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Variation of Seed Traits and Initial Quality among Selected Cowpea, Mungbean, and Soybean Accessions

Sognigbé N'Danikou *, Abdul J. Shango and Jeremiah P. Sigalla

World Vegetable Center, Eastern and Southern Africa, Duluti, Arusha P.O. Box 10, Tanzania
* Correspondence: ndanikou@gmail.com

Abstract: Seed traits are essential quantitative variables to assess seed quality and are also indicators of crop success. The World Vegetable Center Genebank in Arusha, Tanzania, has an important legume collection; however, there is limited information on seed traits per genotype, i.e., 1000-seed weight, the number of seeds per gram, germination percentage (GP), and mean germination time (MGT). In this study, 8 cowpea, 54 mungbean, and 15 soybean accessions were evaluated for the abovementioned parameters. A significant difference was observed among the genotypes of the selected legumes with respect to all the measured parameters. An intraspecific variation was also observed for the measured seed traits. The 1000-seed weight decreased with an increase in the number of seeds per gram. Unlike cowpea, the GP of mungbean and soybean accessions was significantly and negatively influenced by the number of seeds per 1 g. Hence, the smaller-sized seeds (with many seeds in 1 g) exhibited lower GP. It is concluded that the seed traits and initial quality among the selected legumes are strongly influenced by their genetic makeup. These results are useful to inform seed management, exchange, utilization, and have a high potential in breeding programs, and require further research attention.

Keywords: traditional African vegetables; legume crops; genotypes; thousand-seed weight; number of seeds per 1 g

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1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp), Mungbean (*Vigna radiata* (L.) Wilczek), and Soybean (*Glycine max* (L.) Merr) of the Fabaceae family are among the major grain legumes cultivated in East Africa and Southeast Asia. They provide a plant-based source of dietary proteins and other essential micronutrients and hence can play a significant role in the global food system transformation [1]. Since it is essential to conserve plant genetic resources for food and agriculture, large ex situ germplasm collections generally harbor and safeguard a wide range of crop diversity. Thus, among other legumes, the World Vegetable Center is also holding in trust the world's second largest mungbean germplasm collection, with more than 6700 accessions [2]. Given the fact that genebanks are essential for plant genetic resource conservation and use by providing the raw materials for the improvement of crops, they contribute to the sustainable development goals and increase food production to overcome hunger and poverty.

WorldVeg's genebank harbors a substantial Mungbean collection among other legumes, with important intraspecific genetic diversity and many improved varieties from that collection which are already adopted by farmers and commercialized [3]. The seeds in genebanks are irreplaceable vital resources and a heritage that must be conserved to provide future agricultural options in a world facing climate change and other unforeseen challenges [4]. Seeds are thus the central component of the plant life cycle because the establishment of a new generation of plants depends on them [5]. Hence, to ensure seed survival and availability to present and future generations, proper procedures for their handling and research are vital [4]. Therefore, it is necessary to assess the seed parameters such as seed

weight (i.e., 100- and 1000-seed weight), the number of seeds per unit weight (1 g), germination percentage, and germination time of genebank accessions. This helps to establish the information required for seed handling in genebanks but also on the capacity to provide viable and uniform seeds during seed production.

Among other characteristics, seed traits are a potential gear for more research into the genetic control of seed development and are fundamental components of crop improvement [6]. In the context of plant breeding, seed weight and the number of seeds per unit weight (1 g) are among the key quality indicators of numerous crop plants' genotypes. These seed traits are commonly used since they are fast, and less prone to errors, making measuring the weight of a standard number or volume of seeds more informative, and practical [6]. The traits of seeds, their correlation, and their relationships are very important quantitative variables and essential for systematic breeding for seed yield and shape determination [7,8]. The mass, oil, and protein content of seeds are well-associated with seed traits [8–10]. Seed size is an important determinant of seed dispersal, probable loss [11], moisture imbibitions, germination, and grain grading [12], and it is also a consumer trait of crops including legumes in most parts of the world [13]. Seed size has been reported to be a highly correlated and very important grading parameter of seed grains, and the demand for seed grains for human consumption is linked to size and shape in soybean [14]. The chemical composition of plants (i.e., N, P, and K), the quality of protein, and total soluble carbohydrates are positively affected by seed weight (denseness) for example in *Phaseolus vulgaris* [15].

The seed weight of particular legume genotypes is an essential marketing trait since the heavier the seeds, the better the price they command, and it could also be a useful criterion for determining suitability for a particular end-use application [16,17]. For example, in Sri Lanka, some mungbean varieties with larger seeds have fetched much higher prices than the small-size seeds despite possessing similar physical characteristics with a shorter duration of seed maturity [17]. Seed weight is also vital during designing suitable processing machinery that could handle a maximum seed weight [16]. Good-quality seeds contribute 15–20% to yield increase. The size of the grains indicates the quality of the seeds, and consequently, the vegetative growth can be affected by the size of the seeds that were planted [18]. In suitable germination and growth conditions, genotypes with large seeds will definitely produce larger seedlings in comparison with small seeds and can result in increased crop productivity on-farm [19].

