Genotype $\times$ Environmental Effects on Food Quality of Common Bean: Resource-efficient Testing Procedures

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Abstract. Genetic and environmental interactions for bean cooking time, water absorption, and protein content were estimated with 10 dry bean (*Phaseolus vulgaris* L.) cultivars grown at three locations in Rwanda, Africa, during five consecutive harvests. The genotypic variance component was larger than genotype $\times$ environment variance components for the cooking time index and percent water absorption. No significant genotypic effect was observed for seed protein content. The phenotypic correlation (-0.37) between the cooking time index and percent water absorption was not strong enough to justify the use of water absorption as an indirect selection method for cooking time. The most efficient allocation of resources to evaluate the cooking time of common bean cultivars with a 25-pin bar-drop cooker was four field replications over two harvests at two locations. Water absorption was evaluated most efficiently with four field replications over two harvests at a single location.

The protein content and cooking time of common bean are important in many countries in Africa and Latin America where the dry seeds are a dietary staple and firewood is the main fuel source used for cooking (Shellie-Dessert and Bliss, 1990). Dry bean provides more than one-half of the dietary protein, and at least one-quarter of calorie requirements for people in Rwanda, a small country in central Africa (MINIPLAN, 1988). Rwandan household fuel-wood requirements are largely determined by how often beans are cooked and the cooking time required to render the beans palatable. The rate of deforestation in Rwanda currently exceeds that of afforestation programs (Sirven, 1981). An annual savings of 150,000 Mg of wood was estimated for Rwanda if the country's 1.1 million rural households were to adopt cultivars that cooked more quickly than those currently produced and consumed (Shellie-Dessert and Hosfield, 1990).

A wide array of local cultivars is grown throughout Rwanda, each reflecting local consumer preferences and natural selection for types that are relatively successful under variable and often unfavorable conditions (Shellie-Dessert and Hosfield, 1990). The culinary and nutritional quality traits of the dry seeds of *P. vulgaris* can be manipulated by breeders if extant genotypic variability is significant with respect to environmental effects, and if a useful screening method is available for selection of the traits. Variability in culinary and nutritional traits of beans has been reported (Hosfield and Uebersax, 1980; Hosfield et al., 1984). However, information is scarce regarding the heritability of food quality traits over a range of grain types.

Variance components have been used to estimate the most efficient allocation of locations, years, and replications necessary for testing and selecting genotypes with improved plant characteristics. Fernandez and Chen (1989) used variance components to ascertain efficient resource allocation for mungbean (*Vigna radiata* L.) yield trials. Variance components were used by Campbell and Kern (1982) to establish an efficient quality testing program for sugarbeet (*Beta vulgaris* L.).

Evaluation of common bean genotypes for cooking time at the International Center for Tropical Agriculture (CIAT) in Cali, Colombia, is based on a cooking time index derived from a bar-drop cooker (Jackson and Varriano-Marston, 1981). Although this method (Jackson and Varriano-Marston, 1981) is useful and produces reliable data, the technique is laborious and time consuming for many samples. It has been suggested that the amount of water dry beans absorb during soaking before cooking may be indicative of the amount of time required to render them soft enough to eat. Hence, the water absorption of a genotype may be a useful and rapid indirect selection method to screen germplasm for cooking time.

The objectives of this study were to: 1) estimate genotype and genotype $\times$ environmental components of variance for cooking time, protein content, and water absorption; 2) investigate the usefulness of water absorption as a rapid, indirect method of screening for cooking time; and 3) use variance component estimates to determine the most efficient temporal and spatial allocation of resources for testing dry bean food quality.

Materials and Methods

Ten high-yielding, tropically adapted cultivars representing several grain types were obtained from the Institut Scientifique de Recherche Agronomique au Rwanda (ISAR). The cultivars, which were unselected for protein content and culinary quality traits, were Black ('Kibobo' and 'Ikinimba'), Creme/Red Mottled ('Rubona 5', 'Calima', 'Mutiki 2', 'Var 11', and 'Nyrakizungu'), Red Kidney ('Kilyumukwe'), Pinto ('Nsizebashonje 4'), and Cranberry ('GLPX-1124') grain types.

