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Comprehensive phenotyping of seed physical traits in relation to water absorption and cooking quality in Western Himalayan common beans (*Phaseolus vulgaris* L.)

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ABSTRACT

Cooking quality is the major characteristic defining the farmer's acceptance of common bean varieties in the Western Himalayas. In the present study, we evaluated a core set of 254 lines for 21 seed physical traits and cooking time. There was substantial variability in 21 seed physical and water absorption traits as well as cooking time traits in the accessions as depicted by the higher range and CV. Genotypes such as GLP-1, WB-1518, WB-1634, WB-216, and N-4 were easy to cook. Cooking time score was positively correlated with various seed physical traits including seed brilliance (0.871), seed texture (0.772), alkali spreading value (0.771), Water Absorption (0.137), and negatively correlated with hardness value (-0.531). The first three PCs accounted for 54.60 percent variation mainly contributed by sphericity, aspect ratio, seed breadth, seed length, length-breadth ratio (PC1), surface area, seed volume, equivalent diameter, seed breadth, seed length (PC2) and seed brilliance, alkali spreading value, cooking time score, seed texture, hardness value (PC3). Our results indicated huge diversity in seed physical traits and also identified effective surrogates such as seed texture and brilliance as well as hardness and ASV that are quite easier to score especially when dealing with large germplasm sets.

Keywords- *Common beans, cooking quality, hard-to-cook trait, water absorption*

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INTRODUCTION

Common bean is one of the most important pulse crops, especially in Brazil, China, the Indian sub-continent, and Sub-Saharan Africa, with about half of bean production, occurring in Sub-Saharan Africa and Latin America followed by South and South-East Asia (35%). Most of the bean production, amounting to about 26.5 million tons, is used for direct human consumption (1) through local trade as well as international export markets (> 4 million tonnes) (2). On account of its desirable nutritional profile, it is regarded as a "nearly perfect food". Common beans contain a balanced mixture of different health-promoting nutrients such as proteins (22.8%), energy (307 Kcal), and fat (1.6 g). It has a very low glycaemic index value (0.24) as compared to cereals and is also rich in nutrients such as iron (8.8 mg/100g).

A major characteristic defining the farmer's acceptance of common bean varieties is cooking time as it is not only linked to the energy imperatives, especially in resource-poor nations in Sub-Saharan Africa but also implicates the nutritional qualities of bean seeds. The cooking process in beans has been reported to improve digestibility, nutritional and biological value, and also inactivate several anti-nutritional factors. In terms of organoleptic characteristics, cooking confers the characteristic tenderness, textural, sensory, and taste features that define consumer acceptance (3, 4,5). The cooking quality in beans (including cooking time, the proportion of stone seeds, as well as the physical and chemical attributes of cooked beans such as hydration, swelling, and starch gelatinization) modifies its physical, biochemical, and nutritional parameters, while as delayed cooking can potentially reduce the nutritive quality of beans (6). Since cooked grain form is the major consumption pattern of beans, the cooking quality attributes are important for the adoption of bean varieties (7). Among legume crops, varied cooking times ranging from as low as 0.5 hours in mung bean (8) to 1.5 hours in common bean (9), 2.4 hours in cowpea (10), 3-4 hours in Bambara groundnut (11) and 3.6 hours for soybean (12) have been reported.

Cooking time is a major focal area of bean improvement on account of its significance for energy utilization, nutritional value, and gender equity. Bean breeding initiatives have always concentrated on identifying and developing easy-to-cook (ETC) bean cultivars as ETC genotypes preserve more nutrients (13). In addition, ETC genotypes retain more nutrients as compared to delayed cooking and hard-to-cook (HTC) beans (13) On account of scarce firewood bean-growing countries such as Eastern Africa, the amount of energy and time necessary to cook beans is a significant economic factor (14). Therefore, ETC bean cultivars could help such farmers attain food security, conserve firewood and provide adequate nutrition for consumers (15) and are being preferred by consumers in most developing nations, particularly in Africa (16). While commercial bean growers may be more concerned about higher yield and stress resistance, subsistence farmers generally prioritize flavor and ease of cooking. Over the last 50 years, important international and national bean breeding programs (CIAT, EMBRAPA, NDSU, MSU, PABRA) have developed a large number of high-yielding, disease and drought-resistant cultivars in a variety of market classes. Even though cooking time is a crucial customer acceptance factor, there has been little focus on culinary qualities such as cooking time (17). Delayed cooking is a major concern, especially among urban consumers, due to the energy cost and time imperatives (18) as most of these use kerosene and gas as fuel. Fuels such as firewood and charcoal may offer cheaper alternatives for rural and urban consumers respectively, but they have a higher environmental cost (19).

In common bean, three major defects in a cooking trait are usually encountered including "delayed cooking (DC)", "hard-to-cook (HTC) defect" and "hard shell (HS)", which are invariably used to represent similar quality bottlenecks; buat is distinctly defined traits. Delayed cooking (DC) refers to longer cooking times in beans that are produced and stored under ambient conditions. Hard-to-cook (previously termed sclerema) beans are those which do not soften sufficiently upon soaking and do not become tender during a reasonable cooking time (6), while hardshell (HS) refers to beans that fail to imbibe a reasonable amount of water under soaking (20). Delayed cooking and hard-to-cook defects are attributed to various physical and biochemical attributes that define the hydration of cotyledons render the cells unable to separate during the cooking process, whereas, hard shell is attributed to defects in seed coat and failure to absorb water (9). The hard-to-cook phenomenon that results from the production or storage of bean seeds in sub-optimal environments such as high temperature, drought, and high humidity, is characterized by restricted softening of the cotyledons upon cooking even after the cotyledons have imbibed water. Because of the longer cooking time, HTC beans do not attain the cooked texture acceptable to consumers (21). However, the age-related impermeability in soybean was disproved and all aged samples (with varying storage periods), stored at 410 C and 100% relative humidity for 7 days, achieved identical levels of water absorptivity (1.3g water/ g of dry cotyledon) after four hours of soaking(22).