Crop species differ widely in how quick their seeds germinate, whereby the seedling emergence rate is a result of the interaction between the genotype and specific environmental factors [20]. The time taken to the completion of the germination process is a very crucial parameter of seed quality, and also rapid seed germination as an adaptive trait in the life cycle of a plant determines the quick transition to the growth phase. Thus, under diverse environmental conditions, vigorous, rapid, and uniform germination and emergence are desirable for seedling growth and grain yield in various food crops, including legumes [20]. Despite the vast legumes collections in the World Vegetable Center genebank, there is limited information on the influence of genotypes on seed traits such as seed weight (i.e., 1000-seed weight), the number of seeds per unit weight (1 g), GP, and MGT for the selected grain legumes. The current research is based on the hypothesis that seed traits and initial seed quality before storage vary with the genotypes of the same crop. This study, therefore, aimed to generate information on the influence of genotype on seed-traits variation and initial seed quality among the cowpea, mungbean, and soybean accessions in order to inform seed management, exchange, and use.

2. Materials and Methods

Study site and Experimental materials: Seed-traits assessments were carried out at the genebank of the World Vegetable Center in Arusha, Tanzania (36.8° E, 3.4° S, and 1290 masl altitude). Arusha is a cool, wet, medium-altitude area, whereby the average max/min temperatures vary between 12 and 27 °C, with June and July being the coldest months.

The rainfall is bimodal with a mean annual rainfall of 800 mm. The main rainy period is from March to May [21]. Fresh seeds harvested between February and May 2021 after regeneration of 8 cowpea, 54 mungbean, and 15 soybean accessions were used in the evaluations. The number of accessions was based on the limited information on the seed traits but also based on Genebank criteria for regeneration of accessions, i.e., prioritizing new accessions which contained less than the recommended number of seeds for storage in the Genebank, accessions with the number of seeds remaining less than three times the number of seeds used for regeneration of the particular species, and ones that are required urgently for distribution. The evaluation of initial quality, i.e., seed traits (100-seed weight, 1000-seed weight, and the number of seeds per 1 g) for the freshly harvested and well-dried seed lots was carried out at room temperature (22 ± 1 °C) before the rest of the seeds were put in air-tight aluminum packs and stored at 10 °C in the Genebank.

Experimental design: The dried seeds' weight (100-seed weight, 1000-seed weight) and the number of seeds per 1 g were determined according to the International Seed Testing Association (ISTA) rules [22]. The experiment was laid out as a completely randomized design (CRD) with accessions for each test crop used as a treatment. Eight (8) replicates of 1 g pure seeds were drawn randomly, and the number of seeds was counted and recorded. Another eight (8) pure seed replicates of 100 seeds were drawn randomly from each accession. The seed germination test was conducted before storage using dry seeds primed in water overnight at room temperature and then incubated in a germination chamber (Model: Memmert IPP750 Plus, Memmert GmbH + Co. KG, Schwabach, Germany) under a controlled environment. Subsamples (four replicates of 25 seeds each) of the three test crops' accessions were drawn randomly to determine seed germination percentage using the Petri dish (top-of-paper) method [4]. The seeds were placed on two germination tissue papers in 9 cm Petri dishes and incubated in the germination chamber at a constant temperature of 25 °C in the dark. The germination tissue paper in the Petri dishes was moistened with 5 mL of distilled water and monitored daily. After plating, the number of germinated seeds on the first count (i.e., 2 days for mungbean and 5 days for soybean and cowpea) and final count (i.e., 7 days for mungbean and 8 days for soybean and cowpea) [4], germination percentage (GP), and mean germination time (MGT) were established.

Seed weight measurement: For each crop, the weight of 100 seeds was recorded in grams per accession using an electronic weighing balance (Model: ML4002/02, Mettler Toledo, Switzerland) to three decimal places, and the mean weight was determined from the 8 replicates. The variance, standard deviation, and coefficient of variation were calculated, as shown in Equations (1)–(3), respectively. For the case where the coefficient of variation did not exceed 4.0, the mean values were calculated, but where the coefficient of variation exceeded the limit of 4.0, as per ISTA guidelines [22], a further 8 replicates were drawn from the seed lot, weighed, and the standard deviation was calculated for the 16 replicates. Further, any replicate that diverged from the mean by more than twice the calculated standard deviation was discarded. The mean weight of 100 seeds was used to calculate the weight of 1000 seeds, as shown in Equation (4), following the standard ISTA Rules [22].

$$Variance = \frac{N\sum x^2 - (\sum x)^2}{N(N-1)} \quad (1)$$

where x = weight of each replicate in grams; N = number of replicates; Σ = sum.

$$Standard\ deviation\ (sd) = \sqrt{Variance} \quad (2)$$

$$Coefficient\ of\ variation = \frac{sd}{\underline{x}} \times 100 \quad (3)$$

where $\underline{x}$ = mean weight of 100 seeds.

$$1000\ seed\ weight = w_{100} \times 10 \quad (4)$$

where w_{100} = weight of 100 seeds.

