level of 70-80 mg/kg within the range from the minimum concentration of 46.76 mg/kg to the maximum 107.25 mg/kg based on the combined data. Apart from this, about 96 cultivars showed their concentrations above the grand mean of 77.68 mg/kg Fig. 2 (I) and Table 1. For Zn concentration in dry bean, narrow variation among the cultivars was observed by a combined analysis that showed the maximum concentration 42.35 mg/kg and the minimum 21.70 mg/kg with the grand mean 31.19mg/kg. The concentration level of Zn was higher at SARI with value >42.00 mg/kg to some cultivars while at Lyamungo no cultivar reached this concentration Fig. 2 (II). The Fe and Zn concentration levels in seeds showed a positive correlation of 0.51 which means that Fe level is increasing at the same time Zn level increases.

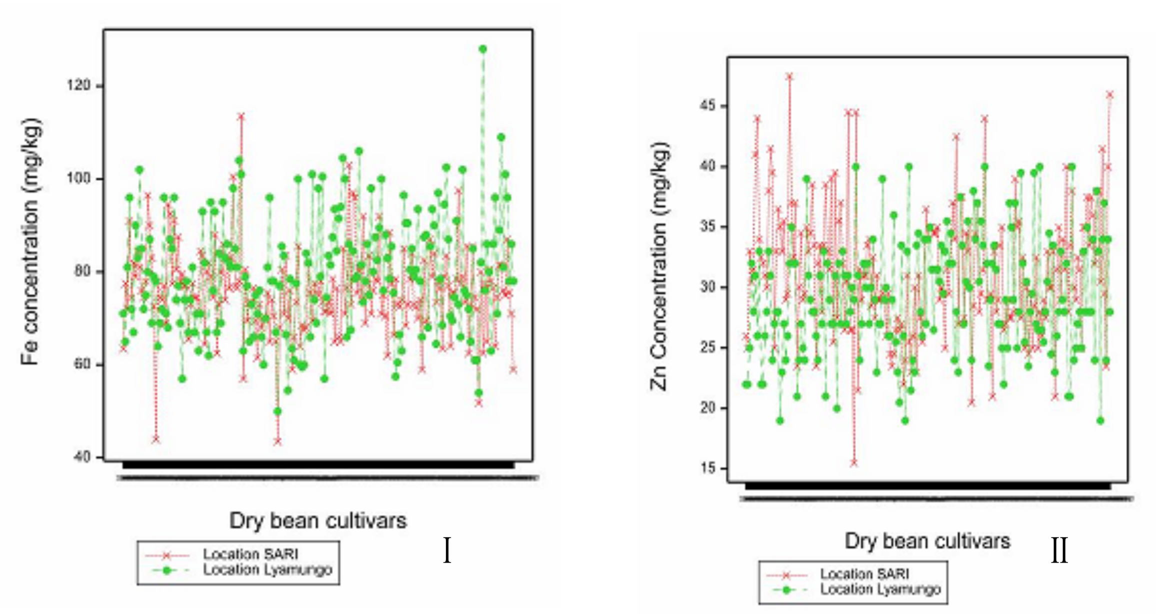

Figure 2: Iron concentration levels for genotype and environmental interactions (I), Zinc concentration levels for interaction of genotype and environment (II)

Water uptake and cooking time

Since the initial level of moisture across the samples ranged from 10-14%, then from the results it showed that the maximum water uptake was 61.54% for G 79 cultivar which is small white market class with sun-dry moisture level of 12.20%. The minimum water uptake was 3.7% for MAZ 37 with sun-dry moisture level of 12.45% which belongs to the large red kidney market class. About 83 cultivars imbibed more water at the proportion of 45% to 58% Fig. 3 (I). This reflected less cooking time compared to commonly grown cultivars Lyamungo 90 and Jesca in Tanzania, which showed water uptake of between 48 and 50%, and cooking time between 35 and 41 min, respectively. The higher the water uptake, the shorter the cooking time it takes among the cultivars tested. Generally, small seed-sized cultivars needed less cooking time due to more water imbibed than large-seeded cultivars. For instance, Awash-1 with 53% water uptake took 14.17 min cooking time, KG 24-43 with 47% water uptake took 18.47 min and Mexican 142 with 50% water uptake took 20.90 min. Cooking time ranged from 14 min to 75 min. About 60 cultivars from this collection took <30 min to cook and most of them were those with small seed size and with higher water uptake. About 73 cultivars ranged from