Various seed physical traits and water absorption parameters have long been used as an indirect criterion to determine bean cooking quality in beans (23,5). The generally held premise is that the various seed physical traits and the amount of water absorbed before cooking are correlated with cooking time (24,6). Seed hardness has a negative impact on both hydration of seeds as well as their cooking time (20,25). One of the earliest and widely accepted causes of delayed cooking and hard-to-cook phenomenon in beans is impaired water permeability (hard shell) which is thought to be a manifestation of hilar dysfunction. There are varied reports that brilliant seeded varieties have poor water imbibition attributes. Breeding for brilliant seeded varieties is based on the premise that brilliant seeds handle the environmental stresses during the growth cycle as well as the inclement storage conditions better than dull seeds (26), but such a misconception could lead to alter consumer preferences. The shiny-seeded genotypes have a comparatively thicker palisade cell layer (27) and also contain more antioxidants such as anthocyanins in their seed coats (13).

There are observed experimental shreds of evidence that have contrasted the generally held premise that water absorption is a reliable indicator of ease of cooking in beans. In soybean, similar tenderness in seeds in partially soaked (100% absorption) and fully hydrated (142% absorption) seeds(28).It was proposed that seed coat thickness and hilum size influence water absorption, particularly during the initial stage of soaking, while protein content was the major factor in the later stages(29). Variations in water absorption have been attributed to the thickness and texture of the seed coat, the strength of seed coat attachment, the size of the hilum and micropyle, and seed size (30, 31,6). However, the barriers to water absorption under soaking are invariably eliminated during cooking, resulting in an inconsistent relationship between water absorption and cooking time. More importantly, in stored beans, the imbibitional characteristics may not be related to their cooking time behavior. There are inconsistent reports on hydration differences of fresh and aged seeds during soaking which are largely attributed to differences in methods used in the measurement of hydration capacity (32). While as, most of the studies based on gravimetric methods have reported similar hydration capacity (33), other methods have reported greater water absorption in aged beans as compared to fresh beans (34,35). A potential reason for such inconsistency could be the lack of correction for solids lost during soaking (32). Similarly, seed texture is an important factor that determines cooking quality in beans with rough-seeded varieties expected to cook faster on account of having a greater surface area that may favorably enhance the uptake of water during the soaking and cooking. Such a premise has also been substantiated by the differential surface area of shiny and rough seeds by scanning electron micrographs (27).

In Western Himalayan Kashmir, the common bean enjoys a niche crop status on account of its globally recognized organoleptic qualities and cooking attributes. The present study was an attempt to conduct comprehensive phenotyping of seed physical traits in relation to cooking characteristics and get an insight into the currently held premises as well as identify natural variation for seed physical traits in the Western Himalayan bean collection.

MATERIALS AND METHODS

Site of the experiment

The experiment was laid in 2021 at the research fields of the Division of Genetics and Plant Breeding, Faculty of Agriculture Wadura, SKUAST-K, Sopore (34o 17′ North and 74o 33′ E at an altitude of 1594 masl). The soil of the experimental site is a typical inceptisol with a clay loam texture. The pH was almost neutral (7.2), with organic carbon of 0.65%, electrical conductivity of 0.18 dS/m, and CEC of 16 meq/kg. All the accessions were grown as single rows of four-meter length, with a spacing of 15 cm x 40 cm, in an augmented block design with four checks. The mean minimum and maximum temperatures (May-September) were 10.63 0C and 22.48 0C, with the lowest (16.43 0C) and highest (25.61 0C) maximum recorded in May and July respectively.

Experimental material

The material for the present study comprised of a core set of 254 lines including four checks (two state-released checks viz., Shalimar Rajmash-1, Shalimar French Bean-1 and two nationally released varieties viz., Arka Anoop and Arka Komal), representing diverse market classes in beans. The accessions belonged to both plain seeded as well as mottled beans ranging across diverse color classes and seed sizes and shapes (Figure 1). All the accessions were grown as single replicates in an augmented block design except the checks that were replicated in each block.

Crop management

The Management practices were uniform and homogeneous and comprised of seed treatment with the fungicide and the insecticide @ 2ml/kg seed, application of the pre-emergent herbicide Pendimethalin at a dose of 1.25l/ha as well as timely manual weeding, the recommended dose of fertilizers (NPK) comprising a basal dose and a topdressing of urea at the V3 stage (first open trifoliate leaf). The crop was irrigated intermittently to avoid drought stress. The pods were harvested manually at the R9 (maturation stage). The harvested pods were sundried and threshed. Bean seeds were hand cleaned to remove field debris, off types, and damaged seeds. The seeds were stored in plastic bottles for two weeks to equilibrate the moisture.

Cooking time score

Cooking of beans freshly harvested, sun-dried, and moisture equilibrated, was done in an autoclave following the method described with modifications. Fifty soaked grains were placed in a glass beaker, filled with 200 ml of distilled water, covered with a watch glass, and cooked under the conditions of 110°C for 5 min. Following scale was used for scoring the cooking properties of bean genotypes(36). The softness/hardness (cookability) of the beans was determined subjectively by pressing the cooked beans between the thumb and forefinger (37). Following scale was used for scoring the cooking properties of bean genotypes.

Scale	Designation	Description
1	Undercooked	Grain is difficult or not able to smash and cotyledon feels hard
2	Slightly undercooked	Grain is less difficult to smash and cotyledon feels slightly hard
3	Average cooked	Grain is firm but smashes easily and cotyledon feels soft
4	Slightly overcooked	There is little resistance to smash grain and cotyledon feels mushy
5	Overcooked	Grain is easily pressed into a mush

Seed physical parameters

All 254 genotypes were harvested and sun-dried and kept in ambient storage in plastic bottles for two weeks to equilibrate moisture content. Then replicate samples were drawn to estimate the following seed physical parameters.

