LRF-717
[1-7]

RESEARCH ARTICLE Legume Research- An International Journal

Association of Seed Amylose Content with Agronomic Traits and *GBSS1a* Haplotypes in Common Bean Landraces

Sookyeong Lee[1], Do Yoon Hyun[1], Kyung Jun Lee[2], Seungbum Lee[1], Gyu-Taek Cho[1], Hee-Jong Koh[3]

10.18805/LRF-717

ABSTRACT

Background: Common bean (*Phaseolus vulgaris* L.) is a valuable source of starch, proteins and minerals in the human diet. Starch, a major component of bean seeds, is composed of amylose and amylopectin and the content of amylose is important for affecting the quality of bean-derived food products. However, limited information is available on amylose assessment in bean.

Methods: In this study, for a total of 300 bean landraces, we evaluated the seed amylose content and characterized twenty agronomic traits and analyzed the granule-bound starch synthase 1a (*GBSS1a*) gene haplotypes, which are likely to be associated with amylose content. Also, the correlation of amylose content with agronomic traits and *GBSS1a* gene haplotypes were determined.

Result: Bean landraces showed diverse phenotypes, highly variable amylose content (39.95-63.57%) and three *GBSS1a* haplotypes (YP, YQ and NP). Correlation analysis revealed that seed size and seed weight were significantly correlated with amylose content and NP-type beans contained more amylose than YQ-type beans. Although these correlations could not fully explain the tremendous amount of variation in seed amylose content in bean germplasm, information on starch composition-related *GBSS1a* haplotypes and agronomic traits would facilitate genetic analyses and breeding research targeted toward improving the composition of bean starch.

Key words: Agronomic trait, Amylose, Common bean, *GBSS1a* gene, Haplotype, Landrace, Starch.

INTRODUCTION

Common bean seeds are a prominent source of complex carbohydrates (50-60%), proteins (20-25%) and minerals (Basavaraja *et al*., 2021; Rasool *et al*., 2019). Starch represents a major nutritional constituent of carbohydrates in the human diet and is composed of amylose and amylopectin (Tayade *et al*., 2019). Amylose is a linear structure linked by α-1,4-glycosidic bonds and is synthesized by granule-bound starch synthases (GBSSs), whereas amylopectin is a branched structure linked by both α-1,4-glycosidic and α-1,6-glycosidic bonds and is synthesized by soluble starch synthases (SSs), branching enzymes (BEs) and debranching enzymes (DBEs) (Dupuis *et al*., 2014). Given their distinct structures, the amylose-to-amylopectin ratio influences the properties of starch (Dupuis *et al*., 2014). Among these two starch components, the content of amylose was known to be highly correlated with the quality of starchy foods, as it determined the pasting and gel-texture properties of starch during cooking (Punia *et al*., 2020). Therefore, evaluating the amylose content of common bean accessions can provide a strong foundation for improving the quality of bean-derived food products.

Despite the importance of common bean in the human diet, most studies on starch and amylose have been conducted on cereal crops and only a few on legumes (Tayade *et al*., 2019). Moreover, among the studies conducted on legume starch, most were performed on pea (*Pisum sativum* L.). For example, Hıbl *et al*. (2001) assessed variation in starch and amylose content in 402 pea accessions; Jha *et al*. (2015) examined allelic variation in genes associated with amylose and total starch contents of 169 pea accessions; and Carpenter *et al*. (2017) performed an association analysis on 25 starch metabolic genes and amylose content of 92 pea accessions. Among the studies conducted on common bean, Rivera *et al*. (2018) evaluated variations in the seed starch and amylose content of 202 common bean accessions, while Isono *et al*. (2003) and Matsui *et al*. (2003) reported that *GBSS1a* and *GBSS1b* genes were mainly involved in the synthesis of amylose in

[1]National Agrobiodiversity Center, National Institute of Agricultural Sciences, RDA, Jeonju 54874, Republic of Korea.
[2]Honam National Institute of Biological Resources, Mokpo 58762, Republic of Korea.
[3]Department of Agriculture, Forestry and Bioresources, Plant Genomics and Breeding Institute, Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea.