Field experiments were conducted at the ISAR research stations at Rubona (1700 m elevation), Karama (1400 m), and Rwerere (2300 m) in Rwanda during the first and second har-

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vests (September to January; March to June, respectively), in 1984, 1985, and the first harvest of 1986. Annual precipitation (1200 mm, 10-year average) and edaphic factors (ultisols, pH 5.5, 3% organic matter) were similar at the Rubona and Rwerere stations. Karama received less annual precipitation (811 mm, 10-year average), and had different edaphic conditions (oxisols, pH 5.0, 1.5% organic matter). Average annual temperature (10-year average) varied inversely with elevation (Rwerere 16C, Rubona 19C, Karama 22C). The cultivars were planted in single-row, 4-m plots and arranged in a randomized complete-block design with three replications. Rows were spaced 45 cm apart, and the within-row spacing was 10 cm.

The moisture content of the seeds was equilibrated to $\approx 11\%$ before protein analysis. Nitrogen determinations were performed on triplicate samples from each field replication in each of three locations after the first season of 1984, and the first season of 1985. Seeds were ground in a hammermill to ≈ 40 μ m particle size and the percent N of the bean flour was determined by an automated micro-Kjeldahl method (Kelly and Bliss, 1975). Percent protein was calculated by multiplying grams of N by 6.25.

The moisture contents of the dry bean samples were equilibrated to each other before analysis of water absorption and cooking time by storing them for 2 weeks in sealed plastic buckets at ambient (≈ 20 C) temperatures and relative humidity ($\approx 70\%$). The percent water absorption was determined by first soaking 30 seeds for 16 h in deionized water at room temperature and dividing the difference in weight before and after soaking by the dry weight of the 30-seed sample. Water absorption determinations were expressed in percentages and made on duplicate samples from each field replication per location in 1985 and 1986. Cooking time was estimated from the cooking time index determined with a 25-seed bar-drop cooker (Jackson and Varriano-Marston, 1981). The 25-seed bar-drop cooker is a miniaturization and modification of the experimental laboratory bean cooker designed by Mattson (1946) and modified by Burr et al. (1968). Twenty-five seeds that did not possess the hard seed coat defect (Gloyer, 1928) were selected from the imbibed, 30-seed sample used for water absorption determinations. The seeds expressing the hard seed coat trait were not evaluated for cooking time and water absorption because the failure to imbibe water masked the genetic potential of these quality traits. The cooking time index was calculated as the elapsed time from initiation of cooking until 13 of the 25 penetrating bars had dropped and perforated seeds in the cooker. Cooking time determinations were made on a single sample from each field replication per location for the first season of 1984, and from duplicate samples from each field replication per location in 1985 and 1986.

Statistical analysis. All data were subjected to analyses of variance (ANOVA) appropriate to a randomized complete-block design. A combined ANOVA following the outline of Miller et al. (1959) was used to calculate variance components. Replications, harvests, and locations were considered to be random effects in the mathematical model. Since the 10 entries were selected because of their yield and adaptation, genotypes were fixed. The ANOVA table and the expected mean squares are presented in Table 1. An approximate F test (Satterthwaite, 1946) was used to test the genotypic main effect. The least significant difference (LSD) test criterion ($P < 0.05$) was used to evaluate differences between genotypic means. Separate estimates of the components of variance were obtained to evaluate the relative magnitude of the different effects by algebraic ma-

Table 1. General form of the combined ANOVA for the cooking time index and water absorption of 10 cultivars grown over multiple locations and harvests in Rwanda.