- ***Seed length (mm):*** Seed length (mm) was measured using a digital vernier caliper and averaged across 10 representative seeds for each genotype.

- ***Seed breadth (mm):*** Seed breadth (mm) was measured using vernier calipers and averaged across 10 representative seeds for each genotype.

- ***Seed thickness (mm):*** Seed thickness (cm) was measured using vernier calipers and averaged across 10 representative seeds for each genotype

- ***100-seed weight (g):*** 100-seed weight of three randomly drawn samples of sun dried seeds from each experimental plot was weighed in grams and averaged.

- ***Length/breadth ratio:*** The length/breadth ratio of seeds (LBR) was calculated using the relationship LBR=L/B (39).The average of ten randomly drawn seeds was taken.

- ***Seed brilliance:*** Seed brilliance was measured visually and seeds were classified as dull, medium, and brilliant.

- ***Seed texture:*** Seed texture was recorded on a scale of 1-2, with one depicting smooth and 2 depicting rough seed coat surface. The visualization of the seed coat was done using a CelestronMicrocapture Pro Digital Microscope (Celestron LLC, USA)

- ***Equivalent diameter:*** The geometric mean diameter, Dm, was calculated using the relationship Dm = (LBT)1/3(38). The average of ten randomly drawn seeds was taken.

- ***Sphericity:*** The sphericity of seeds (Φ) was calculated as a function of the three principal dimensions as shown below using the relationship Φ = [(LBT)1/3/L]×100 (38) and was averaged for ten randomly drawn seeds.

- ***Aspect ratio:*** The aspect ratio of seeds (Ra) was calculated using the relationship Ra = B/L (39). The average of ten randomly drawn seeds was taken.

- ***Seed volume:*** The volume of the seeds V in mm3, was calculated using the relationship V = πb2L2/ 6 (2L-3) where b = (BT)1/2 (37) and was averaged for ten randomly drawn seeds.

- ***Surface area of seeds:*** The surface area of the seeds A in mm2, was calculated using the relationship A=πBL2/2L-B,(37), and was averaged for ten randomly drawn seeds.

- ***Hilum length:*** Measured by CelestronMicrocapture Pro Digital Microscope (Celestron LLC, USA) with inbuilt provision for measuring distances after calibration.

- ***Hilum width:*** Measured by CelestronMicrocapture Pro Digital Microscope Microscope (Celestron LLC, USA) with inbuilt provision for measuring distances after calibration

- ***Coat proportion (%):*** Seed coat proportion was determined on 20 seeds per accession, as the ratio in weight between coat and cotyledon expressed in percentage, after removing the seed coat from the cotyledons, both after soaking and keeping them for 24h at 105o C.

- ***Water absorption capacity (%):*** Water absorption was measured was determined by first soaking 30 seeds for 24 h in de-ionized water at room temperature and dividing the difference in weight before and after soaking by the dry weight of the 30-seed sample.

- ***Hydration capacity:*** Seeds, weighing 100 g, were counted and soaked overnight. After the water was drained, the soaked seeds were blotted dry and weighed. Hydration capacity (Hc) was calculated as a change in weight per number of seeds. Hydration capacity (g/seed) = (Ma – Mb)/N

- ***Swelling capacity:*** Seeds, weighing 100 g, were counted, their volume noted, and soaked overnight. The volume of soaked seeds was noted in a graduated cylinder (40). Swelling capacity (Sc) was calculated as the change in volume per number of seeds. Swelling capacity (ml/seed) = (Va - Vb)/N***Swelling capacity:*** Seeds, weighing 100 g, were counted, their volume noted, and soaked overnight. The volume of soaked seeds was noted in a graduated cylinder (40). Swelling capacity (Sc) was calculated as the change in volume per number of seeds. Swelling capacity (ml/seed) = (Va - Vb)/N

- ***Hardness value:*** It was measured by using a hardness tester i.e., a Durometer on a scale of 1-100 to test the hardness of soaked seeds.

Statistical analysis: The multivariate analysis based on GT and GC*T values was done using the software STAR (Statistical Tool for Agricultural Research) version 2.0.1 developed by IRRI (International Rice Research Institute, Los Banos, Philippines) (IRRI, 2020)

RESULTS AND DISCUSSION

Variability for seed physical traits

In the present study there was substantial variability in 14 seed physical traits in 254 genotypes of common bean indicating significant diversity of the material in respect of studied traits as depicted by higher range and CV values.Seed length had a mean value of 15.27 mm with range of 9.82 to 19.95 mm. The highest seed length was recorded in the case of WB-59 (19.95mm), followed by WB-20-240 (19.94mm) and IPR-20520 (19.93mm) while as lowest value for seed length was recorded in WB-1252 (9.82mm). Seed breadth had a mean value of 7.67 mm with a range of 4.76 to 9.34 mm and the highest seed breadth was recorded in the case of WB-20-277 (9.34 mm) followed by GLY-2 (9.3 mm) and WB-1709 (9.29 mm) while as lowest value for seed breadth was recorded in WB-1707 (4.76mm). Seed width had a mean value of 4.74 with a range of 4.04 to 5.8 mm. The highest seed width was recorded in the case of WB-20-173 (5.8mm), followed by WB-20-243 (5.79mm) and WB-1552 (5.78mm) while as lowest value for seed width was recorded in WB-20-310 (4.04mm). The seed length/breadth ratio had a mean value of 2.03 with a range of 1.15 to 3.83 and. Highest seed length/breadth ratio was recorded in the case of WB-1707 (3.83), followed by WB-243 (3.56) and WB-206 (3.36) while as lowest value for seed length/breadth ratio was recorded in WB-20-243 (1.15). Similar, results were reported in common bean with a range of 11.24-14.35 mm (seed length), 6.57-8.99 mm (seed breadth), and 4.78-5.57 mm (seed thickness)(41).It was also reported a broad range for seed length, breadth, thickness and equivalent diameter of seeds varied significantly in the range of 11.45–16.45 mm, 6.65–7.00 mm, 4.70–6.13 mm and 7.31–9.24 mm respectively(42). In our earlier study, we observed a similar range for seed length, breadth, width, and length-breadth ratio in the range 12.22-19.88,6.22-8.92,2.26-5.53,1.69-3.01 respectively (5). Seed length, breadth, and width are important economic traits that are not only studied in terms of their relationship with cooking quality but also are yield-determining traits. The length, breadth, and width also determine the overall appearance of seed that influences their marketability based on local consumer preferences.