Germination percentage (GP) computation: The percentage of seed germination was computed using periodic counts of the number of germinated seeds for each of the test crops [4], as shown in Equation (5).

$$Germination\ (\%) = \frac{n}{N} \times 100 \quad (5)$$

where n = number of germinated seeds, N = total number of seeds tested.

Mean germination time (MGT) estimation: The germination rate as expressed by mean germination time (MGT) was calculated based on the periodic counts made during the experiment [23], as shown in Equation (6).

$$Mean\ germination\ time\ (MGT) = \frac{\sum(n \times d)}{N} \quad (6)$$

where n = number of seed germinated on each day; d = number of days from the beginning of the test; and N = total number of seeds germinated at the termination of the test

Statistical analysis: Data were statistically examined by one-way analysis of variance (ANOVA) using the "R" software version 4.1.1 [24] to uncover similarities and differences between accessions for the measured variables (i.e., 1000-seed weight and number of seeds per 1 g). The generalized linear model with binomial and quasibinomial (to account for overdispersion) error structures was used to test the effects of the genotypes on GP. Effects of the genotypes on the MGT were compared using the generalized linear model with a quasi-Poisson error structure. Data cleaning was done using the R package "tidyverse" [25], while Tukey's honestly significant difference (HSD) test was used for multiple comparisons to determine significant differences among accession means at a $p = 0.05$ significance level using R package "multcomp" [26] and rcorr() function in "Hmisc" package [27] to obtain both the Pearson's simple correlation coefficients (r) and the significance levels (p-values) among variables.

3. Results

3.1. Variation of Seed Traits among Selected Soybean Genotypes

A significant difference ($p < 2 \times 10^{-16}$) was observed among the selected soybean accessions with respect to the mean weight of 1000 seeds, the number of seeds per 1 g, MGT, and GP ($p = 5.79 \times 10^{-7}$) (Table 1). The highest mean weight of 1000 seeds was exhibited by accession "TGM 442" (401 ± 2.3 g), while the highest mean number of seeds per 1 g was exhibited by accession "TGM 632" (15 ± 0.4 seeds), and the highest mean GP and MGT were observed on accession "TGM 96" (100 ± 0.0%; 6 ± 0.0 days) compared to other accessions. Figure 1 presents the soybean accessions that exhibited the highest (a) and the lowest (b and c) mean weight of 1000 seeds.

Table 1. Mean values of measured seed parameters per soybean genotype.

Genotypes	1000-Seed Weight (in Grams)	Number of Seeds per 1 g	[2] GP (%)	[3] MGT (Days)
TGM 442	401 ± 2.3 a [1]	3 ± 0.1 h	82 ± 0.4 c	5 ± 0.2 c
TGM 459	392 ± 2.1 b	3 ± 0.1 h	91 ± 1.8 b	6 ± 0.0 b
AGS 329	345 ± 2.8 c	3 ± 0.1 h	97 ± 0.5 ab	2 ± 0.0 g
TZA 450	229 ± 2.3 d	5 ± 0.1 g	93 ± 3.2 ab	2 ± 0.1 g
TGM 9	178 ± 1.6 e	8 ± 0.2 f	94 ± 2.6 ab	2 ± 0.1 g
TGM 631	175 ± 1.4 e	7 ± 0.1 f	100 ± 0.5 a	4 ± 0.0 d
TGM 79	158 ± 1.3 f	9 ± 0.2 e	99 ± 1.0 ab	4 ± 0.0 d
TGM 14	149 ± 1.5 g	7 ± 0.1 f	94 ± 0.9 ab	3 ± 0.3 f
TGM 637	142 ± 0.9 gh	9 ± 0.2 cde	98 ± 0.3 ab	2 ± 0.0 g
TGM 651	140 ± 1.5 h	9 ± 0.2 de	96 ± 0.3 ab	2 ± 0.0 g
TGM 629	135 ± 1.1 h	10 ± 0.2 c	98 ± 0.8 ab	2 ± 0.0 g
TGM 802	135 ± 1.8 h	10 ± 0.2 cd	99 ± 0.8 ab	3 ± 0.0 e

Table 1. *Cont.*

Genotypes	1000-Seed Weight (in Grams)	Number of Seeds per 1 g	[2] GP (%)	[3] MGT (Days)
TZA 2888	125 ± 1.9 i	10 ± 0.4 cd	95 ± 2.5 ab	2 ± 0.0 g
TGM 96	108 ± 1.6 j	11 ± 0.3 b	100 ± 0.0 a	6 ± 0.0 a
TGM 632	91 ± 0.9 k	15 ± 0.4 a	98 ± 1.5 ab	2 ± 0.0 g
Grand mean	194.0	8.0	96.3	3.0
Max.	410.0	16.0	100.0	6.0
Min.	90.0	3.0	81.3	2.0

[1] Means (±SE) bearing the same letter(s) within the column are not significantly different ($p > 0.05$) according to Turkey's honest significance test. [2] GP = Germination percentage; [3] MGT = Mean germination time.