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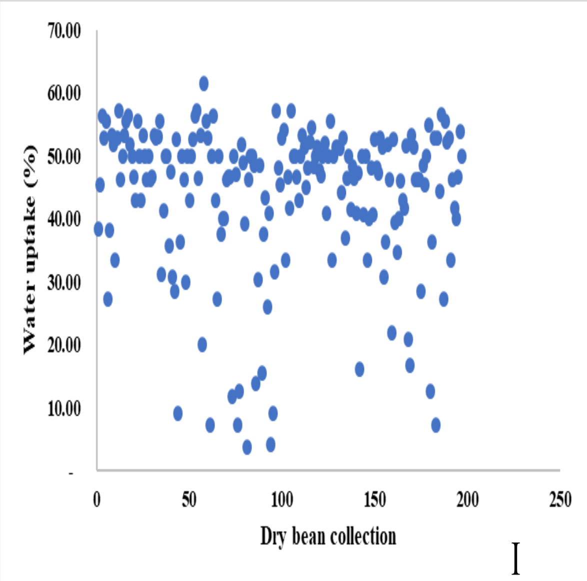
31 to 50 min for cooking time Fig. 3 (II). The analysis showed a negative correlation (r=-0.20) between cooking time and water uptake (p=0.01).



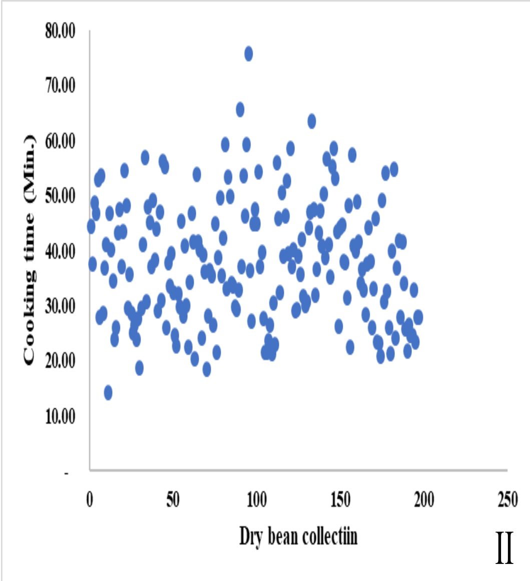


Figure 3: Illustration of Water uptake proportion (I) and Cooking time (II)

Nutrition and food systems are significant terms that are used by agricultural scientists, especially when they are conducting research for development in any field of preference. The 17 Sustainable Development Goals (SDGs) also emphasize food and nutrition through its 2nd and 3rd goals for zero hunger, good health and wellbeing, respectively for people [21]. Among the legumes that contribute to human nutrition, dry beans have been bred for Fe and Zn micronutrient contents. This study was to ascertain the Iron and Zinc contents of 200 cultivars collected from different places to ascertain whether they could contribute to the SDGs. This gave a wider opportunity of identifying specific market classes for specific bean growing region for improved livelihoods among the communities. This study is supported by that of Cichy et al [22] with a wider phenotypic diversity of different 206 accessions and that of Mahajan et al [23], which were looked for linkage disequilibrium based association mapping of micronutrients in different market seed classes of dry beans. In this research study, we identified some of the bean cultivars containing higher Fe and Zn concentrations from seeds although there were variations in different environments. These data are supported by other similar research conducted in Uganda [24] that showed seed concentration of up to 88 mg/kg and 41mg/kg for Fe and Zn, respectively. Also, in Tanzania, biofortified climbing beans with 80.30 mg/kg Fe [25], India [23], Rwanda and Democratic Republic of Congo with 94 mg/kg Fe [26] were discovered and now are being used by end users. Based on these results, there is a positive correlation between Fe and Zn concentrations in seeds, which implied that genetic factors for high Fe concentration are co-segregated with genetic factors for high Zn concentration. It could lead to a good strategy to select higher concentrations of both Fe and Zn in the dry bean breeding programs. It has also been proven that selecting for a higher Fe level in bean seeds also tends to select for increased Zn levels in crop seeds [27, 28]. The identified cultivars with high Fe and Zn can be used