Source of variation	df*	MS	Mean square expectation ^y
Harvest (H)	(h-1)		
Location (L)	(l-1)		
H $\times$ L	(h-1)(l-1)		
Rep/(H $\times$ L)	(r-1)hl		
Genotype (G)	(g-1)	M_5	$\sigma_e^2 + r\sigma_{ghl}^2 + rh\sigma_{gl}^2 + rl\sigma_{gh}^2 + rhl\sigma_g^2$
G $\times$ H	(g-1)(h-1)	M_4	$\sigma_e^2 + r\sigma_{ghl}^2 + rl\sigma_{gh}^2$
G $\times$ L	(g-1)(l-1)	M_3	$\sigma_e^2 + r\sigma_{ghl}^2 + rh\sigma_{gl}^2$
G $\times$ L $\times$ H	(g-1)(l-1)(h-1)	M_2	$\sigma_e^2 + r\sigma_{ghl}^2$
Error	(g-1)(r-1)hl	M_1	σ_e^2

*h, l, g, and r are numbers of harvests, locations, genotypes, and replicates, respectively; harvests and locations are random variables and genotypes are fixed in the model.

^y σ_e^2 = error variance; σ_{ghl}^2 = variance due to interaction of genotypes, harvests, and locations, etc.

Table 2. Mean squares from the combined ANOVA for cooking time index, water absorption, and protein content of 10 dry bean cultivars grown in Rwanda.

Source ^z	Cooking time		Water absorption		Protein content	
	df	MS	df	MS	df	MS
H	3	1830*	2	68	1	118
L	2	1152	2	2104*	2	320
H $\times$ L	6	260**	4	317	2	28.4**
R/(L $\times$ H)	24	67	18	157	12	2.8
G ^y	9	647**	9	4521**	9	5.2
G $\times$ H	27	103	18	337**	9	7.8**
G $\times$ L	18	60	18	154	18	2.7
G $\times$ L $\times$ H	54	66**	36	139	18	2.2**
Error	216	31	162	105	108	0.9

^zH, L, G, and R are numbers of harvests, locations, genotypes, and replicates, respectively.

^ySignificance determined by Satterthwaite's (1946) quasi F ratio.

***Significant at $P = 0.05$ and 0.01 , respectively.

nipulation of terms comprising the expected mean squares. The standard error of variance components was computed according to Christie et al. (1988). Variance component estimates were used to calculate the theoretical variance of a genotypic mean (G_x) for different combinations of harvests (1 to 5), locations (1 to 5), and replications (2 to 5) using the following equation:

$$G_x = [(\sigma_{gh}^2/h) + (\sigma_{gl}^2/l) + (\sigma_{ghl}^2/hl) + (\sigma_e^2/rlh)]$$

where h, l, and r are the number of harvests, locations, and replications, respectively. Pearson correlation coefficients for water absorption and cooking time were calculated based on 270 observations using the software program SAS (SAS, 1985).

Results and Discussion

Genotype $\times$ environment interactions

The second harvest in 1984 was eliminated from the analysis because drought caused a loss of $> 30\%$ of the trial. Genotypic mean squares from the combined ANOVA for cooking time and water absorption were highly significant despite significant environmental effects (Table 2). The variance components for genotypes accounted for 25% and 52% of the total variance for

cooking time and water absorption, respectively (Table 3). The genotypic effect for protein content was not significant (Table 2), and the genotypic variance component was zero (Table 3). Environmental effects were unique for each of the culinary and nutritional traits measured.

Cooking time. The genotypic variance component exceeded that of the genotype $\times$ location and genotype $\times$ harvest interaction components (Table 2). Genotype $\times$ location and genotype $\times$ harvest variance components accounted for only 0% and 7% of total variance, respectively (Table 3), indicating no consistent location or harvest effect on genotypic response. The significant second order interaction of genotypes, locations, and harvests (19% of total variance) demonstrated that location and harvest caused changes in the relationships among cultivars for cooking time (Tables 2 and 3). However, the genotypic variance component (24% of total variance) exceeded that of first and second order interaction variance components. The relatively consistent genotypic response observed among multiple locations and harvests indicated that general genotypic recommendations can be made for the region and for future harvests.

A 12-min range among cultivars for the cooking time index was observed (Table 4). Shellie-Dessert and Hosfield (1990) showed an average fuelwood savings of 1.3 kg per cooking session associated with a 15-min reduction in cooking time. A 12-min range is of sufficient magnitude to indicate that fast-cooking cultivars could lead to fuel-wood conservation.