(**a**) (**b**) (**c**)

Figure 1. Soybean genotypes with varying seed sizes: highest mean weight of 1000 seeds (**a**) TGM 442; lowest mean weight of 1000 seeds (**b**) TGM 632; (**c**) TGM 96.

3.2. Variation of Seed Traits among Selected Cowpea Genotypes

A significant difference ($p < 2 \times 10^{-16}$) was observed among the selected cowpea accessions with respect to the average weight of 1000 seeds, the number of seeds per 1 g, GP ($p = 1.321 \times 10^{-5}$), and MGT ($p = 8.177 \times 10^{-15}$) (Table 2). Accession "TZA 4967A" (143 ± 1.6 g) exhibited the highest mean 1000-seed weight, while accession "CP17" and "TZA 167" had the highest mean GP (99 ± 0.4%; 98 ± 2.0%); accession "TZA-NA-R1" had the highest mean number of seeds per 1 g (10 ± 0.5 seeds) followed by "CP17" (9 ± 0.3 seeds). Accession "TZA 4967A" had the lowest MGT (2 ± 0.0 days), which means it germinated the most rapidly compared to the other accessions. Figure 2 presents the cowpea accessions that exhibited the highest (a) and the lowest (b and c) mean weight of 1000 seeds.

Table 2. Mean values of measured seed parameters per cowpea genotype.

Genotypes	1000-Seed Weight (in Grams)	Number of Seeds per 1 g	[2] GP (%)	[3] MGT (Days)
TZA 4967A	143 ± 1.6 a [1]	9 ± 0.2 b	84 ± 1.7 b	2 ± 0.0 b
TZA-NA-WHIB	139 ± 1.3 ab	8 ± 0.2 b	84 ± 1.6 b	3 ± 0.0 a
TZA-NA-WHI	138 ± 1.3 ab	9 ± 0.2 b	76 ± 1.0 b	3 ± 0.0 a
TZA 167	135 ± 1.9 b	9 ± 0.2 b	98 ± 2.0 a	3 ± 0.0 a
IT94K-410-1(ILO)	134 ± 1.8 bc	9 ± 0.3 b	79 ± 5.0 b	3 ± 0.0 a
IT97K-499-38	128 ± 1.4 cd	9 ± 0.5 b	82 ± 0.8 b	3 ± 0.1 a
CP17	123 ± 1.5 d	9 ± 0.3 ab	99 ± 0.4 a	3 ± 0.0 a
TZA-NA-R1	87 ± 0.9 e	10 ± 0.5 a	86 ± 3.5 b	3 ± 0.0 a

Table 2. *Cont.*

Genotypes	1000-Seed Weight (in Grams)	Number of Seeds per 1 g	[2] GP (%)	[3] MGT (Days)
Grand mean	128.4	9.0	85.9	3.0
Max.	150.0	14.0	100.0	4.0
Min.	83.1	7.0	64.0	2.0

[1] Means (±SE) bearing the same letter(s) within the column are not significantly different ($p > 0.05$) according to Turkey's honest significance test. [2] GP = Germination percentage. [3] MGT = Mean germination time.



(**a**) (**b**) (**c**)

Figure 2. Cowpea genotypes with varying seed sizes: highest mean weight of 1000 seeds (**a**) TZA 4967A; lowest mean weight of 1000 seeds (**b**) CP17; (**c**) IT97K-499-38.

3.3. Variation of Seed Traits among of Selected Mungbean Genotypes

The results indicated that the average weight of 1000 seeds, the number of seeds per 1 g, GP, and MGT differed significantly ($p < 2 \times 10^{-16}$) among the selected mungbean accessions (Table 3). The highest mean weight of 1000 seeds was exhibited by accession "VC 3909A" (73 ± 0.5 g), while the highest mean number of seeds per 1 g was exhibited by accession "VI003252BG" (44 ± 1.2 seeds). The highest mean GP (100 ± 0.0%) was exhibited by accessions "VI00578AG", "VI00537BG", and "VI004691AG", while accession "VI000947AG" had the lowest MGT (1 ± 0.0 days) followed by accessions "VI003172BG" (1 ± 0.2 days) and "VI001126BG" (1 ± 0.1 days). Figure 3 presents the mungbean accessions that exhibited the highest weight of 1000 seeds (a), the lowest 1000-seed weight and the highest number of seeds per 1 g (b, c and d), the highest GP (e), and the highest GP and MGT (f).

Table 3. Mean values of measured seed parameters per mungbean genotype.

