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Phenotypic diversity of mung bean [*Vigna radiata* (L.) Wilczek] genotypes based on qualitative traits, in South Ethiopia

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Abstract

Understanding genetic diversity is vital for the conservation and utilization of genetic resources of crops. To date, the available information on genetic resource collection, phenotypic characterization, and conservation of mung bean genotypes in Ethiopia is limited. To fill these knowledge gaps, the present study was conducted at Jinka Agricultural Research Center during the 2018 cropping season. The objective of the study was to determine the extent of genetic diversity among mung bean genotypes using qualitative morphological traits. A total of 60 mung bean genotypes were evaluated using a 6×10 alpha lattice design replicated twice. Data on 30 qualitative traits were collected and subjected to the Shannon-Weaver diversity index (H') and multivariate analyses. The estimate of H' ranged from 0.00 for germination type to 0.96 for seed shape. The first seven principal components explained 94.0% of the total variation. In general, leaf pubescence, attachment of primary leaves, pod attachment to the peduncle, and seed shape were prominent genetic variations among mung bean genotypes, and were the most effective traits for distinguishing among mung bean genotypes and could be used as selection criteria for improvement. Cluster analysis based on qualitative traits revealed four distinct groups. The highest inter-cluster distance was found between Cluster I and IV (44.67). This investigation revealed the existence of enormous genetic diversity of mung bean genotypes which should be exploited by comprehensive mung bean variety development programs.

Keywords: Divergence, Diversity index, Genetic resources, Qualitative traits

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INTRODUCTION

Mung bean [*Vigna radiata* (L.) Wilczek] is an important legume crop (Rahim et al., 2010). It is a short-duration legume and belongs to the third largest family of flowering plants, with approximately 650 genera and nearly 20,000

species (Doyle, 2003). Cytological studies have indicated that mung bean has a diploid chromosome number of $2n = 2x = 22$ (Kang et al., 2017).

The study of genetic diversity is important for the development of crop varieties. The study of plant genetic resources as a part of biodiversity conservation realizes cultivated varieties in current use and obsolete cultivars as well as wild and weedy species as useful biological resources for food security (Ulukan, 2011; Ogwu et al., 2014). As suggested by Mohan et al. (2014), the characterization of germplasm helps to form the groups with specific traits and also provides the idea about those traits that help for distinguishing the genotypes from each other. An emphasis has been placed on the morphological characterization to assess the variability among genotypes and some of the agro-morphological traits which could be used as a morphological marker in crop improvement (Tantasawat et al., 2010).

The grouping of genotypes based on agro-morphological traits can easily be detected by the naked eyes and can be used in the mung bean breeding program for improving the physical quality of seed. Morphological traits can be used to assess phenotypic variation in growing environments and are also used as tools for the indirect analysis of genetic variability and diversity. Tabasum et al. (2010) reported the genetic variability in ten green gram genotypes and the extent to which the traits associated with yield could be useful for establishing selection criteria for high seed yield in mug bean breeding.

Genetic diversity is an important factor and a prerequisite in any breeding program. Quantifying genetic diversity is indispensable for designing and accelerating breeding programs. Therefore, the collection, conservation, characterization, and evaluation of available germplasm is important to determine the magnitude of genetic diversity for further breeding programs. Information on the nature and degree of genetic diversity can assist plant breeders in choosing the best genotypes as parents for hybridization (Denton and Nwangburuka, 2011; Nwosu et al., 2013).

Characterization of germplasm is important to classify genotypes based on morphological traits that might be used to assess the variability among

germplasm (Tantasawat et al., 2010). Some of the agro-morphological traits may be used as morphological markers in crop improvement. It also helps in the assessment of genetic variability and diversity present in available germplasm. Therefore, morphological characterization helps in the effective utilization of germplasm in crop improvement programs. This trait can also be used as a morphological marker for screening breeding material at the seedling stage. Similarly, stem color, petiole color, and pod color can also be used for the identification of material at the post seedling stage.

Information on qualitative traits based on genetic diversity and characterization of mung bean genotypes in Ethiopia is absent. Therefore, the present study was undertaken with the specific objectives of determining the extent of genetic diversity and identifying the phenotypic patterns using qualitative traits.

MATERIALS AND METHODS

Study Area and Design

The field experiment was conducted from March to June 2018 at Jinka Agricultural Research Center (JARC) during the main cropping season. Jinka Agricultural Research Center is located 729 km southwest of Addis Ababa at 360 33' 02.7" E, 050 46' 52.0" N, and at an altitude of 1420 m above sea level. The maximum, minimum, and average temperatures of the center are 27.680C, 16.610C, and 22.14oC, respectively while the mean annual rainfall is 1381 mm. The soil type of the center is Cambisols (Mesfin et al., 2017).

Experimental Materials

A total of sixty mung bean genotypes were used in this study. Out of these, forty-four genotypes were obtained from Melkassa Agricultural Research Center (MARC), and sixteen genotypes were collected from the Southern Nations, Nationalities, and Peoples (SNNP) region of Ethiopia.

Table 1. List of genotypes used for this diversity study.

| Genotype | Code | Genotype | Code | Genotype | Code | Genotype | Code |
|-----------------|------|-----------------|------|-----------------|------|-------------|------|
| VC6489-9-1 | G38 | N-26 | G43 | NLLP-MGC-04 | G4 | Acc003 | G47 |
| NLLP-MGC-10 | G10 | NLLP-MGC-16 | G16 | NVL-1 | G44 | Acc004 | G48 |
| NLLP-MGC-06 | G6 | VC2778A(KPS2) | G29 | NLLP-MGC-15 | G15 | Acc005 | G49 |
| NLLP-MGC-20 | G20 | VC6469-12-34A | G37 | HARSHA | G39 | Acc006 | G50 |
| NLLP-MGC-14 | G14 | NLLP-MGC-09 | G9 | NLLP-MGC-08 | G8 | Acc007 | G51 |
| NLLP-MGC-19 | G19 | VC6368(46-40-4) | G34 | NLLP-MGC-05 | G5 | Acc008 | G52 |
| NLLP-MGC-21 | G21 | NLLP-MGC-01 | G1 | NM92(VC6370-92) | G31 | Acc009 | G53 |
| NLLP-MGC-11 | G11 | NLLP-MGC-24 | G24 | NLLP-MGC-23 | G23 | Acc0010 | G54 |
| NLLP-MGC-12 | G12 | VC6492-59A | G35 | V2709 BG | G42 | Acc0011 | G55 |
| VC1973A | G28 | VC6370(30-65) | G33 | NLLP-MGC-27 | G27 | Acc0012 | G56 |
| VC6510-151-1 | G36 | NLLP-MGC-07 | G7 | VC3890A | G30 | Acc0013 | G57 |
| CN9-5 | G41 | NLLP-MGC-26 | G26 | NLLP-MGC-25 | G25 | Acc0014 | G58 |
| NLLP-MGC-02 | G2 | NLLP-MGC-17 | G17 | NLLP-MGC-22 | G22 | Acc0015 | G59 |
| NM94(VC6371-94) | G32 | NLLP-MGC-18 | G18 | Acc001 | G45 | Acc0016 | G60 |
| BARI-MUNG 2 | G40 | NLLP-MGC-03 | G3 | Acc002 | G46 | NLLP-MGC-13 | G13 |

Genotypes with initial “Acc”, that is accession, are from Southern Nations, Nationalities, and Peoples Region (SNNPR) and other genotypes are obtained from Melkassa Agricultural Research Center (MARC).