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as parents in the breeding program for increasing Fe and Zn levels in specific market classes through either classical breeding or genetic engineering technologies.

In the same market class, some cultivars could be cooked in a shorter time than others. In this study, the shortest cooking time was ~15 min while the longest was ~ 76 min. This suggested that, in this pool of germplasm, there are cultivars of fast cooking that can be used to improve those hard cooking cultivars though their physiological and genetic mechanisms are needed to be explored. Furthermore, some cultivars were identified as possessing both desirable traits, short cooking time and higher micronutrient levels, in this study. For instance, Ranjonomby took ~24 minutes and had 90.50 mg/kg of Fe and 31.65 mg/kg of Zinc, while DOR 711took ~56 minutes and had 73.25mg/kg of Fe and 27.53mg/kg of Zn (Table 1). This result was also supported by some studies that proved that the fast cooking seeds contained 20% more protein, 10% more iron and 10% more zinc than those that took twice as long to prepare [30] and the same study demonstrated that the iron bioavailability was higher in the quicker cooking cultivars. Seeds having a longer cooking time might cause the loss of their nutritional values during overtime cooking. A study conducted by Cichy *et al.*[22] analyzed the nutritional value of twelve dry bean cultivars with fast, moderate, and slow cooking time selected from four classes of yellow, cranberry, light red kidney and red mottled. Other researchers explained that the fast cooking bean cultivars maintain nutrients including protein and mineral contents after they are prepared than the moderate and slow cooking cultivars [16].

CONCLUSION

The selection of high Fe content in dry bean breeding could indirectly select high Zn concentration due to their positive correlation. Small seeds quickly imbibe water and lead to a short cooking time compared to large seeds. Cultivars possessing both high micronutrient levels and short cooking time were screened out by farmers. The resulting information in this study provides the benefit to consumers and dry bean breeding program for a better source of materials. These cultivars can be used as parents for Fe and Zn sources to improve micronutrient levels in the breeding program. From this study, the future research suggested will be to consider Fe bioavailability and protein contents for the few identified cultivars with enhanced Fe and Zn concentrations and fast cooking time.

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Table 1: Combined data for Fe and Zn grain concentration, water uptake and cooking time