Genotypes	1000-Seed Weight (in Grams)	Number of Seeds per 1 g	[2] GP (%)	[3] MGT (Days)
VC 3909A	73 ± 0.5 a [1]	11 ± 0.4 y	99 ± 1.0 ab	2 ± 0.0 u–y
VC 5809A	59 ± 0.6 b	13 ± 0.2 x	94 ± 2.000 a–i	2 ± 0.0 n–u
VC 2778A(KPS2)	58 ± 0.2 bc	14 ± 0.4 x	90 ± 1.6 c–n	3 ± 0.1 h–s
VI00221AG	57 ± 0.5 bcd	16 ± 0.8 u–x	98 ± 0.6 abc	3 ± 0.0 d–j
VC 6368(46-40-4)	57 ± 0.5 cd	15 ± 0.2 wx	86 ± 2.0 i–n	3 ± 0.0 g–o
VC 6370(30-65)	55 ± 0.5 de	15 ± 0.2 vwx	88 ± 0.5 f–n	4 ± 0.1 bc
AVMU 1615	54 ± 0.4 ef	16 ± 0.3 u–x	91 ± 1.0 b–m	2 ± 0.0 q–x
VI003886B-BR	54 ± 0.2 ef	15 ± 0.2 vwx	98 ± 1.0 abc	1 ± 0.1 wxy
VI002647AG	54 ± 0.5 ef	18 ± 0.2 s–v	98 ± 0.7 a–d	2 ± 0.1 l–t
VI001124AG	53 ± 0.2 f	15 ± 0.1 vwx	98 ± 0.3 abc	2 ± 0.1 p–x

Table 3. *Cont.*

Genotypes	1000-Seed Weight (in Grams)	Number of Seeds per 1 g	[2] GP (%)	[3] MGT (Days)
VI000947AG	51 ± 0.4 g	18 ± 0.2 r–u	98 ± 1.2 abc	1 ± 0.0 y
VC 2802A-BR	50 ± 0.4 gh	17 ± 0.4 t–w	98 ± 1.4 abc	3 ± 0.0 g–o
VI001244	48 ± 0.6 hi	18 ± 0.4 r–u	93 ± 3.0 a–k	2 ± 0.0 o–w
VI003440AG	47 ± 0.3 i	18 ± 0.2 s–v	95 ± 1.1 a–g	2 ± 0.1 u–y
VI003907AG	43 ± 0.3 j	20 ± 0.2 q–t	98 ± 0.5 abc	2 ± 0.1 r–x
VI001633	43 ± 0.3 j	19 ± 0.4 q–t	86 ± 2.2 i–n	4 ± 0.1 bcd
VI004691AG	42 ± 0.3 j	20 ± 0.4 qrs	100 ± 0.0 a	3 ± 0.4 e–m
VI000466	42 ± 0.5 jk	21 ± 0.3 opq	97 ± 1.0 a–e	2 ± 0.0 n–u
VC 0022709	41 ± 0.3 jkl	21 ± 0.3 opq	97 ± 1.0 a–e	2 ± 0.1 n–v
VI000852	41 ± 0.3 jkl	24 ± 1.2 k–o	89 ± 1.2 e–n	3 ± 0.1 l–t
VI001406BG	41 ± 0.5 jkl	26 ± 0.2 h–n	89 ± 1.0 d–n	2 ± 0.0 s–x
VI002173AG	40 ± 0.4 kl	23 ± 0.3 nop	98 ± 0.9 a–d	3 ± 0.0 g–q
VI000942AG	40 ± 0.3 lm	21 ± 0.4 pqr	95 ± 0.8 a–h	2 ± 0.0 n–w
VI001520A-BLM	39 ± 0.4 lm	21 ± 0.4 opq	95 ± 0.4 a–g	2 ± 0.1 q–x
VI00625B-BR	38 ± 0.2 mn	23 ± 0.5 nop	87 ± 1.5 h–n	3 ± 0.2 d–h
VI00332AG	38 ± 0.4 mn	26 ± 0.6 h–m	100 ± 0.0 a	5 ± 0.2 a