Water absorption. The location main effect for water absorption was significant (Table 2), and the amount of water absorbed

Table 3. Estimates of variance components scaled to sum to 100 for quality traits of 10 dry bean cultivars grown in Rwanda at three locations over multiple harvests.

Trait	Component ^z					Total
	g	gh	gl	ghl	e	
Cooking time	24 ^y	7	0 ^x	19 ^y	50	61.75
Water absorption	52 ^y	8	1	4	36	294.53
Protein content	0 ^x	31	5	22	42	2.03

^zg = genotype, l = location, h = harvest, e = error.

^yVariance component estimate equal to or greater than twice the SE of the estimate.

^xSmall negative values or zero estimates of variance components.

Table 4. Mean performances of 10 dry bean cultivars grown in Rwanda for three food quality traits evaluated in three locations during multiple seasons.

Cultivar	Cooking time index ^z (min)	Water absorption ^y (%)	Protein ^x (%)
Kibobo	46	76.5	21.1
Rubona 5	45	106.8	21.4
Ikinimba	44	67.7	21.4
Kilyumukwe	41	102.5	21.5
Nsizebashonje 4	40	89.9	20.3
GLPX-1124	39	93.4	20.0
Mutiki 2	36	100.0	21.7
Var 11	36	98.8	20.8
Nyrakizungu	35	103.5	20.8
Calima	34	104.3	21.1
LSD (0.05)	5	10.7	NS

^zCooking time index determinations are based on four seasons (1984a, 1985a, 1985b, 1986a).

^yWater absorption based on three seasons (1985a, 1985b, 1986a).

^xProtein content based on two seasons (1984a, 1985a).

corresponded with the average mean temperature and precipitation of a particular location (data not shown). Seed grown at Karama, the driest and warmest location, absorbed the largest amount of water; and seed from Rwerere, the coolest, moistest location, absorbed the least. Morris et al. (1950) found that the amount of water absorbed after overnight soaking was similar in samples stored at low and high humidities. This finding suggested that the location effect on water absorption was due to some factors intrinsic to the seed and not to the moisture content of the seed at the beginning of this experiment.

The genotype $\times$ harvest interaction for water absorption was significant (Table 2), although the variance component only accounted for 8% of the total variance (Table 3). Since the genotypic variance component was 52 and seven times as large as the genotype $\times$ location and genotype $\times$ harvest components, respectively, it is reasonable to make general cultivar recommendations for water absorption and harvests for the region.

Relationship between cooking time index and water absorption. The significant ($P < 0.01$), negative phenotypic correlation (-0.37) between the cooking time index and percent water absorption for 270 observations indicated that slow-cooking beans tended to imbibe less water than fast-cooking beans. Since the seeds that did not imbibe water after overnight soaking (hard seed) were eliminated from the cooking evaluation, the negative correlation between water absorption and cooking time observed in this study may have been larger if selection against the hard seed coat trait had not been made. However, the hard seed coat trait biases the assessment of the genetic potential of a cultivars cooking time. The genetic potential for cooking time and for the hard seed coat trait are unique quality characteristics and require independent evaluation.

Agbo et al. (1987) showed differences in bean seed micropyle orifice dimension, the presence and number of seed coat pores, and microstructural differences that were related to seed coat water uptake. The microstructural differences were related to water imbibition and textural characteristics of cooked-bean genotypes. When beans are cooked, native protopectin within the middle lamella forms a soluble pectin that depolymerizes rapidly during heating and allows water to quickly enter and migrate throughout cotyledonary cells (Stanley and Aguilera, 1985). A high state of cellular hydration and heating thus allows cells to soften and separate. Reduced imbibition and/or compositional differences in pectins could be major factors affecting cooking time.