Corresponding Authors: Gyu-Taek Cho and Hee-Jong Koh, National Agrobiodiversity Center, National Institute of Agricultural Sciences, RDA, Jeonju 54874, Republic of Korea; Department of Agriculture, Forestry and Bioresources, Plant Genomics and Breeding Institute, Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea. Email: gtcho@korea.kr; heejkoh@snu.ac.kr

How to cite this article: Lee, S., Hyun, D.Y., Lee, K.J., Lee, S., Cho, G.T., Koh, H.J. (2022). Association of Seed Amylose Content with Agronomic Traits and *GBSS1a* Haplotypes in Common Bean Landraces. Legume Research. DOI: 10.18805/LRF-717.

Submitted: 24-08-2022 **Accepted:** 10-11-2022 **Online:** 24-11-2022

storage starches in seeds and amylose in transitory starches in leaves, respectively. However, the relationship between *GBSS1a* haplotype and seed amylose content in common bean has not been investigated.

Therefore, the aims is to (i) evaluate the diversity of seed amylose content in common bean; (ii) elucidate the relationship between amylose content and agronomic traits; and (iii) analyze the *GBSS1a* gene haplotypes, which are likely to be associated with amylose content. The results improve the understanding of the genetics of seed amylose content in common bean. Additionally, correlation analysis of amylose content and agronomic traits provides sophisticated information about the breeding material that could be used for improving the starch composition of common bean.

MATERIALS AND METOHDS

Plant materials

A total of 300 common bean landraces originating from six countries, including Korea (44), China (43), Georgia (64), Ukraine (38), Bulgaria (66) and El Salvador (45), were obtained from the National Agrobiodiversity Center (NAC) of the Rural Development Administration (RDA), Republic of Korea (http://genebank.rda.go.kr). The common beans were sown in plug trays on April 9, 2020 and the seedlings were transplanted into the experimental field of NAC on April 29, 2020. The planting distances were 110 cm between the rows and 40 cm between each other in a row. Each accession consisted of 10 plants and twenty agronomic traits were recorded in all of the plants during various stages of their growth. Leaf samples were collected from ten 14-day-old plants of each accession and used for DNA extraction. At maturity, the pods were harvested and threshed before determining the amylose content in the seeds.

Agronomic trait characterization

Twenty agronomic traits (9 qualitative and 11 quantitative traits) were characterized according to the NAC descriptors (Table S1). Qualitative traits were recorded as representative values of 10 plants per accession and quantitative traits were recorded as their average. The average values of measured pod length (PL), pod width (PW) and seed number per pod (SNPP) were taken from 10 pods. The seed area (SA), perimeter (SP), length (SL), width (SW) and length/width ratio (L/W) of 20 seeds per accession were measured using ImageJ software (Schneider *et al*., 2012).

Determination of seed amylose and starch contents

The experimental design was completely randomized and incorporated biological duplicates. Seed starch content (mg/100 mg flour) was determined using the rapid total starch (RTS) method (Megazyme International Ireland, Ltd., Ireland). Seed amylose content (mg/100 mg flour) was determined with the colorimetric method using I_2/KI, as described by Juliano (1971). The percent amylose content of seeds was estimated based on their amylose and starch contents (mg/100 mg flour), as follows:

$$\text{Percent amylose content} = \frac{\text{Amylose content}}{\text{Starch content}} \times 100$$