Among various parameters derived from seed length, seed breadth, and width, the equivalent diameter had a mean value of 8.17 with a range of 6.29 to 9.72 mm. Highest seed equivalent diameter was recorded in the case of WB-20-173 (9.72mm), followed by WB-377 (9.6 mm) and WB-451 (9.5 mm) while as lowest value for equivalent diameter was recorded in WB-1252 (6.29 mm). Equivalent diameter of seeds varied significantly in the range of 7.31–9.24 mm (42). Seed Sphericity had a mean value of 54.54 with a range of 39.95 to 76.59 percent. Highest seed Sphericity was recorded in the case of WB-20-230 (76.59 percent), followed by WB-20-243(75.6 percent) and DARS-10 (73.24 percent) while as the lowest value was recorded in WB-1707(39.95 percent). Seed aspect Ratio had a mean value of 0.52 with a range of 0.26 to 0.87. Highest aspect ratio was recorded in the case of WB-20-243 (0.87), followed by WB-20-230(0.83) and DARS-10 (0.82) while as lowest value for aspect Ratio was recorded inWB-1707 (0.26). Similar results were reported(43).They proposed that sphericity is an important indicator of seed moisture and that the sphericity of seeds decreased nonlinearly with an increase in moisture content and small-seeded beans have invariably higher sphericity and aspect ratio as compared to large seeds. It was reported that the sphericity in bean seeds ranged between 54.4 - 58.9 percent and aspect ratio had a range of 0.49 - 0.55(44). The shape size and volume are depicted in terms of parameters derived from various ratios of length, breadth, and width. The seeds that are longer in length with smaller equivalent diameters are invariably kidney-shaped and are preferred as pulse type whereas the seeds with smaller length, relatively more width, and thickness are preferred as shelled beans. Seeds that are cylindrical with narrow width and thickness are mostly used as vegetable type as small seeds are embedded in succulent pods with a greater pod value ratio. In terms of cooking quality, the seeds that have greater sphericity are invariably easy to cook on account of greater surface area (42).

Seed volume had a mean value of 265.77 mm3 with a range of 92.42 to 445.31 mm3. Highest seed Volume was recorded in the case of WB-20-240 (445.31 mm3), followed by GLP-1(436.24 mm3) and IC-252254 (426 mm3) while as lowest value for seed volume was recorded inWB-303 (92.42 mm3). Surface area had a mean value of 248.54 mm2 with a range of 122.59 mm2 to 357.63 mm2. Highest seed surface area was recorded in the case of WB-2020-240 (357.63 mm2), followed by GLP-1(352.5 mm2) and WB-20-278 (344 mm2) while as lowest value for the surface area was recorded inWB-303 (122.59 mm2).It was reported that the surface area in beans ranged from 127 mm2 - 118 mm2) and volume was in the range of 147 mm3-. 242 mm3)(44). It was reported that the volume of Bambara groundnut seeds increased with an increase in moisture content. The dimensions of the seed, namely seed surface area and seed volume, increased nonlinearly with an increase in moisture content. It was observed that on water absorption the seeds expanded in length, width, thickness, and geometric diameter within the moisture(45).

Seed weight had a mean value of 0.35 g with a range of 0.20 –0.51g. Highest seed weight was recorded in the case of WB-45 (0.51g), followed by WB-20-298 (49.9g) and WB-377 (49.6g) while as lowest value for seed weight was recorded in PBG-569 (0.20 g). Various authors have reported broad ranges of seed weight common bean; 3.5–96.3 g (46), 15.59-66.75 (47), 17.18–53.17 g (48), 12.60-59.94 g (49), 21.50-50.82 g (5).

Seed brilliance had a mean value of 1.50 with a range of 1 (brilliant) to 3 (dull). Highest seed brilliance with a score of 3 was recorded in the case of dull-seeded genotypes WB-966, WB-1634, and WB-216 while as lowest value for seed brilliance was recorded in shiny genotypes like WB-1282 (Figure 3). Similar results were observed that brilliant or shiny seeded varieties have poor water imbibition attributes and demonstrated it in the case of WB-1282 (delayed cooking) and WB-1634 (ETC)(5). WB-1282 has a shiny seed and WB-1634 had a dull seed coat. Shiny seeded genotypes have a comparatively thicker palisade cell layer (27) and also contain more antioxidants such as anthocyanins in their seed coats (13).

There was substantial variation for hilum length and breadth in the genotypes (Figure 2). Highest hilum length was recorded in the case of WB-2020-173(4.1mm), followed by IC-285575 (4.05mm) and WB-20-110 (3.85 mm) while as lowest value for hilum length was recorded in WB-102 (1.11mm).Hilum breadthhad a mean value of 1.45 mm with range of 1.01mm to 2.57mm. Highest hilum breadth was recorded in case of WB-1518 (2.57 mm), followed by WB-401 (2.30 mm) and WB-1182 (1.99mm) while as lowest value for hilum breadth was recorded in N-5 (1.01 mm). Similar results were reported that significant differences in the dimensions of hilum in genotypes with contrasting water absorption, swelling as well as cooking qualities(50,51).In the present study genotype WB-1634 having better attributes of water absorption, and volume expansion and was easy to cook had hilum dimensions of 2.83 × 1.37 mm, compared to WB-1282, which has poor water absorption, volume expansion, and hard to cook, having hilum dimensions of 2.3 × 0.91 mm. The role of the hilum in water uptake, as indicated by the presence of vascular bundles and honeycomb-like structures containing cavities in the hila of lima bean(52). The seed coat thickness and hilum sizetogether influence water absorption, particularly during the initial stage of soaking, while protein content was the major factor in the later stages(29).