Water absorption explained only 14% of the variability in cooking time ($R^2 = 0.137$); thus, the phenotypic correlation (-0.37) between water absorption and cooking time index was too low to justify the use of percent water absorption as an estimate of cooking time. For example, the average water absorption for the five slowest-cooking cultivars was 89%, whereas the five fastest cultivars absorbed 100% of their dry weight (Table 4). If selection for fast cooking were made on the basis of percent water absorption, 'Rubona 5', the second longest-cooking cultivar, would have been selected with 'Calima', the fastest-cooking cultivar. 'Kibobo' and 'Rubona 5' had an average cooking time of 46 min and water absorption of 76.5% and 106.8%, respectively. A similar overlap with a fast- and slow-cooking cultivar for water absorption was noted for 'Kilyumukwe', a bean with an intermediate cooking time (41 min). 'Rubona 5', 'Kilyumukwe', and 'Calima' all had similar water absorption, yet they differed significantly in cooking time.

Protein content. There were no significant differences noted

among cultivars for protein content, but significant genotype $\times$ environment interactions were observed (Table 2). The effect of environmental influences on protein content we found in this study agrees with other findings of genotype $\times$ environment interactions on protein content (Kelly and Bliss, 1975; Leleji et al., 1972; Rutger, 1970). Seed protein of beans has been reported to vary from a low of 19% (Rutger, 1970) to a high of 34% (Woolfe and Hamblin, 1974). The mean and range of protein content among the cultivars used in this study (Table 4) were similar to the observations reported by Rutger ($\approx 20\%$), but the range in protein content was expected to be greater, given the diversity of grain types and cultivars we used.

The large environmental influences on bean seed protein indicate that selection for high protein will be difficult in this material. A more useful goal may be to maintain an acceptable level of seed protein (20% to 22%) while selecting for agronomically superior, fast-cooking cultivars. Increasing the yield of common bean will increase calorie and protein availability if the protein content is maintained at its current level.

Resource-efficient testing procedures

Variance component estimates of genotypic means for water absorption and the cooking time index were used to illustrate the decrease in the expected variance of a cultivar mean as the number of locations, harvests, and replications increased (Fig. 1). The decrease was plotted until it reached a point beyond which additional locations, harvests, and replications had no further pronounced effect on reduction of the variance. It is desirable to select the minimum number of replications, locations, and harvests needed to obtain a reasonable level of precision, yet remain within the resource constraints of a crop improvement program.

The evaluation of cultivars for percent water absorption over two instead of one harvest when the number of replications and locations was kept constant at three and one, respectively (Fig. 1, Δ vs. $\square$), increased precision $\approx 50\%$ ($70 G_x$ to $36 G_x$). Evaluation of water absorption over three harvests (Fig. 1, $\square$ vs. $\blacksquare$) increased precision by $\approx 30\%$, but this increase in precision was not of sufficient magnitude to warrant the additional required resources. When the number of locations and harvests was kept constant at one and two, respectively, the reduction in variance due to increasing the number of replications from two to three was $\approx 20\%$ ($45 G_x$ to $36 G_x$). The addition of a fourth replication increased precision only 11% ($36 G_x$ to $32 G_x$).

Fig. 1. Expected variance of a genotypic mean for water absorption and cooking time. The letters h and r indicate harvests and replications, respectively.

G_x). A four-replication experiment for the evaluation of water absorption would be advantageous from the standpoint of ensuring that an experimenter obtained data from at least three replications. Unpredictable conditions and climatic factors in many tropical environments often lead to missing data. A four-replication experiment would ensure the salvage of three replications and a sufficient degree of experimental precision to warrant reliable conclusions. When the number of locations was increased from one to two, with two harvests and four replications, the increased precision for evaluating the water absorption trait was $\approx 15\%$ ($36 G_x$ to $21 G_x$). This result indicated that an additional replication increased precision more efficiently than an additional location, because adding a replication to a study is less costly than adding a test site.

The substantial genotype $\times$ harvest $\times$ location interaction for the cooking time index indicated that it is best to evaluate the cooking time of cultivars over a minimum of two harvests and two locations. When the replication number was kept constant at four, the reduction in variance due to increasing the number of harvests and locations from one to two was 50% ($24 G_x$ to $12 G_x$) and 42% ($24 G_x$ to $14 G_x$), respectively. The increased precision for cooking time index with four vs. three replications was small, but as with the water absorption trait, four replications are recommended as a safety factor for field plot procedures.

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