GBSS1a haplotype analysis

Genomic DNA was extracted from 20 mg freeze-dried leaves of each accession using the Gentra Puregene Tissue Kit (QIAGEN, Inc., USA). Six primer sets flanking different regions of the *GBSS1a* reference gene sequence (Phvul.001G082500; source, *P. vulgaris* cv. 'G19833,), from the 5' untranslated region (5′UTR) to 3′UTR (3,919 bp), were designed using the NCBI primer designing tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) (Table S2). PCR was performed in a 20 μL reaction volume containing 1 μL genomic DNA (50 ng/μL), 1 μL each of forward and reverse primers (10 pmoles/μL), 10 μL of 2 × Direct PCR premix (Inclone Biotech, Republic of Korea) and 8 μL distilled water. PCR was performed on the SimpliAmp Thermal Cycler (Thermo Fisher Scientific, USA) under the following conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 15 s, 58°C for 30 s and 72°C for 1 min, followed by a final extension at 72°C for 5 min. Then, 20 μL of the PCR product of each reaction was mixed with 4 μL Dyne LoadingSTAR (Dyne Bio, Republic of Korea) and samples were electrophoresed on a 1% agarose gel in 0.5× TBE buffer at 135 V for 40 min. DNA-containing gel slices were excised using a razor blade under a UV transilluminator and DNA was purified using the QIAquick Gel Extraction Kit (QIAGEN, Inc., USA). The purified DNAs were sequenced by GenoTech, Corp. (Daejeon, Republic of Korea). Multiple DNA sequences were aligned with the reference gene sequence using BioEdit version 7.0.5.3 (Hall, 1999) to identify polymorphisms.

Statistical analysis

The R software (version 4.0.2) was used for all statistical analyses. Analysis of variance (ANOVA) was performed to compare the amylose content of various genotypes. The Pearson correlation coefficient was determined to test the correlation of amylose content with various quantitative traits. K-means clustering analysis was performed using 11 quantitative agronomic traits.

RESULTS AND DISCUSSION

Variation in agronomic traits

Nine qualitative traits were evaluated among the 300 bean landraces and diverse phenotypes were observed (Fig 1). Approximately 50% of the germplasm showed an indeterminate upright growth habit, while the remaining 50% showed determinate and indeterminate bush growth habits. The banner and wings of flowers showed both solid and patterned colors; however, only a few accessions displayed patterned colors in the wings. Pods showed more diverse colors at maturity than at harvest stage. Most bean accessions produced slightly curved or straight pods containing weak- or medium-brilliance seeds and the seeds showed eight different colors and five different shapes. All

11 quantitative traits examined in this study showed wide variation between the bean landraces (Table 1). DTF (days to flowering, 53.74±5.22 days) and DTM (days to maturity, 87.42±11.47 days) were approximately 1 month apart. The average PL and PW were 11.51±2.44 cm and 12.09±2.88 mm, respectively. Among the seed-related traits, SA (79.00±21.81 mm^2) and 100SW (100-seed weight, 38.04±12.86 g) showed the greatest variation. The K-means clustering results revealed three clusters (Fig S1). The clusters 1, 2 and 3 consisted of 132, 75 and 93 accessions, respectively. Average values of SA, SP, SL, SW, 100SW and PW increased in the order of 1, 2 and 3, whereas average values of SNPP decreased, indicating that these traits were the primary clustering determinants. Also, cluster 3 had higher DTF and DTM values than clusters 1 and 2. The average values of PL and L/W increased in the order of cluster 2, 3 and 1.

Variation in seed amylose and starch contents

The starch, amylose and percent amylose contents of bean seeds varied considerably between the landraces. The average values and ranges of starch, amylose and percent amylose contents were 32.21±3.32 mg/100 mg flour (range, 22.20-43.36 mg/100 mg flour), 15.51±1.67 mg/100 mg flour (range, 10.03-19.70 mg/100 mg flour) and 48.24%±3.19% (range, 39.95-63.57%), respectively (Table 2). Previous studies on common bean cultivars revealed variation in seed starch and amylose contents, ranging from 15.4 to 60 mg/

Table S1: Summary of 20 agronomic traits in common bean according to National agrobiodiversity center (NAC) descriptors.