The range of trait dispersion is depicted by mean, rangeand coefficient of variation (C.V.). in the present study, highest C.V. value was observed in case seed volume (29.96) of followed by length-breadth ratio (22.87), aspect ratio (22.7), seed brilliance (21.93), surface area (20.68), seed weight (20.02), seed length (16.63), hilum breadth (14.97), seed breadth (13.66), sphericity (12.99) and lowest was reported in hilum length (9.28), seed width (7.85), equivalent diameter (7.7).

Variability for water absorption traits and traits related to cooking time score

There was substantial variability in eighttraits related to water absorption and cooking time in 254 genotypes of common bean indicating significant diversity of the material in respect of studied traits. Water absorption had a mean value of 97.71 percent with a range of 49.24-143.48 percent. Highest seed water absorption was recorded in the case of N-3 (143.48 percent), followed by G-3 (109.23 percent) and WB-418 (108.5 percent) while as lowest value for water absorption was recorded in WB-1006 (49.24 percent). In various earlier studies on water absorption traits in the common bean from Kashmir Himalayas, broader ranges of water absorption havebeen reported; 25.90 - 183.09 percent (23), 21.80 - 294.01 percent (6), 100.66-204.86 percent (5).The water absorption and cooking kinetics of common bean seeds are influenced by their intrinsic (i.e., physical and chemical) properties(45). Variations in water absorption have been attributed to the thickness and texture of the seed coat, the strength of the seed coat attachment, size of the hilum and micropyle, and seed size (30,31,6). Water absorption has been used as an effective surrogate for bean cooking quality (23). The general assumption is that the greater the water absorbed before cooking less will be the cooking time (6). There are, however, observed experimental evidences, that have contrasted this theory. The tenderness in soybean seeds in partially soaked (100% absorption) and fully soaked (142% absorption) seeds(28).

Swelling capacityhad a mean value of 0.32 with range of 0.04 ml/g seed to 0.54 ml/g seed. Highest swelling capacity was recorded in case of WB-195(0.54 ml/g seed), followed by WB-650 (0.54 ml/g seed) and WB-2020-259 (0.538 ml/g seed) while the lowest value was recorded in WB-341 (0.04 ml/g seed). Hydration capacityhad a mean value of 0.68 with a range of 0.39 g/g seed to 0.99 g/g seed. Highest hydration capacity was recorded in case of WB-20-298(0.99 g/g seed), followed by WB-377 (0.98 g/g seed) and WB-966 (0.97 g/g seed) while as lowest value for hydration capacity was recorded in WB-1006 (0.39 ml/g seed). Similar results were reported in a range of 0.12 – 1.02 g/g seed for hydration capacity and 1.02 - 1.56 g/g seed for swelling capacity(23). It was also reported in common beans that the hydration capacity and swelling capacity of the seeds varied significantly in the range of 0.12–0.42 g/seed and 0.09–0.28 mL/seed, respectively. (percent)(42). The CV was highest for swelling capacity (18.62) followed by water absorption (16.28), and hydration capacity (13.61) (23).

Electrical conductivity (EC)had a mean value of 638.63ds/cm with range of 197 to 1906 and ds/cm and C.V. of 28.59 per cent. Highest electrical conductivity was recorded in case of WB-1518-2 (1906µs/cm), followed by WB-1518 (1821µs/cm) and WB-1678 (1607µs/cm) while as lowest value for electrical conductivity was recorded in KD-16 (197µs/cm). Similar results were reported that wide variation in electrical conductivity with a range of 1.99 – 5.87 ms/g of seed(41,42). The response of EC under soaking and found that the EC increased with time both undersoaking as well as cooking(53).

Coat proportion, which is an important trait implicating water imbibition had a mean value of 13.98percent with a range of 9.52 - 19.83 percent. Highest coat proportion was recorded in case of WB-20-182 (19.83), followed by WB-1634 (18.76 percent) and WB-489 (18.29 percent) while as lowest value for coat proportion was recorded in WB-2020-39 (9.52 percent). Various workers have reported broad variation in coat proportion in common bean ; 2.70 – 25.14 percent (23), 4-8 percent (54), 11.12 – 15.39 percent (5). The bean seed coats revealed that seeds with thinner coats have a faster rate of water absorption during the initial soaking period (0–6 hours)(45). Delayed cooking and HTC in legumes is associated mainly with cotyledon and seed coat modifications impairing water absorption. Evidence for the role of seed coat in water imbibition and the prominent pores in beans as well as the cracks in the hydrophobic cuticle layer help in water imbibition leading to seed softening upon soaking(55). Cooking times were sharply reduced after 3h of soaking and plateaued after 6h of soaking. Interestingly, soaking did not make a significant difference in cooking time. Cotyledon cell wall thickness was associated with longer cooking times of soaked beans. The macro-sclereid and osteo-sclereid layers of seed coat contributed to longer cooking times of unsoaked beans suggesting that cotyledon cell wall thickness and composition have a significant role in the genetic variability for the cooking time of soaked beans and variability for seed coat proportion and thickness contribute to the genetic variability for the cooking time in beans(56).

Hardness valuehad a mean value of 20.76 with range of 10.12 to 56.2. Highest hardness value was recorded in case of WB-20-168 (56.2), followed by WB-20-180 (51.2) and WB-1282 (50.1) while as lowest value for hardness value was recorded in WB-1678 (10.12).Seed hardness is a genetically determined character or can be imposed by production and/or storage under harsh environments. Hardness is usually conferred by inability of seed cotyledons to imbibe water upon soaking or under cooking leading to lowered softening of cotyledons. This trait is often exhibited in form of hard shells (Figure 4a and 4b). The increasing storage temperatures from 8.5 to 40 °C increased seed hardness(57).