AVMU 1642	38 ± 0.4 mn	24 ± 0.6 k–o	87 ± 1.0 h–n	3 ± 0.0 d–i
VI005030BY	37 ± 0.5 no	23 ± 0.2 m–p	92 ± 2.2 a–l	4 ± 0.0 b–e
AVMU 1637	37 ± 0.2 no	26 ± 0.6 h–l	85 ± 0.5 k–n	4 ± 0.2 b
VC 4442A	36.438 ± 0.4 no	25 ± 0.6 k–n	86 ± 0.8 i–n	3 ± 0.2 d–l
VI001126BG	36 ± 0.3 no	23 ± 0.4 l–p	96 ± 0.6 a–f	1 ± 0.1 xy
VI003699	36 ± 0.5 no	26 ± 0.4 h–n	89 ± 3.4 d–n	3 ± 0.2 d–l
VI001557BG	36 ± 0.4 no	23 ± 0.8 l–p	84 ± 2.2 lmn	3 ± 0.2 h–s
VI001490AG	35 ± 0.3 op	24 ± 0.4 k–n	86 ± 1.3 h–n	3 ± 0.1 f–n
VI00164BG	35 ± 0.3 opq	24 ± 0.7 k–n	86 ± 1.9 h–n	2 ± 0.1 t–y
VC 4718AG	34 ± 0.3 opq	25 ± 0.5 k–n	85 ± 1.1 j–n	2 ± 0.0 m–u
VI00253AG	34 ± 0.2 opq	25 ± 0.3 j–n	85 ± 2.0 k–n	3 ± 0.0 f–n
VI001514AG	34 ± 0.2 pq	25 ± 0.4 i–n	90 ± 3.5 c–n	2 ± 0.1 n–u
VI00537BG	33 ± 0.3 pqr	26 ± 0.3 h–k	100 ± 0.0 a	3 ± 0.0 d–i
VI002986AG	33 ± 0.4 pqr	26 ± 0.5 h–m	88 ± 2.3 f–n	3 ± 0.2 g–p
VI001535 BG	33 ± 0.5 qrs	28 ± 0.4 fgh	85 ± 0.9 k–n	2 ± 0.0 i–s
VI005066AGM	32 ± 0.3 rst	28 ± 0.5 ghi	97 ± 1.3 a–e	3 ± 0.1 d–k
VC 001562AG	31 ± 0.2 r–u	28 ± 0.4 g–j	82 ± 1.4 n	2 ± 0.2 l–t
VI003958B-BLM	31 ± 0.7 s–v	30 ± 0.4 d–g	94 ± 0.9 a–j	2 ± 0.2 k–t
VI001482AG	30 ± 0.5 t–w	29 ± 0.8 efg	99 ± 0.4 ab	2 ± 0.1 j–t
VI002894B-BR	29 ± 0.3 u–x	30 ± 0.2 efg	93 ± 1.6 a–l	3 ± 0.1 g–r
VI003062	29 ± 0.4 u–x	31 ± 0.7 c–f	85 ± 1.9 k–n	3 ± 0.2 d–i
VI004718AG	29 ± 0.3 vwx	33 ± 0.5 bcd	85 ± 1.1 j–n	2 ± 0.0 m–u
VI00578AG	28 ± 0.4 wxy	31 ± 0.6 c–f	100 ± 0.0 a	3 ± 0.2 b–f
VI003720	27 ± 0.4 w–z	34 ± 0.5 bc	87 ± 1.5 g–n	3 ± 0.1 c–g
VI003172BG	27 ± 0.4 xyz	33 ± 0.7 bcd	87 ± 1.0 g–n	1 ± 0.2 xy
VI003251A-BL	26 ± 0.1 yz	32 ± 0.7 cde	91 ± 1.7 b–m	1 ± 0.0 v–y
VI001509AG	26 ± 0.4 z	35 ± 0.4 b	83 ± 1.2 mn	3 ± 0.1 d–i
VI003252BG	23 ± 0.4 A	44 ± 1.2 a	84 ± 2.0 lmn	2 ± 0.1 i–s
Grand mean	39.8	24.0	92.0	3.0
Max.	75.4	49.0	100.0	5.0
Min.	21.1	10.0	80.0	1.0