S/N	Cultivar	Fe (mg/kg)	Zn (mg/kg)	W/uptake (%)	C/time (min)
1	217/2	67.25	24.11	38.46	44.26
2	222/1	71.25	23.56	56.25	48.63
3	296/6	76.50	29.44	52.94	46.70
4	A 686	93.50	31.33	55.56	52.90
5	A 774	73.25	29.83	27.27	27.86
6	A 797	74.50	36.58	38.10	53.55
7	ALS 3	84.50	37.07	53.33	28.52
8	AMENDON	83.75	33.53	51.85	36.84
9	Awash Meka	91.50	28.09	33.33	41.03
10	Awash-1	80.50	27.76	52.94	14.17
11	BAT 332	73.25	28.66	57.14	46.82
12	C.2014/Hu/11	76.50	29.04	46.15	40.03
13	C.2018/Hu/11	88.25	35.64	50.00	34.48
14	C.2019/Hu/11	88.50	36.89	53.33	23.70
15	C.202/Hu/3	76.00	33.41	56.25	43.21
16	CAL 113	75.75	26.12	51.72	47.42
17	CANPSULA	61.00	30.65	50.00	37.00
18	CC 13	71.00	32.70	46.67	43.28
19	CC 547	71.75	29.08	42.86	54.47
20	CC 814	73.00	28.73	55.56	48.12
21	CC 906	86.50	31.72	50.00	29.40
22	CIM 9313-1	70.00	26.67	42.86	35.57
23	CN Bunsi (60)	81.25	27.83	53.33	28.42
24	CN Bunsi (62)	89.75	41.08	50.00	25.10
25	CN Bunsi (63)	81.25	36.04	46.15	26.30
26	CN Bunsi (64)	93.50	32.28	50.00	23.89
27	CN Bunsi (66)	82.25	22.30	46.67	18.58
28	CN Bunsi (68)	73.50	27.25	53.33	29.39
29	CORNELL 49822	68.25	31.11	52.94	41.11
30	CZ 102-24	75.50	27.10	53.13	56.94
31	CZ 108-27	77.50	26.96	55.56	30.66
32	CZ 114-46	66.25	37.21	31.25	47.83
33	CZ 114-50	73.50	32.04	41.18	45.07
34	CZ 114-51	79.25	31.93	50.00	37.13
35	CZ 114-8	70.75	33.94	50.00	49.11
36	DONTIMOTEO	72.75	30.94	47.37	43.76
37	DOR 662	67.25	24.91	30.77	28.96
38	DOR 708	77.75	28.45	28.57	46.88

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39	DOR 710	88.25	32.29	52.63	30.98
40	DOR 711	73.25	27.53	9.09	56.18
41	DOR 755	73.50	33.28	36.36	55.20
42	DOR 766	72.00	32.03	35.71	38.16
43	F1POPULATION	87.00	32.53	50.00	25.85
44	FEB 181	75.25	30.30	46.15	37.65
45	FEB 189	90.50	33.93	30.00	33.45
46	FLO DE MAYO	64.75	26.34	50.00	39.47
47	G 100	78.50	35.67	42.86	32.20
48	G 23	74.50	24.20	50.00	24.49
49	G 30	90.50	33.82	52.63	22.56
50	G 31	78.50	32.65	56.25	31.99
51	G 5	84.75	32.56	57.14	29.82
52	G 5686	79.25	29.05	46.43	45.34
53	G 60	82.00	28.83	53.33	28.13
54	G 78	99.25	38.80	20.00	40.84
55	G 79	81.00	27.34	61.54	29.84
56	G 87	78.75	28.47	55.56	22.43
57	G 90	91.00	24.68	52.94	34.11
58	IBC 2	107.25	42.35	7.14	46.61
59	JESCA	60.00	27.35	50.00	41.52
60	KABALABALA	79.75	28.14	56.25	20.20
61	KG 114-177	73.25	28.04	42.86	53.83
62	KG 114-178	69.25	31.57	27.27	41.62
63	KG 114-179	73.25	30.53	50.00	40.17
64	KG 114-182	71.25	32.78	37.50	24.00
65	KG 114-185	72.25	30.81	40.00	39.25
66	KG 15-6	66.25	29.34	40.00	36.19
67	KG 24-43	74.50	33.33	46.15	18.47
68	KG 30-29	67.00	28.34	46.67	28.02
69	KG 4-20	65.25	27.47	46.67	36.26
70	KG 4-3	69.75	28.04	11.76	35.44
71	KG 67-10	78.25	28.04	50.00	26.31
72	KG 67-11	80.50	35.10	47.06	44.85
73	KG 67-5	76.00	29.53	7.14	21.53
74	KG 71-4	74.75	28.00	28.57	56.25
75	KG 75-5	74.25	28.26	12.50	38.67
76	KG 97-11	66.00	27.83	51.72	49.48
77	LYAMUNGO 90	46.76	25.35	48.84	35.43
78	MAZ 37	72.50	26.70	3.70	59.25
79	MAZ 41	83.00	31.60	46.15	33.10