In the present study we standardised the normal alkali spreading test routinely used in case of rice in our germplasm set using 3% KOH solution as against 1.7% in case of rice. The ASV had a mean value of 3.30 with range of 1 to 7. Highest Alkali spreading value (7) was recorded in case of ETC genotypes such as GLP-1, WB-1518, WB-216 and WB-1634 while as lowest value for alkali spreading value (1) was recorded in delayed cooking genotype such as WB-1282.Similar results shows ASV as a rapid and easy method to evaluate the GT of rice starch granules, the alkali degradation test that relies on visual observation of the degree of dispersion of 6 grains of milled rice after immersion in 1.5 or 1.7 percent KOH overnight. The degree of degradation, is expressed by a numerical score ranging from 1 (kernels not affected by alkali) to 7 (kernels completely dispersed and intermingled)(58). Genetic studies on the inheritance of seed coat texture in cowpea have been few and, in most cases, only two categories of testa textures (smooth and rough) are involved (14).

Seed texturehad a mean value of 1.18 with range of 1 (smooth) to 2 (rough). As in case of ASV, seed texture was rough in case of GLP-1, WB-1518, WB-216 and WB-1634 while as seeds of delayed cooking beans are smooth as in case of WB-1282, WB-112, N-7 and WB-341. Seed texture implicates water absorption by way of effecting seed coat surface area (Figure 3). The significant variation in seed texture in an andean diversity panel of 373 genotypes(56). The range of trait dispersion is depicted by range and C.V. and C.V.value showed that highest C.V. value was observed in case of Alkali spreading value (35.66) followed by Swelling capacity (33.32), hardness value (31.1), EC (28.59), seed texture (23.5), Hydration capacity (20.14) and lowest was observed in coat proportion (12.61), water absorption (6.67).

Variability for cooking time score

There was substantial variability in cooking time score in 254 genotypes of common bean indicating significant diversity of the material in respect of studied traits. Cooking time scored a mean value of 2.61 with a range of 1 to 5 and a C.V of 23.58 percent. Highest cooking time score (5) was recorded in the case of GLP-1, WB-1518, WB-1634, and WB-216 while as lowest value for cooking time score was recorded in delayed cooking beans such as WB-1282.The cooking time is an important trait with implications for gender equity, nutritional value of diets, and energy utilization(18). The cooking time of common beans is influenced by genotype and storage conditions(65). The delayed and hard-to-cook (HTC) traits result in the extended cooking time to confer desired texture. A large number of workers have reported significant variability in common bean germplasm in relation to cooking time score in terms of the time taken to cook or the softening level under cooking(51). 30 bean lines along with 2 checks for cooking time were evaluated. In this experiment, two different methods (Matson and Bags method) were compared. Small-seeded seeds absorbed more water compared to large-seeded seed(59). The variations in the cooking quality traits in the pre-breeding collection of 60 Phaseolus sp landraces from Western Anatolia(60). There was a considerable variation in the variables tested within the germplasm of cooking time (26.0-100.0 min) indicating about fourfold variation in cooking time. There were significant differences between tested dry bean local populations in all evaluated traits. evaluated 15 common bean genotypes on the basis of yield, seed size, hydration properties, and cooking time within market classes recognized by consumers along with three farmers' checks at nine on-farm locations in Uganda for two seasons(66). Cooking time varied from 19 to 271 minutes with the genotypes Cebo Cela and Ervilha consistently cooking fastest in 24 and 27 minutes respectively. Comparatively, the local checks (NABE-4, NABE-15, and Masindi yellow) took 35 to 45 minutes to cook. Cooking time was largely controlled by genotype (40.6 percent of TSS)(61). It was found that the significant variation in cooking time score in Western Himalayan beans indicates the natural variation can be utilised for improving cooking quality in beans(5).

Trait association of seed physical traits and cooking time score

A great deal of phenotyping effort for complex traits like cooking time goes into understanding the trait relationships driven by evolutionary relationships, linkage as well as pleiotropy to identify effective surrogate traits. In the present study, 22 traits were evaluated to get an insight into such relationships and the results (Figure 5) revealed that cooking time score was positively correlated with various seed physical traits including seed brilliance (0.871), followed by seed texture (0.772), Alkali Spreading Value (0.771), Water Absorption (0.137) and negatively correlated with hardness value (-0.531). There was no significant relationship with other seed physical traits. Among other traits, seed texture was positively correlated with seed brilliance (0.895) followed by Alkali spreading value (0.829), hilum breadth (0.130), water absorption (0.125), and negatively correlated with hardness value (-0.422). Akali spreading value was positively correlated with seed brilliance (0.895) and negatively correlated with hardness value (-0.477). It was reported that there were significant differences between tested dry bean local populations in all evaluated traits cooking time was negatively correlated with the conductivity of soaking water as well as the hydration index(66). It was evaluated cooking quality and seed traits in twenty genotypes of common beans and found that oat proportion was negatively correlated with water absorption percentage, swelling coefficient, hydration coefficient as well as cooking time score(23). Cooking time score was positively correlated with hydration and swelling coefficients as well as seed weight but negatively correlated with coat proportion. In a recent study with 40 genotypes using a modified GY*T approach, it was also reported that cooking time score was negatively correlated with seed hardness and positively correlated with hydration parameters like water absorption(5).