[1] Means (±SE) bearing the same letter(s) within the column are not significantly ($p > 0.05$) different according to Turkey's honest significance test. [2] GP = Germination percentage. [3] MGT = Mean germination time.

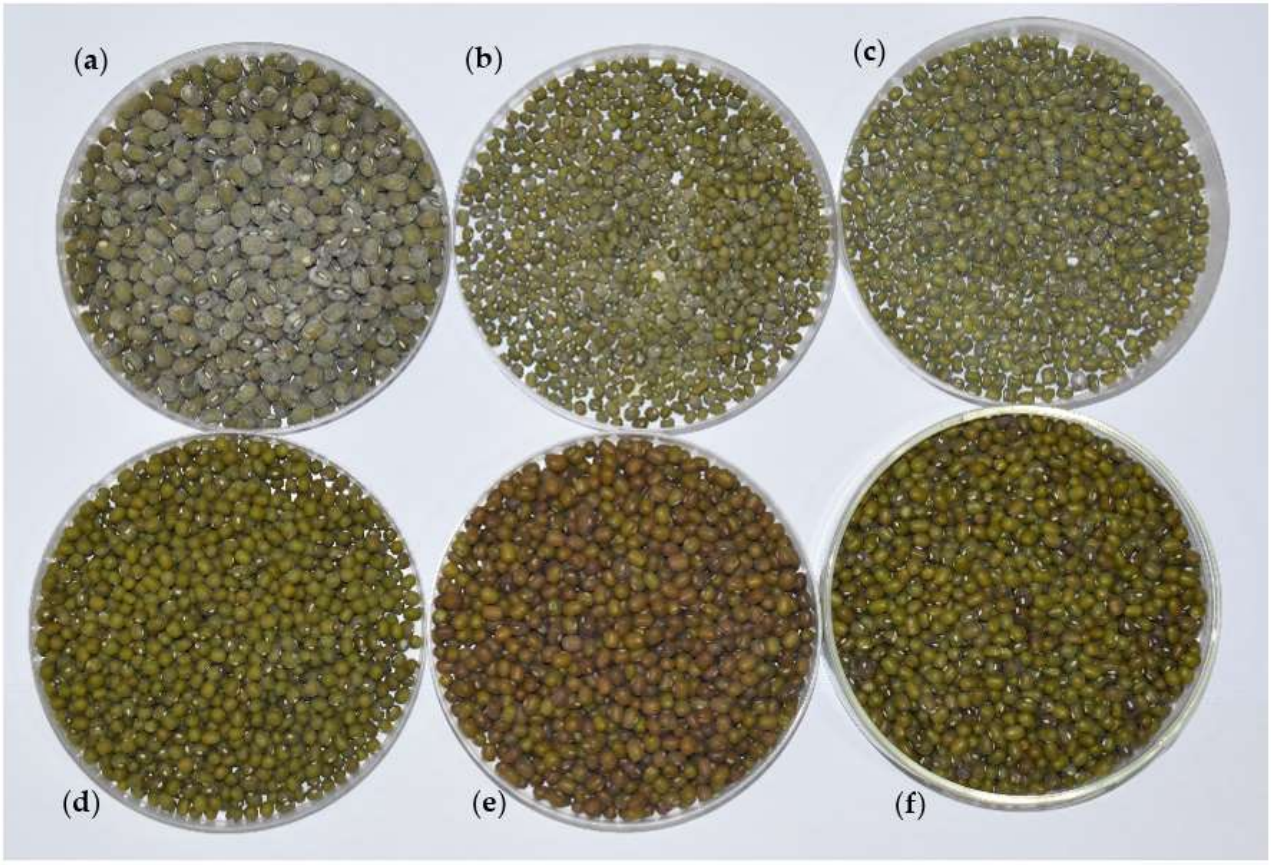


Figure 3. Mungbean genotypes with varying traits: highest weight of 1000 seeds (**a**) VC 3909A; the lowest 1000 seed weight and the highest number of seeds per 1 g (**b**) VI003252BG; (**c**) VI00578AG; (**d**) VI00537BG; the highest GP (**e**) VI004691AG; the highest GP and MGT (**f**) VI00332AG.

3.4. Relationship between the Measured Seed Traits

There was a significant negative correlation between the number of seeds per unit weight (1 g), and 1000-seed weight within the selected cowpea ($r = -0.62$, $p = 2.81 \times 10^{-8}$), mungbean ($r = -0.90$, $p = 3.74 \times 10^{-44}$), and soybean ($r = 0.89$, $p = 1.71 \times 10^{-41}$) accessions. The weight of 1000 seeds decreased with an increase in the number of seeds per unit weight (1 g). This implies that large-sized seeds (with a few numbers of seeds in 1 g) had a higher 1000-seed weight, while small-sized seeds (with many seeds in 1 g) had a lower 1000-seed weight. The GP of mungbean and soybean accessions was significantly and negatively correlated with the number of seeds per 1 g ($r = -0.965$, $p < 0.001$; $r = -0.930$, $p < 0.001$, respectively), and with the 1000-seed weight ($r = -0.967$, $p < 0.001$; $r = -0.891$, $p < 0.001$, respectively). In fact, in mungbean and soybean accessions, the GP decreased with an increase in the number of seeds per unit weight (1 g). This means that the smaller-sized seeds (with many seeds in 1 g) exhibited lower GP. However, there was a weak relationship between GP, the number of seeds per 1 g, 1000-seed weight, and MGT of cowpea accessions.

4. Discussion

Seed traits, i.e., 1000-seed weight, the number of seeds per gram, GP, and MGT, among other characteristics, help to distinguish between particular species and varieties within species, as well as help in the selection of suitable varieties for the purpose of cultivation [17]. Based on the 100-seed weight, previous studies have indicated that the soybean seed size classes are small (<13 g), medium (13–24 g), and large (>24 g) [28], while seed sizes for cowpea are small (<15 g), medium (15–20), and larger (>20 g), [29], and seed size for mungbean are small (<4 g), medium (4–6 g), and large (>6 g) [30]. The findings in the present study indicate that 1000-seed weight, GP, and MGT differ significantly among

accessions of the studied legume crops, whereby the variation is highly associated with the genotype. As the seed size increases for a particular genotype, the lesser the number of seeds per 1 g, and consequently, higher weights of 1000 seeds, is observed.