Experimental Design and Procedures

The experiment was laid out using a 6 × 10 alpha lattice design. The plot size was 4.5 m² with 3 m length and 0.3 m width, and the distance was 0.5 m between rows, and 0.05 m between plants, respectively. The distances between plots, intra - blocks, and replications were 1, 1.5, and 2 m, respectively. Two seeds per hill were planted and one seed per hill was left after thinning, and one row accommodated 12 plants. For this experiment, 100 kg ha⁻¹ NPS and 50 kg ha⁻¹ urea fertilizer were applied, and weeding was carried out twice.

Data Collection

The descriptor of mung bean developed by the International Board for Plant Genetic Resources (IBPGR, 1980) was followed for data collection. A total of 30 qualitative traits were collected on a plot basis. The qualitative traits used and their phenotypic classes were: seed germination habit (epigeal, hypogeal), growth pattern (determinate,

indeterminate), attachment of primary leaves at two leaf stage (sessile, sub-sessile, petiolate), growth habit (erect, semi-erect, spreading, semi-prostrate, prostrate, climbing at pod maturity), stem color (light green, dark green, light purple, dark purple, others), leafiness at 50% flowering (sparse, intermediate, abundant) leaf pubescence (glabrous, very sparsely, sparsely pubescent, moderately pubescent, densely pubescent), petiole pubescence (glabrous, pubescent, moderately pubescent, densely pubescent), lobbing of terminal leaflet (unlobbed, shallow, intermediate, deep, very deep at first pod maturity), terminal leaflet lobe shape (lanceolate, broadly ovate, ovate, rhombic, others), stipule size (small, medium, large), stipule shape (ovate, lanceolate, others), stem pubescence (glabrous, sparsely, moderately pubescent, highly pubescent), raceme position at first pod mostly above, in upper canopy, throughout canopy), calyx colour (green, purplish green, greenish purple, others), corolla color (yellow, greenish yellow, yellowish green, green-purplish yellow,

others), bracteole size (small, intermediate, large), bracteole shape (linear, lanceolate, others), flowering period (asynchronous, intermediate, synchronous), pod attachment to peduncle (erect, horizontal, horizontal-pendent, pendent, others), pod pubescence (glabrous, sparsely, moderately pubescent, densely pubescent), pod curvature (straight, slightly curved, curved), pod beak shape, (pointed, blunt, others), constriction of pod between (absent, slight, pronounced), pod cross section (semi flat, round, others), seed shape (globose, ovoid, narrowly ellipsoid, cubical to oblong, kidney shaped, drum shaped, others), seed colour (white, cream, light brown, intermediate brown, dark brown, grey, mottled grey, mottled brown, mottled cream, light cream, green brown, chocolate, black), lusture on seed surface (absent, present), mottling on seed surface (absent, slight, intermediate, heavy), hilum shape (concave, plain, convex, others).

Data Analyses

Phenotypic frequency distribution for the traits was computed for all genotypes. The Shannon-Weaver diversity index (H') was computed using the phenotypic frequencies of each qualitative trait as described by Hennink and Zeven (1990) by using the following formula

$$H' = -\frac{\sum_{i=0}^n P_i \ln P_i}{\ln(n)}$$

Where P_i = the proportion of individuals (genotypes) in the i th class and n -class trait and n is the number of phenotypic classes for a given trait, $\ln(n)$ is the natural logarithm $\ln(n)$. H' was estimated for each trait and standardized by dividing it by \log_{10} and normalized to keep the H' values between 0.00 and 1.00. The diversity index was classified as high ($H' \geq 0.60$), intermediate ($0.40 \leq H' \leq 0.60$), or low ($0.10 \leq H' \leq 0.40$), as described by Firdissa et al. (2005). All diversity index analyses were conducted using the Microsoft Excel computer program. The non-normalized 'H' values were used for the analysis of variance. Hierarchical (Ward, 1963) clustering was performed using frequency distribution of phenotypic classes. The standard genetic distances from the portion of phenotypic classes were used to construct a dendrogram by ward method subjected to Minitab Statistical Software

version 17. The average intra and inter-cluster distances were calculated using the generalized Mahalanobis's D2 statistics (Mahalanobis, 1936). The pseudo-F statistics (PSF), and pseudo-T2 statistics were considered for defining optimum cluster numbers (Milligan and Cooper, 1988). The contribution of each trait to divergence as described by Sharma (1998) with the formula [$CTIC = \frac{SD}{x} \chi 100$] where SD and x are the standard deviation and mean performance of each trait, respectively. Principal components analysis is expected to reduce the observed variables into a small number of components and was computed using Minitab Software Version 17. In a principal component analysis, the values were standardized to have unit variance and a mean of zero. .

RESULTS

Morphological Diversity Analysis based on Qualitative Traits

Frequency Distribution

Morphological characterization of genotypes based on qualitative traits indicated a high level of variation among the genotypes (Table 2), and that the studied morphological traits were polymorphic with different levels of variability. This result is supported by the earlier report of Hapsari *et al.* (2018) among one hundred and twelve mung bean genotypes. On the contrary, Kaur *et al.* (2017) reported that no significant differences were observed among the studied morphological traits (plant habit, stem color, stem pubescence, petiole color, premature pod color, pod pubescence, pod curvature, seed color, and seed shape) on mung bean genotypes.

In this study, all the genotypes showed an epigeal germination habit (Table 2), which is in agreement with the previous results reported by Bisht *et al.* (2005) on mung bean. Similarly, Harouna *et al.* (2020) reported that among the 160 accessions of wild *Vigna* legumes, cowpea and the landrace of *Vigna vexillata* had, an epigeal germination habit, which is also shared by most accessions of *Vigna reticulata* and *Vigna racemosa*.

The growth pattern showed that 96% of the phenotypic classes were determinate type followed by 4% indeterminate type (Table 2).

This finding contradicts the earlier reports by Sowmya *et al.* (2019) who observed that out of 76 mung bean accessions, 66 accessions (86.84%) showed indeterminate growth patterns while 10 accessions (13.16%) showed determinate growth pattern. Similarly, Popoola *et al.* (2017) reported that all the 26 *Vigna vexillata* (L.) accessions, showed an indeterminate growth pattern. For growth habit, semi-erect was the most frequently observed phenotypic class at 85%, followed by erect at 4%, spreading at 4%, semi-prostrate at 4% and prostrate at 2% (Table 2). Contrastingly, the climbing type was the least observed phenotypic class, with a mean frequency of 1% (Table 2). The present study showed that the semi-erect type was the most predominant phenotypic class. Singh *et al.* (2014) similarly observed that among the 104 mung bean genotypes, 90.38% had semi-erect growth habits, while 5.77% had a spreading type of growth habit, and 3.85% had shown an erect growth habit. Likewise, Patel *et al.* (2019) reported that out of the 44 mung bean genotypes, 47.73% had semi-erect growth habits, 15.91% had erect, and 13.64% showed the spreading type of growth habit. Correspondingly, Gonné *et al.* (2013) reported that semi-erect was the most frequently observed phenotypic class on cowpea genotypes. Sessile type of primary leaf attachment (55%) was the most frequent phenotypic class followed by sub-sessile 33% while the least frequently observed phenotypic class was petiolate with a mean frequency of 12% (Table 2). Bisht *et al.* (2005) also recorded that sessile 100% types in all the species of mung bean, which coincides with the work of Harouna *et al.* (2020) who reported that among the 160 accessions of wild *Vigna* legumes, the *Vigna vexillata* landrace 100% showed sessile, *Vigna racemosa* accessions 83.33% showed the sub-sessile type of primary leaf attachment, the landrace of *Vigna vexillata* also exhibited 81.67% was sub-sessile type of primary leaf attachment, while the *Vigna reticulata* accessions shared 46.15%, sub-sessile phenotypic class.