PRINCIPAL COMPONENT ANALYSIS

The PCA is a useful data reduction technique that helps plant breeders toreduce the data dimensions and exclude the traits that either have non-significant contributions towards variation or have non-significant correlations with the trait of interest. In the present study, PCA was done based on 22 seed physical traits (Table 3) scored in the lab experiment. The number of PCA is calculated from-correlation matrix and is equal to the number of traits. Based on the eigen value and the cumulative variance accounted, the PCA concentrated about 80 percent of the variability in the first seven PC with eigenvalue> unity. The first three PCs accounted for 54.60 percent variation (19.90, 18.30, and 16.40 percent respectively) mainly contributed by sphericity, aspect ratio, seed breadth, seed length, length-breadth ratio (PC1), surface area, seed volume, equivalent diameter, seed breadth, seed length (PC2) and seed brilliance, alkali spreading value, cooking time score, seed texture, hardness value (PC3). The eigenvalue ranges from 4.377 for PC1 to 1.073 for PC7.

The PCA biplot (Figures 6 and 7) can be effectively used as independent selection criteria based on multiple traits with a focus on the target trait (cooking quality in the present case) evaluation (62). The distance to the biplot origin (vector length) of a trait indicates how well the trait is represented in the biplot; a relatively short vector indicative of weak or lack of correlation with other traits (63), invariably due to poor goodness of fit of the biplot as the two PCs (PC1 and PC2) account for only a part of total variation (the goodness of fit of the GT biplot in Fig. 6 is 38.29 percent). Similar results have been reported in beans(64,65). However, due to the obvious limitations of PCA biplots in elucidating the trait associations in selection on account of the dispersal of traits in all directions, the use of a modified GYT biplot for cooking quality based on seed physical traits(5).

Table 1: Descriptive Statistics for 14 seed physical traits in 254 common bean genotypes

Variable	Min	Max	Mean + SE	CV %)
SL	9.82	19.95	15.27 + 0.16	16.63
SB	4.76	9.34	7.67 + 0.07	13.66
SWD	4.04	5.80	4.74 + 0.02	7.85
EQD	6.29	9.72	8.17 + 0.04	7.70
SPH	39.95	76.59	54.54 + 0.44	12.99
L/B	1.15	3.83	2.03 + 0.03	22.87
ASP	0.26	0.87	0.52 + 0.01	22.70
VOL	92.42	445.31	265.77 + 5.00	29.96
SA	122.59	357.63	248.54 + 3.23	20.68
SDW	0.20	0.51	0.35+ 0.004	20.02
HL	1.11	4.12	3.63 + 0.28	9.28
HB	1.01	2.57	1.45 + 0.01	14.97
BRL	1.00	3.00	1.50 + 0.05	21.93
TXT	1.00	2.00	1.18 + 0.02	23.50

Trait legend: *SL: Seed Length, SB: Seed Breadth, SWD: Seed Width, EQD: Equivalent diameter, SPH: Sphericity, L/B: Length/breadth ratio, ASP: Aspect ratio, VOL: Seed Volume, SA: Surface area, SDW: Seed weight, HL: Hilum Length, HB: Hilum Breadth, BRL: Seed brilliance, TXT: Seed texture*

Table 2: Descriptive Statistics for eight seed water absorption traits in 254 common bean genotypes

Variable	Min	Max	Mean + SE	CV %)
WA.	49.24	143.48	97.71 + 0.41	6.67
SWC	0.04	0.54	0.32 + 0.01	33.32
HYC	0.39	0.99	0.68 + 0.01	20.14
CP	9.52	19.83	13.98 + 0.11	12.61
HRDV	10.12	56.20	20.76 + 0.67	31.10
EC	197.00	1906.00	638.63 + 19.47	28.59
ASV	1.00	7.00	3.30 + 0.09	35.66
CTS	1.00	5.00	2.61 + 0.06	23.58

Trait legend: *WA: Water absorption capacity, SWC: Swelling capacity, HYC: Hydration capacity, CP: Coat proportion, HL: Hilum Length, HB: Hilum Breadth, HRDV: Hardness value, EC: Electrical conductivity, ASV: Alkali spreading value, TXT: Seed texture, CTS: Cooking time Score*

Table 3: Principal Component Analysis

Component	Eigenvalue	Proportion var. (%)	Cumulative Variance (%)
Component 1	4.38	19.90	19.90
Component 2	4.02	18.30	38.20
Component 3	3.61	16.40	54.60
Component 4	1.93	8.80	63.40
Component 5	1.36	6.20	69.60
Component 6	1.26	5.70	75.30
Component 7	1.07	4.90	80.20

Figure 1: Variability for seed length, breath, shape, colour and mottling pattern in Western Himalayan beans