Seed size in grain legumes such as cowpea is important since it directly influences productivity and determines grain quality for commercialization [31]. Therefore, understanding the genetic factors responsible for the inheritance of seed traits (like seed size) is essential for breeding programs that constitute diverse genetic variability in germplasm collections. According to Lopes et al. [31], five genes are involved in the inheritance of the character studied and are concentrated in the large-seeded parent, whereby each gene contributes to an increase in the 100-grain weight by 1.1 g. The 100-seed weight is an important measure of yield and a useful indicator to monitor the inheritance of quantitative traits affected by genotype and environmental conditions [32]. A total of 118 quantitative trait nucleotides (QTNs) controlling 100- or 1000-seed weight across multiple environments and three potential candidate genes associated with 100- or 1000-seed weight among the four-way recombinant soybean inbred lines have already been identified [32]. Moreover, a higher percentage of superior alleles was found in the large-seed lines than in small-seed lines [32].

Cowpea genotypes grown in Ghana exhibited 100- or 1000-seed weights ranging between 11.20 to 22.58 g, whereby eight genes-controlled seed size, and small seeds were partially dominant over large seed [13]. Cowpea grain production components including 100- or 1000-seed weight are highly correlated with gas exchange, carboxylation instantaneous efficiency, and water-use efficiency; hence, they very crucial factors for selecting cowpea populations with tolerance to drought [33]. Moreover, the production component (i.e., 100- or 1000-seed weight), especially in autogamous species, such as cowpea, is influenced by the combination of alleles present in the parents because the predominant allelic interaction is additive, but dominance also occurs as a minor contribution; hence, this is crucial for breeding programs since the selection is facilitated by superior individuals that frequently segregate with better performance [31,33]. It was previously reported that there are significant variations between super early maturity (<75 days) and early maturity (76–79 days) soybean genotypes in the 100- or 1000-seed weight [34]. The super early maturity genotypes ranged from 15.27–20.18 g, compared to the early maturity group's range of 13.90–20.23 g. It was also vivid that mungbean genotypes that produce higher seed yield and the related yield characters including 100- or 1000-seed weight also have higher leaf area (LA), total dry mass (TDM), and crop growth rate (CGR) [35].

Several recent studies also indicate that the seed size of legume genotypes is influenced by the genetic make-up [17] and slightly impacted by the environment. The 1000-seed weight of mungbean varied significantly among cultivars as well as the nitrogen, phosphorus, and sulfur (NPS) fertilizer rates, indicating that this trait is also attributed to both the genotypic variation in mungbean cultivars and management [36]. Legumes including mungbean have a high P requirement, as the main storage site of P is the seed; hence, the 1000-seed weight increased with the increased rates of NPS fertilizer [36]. Similarly, Imran et al. [37] also reported that mungbean genotypes, phosphorus levels in soil, and tillage systems significantly affect the 1000-seed weight. However, on the contrary, a study on soybean [38] indicated that the 100- and/or 1000-seed weight trait is mainly controlled by genetic factors rather than the management since the NPS application rates and the interaction effect were nonsignificant.

The difference between the smaller-, medium-, and large-sized seeds is that the latter exhibits the superior genetic potential in 1000-seed weight which indicates more food storage in the seed; hence, a high GP is expected [39]. The genotypes characterized with the lowest value of MGT (i.e., the seeds germinated most rapidly) signified that the seed vigor is better than other accessions. Despite the fact that the actual rate of seed germination among the crop species and varieties relies on the desired amount of moisture and temperature during the germination [4], the GP of mungbean and soybean accessions significantly decreased in the smaller-sized seeds (with higher seeds in 1 g) and larger-sized seeds (with a higher 1000-seed weight) compared to the medium-sized seeds. These results are in line

with the findings whereby soybean with medium-sized seeds had a higher germination percentage than that with large-sized and small-sized seeds [40]. Since soybean is an oily crop and the increase in seed weight from medium seed size can lead to oxidative stress due to the oily components in the seed, it can cause the seed to become hard but liable to break easily (brittle) and lead to a decline in GP of seeds [40].

5. Conclusions

The seed traits, i.e., 1000-seed weight, the number of seeds per gram, GP, and MGT evaluated in this study presented significant variations among genotypes of the selected legume grains, i.e., cowpea, mungbean, and soybean. This indicates the strong influence of the genetic makeup of the accessions on seed traits and initial quality. These results are useful to inform seed management, exchange, and utilization, and have a high potential in breeding programs. The seed weight of particular legume genotypes could be a useful criterion for determining suitability for a particular end-use application because some varieties of legumes with larger seeds can fetch much higher prices than the small-size seeds despite possessing similar physical characteristics and duration of seed maturity. To meet the needs of these grain legumes, there is a need for essential efforts to increase production, especially through the use of genotypes with appropriate-quality seeds and high yield. Therefore, in suitable germination and growth conditions, genotypes with large seeds will definitely produce larger seedlings in comparison with small seeds and therefore can result in increased crop productivity. Multi-season or -location trials are recommended so as to investigate the effects of environmental conditions of the sites and their interactions with the genotypes on seed size and weight. Genotypic variability and heritability can be analyzed to determine the worthwhileness of these traits for crop improvement. In addition, the genetic relationship among traits will be needed, as it is efficient for the systematic selection of coinherited traits.

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