Light green stem color 88% was the most frequent phenotypic class followed by dark green

9% and light purple 3%, respectively (Table 2). Dark purple stem color was the least observed phenotypic class with the mean frequency of 0%. Singh *et al.* (2014) similarly reported that out of the 104 mung bean genotypes, about 95.19% were found to be green stem color. Patel *et al.* (2019) also reported that among the 44 mung bean genotypes, 38.64% showed green stem color, whereas 61.36% had green with purple stem color. This finding is also supported by the previous works on different pulse crops based on stem color (Jain *et al.*, 2002; Katiyar *et al.*, 2008; Singh *et al.*, 2014; Kaur *et al.*, 2017) in mung bean, Chakrabarthy and Agarwal (1989) in black gram; Yadav and Srivastava (2015), Gnyandev (2009), Joshi and Yasin (2014) in chickpea; Durga *et al.* (2015) in horse gram; and Kumar and Shrikant (2016) in cowpea. Kaur *et al.* (2017) reported that anthocyanin coloration recorded at the seedling stage was present in all the varieties and hence indicated no variation.

Leafiness at 50% flowering was intermediate 76% followed by sparse 13% and abundant 11% (Table 2). The intermediate type of leafiness was the most frequently observed phenotypic class in this study in line with Harouna *et al.* (2020) on *Vigna racemosa*, *Vigna reticulata* accession, and cowpea, while it contradicts the observations on rice bean and the landrace of *Vigna vexillata* that showed an abundant leafiness (Harouna *et al.*, 2020).

About 75% of the studied mung bean genotypes showed sparsely pubescent type of leaf while the glabrous type was the least frequently observed phenotypic class with the mean frequency of 2% (Table 2). This finding contradicts with the results of Harouna *et al.* (2020) who reported that among 160 accessions of wild *Vigna* legumes, *Vigna vexillata* land race showed 100% very sparsely pubescent type of phenotypic class, and rice bean showed 100% moderately pubescent type of phenotypic class, cowpea showed 100% glabrous type of phenotypic class, *Vigna racemosa* accessions showed 33.33% glabrous, 33.33% moderately pubescent and 33.33% sparsely pubescent type of phenotypic class. *Vigna reticulata* accessions showed 28.85% very sparsely pubescent, 17.3% sparsely pubescent, 25% moderately pubescent and 28.85% densely

pubescent type of phenotypic class, *Vigna vexillata* 26.67% very sparsely pubescent, 22.06% sparsely pubescent, 31.67% moderately pubescent and 20% densely pubescent type of phenotypic class, *Vigna ambacensis* showed 30.95% very sparsely pubescent, 4.77% sparsely pubescent, 59.52% moderately pubescent and 4.76% densely pubescent type of phenotypic class were observed.

Around 74% of the studied mung bean genotypes had moderately pubescent petiole; while the glabrous type of petiole was the least frequently observed on the studied mung bean genotypes (Table 2). Similarly, Harouna *et al.* (2020) reported that among the 160 accessions of wild *Vigna* legumes studied for the trait petiole pubescence, rice bean showed 100% moderately pubescent type of phenotypic class. Contrastingly, Harouna *et al.* (2020) reported that *Vigna vexillata* landrace and cowpea showed 100% glabrous type of phenotypic class, *Vigna racemosa* accessions showed 33.33% glabrous, 33.33% pubescent and 16.67% moderately pubescent, and 16.67% densely pubescent type of phenotypic class, *Vigna reticulata* accessions showed 7.69% glabrous, 13.46% pubescent, 36.54% moderately pubescent and 42.3% densely pubescent type of phenotypic class, *Vigna vexillata* 5% glabrous, 50% pubescent, 43.33% moderately pubescent and 1.67% densely pubescent type of phenotypic class, *Vigna ambacensis* showed 30.95% very sparsely pubescent, 1.9% glabrous, 14.29% pubescent and 23.81% densely pubescent type of phenotypic class were observed. A similar result was reported by Singh *et al.* (2014), who found that out of the 104 mung bean genotypes, the glabrous type was not observed. Lobbing of the terminal leaflet was intermediate with the mean frequency of 80% while very deeply lobbed 1% was the least observed phenotypic class on the studied mung bean genotypes (Table 2). This result is in agreement with the report of Harouna *et al.* (2020) among the 160 accessions of wild *Vigna* legumes, rice bean showed 100% an intermediate type of phenotypic class, while it contrasts with that of *Vigna vexillata* landrace, cowpea and *Vigna racemosa* accessions showed 100% unlobed type of phenotypic class, *Vigna*

reticulata accessions showed 96.15% unlobed and 3.85% intermediate type of phenotypic class, *Vigna vexillata* 95% unlobed, 1.67% intermediate and 3.33% was deep. For the trait lobbing of the terminal leaflet *Vigna ambacensis* showed 97.62% unlobed and 2.83% intermediate types of phenotypic class were observed.

Terminal leaflet lobe shape was ovate with the mean frequency of 83% while lanceolate 2% was the least observed phenotypic class on the studied mung bean genotypes (Table 2). This finding contradicted the report of Popoola *et al.* (2017) who observed that among the 26 *Vigna vexillata* (L.) accessions, lanceolate leaflet shape was dominant at 50% in thirteen accessions, ovate leaflet shape 34.61% in nine accessions, and ovate-elliptic shape 11.53% in three accessions while only one accession had heterophytic shape (ovate-lanceolate leaflet) with the mean frequency of 3.84%. This result is supported by the report of Harouna *et al.* (2020) among the 160 accessions of wild *Vigna* legumes, cowpea showed 100% ovate phenotypic class, *Vigna ambacensis* showed 83.33% ovate and 16.67% lanceolate, *Vigna racemosa* accessions showed 66.67% ovate and 33.33% lanceolate, *Vigna reticulata* accessions showed 53.85% ovate, 38.46% lanceolate and 7.69% other types of phenotypic class. On the contrary, rice bean showed 100% other types of phenotypic class. Medium stipule size with the frequency 80% followed by small 12% and large 8%, respectively were observed on mung bean genotypes under study (Table 2). This finding conforms to the report of Harouna *et al.* (2020) among the 160 accessions of wild *Vigna* legumes, 67% of *Vigna racemosa* had the medium stipule size variant, as well as 56% of the *Vigna reticulata* accessions. Contrastingly, *Vigna vexillata* landrace, cowpea rice bean had 100% large variant of the stipule size trait, while the small size variant was observed in 45% and 52% of *Vigna ambacensis* and *Vigna vexillata*, respectively.

The glabrous pubescent type had a mean frequency of 82% while highly pubescent type (1%) was the least observed phenotypic class (Table 2). This result is in agreement with

Harouna *et al.* (2020) who reported that among the 160 accessions of wild *Vigna* legumes, *Vigna vexillata* landrace and cowpea showed (100%) glabrous type of phenotypic class; on the other hand; rice bean showed 100% highly pubescent type of phenotypic class, *Vigna racemosa* accessions showed 50% sparsely pubescent, 33.33% glabrous and 16.67% moderately pubescent, *Vigna reticulata* accessions showed 55.77% medium, 25% small, 19.23% sparsely pubescent and 17.31% glabrous, *Vigna vexillata* showed 33.33% moderately pubescent, 33.33% sparsely pubescent and 18.33% highly pubescent, *Vigna ambacensis* showed 35.71% sparsely pubescent, 33.33% highly pubescent and 30.95% moderately pubescent was the observed phenotypic class.