Qualitative traits	Organ	Description
Growth habit	-	1: determinate bush; 2: indeterminate bush; 3: indeterminate upright
Banner color	Flower	1: white; 2: green; 3: purple; 4: white with purple dots or red stripes; 5: dark purple with purple stripes; 6: orange; 7: red purple
Wing color	Flower	1: white; 2: green; 3: purple; 4: orange; 5: white with purple dots or red stripes; 6: red; 7: dark purple with purple stripes; 8: red purple
Pod color at maturity	Pod	0: light yellow; 1: dark red purple; 2: orange; 3: green with red purple stripes; 4: green with red stripes; 5: dark red; 6: green; 7: grey green; 8: yellow
Pod color at harvest	Pod	1: yellow; 2: brown; 3: dark green; 4: dark brown; 5: others
Pod curvature	Pod	1: straight; 5: slightly curved; 7: curved; 9: recurving
Seed brilliance	Seed	0: matt; 3: weak; 5: medium; 7: shiny
Seed color	Seed	1: white; 2: yellow; 3: green; 4: red; 5: red purple; 6: navy; 7: brown; 8: black; 9: pink; 10: bicolor
Seed shape	Seed	1: round; 2: oval; 3: cuboid; 4: kidney shaped; 5: truncate fastigiate
Quantitative traits	**Organ**	**Description**
Days to flowering	Flower	Days from sowing to flowering in 2-3 plants
Days to maturity	Pod	Days from sowing to pod maturity in 2-3 plants
Pod length	Pod	Average length (cm) of 10 fully expanded immature pods measured parallel to the pod structure
Pod width	Pod	Average length (mm) of 10 fully expanded immature pods measured orthogonally to the pod structure
Seed number per pod	Seed	Average number of seeds in 10 mature pods
Seed area	Seed	Average area (mm^2) of 20 seeds when placed on a horizontal surface
Seed perimeter	Seed	Average perimeter (mm) of 20 seeds when placed on a horizontal surface
Seed length	Seed	Average of the longest length (mm) of 20 seeds placed on a horizontal surface and measured parallel to the seed structure
Seed width	Seed	Average of the shortest length (mm) of 20 seeds placed on a horizontal surface and measured orthogonally to the seed structure
Length/width ratio	Seed	Calculated by dividing seed length by seed width
100-seed weight	Seed	Average weight (g) of 100 seeds

Table S2: List of six primer sets used to amplify *GBSS1a* gene fragments.

Forward primer (5′→3′)	Reverse primer (5′→3′)	Amplified fragment size (bp)
GCTCCGCACTGCTACAAATAC	TCAATAATCCACTTACGGCCAAAGC	707
AGATCGAGTGCGGGATGAAC	ACACGTGAAGTTACCTGGCA	947
GGTTCAAAACTCTACGGCCC	TTCGGAACAGAGAACGGTGTG	905
ACACTGCTCTTCTTCCGTGC	AGGGATTGAACCATGAGCTTAAC	985
AGCCATCCCAAAGTTCATTGAC	ATTCCAGCTTCACTGCGACC	997
CTACCGTAAGGAGAGCCCTTG	AGAACGGTGAGACCAAAGCCAA	700