Figure 2: Variability in hilum dimensions in common bean

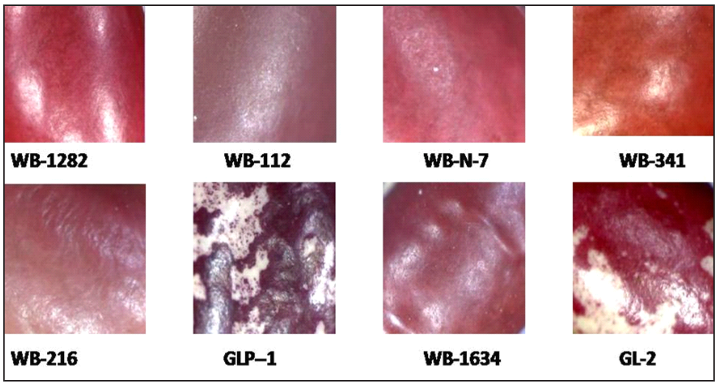

Figure 3: Variability in seed coat texture in common beans

Figure 4a: Easy to cook genotypes without hard shell

Figure 4b: Delayed cooking genotypes with hard shell

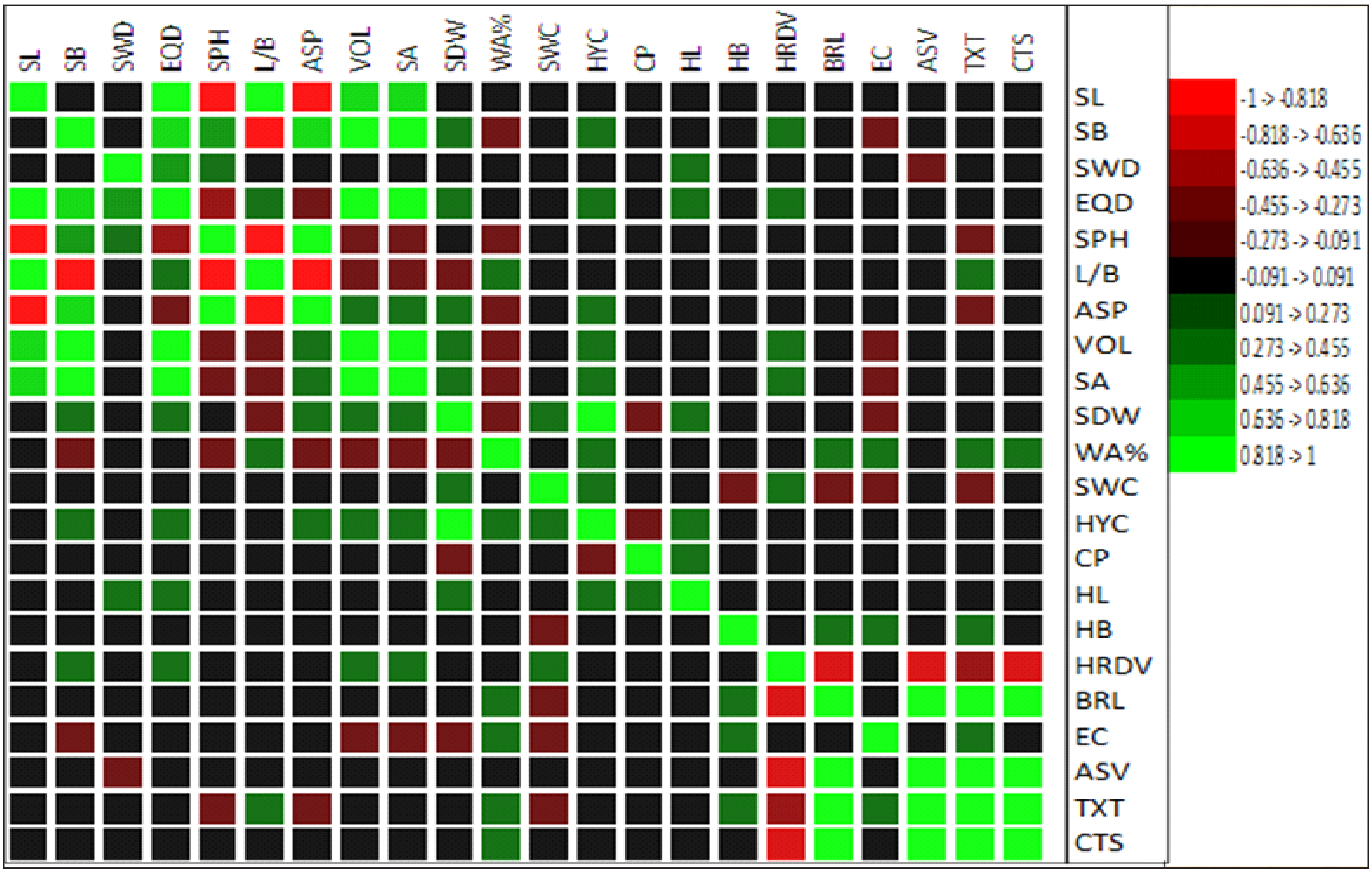

Figure 5: Correlation heat map of 22 seed physical and water absorption traits in relation to cooking quality

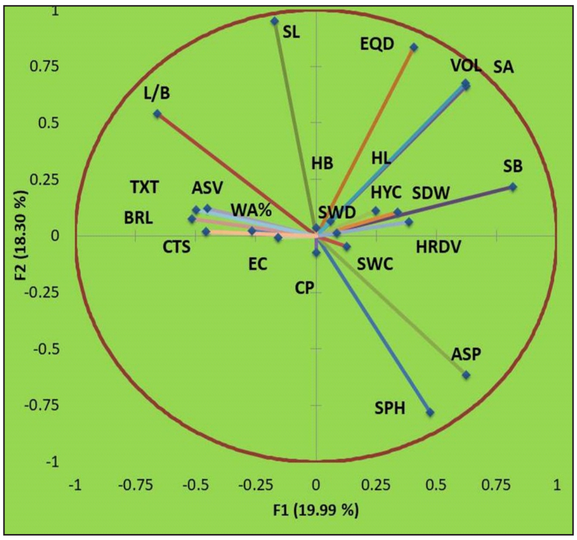

Figure 6: PCA biplot of seed physical, water absorption and cooking traits in common bean

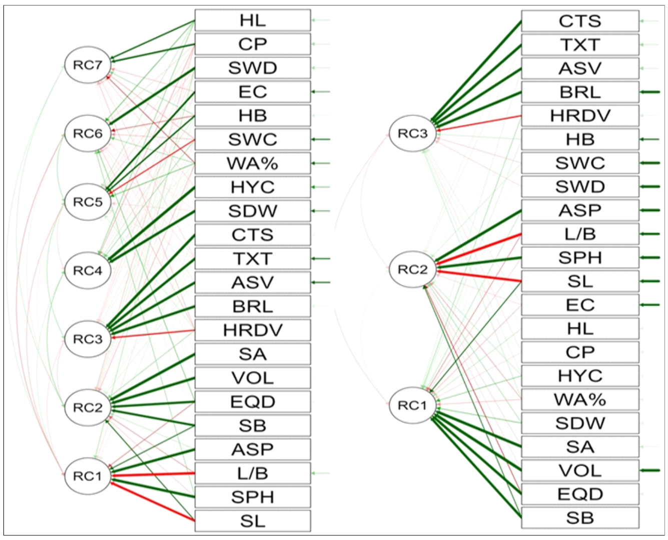

Figure 7: Path analysis showing trait relationship with Principal components

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