Phenotypic observations of raceme position showed 76% of the mung bean genotypes had their raceme positions on the upper canopy (Table 2). Green calyx and yellow corolla colors were the most frequently observed phenotypic classes with the frequency of 90% and 89%, respectively for mung bean genotypes under study. Similar findings were reported by several researchers on different pulse crops based on flower morphological characters (Singh *et al.*, 2014; Kaur *et al.*, 2017; Patel *et al.*, 2019) in mung bean; Chandrashekhar (2008) and Das *et al.* (2014) in French bean; Yadav and Srivastava (2002), Gnyandev (2009) and Joshi and Yasin (2014) in chickpea, also on cowpea by (Basavaraj *et al.*, 2013; Kumar and Shrikant, 2016), Kumar *et al.* (2013) in Guar; and Durga *et al.* (2015) in horse gram.

Intermediate bracteole size was the most frequently observed phenotypic class with the frequency 85%; whereas, among the three bracteole shape phenotypic classes, linear bracteole shape was the most frequently observed with the frequency 90% in the studied mung bean genotypes (Table 2). Among the three flowering periods, asynchronous (flowering greater than 30 days) was the most frequent with a frequency of 85% for the mung bean genotypes under study.

The pendent type was the most frequently observed phenotypic class with a frequency of

66% and the erect type was the second most frequent phenotypic class with the frequency 28% among the listed phenotypic classes observed for pod attachment to peduncle for the mung bean genotypes under study (Table 2). This finding is in agreement with the result of Hapsari *et al.* (2018), who reported that among 122 mung bean genotypes, 86 genotypes 70.49% showed the pendent type of phenotypic class, and 36 genotypes 29.51% showed an erect type of phenotypic class.

Glabrous pod pubescent was the most frequent phenotypic class with the frequency of 84%, while dense pod pubescent was the least observed phenotypic class with the frequency 3% for mung bean genotypes under study (Table 2). This finding is in agreement with Harouna *et al.* (2020) who reported that among the 160 accessions of wild *Vigna* legumes, *Vigna vexillata* landrace, cowpea, and rice bean showed 100% glabrous type of phenotypic class, on the other hand; *Vigna racemosa* accessions showed 50% glabrous, 33.33% sparsely pubescent and 16.67% moderately pubescent, *Vigna reticulata* accessions showed 42.3% densely pubescent, 28.85% sparsely pubescent, 26.92% moderately pubescent and 9.61% erect, *Vigna vexillata* showed 48.33% horizontal, 33.33% others and 1.92% glabrous, *Vigna ambacensis* showed 85.71% sparsely pubescent, 9.52% densely pubescent and 4.76% moderately pubescent type of phenotypic class observed.

Approximately 86% of the genotypes had slight pod curvature while 6% genotypes had curved (sickle-shaped). This result is in agreement with Harouna *et al.* (2020) who showed that among the 160 accessions of wild *Vigna* legumes, more than 50% of the studied wild accessions showed the “slightly curved” form of the pod curvature trait, which was similar to cowpea 100% slight pod curvature. While; contradicting results were observed for rice bean and the *Vigna vexillata* landrace commonly shared the 100% straight form of the trait with 14% of *Vigna ambacensis* 10% of *Vigna vexillata* and (48%) of *Vigna reticulata* accessions showed straight pod curvature, *Vigna vexillata* 38% and *Vigna*

racemosa 33% accessions showed the curved form of the pod curvature trait.

Among the evaluated genotypes, about 97% of them had a slight constriction of pod between seeds (Table 2). Similarly, Harouna *et al.* (2020) reported that among the 160 accessions of wild *Vigna* legumes, a slight constriction of the pod between seeds was the form found in cowpea 100% and 24% of *Vigna ambacensis*, 23% of *Vigna reticulata*, and 33% of *Vigna racemosa* accessions. While most of the studied wild *Vigna* accessions (more than 50%) had no constriction of the pod between seeds (variant: “absent”), as found in the *Vigna vexillata* landrace. The pronounced form of the trait was observed only in rice bean and 15% of *Vigna ambacensis*, as well as 12% of *Vigna reticulata* accessions.

For seed shape, ovoid seed shape was the most frequently observed phenotypic class with frequency 75% for the mung bean genotypes under study. Sowmya *et al.* (2019) reported that out of 76 mung bean accessions, 43 accessions 56.58% showed oval seed shape while 33 accessions 43.42% showed drum seed shape. For seed color, the green-brown phenotypic class was the most phenotypic class with a frequency of 87% while white cream, light brown, intermediate brown, dark brown, mottled brown, mottled cream, light cream, chocolate, and black were the least observed phenotypic class on mung bean genotypes under study (Table 2). It indicates that green-brown seed color was the most frequent occurring in 87% among 60 mung bean genotypes (Table 2). In contrast, Singh *et al.* (2014) reported that the proportion of genotypes with greenish-purple was found to be 94.23% among the 108 mung bean genotypes. Wang *et al.* (2018) reported that among 184 mung bean accessions, green seeds accounted for 78%. Similarly, Hapsari *et al.* (2018) reported that among 122 mung bean genotypes, 61 genotypes 50% showed green seed color, 57 genotypes 46.72% showed mixed seed color, and 4 genotypes 3.28% showed brown seed color.

The phenotypic class mottling on seed surface was not observed on 85% of mung bean genotypes while slight and intermediate

phenotypic classes were observed on around 8 and 7% of mung bean genotypes under study (Table 2). In this study, it was possible to classify genotypes based on seed physical features. Singh *et al.* (2014) also suggested that seed physical characters may also serve as morphological markers to characterize mung bean genotypes. Plain, concave, and convex hilum shapes were the most frequently observed phenotypic classes (Table 2).

Shannon-Weaver Diversity Indices

Assessment of genetic diversity is vital in any crop improvement program to identify high yielding genotypes (Rhman and Munur, 2009). Shannon-Weaver diversity indices (H') are used to compare phenotypic diversity among qualitative characters. In the present study, the Shannon-Weaver diversity index (H') was adopted to compute the diversity of mung bean genotypes based on the diversity frequency of 30 qualitative morphological traits. The estimates of the Shannon-Weaver diversity index (H') depicted the observed phenotypic characters, the (H') values ranged from 0.15 for constriction of pod between seeds to 0.96 for seed shape (Table 2). Traits such as growth habit, attachment of primary leaves, leafiness at 50% flowering, leaf pubescence, lobbing of the terminal leaflet, terminal leaflet lobe shape, stipule size, stem pubescence, raceme position (at first pod), pod attachment to the peduncle, and seed shape showed Shannon diversity values of 0.65, 0.95, 0.72, 0.88, 0.73, 0.67, 0.64, 0.63, 0.70, 0.84 and 0.96, respectively exhibited high percentages contribution to the total variation compared to others. The overall mean value for (H' = 0.54) confirmed the existence of certain level of diversity among the mung bean genotypes. All phenotypic classes showed a high diversity index except germination type (H' = 0.00), indicating that there was a balanced frequency distribution of phenotypic classes and high genetic diversity for the studied traits. Similarly, Borines *et al.* (2019) reported that 13 out of 29 traits or 44.83% have $H' > 0.50$, hence considered as moderately to highly diverse. A low H' indicates unbalanced frequency classes for an individual trait and lack of diversity for the

trait. A high level of phenotypic diversity among the 60 mung bean genotypes was recorded which gives a key to look for variety development through direct selection.