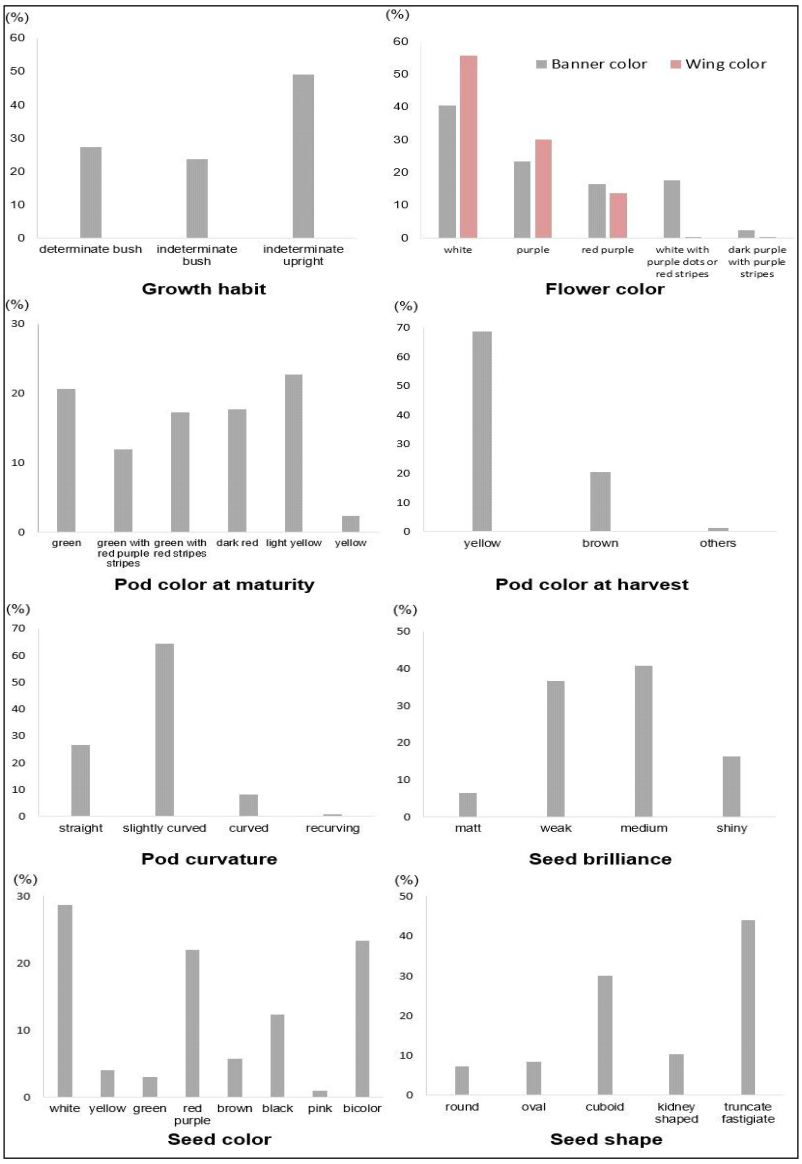

Fig 1: Distribution of nine qualitative traits among 300 common bean germplasms. Bar graphs show the percentage distribution of each phenotype.

Table 1: Summary of 11 quantitative traits evaluated in 300 common bean germplasms.

Trait	Range	Mean±SD	CV (%)
Days to flowering (d)	44-75	53.74±5.22	9.71
Days to maturity (d)	75-134	87.42±11.47	13.12
Pod length (cm)	5.56-21.57	11.51±2.44	21.20
Pod width (mm)	5.80-28.67	12.09±2.88	23.82
Seed number per pod	2.33-8.60	4.95±0.95	19.19
Seed area (mm^2)	27.59-136.22	79.00±21.81	27.61
Seed perimeter (mm)	20.04-48.52	35.47±5.30	14.94
Seed length (mm)	6.52-18.54	12.86±2.25	17.50
Seed width (mm)	5.37-10.15	7.69±0.98	12.74
Length/width ratio	1.22-2.48	1.68±0.22	13.10
100-seed weight (g)	13.12-76.64	38.04±12.86	33.81

SD-Standard deviation; CV-Coefficient of variation in percentage.

Fig S1: Clustering of 300 common bean accessions based on 11 quantitative agronomic traits.

Table 2: Variation in starch, amylose and percent amylose contents of bean seeds between 300 landraces.

Trait	Range	Mean±SD	CV (%)
Starch content (mg/100 mg flour)	22.20-43.36	32.21±3.32	10.31
Amylose content (mg/100 mg flour)	10.03-19.70	15.51±1.67	10.77
Percent amylose content (%)	39.95-63.57	48.24±3.19	6.61

SD-Standard deviation; CV-Coefficient of variation in percentage.

100 mg flour and 10.2% to 51.1% amylose content (Keskin *et al*., 2022; Tayade *et al*., 2019). Whereas the range of starch content of 300 common bean landraces investigated in the current study (22.20-43.36 mg/100 mg flour) was within that reported previously, the range of amylose content of these landraces (39.95-63.57%) was not completely consistent with previous data. Among the 300 landraces examined, the amylose content of 22 landraces was more than 52%. This extreme variation in seed amylose content observed between the 300 landraces could be explained by the diverse geographical origins of the landraces (Kanwar and Mehta, 2018; Murube *et al*., 2021). Moreover, common bean starch contains the highest amylose content compared with other legume starches (Punia *et al*., 2020); therefore, finding bean accessions with high amylose content is not difficult.