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80	MAZ 42	77.25	26.03	50.00	53.23
81	MAZ 44	75.00	25.39	50.00	49.72
82	MAZ 46	62.25	23.78	48.57	34.05
83	MAZ 47	77.25	30.76	13.79	33.49
84	MAZ 48	61.25	24.30	30.23	29.80
85	MAZ 49	63.50	21.70	48.48	29.31
86	MAZ 50	75.50	32.11	15.38	32.85
87	MAZ 52	92.75	34.64	37.50	65.62
88	MAZ 56	62.00	22.28	43.33	36.93
89	MAZ 57	64.00	25.03	26.09	53.50
90	MAZ 59	69.00	29.00	23.53	53.00
91	MAZ 70	63.75	26.85	40.91	46.17
92	MAZ 72	76.00	29.47	4.00	59.13
93	MAZ 74	82.00	32.94	9.09	75.75
94	MEX 54	70.75	27.80	31.58	36.34
95	MEXICAN 142	90.00	39.00	50.00	20.90
96	MICHETTE	85.25	25.80	57.14	27.16
97	MLB 17-89A	75.75	31.07	48.15	44.75
98	MLB 40-89A	72.25	32.33	45.45	47.39
99	MLB 48-89A	86.50	34.04	52.94	44.71
100	MONT-CALM	78.50	35.04	54.05	54.26
101	Navy line 15	87.00	33.32	46.67	39.62
102	Navy line 19	64.25	30.92	41.67	27.63
103	Navy line 22	97.00	31.50	45.45	52.02
104	Navy line 25	73.00	33.06	57.14	21.50
105	Navy line 38	78.50	33.32	50.00	21.49
106	Navy line 40	76.30	29.84	33.33	37.03
107	Navy line 43	83.00	31.95	50.00	23.62
108	Navy line 48	79.25	29.54	46.67	26.44
109	Navy line 5	65.50	27.00	55.00	24.37
110	Navy line 51	80.50	29.75	42.86	21.16
111	Navy line 52	78.75	33.73	50.00	30.30
112	Navy line 54	79.50	30.89	53.33	22.79
113	NUA 11	94.75	33.38	51.43	55.95
114	NUA 110	85.50	34.64	44.90	45.70
115	NUA 116	71.25	29.71	52.17	50.55
116	NUA 117	94.50	36.55	54.55	39.00
117	NUA 125	70.25	25.11	48.39	46.25
118	NUA 129	90.75	37.54	50.00	52.51
119	NUA 13	83.50	35.00	52.63	38.75
120	NUA 130	87.25	35.59	51.43	39.30