Overall mean diversity index (H') was fairly high for all the studied traits except in some of the traits (Table 2). The highest overall mean diversity index (H') was recorded for seed shape ($H'=0.96$, followed by attachment of primary leaves ($H'=0.95$) and leaf pubescence ($H'=0.88$), respectively. The high level of diversity index (H') indicated the availability of high genetic potential of mung bean genotypes and also the presence of many important desirable genes for mung bean improvement for the intended traits and further genetic studies. The low level of

diversity for some of the phenotypic classes such as germination type, growth pattern, stem color, calyx color, petiole pubescence, bracteole shape, flowering period, constriction of pod between seeds, seed color, lusture on the seed surface, and hilum shape had a low level of diversity might indicate the existence of a narrow genetic base and a small sample size highly contributed to a low level of diversity index. This finding is in line with the previous results by Wuletaw and Endeshaw (2003) on grass pea, who reported that the low level of a diversity index noted was associated with poor sampling during collection and other natural and artificial selection.

Table 2. Frequency distribution and Shannon-Weaver Diversity Indices ('H') of 30 qualitative traits of Mung bean genotypes at Jinka, in 2018.

| Character & description | Frequency (%) | H' | Character & description | Frequency (%) | H' |
|--|---------------|-------------|---|---------------|-------------|
| 1. Germination | | 0 | 9. Lobbing of the terminal leaflet | | 0.73 |
| Epigeal | 100 | | Unlobbed | 9 | |
| Hypogeal | 0 | | Shallow | 7 | |
| 2. Growth pattern | | 0.17 | Intermediate | 80 | |
| Determinate | 96 | | Deep | 3 | |
| Indeterminate | 4 | | Very deep (at first pod maturity) | 1 | |
| 3. Growth habit | | 0.65 | 10. Terminal leaflet lobe shape | | 0.67 |
| Erect | 4 | | Lanceolate | 2 | |
| Semi-erect | 85 | | Broadly ovate | 4 | |
| Spreading | 4 | | Ovate | 83 | |
| Semi-prostrate | 4 | | Rhombic | 8 | |
| Prostrate | 2 | | Others | 3 | |
| Climbing | 1 | | 11. Stipule size | | 0.64 |
| 4. Attachment of primary leaves | | 0.95 | Small | 12 | |
| Sessile | 33 | | Medium | 80 | |
| Sub-sessile | 55 | | Large | 8 | |
| Petiolate (at the two-leaf stage) | 12 | | 12. Stipule shape | | 0.5 |
| 5. Stem color | | 0.43 | Ovate | 80 | |
| Light green | 88 | | Lanceolate | 20 | |
| Dark green | 9 | | Others | 0 | |
| Light purple | 3 | | 13. Stem pubescence | | 0.63 |
| Dark purple | 0 | | Glabrous | 82 | |
| 6. Leafiness (at 50% flowering) | | 0.72 | Sparsely | 9 | |
| Sparse | 13 | | Moderately pubescent | 8 | |
| Intermediate | 76 | | Highly pubescent | 1 | |
| Abundant | 11 | | 14. Raceme position (at first pod) | | 0.7 |
| 7. Leaf pubescence | | 0.88 | Mostly above | 17 | |
| Glabrous | 2 | | In upper canopy | 76 | |
| Very sparsely | 8 | | Throughout canopy | 7 | |
| Sparsely pubescent | 75 | | 15. Calyx color | | 0.39 |
| Moderately pubescent | 9 | | Green | 90 | |
| Densely pubescent | 6 | | Purplish green | 5 | |
| 8. Petiole pubescence | | 0.16 | Greenish purple | 5 | |
| Glabrous | 2 | | Others | 0 | |
| Pubescent | 20 | | | | |
| Moderately pubescent | 74 | | | | |
| densely pubescent | 4 | | | | |

Table 2. Continued.

| Character & description | Frequency (%) | H' | Character & description | Frequency (%) | H' |
|---|---------------|-------------|--|---------------|-------------|
| 16. Corolla color | | 0.45 | 24. Constriction of pod between seeds | | 0.15 |
| Yellow | 1 | | Absent | 2 | |
| Greenish-yellow | 5 | | Slight | 97 | |
| Yellowish green | 5 | | Pronounced | 1 | |
| 17. Bracteole size | | 0.52 | 25. Pod cross-section | | 0.58 |
| Small | 6 | | Semi-flat | 27 | |
| Intermediate | 85 | | Round | 73 | |
| Large | 9 | | Others | 0 | |
| 18. Bracteole shape | | 0.39 | 26. Seed shape | | 0.96 |
| Linear | 90 | | Globose | 4 | |
| Lanceolate | 5 | | Ovoid | 75 | |
| Others | 5 | | Narrowly ellipsoid | 5 | |
| 19. Flowering period | | 0.42 | Cubical to oblong | 6 | |
| Asynchronous | 85 | | Kidney shaped | 5 | |
| Intermediate | 15 | | Drum shaped | 5 | |
| Synchronous | 0 | | Others | 0 | |
| 20. Pod attachment to the peduncle | | 0.84 | 27. Seed color | | 0.47 |
| Erect | 28 | | White | 0 | |
| Horizontal | 2 | | Cream | 0 | |
| Horizontal-pendent | 4 | | Light brown | 0 | |
| Pendent | 66 | | Intermediate brown | 0 | |
| Others | 0 | | Dark brown | 0 | |
| 21. Pod pubescence | | 0.59 | Grey | 5 | 0.5 |
| Glabrous | 84 | | Mottled grey | 8 | |
| Sparsely | 9 | | Mottled brown | 0 | |
| Moderately pubescent | 4 | | Mottled cream | 0 | |
| Densely pubescent | 3 | | Light cream | 0 | |
| 22. Pod curvature | | 0.5 | Green brown | 87 | |
| Straight | 8 | | Chocolate | 0 | |
| Slightly curved | 86 | | 28. Lusture on the seed surface | | 0.42 |
| Curved (sickle-shaped) | 6 | | Absent | 15 | |
| 23. Pod beak shape | | 0.54 | Present | 85 | |
| Pointed | 23 | | | | |
| Blunt | 77 | | | | |
| Others | 0 | | | | |

Table 2. Continued.

| Character & description | Frequency (%) | H' |
|---|---------------|-------------|
| 29. Mottling on the seed surface | | 0.53 |
| Absent | 85 | |
| Slight | 8 | |
| Intermediate | 7 | |
| 30. Hilum shape | | 0.36 |
| Concave | 44 | |
| Plain | 52 | |
| Convex | 4 | |
| Others | 0 | |
| Densely pubescent | 6 | |
| Overall mean | | 0.53 |