GBSS1a haplotype analysis

Relative to the *GBSS1a* reference gene sequence of 'G19833', the common bean germplasm investigated in this study showed two non-synonymous single nucleotide polymorphisms (SNPs) in exon 10 of the *GBSS1a* gene at 2,748 and 2,878 bp positions (Fig 2). The SNPs at 2,748 and 2,878 bp were predicted to cause tyrosine (Y, TAT) to asparagine (N, AAT) and proline (P, CCA) to glutamine (Q, CAA) substitutions, respectively. Three different SNP haplotypes were identified in the germplasm, which were named according to the combination of amino acids: YP haplotype, which was identical to the reference sequence and YQ and NP haplotypes, which carried one amino acid substitution relative to the reference sequence (Fig 2). Notably, the NQ haplotype, carrying two amino acid substitutions relative to the reference, was not found among the 300 common bean landraces investigated in this study.

Correlation analysis between amylose content and agronomic traits

We examined the correlation between seed amylose content and 11 quantitative agronomic traits by determining the Pearson correlation coefficient at $p<0.05$ (Fig 3). The results showed that amylose content was positively correlated with PW, SW, SA, 100SW, SP and SL. Purwanti *et al*. (2019) reported a significant positive correlation between amylose content and seed size in lablab bean (*Dolichos lablab* L.) seeds, consistent with the current study on common bean seeds. On the other hand, we also found that SNPP showed a negative correlation with amylose content and seed size, implying that an increase in SNPP is accompanied by a decrease in seed size, resulting in lower amylose content. Furthermore, analysis of the correlation between amylose content and seed weight in previous studies produced different results, depending on the plant species or germplasm under investigation. For example, Rivera *et al*. (2018) reported no correlation between amylose content and 100-seed weight in a Spanish core collection of common bean accessions, whereas Tahir *et al*. (2011) reported a negative correlation between amylose content and 1,000-seed weight in lentil (*Lens culinaris* Medik.). In contrast to these studies, we identified a positive correlation between

amylose content and 100SW in a collection of 300 common bean landraces.

Correlation analysis between amylose content and *GBSS1a* Haplotype

Common bean landraces with the YP, YQ and NP haplotypes of the *GBSS1a* gene contained 48.31%±3.52%, 47.57%±2.76% and 49.37%±3.08% amylose, respectively. Landraces with the NP haplotype showed higher amylose content than those with YQ haplotype (p<0.05) (Table 3). However, this correlation could not fully explain the tremendous amount of variation in seed amylose content in bean germplasm, demonstrating amylose content is a quantitative trait influenced by numerous genes.

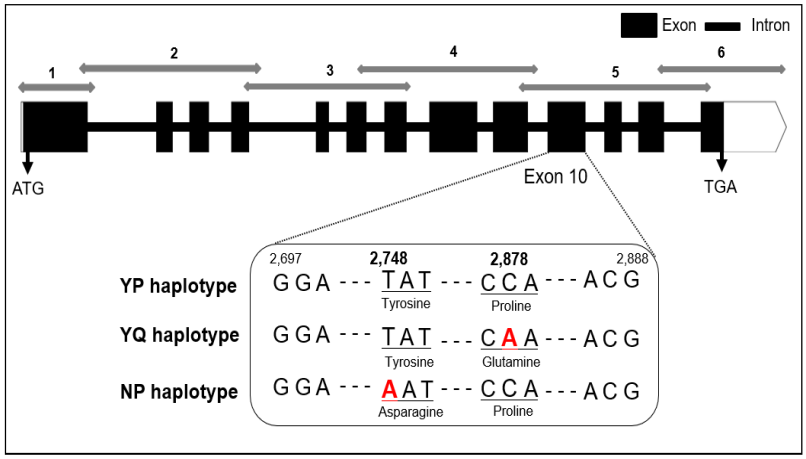

Fig 2: *GBSS1a* gene structure and haplotypes identified in the common bean germplasm. The gene structure was visualized with the Exon-Intron Graphic Maker (http://wormweb.org/exonintron). White boxes at the ends of the gene diagram represent the untranslated regions: 5′UTR (left) and 3′UTR (right). Six double-headed arrows above the gene diagram indicate the primer sets used to amplify gene fragments. Three *GBSS1a* gene haplotypes identified in this study are outlined below the gene diagram and nucleotide polymorphisms relative to the reference gene are highlighted in red.