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121	NUA 134	77.25	27.28	47.62	58.60
122	NUA 137	93.25	32.59	46.88	37.05
123	NUA 145	77.75	34.38	50.00	40.10
124	NUA 15	82.75	32.93	52.00	28.94
125	NUA 152	73.50	22.52	40.91	29.25
126	NUA 156	81.75	34.13	50.00	38.90
127	NUA 158	79.25	34.28	55.56	35.70
128	NUA 16	84.50	37.04	33.33	42.05
129	NUA 160	78.00	29.39	50.00	31.65
130	NUA 161	83.00	32.92	51.35	30.00
131	NUA 163	80.25	32.61	51.35	30.60
132	NUA 165	90.75	42.08	51.22	44.10
133	NUA 17	85.50	30.67	44.12	46.95
134	NUA 18	75.50	26.46	52.94	63.54
135	NUA 19	79.25	29.28	37.04	47.40
136	NUA 200	72.50	27.91	46.43	31.94
137	NUA 204	83.50	33.30	50.00	36.45
138	NUA 207	81.50	29.96	41.46	43.15
139	NUA 209	71.00	28.04	48.39	47.20
140	NUA 210	68.00	30.52	46.43	40.85
141	NUA 211	66.75	30.69	40.91	50.30
142	NUA 212	69.50	24.39	47.22	38.60
143	NUA 213	68.50	28.12	16.00	56.70
144	NUA 224	90.75	27.10	50.00	41.10
145	NUA 225	79.50	33.15	40.74	35.05
146	NUA 226	81.75	31.87	50.00	55.10
147	NUA 229	88.50	29.50	46.43	38.80
148	NUA 23	79.50	28.57	33.33	58.60
149	NUA 231	84.50	38.04	40.00	53.00
150	NUA 232	76.00	30.69	48.15	43.35
151	NUA 233	76.50	32.10	40.74	26.20
152	NUA 235	81.75	34.82	52.63	44.00
153	NUA 236	76.00	32.28	48.15	44.60
154	NUA 238	62.50	25.30	47.17	38.05
155	NUA 240	78.75	24.03	51.35	31.45
156	NUA 244	68.25	28.78	30.77	48.15
157	NUA 245	86.25	27.57	36.36	22.45
158	NUA 256	86.25	35.11	51.72	57.40
159	NUA 257	87.00	29.94	46.15	40.75
160	NUA 272	65.50	34.00	52.63	42.65
161	NUA 273	69.00	25.84	21.95	39.90

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162	NUA 30	87.50	35.30	52.63	48.74
163	NUA 31	77.50	26.30	39.39	41.60
164	NUA 39	66.00	27.33	34.62	33.85
165	NUA 40	86.75	27.80	40.00	36.55
166	NUA 48	93.00	31.79	45.95	32.75
167	NUA 57	82.00	32.53	42.86	28.25
168	NUA 64	67.25	27.45	41.67	37.40
169	NUA 66	112.00	37.00	51.52	39.66
170	NUA 67	72.50	33.30	51.61	44.20
171	NUA 9	75.00	22.14	20.83	37.95
172	PAN 72	84.75	28.95	16.67	26.00
173	PI 207262	85.25	31.80	53.33	32.98
174	R.K. MICHIGA	71.75	31.24	51.35	45.82
175	RANJONOMBY	90.50	31.65	46.15	23.42
176	RAZ 36	74.75	29.83	46.15	23.19
177	RAZ 44	80.25	36.36	46.15	20.69
178	RRN 47	67.25	24.88	28.57	49.03
179	RRN 48	69.00	28.47	48.48	30.76
180	RWR 1059	85.25	39.09	45.45	54.12
181	RWR 2075	67.75	27.25	50.00	32.52
182	SAB 662	66.50	27.10	55.00	25.97
183	SELIAN 05	52.90	29.94	12.50	21.17
184	SM 133	81.00	29.83	36.36	39.87
185	SWP 09	95.25	25.04	52.94	54.83
186	SWP 10	76.75	34.04	7.14	24.08
187	SWP 12	75.50	31.80	52.94	36.76
188	TU	80.25	36.29	44.44	41.63
189	VAX 1	70.50	30.94	56.52	27.76
190	VAX 2	81.25	33.32	27.27	41.55
191	ZABR 16573-78F22	80.00	35.04	55.56	33.98
192	ZABR 16575-11F22	78.50	37.19	52.94	21.58
193	ZABR 16575-17F22	85.25	38.04	33.33	26.43
194	ZABR 16575-24F22	95.25	35.64	46.15	24.51
195	ZABR 16575-39F22	78.50	25.87	41.67	24.40
196	ZABR 16575-46F22	72.75	28.45	52.17	25.61
197	ZABR 16575-51F22	88.00	38.05	40.00	32.70
198	ZABR 16575-60F22	91.50	33.84	46.67	23.40
199	ZABR 16575-86F22	76.75	23.80	53.85	27.75
200	ZABR 16577-51F22	68.50	38.96	50.00	27.86
	Mean	77.68	31.19	43.63	37.74
	SGE (5%)	<0.001	<0.001	0.01	0.01

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