Principal Component Analysis

The principal component analysis is a multivariate technique used to observe relationships among several variables. It involves a mathematical procedure that transforms some possibly correlated variables into a smaller number of uncorrelated variables and studies the patterns of variation and the relative importance of each trait in explaining the observed variability. In the present study, the principal component analysis based on the 30 qualitative traits was computed. Hair *et al.* (1998) suggested that the principal component (PCs) with eigenvalues greater than unity, and component loadings greater than ± 0.3 were considered to be meaningful and valuable. Therefore, the first four principal components with eigenvalues greater than unity explained 90.0% of the total variations (Table 3). Principal component 1 (PC1) alone accounted for 75% of the total variation. Attachment of primary leaves, terminal leaflet lobe shape, stipule size, stipule shape, stem pubescence, bracteole shape, pod attachment to the peduncle, pod curvature, pod beak shape, mottling on seed surface, and hilum shape had the highest loadings on PC1. Accordingly, the attachment of primary leaves, stipule size, stipule shape, stem pubescence, raceme position (at first pod), bracteole shape, pod attachment to the

peduncle, pod curvature, pod beak shape, mottling on seed surface, and hilum shape had a relatively better value for variation coefficient on PC1 axis. These were the most distinctive traits that had significantly brought the variations among the genotypes. The second principal component (PC2), explained 8% of the total variations and was highly positively correlated with corolla color, bracteole size, flowering period, lusture on the seed surface, and it was also highly negatively correlated with seed color, constriction of pod between seeds and leaf pubescence. Growth pattern, leafiness (at 50% flowering) lusture on the seed surface, flowering period, bracteole size, and corolla color were the most distinctive characteristics on the PC2 axis, while PC3 explained 4% of the total variations and correlated highly positively with growth habit, petiole pubescence, lobbing of the terminal leaflet, corolla color, bracteole size, flowering period and pod pubescence, pod cross-section and lusture on seed surface while it correlated highly negatively with growth pattern.

Consequently, on the PC3 axis, the most distinctive characteristics causing the variation among the genotypes were growth habit (0.446), petiole pubescence (0.302) and pod pubescence (0.302). . Meza *et al.* (2013) indicated that the first three principal components explained 34.18% of the total variation and reported that the

first flowering time, ripened pod color, and pod harvest time of common beans were considered as the most distinctive characteristics.

Based on the first three PCs, growth habit, leafiness (at 50% flowering), corolla color, bracteole size, flowering period, pod cross-section and lusture on a seed surface were prominent characteristics in the screening of mung bean genotypes. Similarly, Ekbiç and Hasancaoğlu (2019) suggested that seed main

color, seed secondary color, pod color, pod cross-section, and stringiness were prominent characteristics in the screening of common bean genotypes. The remaining PCs explained 7% of the total variation that was mainly associated with growth habits. Therefore, from all the characters, growth habit was found as the most discriminative trait differentiating genotypes collected from southern Ethiopia.

Table 3. Principal Component Analysis (PCA) in Qualitative Traits of 60 Mung Bean Genotypes.

| Traits | PC1 | PC2 | PC3 | PC4 |
|--------------------------------|--------|--------|--------|--------|
| Growth pattern | -0.163 | 0.215 | -0.268 | -0.033 |
| Growth habit | 0.043 | 0.074 | 0.446 | 0.747 |
| Attachment of primary leaves | 0.201 | 0.16 | -0.119 | 0.065 |
| Stem color | 0.18 | -0.077 | 0.291 | 0.029 |
| Leafiness (at 50% flowering) | 0.185 | 0.25 | 0.124 | -0.067 |
| Leaf pubescence | 0.191 | -0.249 | -0.014 | -0.043 |
| Petiole pubescence | 0.178 | -0.205 | 0.302 | -0.073 |
| Lobbing of terminal leaflet | 0.192 | -0.212 | 0.082 | -0.081 |
| Terminal leaflet lobe shape | 0.202 | -0.077 | -0.153 | 0.026 |
| Stipule size | 0.203 | 0.082 | -0.152 | 0.067 |
| Stipule shape | 0.202 | -0.08 | -0.155 | 0.041 |
| Stem pubescence | 0.2 | -0.092 | -0.169 | 0.046 |
| Raceme position (at first pod) | 0.202 | -0.012 | -0.18 | 0.055 |
| Calyx color | 0.198 | -0.184 | -0.037 | -0.029 |
| Corolla color | 0.175 | 0.279 | 0.209 | -0.088 |
| Bracteole size | 0.185 | 0.248 | 0.117 | -0.043 |
| Bracteole shape | 0.2 | 0.154 | -0.135 | 0.079 |
| Flowering period | 0.175 | 0.279 | 0.209 | -0.088 |
| Pod attachment to peduncle | 0.2 | 0.158 | -0.125 | 0.074 |
| Pod pubescence | 0.178 | -0.205 | 0.302 | -0.073 |
| Pod curvature | 0.203 | 0.083 | -0.151 | 0.074 |
| Pod beak shape | 0.201 | -0.022 | -0.193 | 0.072 |
| Constriction of pods b/n seeds | 0.19 | -0.259 | -0.017 | -0.043 |
| Pod cross section | 0.104 | 0.182 | 0.243 | -0.586 |
| Seed shape | 0.194 | -0.216 | -0.069 | -0.017 |
| Seed color | 0.189 | -0.263 | -0.018 | -0.042 |
| Lusture on seed surface | 0.167 | 0.249 | 0.046 | -0.038 |
| Mottling on seed surface | 0.2 | 0.158 | -0.125 | 0.074 |
| Hilum shape | 0.2 | 0.157 | -0.128 | 0.076 |
| Eigen value | 21.8 | 2.36 | 1.27 | 1.02 |
| Proportion | 0.75 | 0.08 | 0.04 | 0.03 |
| Cumulative | 0.75 | 0.83 | 0.87 | 0.90 |