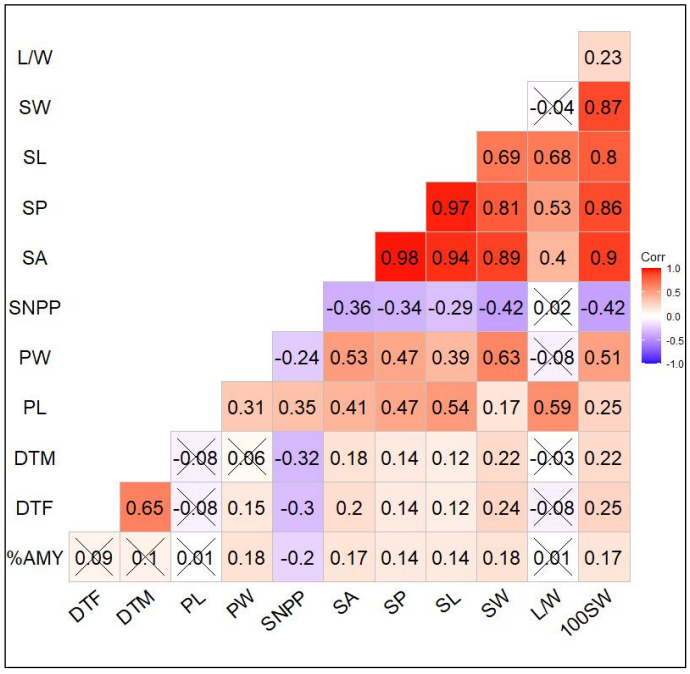

Fig 3: Correlation analysis of percent amylose content and quantitative agronomic traits. Positive and negative correlations between traits are indicated by red and blue backgrounds, respectively. The correlation is stronger when the hue is darker. Non-significant correlation coefficients (p>0.05) are marked as "X". %AMY-Percent amylose content; DTF-Days to flowering; DTM-Days to maturity; PL-Pod length; PW-Pod width; SNPP-Seed number per pod; SA-Seed area; SP-Seed perimeter; SL-Seed length; SW-Seed width; L/W-Length/width ratio; 100SW-100-seed weight.

Table 3: Percent amylose content of common bean landraces with different *GBSS1a* haplotypes.

GBSS1a haplotype	Number of accessions	Amylose content (%)[1]
YP	105	48.31±3.52 [ab]
YQ	126	47.57±2.76 [b]
NP	69	49.37±3.08 [a]

[1]Data represent mean±standard deviation; Different lowercase superscript letters indicate significant differences (p<0.05).

CONCLUSION

In this study, we evaluated the range of diversity in the seed amylose content, agronomic traits and *GBSS1a* gene haplotypes of 300 common bean landraces and examined potential correlations between these variables. The bean landraces showed a wide variation in amylose content. Although this diversity in amylose content was not fully explained by its correlation with agronomic traits or *GBSS1a* gene haplotypes, this study contributes toward improving the amount of information currently available on starch content and composition in legumes. Haplotype analysis of other starch synthesis genes, such as *BE*s and *DBE*s, in common bean would pave the way for further advancements in research on legume starch.

ACKNOWLEDGEMENT

This research was funded by the Research Program for Agricultural Science and Technology Development, National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea, grant number [PJ01424201].

Conflict of interest: None.

REFERENCES

Basavaraja, T., Manjunatha, L., Chandora, R., Gurumurthy, S., Singh, N.P. (2021). Assessment of genetic variability, diversity and trait correlation analysis in common bean (*Phaseolus vulgaris* L.) Genotypes. Legume Research. 44(3): 252-260.

Carpenter, M.A., Shaw, M., Cooper, R.D., Frew, T.J., Butler, R.C., Murray, S.R., Moya, L., Coyne, C.J., Timmerman-Vaughan, G.M. (2017). Association mapping of starch chain length distribution and amylose content in pea (*Pisum sativum* L.) using carbohydrate metabolism candidate genes. BMC Plant Biology. 17(1): 132.