PC= Principal component

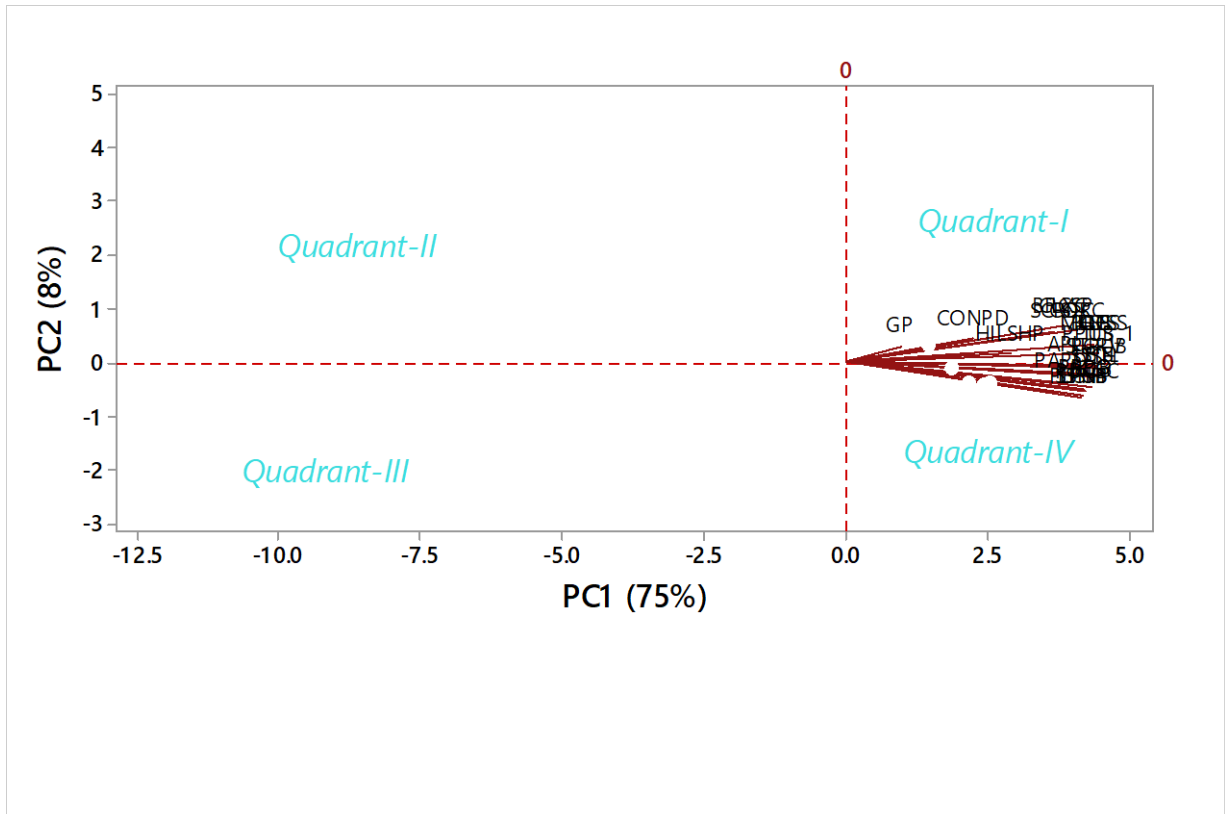


Figure 1. Biplot showing an Association of 30 Qualitative Traits of Genotypes.

Cluster Analysis

Cluster analysis was done based on similarity among the genotypes which gave the relative position of genotypes in the group. A study was conducted to determine the morphological genetic diversity among the 60 mung bean genotypes based on the qualitative traits (Table 4, 5, 6, and; Figure 2). The genotypes were grouped into four different clusters based on the studied 30 qualitative traits (Table 4), indicating the existence of the genetic divergence among the genotypes which might give a better chance to select the genotypes with different weights for mung bean improvement. Based on the **cluster** data, several researchers emphasized the importance of involving diverse parents in crossing programs to achieve high heterotic responses and transgressive segregants in early generations for improved seed yield and other targeted traits. . The result showed that there were significant variations observed among the mung bean genotypes for the studied qualitative traits. Singh *et al.* (2010) suggested that emphasis must be given to the genetic diversity of mung bean genotypes. Therefore, genotypes may be selected from a dendrogram based on genetic diversity, and assembling the genotypes based on their similarity is of paramount importance. Basnet *et al.* (2014) reported that the multivariate analysis involving the qualitative traits clearly showed the alignment of the different genotypes into different clusters according to the similarity indices. The use of diverse parents to increase the chance of getting superior varieties has been reported by (Katiyar *et al.*, 2009; Tantasawat *et al.*, 2010; Narasimhulu *et al.*, 2013).

A dendrogram condenses genetic similarity among mung bean genotypes based on the 30 qualitative traits (Figure 2). The number of genotypes varied from three in (Cluster III) to forty-five in Cluster I (Table 4). The first cluster was the largest, consisting of forty-five genotypes, and accounts for 75% of the total genotypes. The second and fourth clusters consisted of six genotypes each and accounted for 20%, while the third cluster consisted of three genotypes and accounts for 5%. In this study, cluster I and cluster IV have the largest inter-cluster distance of 44.67 units (Table 5), indicating that more emphasis should be given to cluster I for selecting genotypes as parents for crossing with the genotypes of Cluster IV for the studied traits.

As per the contribution of traits for inter-cluster analysis, the traits were classified as high contributors ($CTIC \geq 74\%$), intermediate contributors ($70\% \leq CTIC \leq 74\%$), and low contributors ($CTIC < 70\%$). Accordingly, traits such as leafiness at 50% flowering, leaf pubescence, petiole pubescence, pod attachment to the peduncle, pod beak shape, constriction of pod between seeds, and seed shape were the major contributors of genetic divergence in the entire genotypes, while growth pattern, attachment of primary leaves and hilum shape were low contributors to the divergence on mung bean genotypes (Table 6). Likewise, Toscana *et al.* (2017) reported growth habits, plant pigmentation, terminal leaflet shape, twinning tendency, pod attachment to the peduncle, and seed shape were the major traits with major contributors for inter-cluster of 30 cowpea genotypes.

Table 4. Distribution of the 60 Mung bean Genotypes in Four Cluster Groups.

| Cluster | Number of genotypes | Genotypes Included Under Clusters 1-4 |
|-------------|---------------------|---|
| Cluster I | 45 | G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13, G14, G15, G16, G17, G18, G20, G21, G22, G23, G24, G25, G26, G27, G28, G29, G30, G31, G32, G33, G34, G35, G36, G37, G38, G39, G40, G41, G42, G43, G44, G45 |
| Cluster II | 6 | G46, G47, G48, G49, G50, G51 |
| Cluster III | 3 | G52, G53, G54 |
| Cluster IV | 6 | G57, G58, G55, G56, G59, G60 |

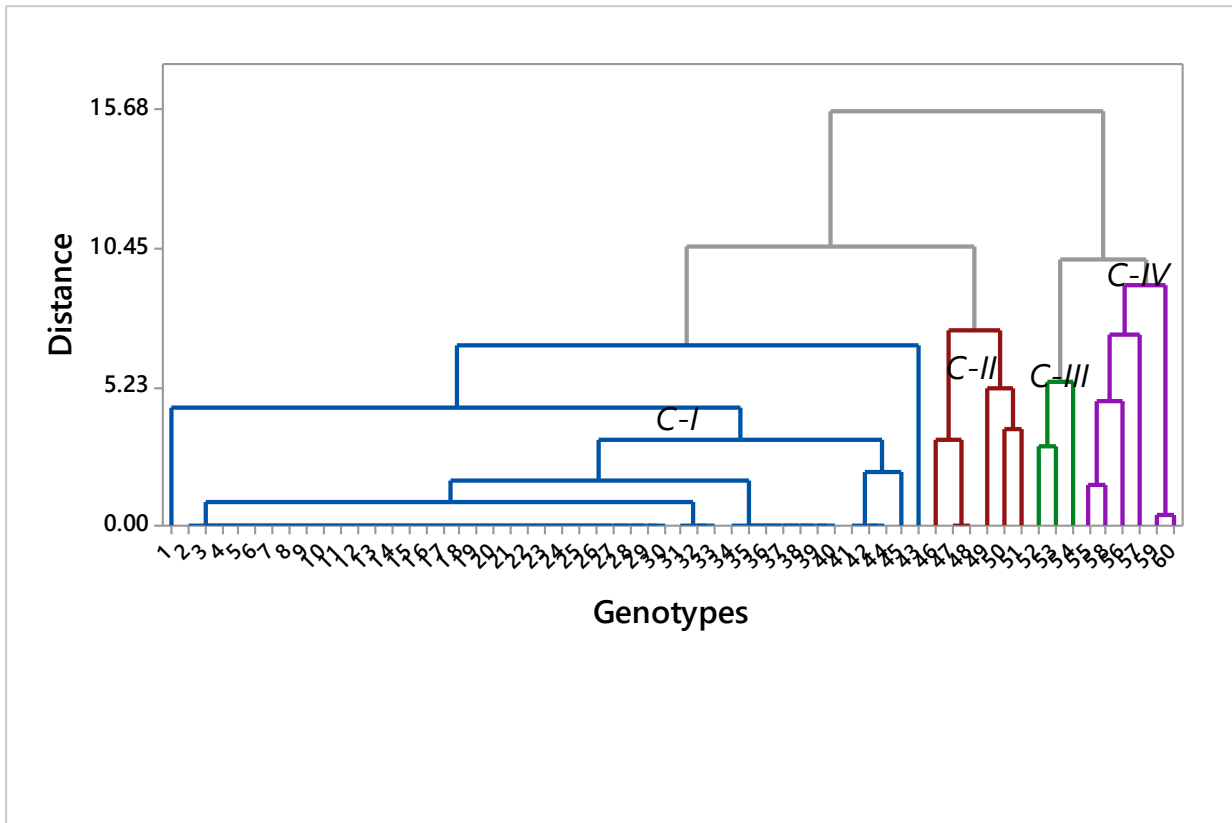


Figure 2. Hierarchical Clustering of the pattern of 60 mung bean genotypes (UPGMA) based on 30 qualitative characters.