Dupuis, J.H., Liu, Q., Yada, R.Y. (2014). Methodologies for increasing the resistant starch content of food starches: A review. Comprehensive Reviews in Food Science and Food Safety. 13(6): 1219-1234.

Hall, T.A. (1999). BioEdit: A User-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series. 41: 95-98.

Hıbl, M., Urban, J., Václavíková, J., Griga, M. (2001). Evaluation of pea genetic resources collection for seed starch, amylose/amylopectin and protein content. Czech Journal of Genetics and Plant Breeding. 37: 114-123.

Isono, N., Ito, H., Senoura, T., Yoshikawa, M., Nozaki, K., Matsui, H. (2003). Molecular cloning of a cdna encoding granule-bound starch synthase i from kidney bean seeds and expression in escherichia coil. Journal of Applied Glycoscience. 50(3): 355-360.

Jha, A.B., Tar'an, B., Diapari, M., Warkentin, T.D. (2015). SNP variation within genes associated with amylose, total starch and crude protein concentration in field pea. Euphytica. 206(2): 459-471.

Juliano, B.O. (1971). A simplified assay for milled-rice amylose. Cereal Science Today. 16: 334-340.

Kanwar, R., Mehta, D.K. (2018). Survey, collection and seed morphometric characterization of French bean (*Phaseolus vulgaris* L.) landraces of Himachal Pradesh. Legume Research. 41(3): 333-341.

Keskin, S.O., Ali, T.M., Ahmed, J., Shaikh, M., Siddiq, M., Uebersax, M.A. (2022). Physico-chemical and functional properties of legume protein, starch and dietary fiber-A review. Legume Science. 4(1): e117.

Matsui, H., Senoura, T., Isono, N., Sakurai, Y., Hamada, S., Ito, H. (2003). Granule-bound starch synthase i isozyme localized predominantly in kidney bean leaves. Journal of Applied Glycoscience. 50(4): 493-497.

Murube, E., Beleggia, R., Pacetti, D., Nartea, A., Frascarelli, G., Lanzavecchia, G., Bellucci, E., Nanni, L., Gioia, T., Marciello, U., Esposito, S., Foresi, G., Logozzo, G., Frega, G.N., Bitocchi, E., Papa, R. (2021). Characterization of nutritional quality traits of a common bean germplasm collection. Foods. 10(7): 1572.

Punia, S., Dhull, S.B., Sandhu, K.S., Kaur, M., Purewal, S.S. (2020). Kidney bean (*Phaseolus vulgaris*) starch: A review. Legume Science. 2(3): e52.

Purwanti, E., Prihanta, W., Fauzi, A. (2019). The diversity of seed size and nutrient content of lablab bean from three locations in indonesia. International Journal of Advanced Engineering, Management and Science. 5: 395-402.

Rasool, S., Nazir, M., Sofi, P.A., Murtaza, I., Shikari, A.B., Shah, M.D., Nazir, N., Nehvi, F.A., Zargar, S.M. (2019). Mineral profiling of common bean (*Phaseolus vulgaris* L.) germplasm. Indian Journal of Agricultural Research. 53(3): 270-276.

Rivera, A., Plans, M., Sabate, J., Casanas, F., Casals, J., Rull, A., Simo, J. (2018). The spanish core collection of common beans (*Phaseolus vulgaris* L.): An important source of variability for breeding chemical composition. Frontiers in Plant Science. 9: 1642.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods. 9(7): 671-675.

Tahir, M., Lindeboom, N., Baga, M., Vandenberg, A., Chibbar, R.N. (2011). Composition and correlation between major seed constituents in selected lentil (*Lens culinaris*. Medik) genotypes. Canadian Journal of Plant Science. 91(5): 825-835.

Tayade, R., Kulkarni, K.P., Jo, H., Song, J.T., Lee, J.D. (2019). Insight into the prospects for the improvement of seed starch in legume-A review. Frontiers in Plant Science. 10: 1213.