Table 5. Average intra-cluster (bolded diagonal) and inter-cluster (off-diagonal) generalized squared distance (D²) values for qualitative traits.

| Clusters | I | II | III | IV |
|----------|-------------|-------------|-------------|-------------|
| I | 4.81 | 17.00 ns | 30.56 ns | 44.67* |
| II | | 3.76 | 13.63 ns | 28.40ns |
| III | | | 3.67 | 16.15 ns |
| IV | | | | 7.15 |

**, * indicates significant at 1% and 5% level of significant; $\chi^2_{28} = 41.34$ and 48.28 at 5% and 1%, probability level, respectively.

Table 6. Levels of trait contribution for inter-cluster analysis of 60 mung bean genotypes.

| Traits | CI | CII | CIII | CIV | Mean | Std | CTIC (%) |
|--------|------|------|------|------|------|------|----------|
| GP | 0.18 | 0.18 | 0.28 | 0.21 | 0.21 | 0.05 | 22.20 |
| GH | 0.42 | 0.42 | 0.42 | 1.37 | 0.66 | 0.48 | 72.24 |
| APL | 0.74 | 0.82 | 0.64 | 1.33 | 0.88 | 0.31 | 34.82 |
| STC | 0.36 | 0.36 | 0.36 | 1.18 | 0.57 | 0.41 | 72.57 |
| LFNS | 0.57 | 0.57 | 0.38 | 1.72 | 0.81 | 0.61 | 75.71 |
| LPUB | 0.57 | 0.57 | 0.38 | 1.72 | 0.81 | 0.61 | 75.71 |
| PPUB | 0.48 | 0.59 | 0.42 | 1.65 | 0.79 | 0.58 | 74.01 |
| LBTL | 0.50 | 0.50 | 0.50 | 1.63 | 0.78 | 0.57 | 72.20 |
| TLLS | 0.44 | 0.44 | 0.44 | 1.46 | 0.70 | 0.51 | 73.38 |
| STS | 0.50 | 0.50 | 0.50 | 1.63 | 0.78 | 0.57 | 72.20 |
| STSH | 0.50 | 0.50 | 0.50 | 1.63 | 0.78 | 0.57 | 72.20 |
| STPUB | 0.47 | 0.47 | 0.47 | 1.54 | 0.74 | 0.54 | 72.54 |
| RPO | 0.55 | 0.55 | 0.55 | 1.79 | 0.86 | 0.62 | 72.09 |
| CLXC | 0.33 | 0.33 | 0.33 | 1.09 | 0.52 | 0.38 | 73.08 |
| CORC | 0.36 | 0.36 | 0.36 | 1.18 | 0.57 | 0.41 | 72.57 |
| BRCS | 0.42 | 0.42 | 0.42 | 1.37 | 0.66 | 0.48 | 72.24 |
| BRCS | 0.33 | 0.33 | 0.33 | 1.09 | 0.52 | 0.38 | 73.08 |
| FLP | 0.42 | 0.42 | 0.42 | 1.37 | 0.66 | 0.48 | 72.24 |
| PAPD | 0.58 | 0.68 | 0.09 | 1.44 | 0.70 | 0.56 | 80.02 |
| PPUB | 0.44 | 0.44 | 0.44 | 1.46 | 0.70 | 0.51 | 73.38 |
| PCR | 0.47 | 0.47 | 0.47 | 1.54 | 0.74 | 0.54 | 72.54 |
| PBSHP | 0.57 | 0.57 | 0.38 | 1.72 | 0.81 | 0.61 | 75.71 |
| CONPS | 0.18 | 0.18 | 0.18 | 0.61 | 0.29 | 0.22 | 74.78 |
| PDCSC | 0.55 | 0.55 | 0.55 | 1.80 | 0.86 | 0.63 | 72.46 |
| SSHP | 0.57 | 0.57 | 0.38 | 1.71 | 0.81 | 0.61 | 75.33 |
| SCRL | 0.23 | 0.39 | 0.39 | 1.06 | 0.52 | 0.37 | 71.39 |
| LSS | 0.42 | 0.42 | 0.42 | 1.37 | 0.66 | 0.48 | 72.24 |
| MOTSS | 0.42 | 0.42 | 0.42 | 1.37 | 0.66 | 0.48 | 72.24 |
| HSP | 0.55 | 0.42 | 0.30 | 0.94 | 0.55 | 0.28 | 50.28 |

CTIC = Contribution Inter Cluster Divergence, GP= growth pattern, APL= attachment of primary leaves, STC= stem color, LFNS= leafiness at 50% flowering, LPUB= leaf pubescence, PPUB= petiole pubescence, LBTL= lobbing of the terminal leaflet, TLLS= terminal leaflet lobe shape, STS= stipule size, STSH= stipule shape, STPUB= stem pubescence, RPO= raceme position (at first pod), CLXC= calyx color, CORC= corolla color, BRCS= bracteole size, BRCS= bracteole shape, FLP= flowering period, PAPD= pod attachment to peduncle, PPUB= pod pubescence, PCR= pod curvature, PBSHP= pod beak shape, CONPS= constriction of pod between seeds, PDCSC= pod cross-section, SSHP= seed shape, SCRL= seed color, LSS= lusture on the seed surface, MOTSS= mottling on the seed surface, HSP= hilum shape

CONCLUSIONS

Morphological characterization of mung bean genotypes based on qualitative traits significantly contributes for better assessment of the genotypes and identification of the best genotypes with desirable traits for the further breeding program. In the present study, a wide range of phenotypic

diversity was recorded among tested mung bean genotypes for genetic enhancement through direct selection for desirable traits. The highest diversity index (H') was obtained for seed shape, while the lowest value was recorded for constriction of pod between seeds from the entire genotypes. The first seven principal components explained 94% of the total variation. In general, the presence of maximum

genetic diversity among mung bean genotypes based on qualitative traits leads to a high chance for hybridization for future variety improvement. Cluster analysis identified four distinct groups based on qualitative traits. Hence, future studies should consider the distribution of genetic diversity, serving as a benchmark for the collection, characterization, and conservation of genotypes. .

Moreover, the conventional approaches of characterization as adopted in this study have certain limitations in identifying duplicates; therefore, adoption of advanced diversity analysis tools like biochemical and molecular approaches which could accurately contribute to estimating the level of genetic diversity should be used.

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CONFLICTS of INTEREST

The authors declare that there is no conflict of interest.

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Correlation and Path Coefficient Analyses of Mung Bean [*Vigna radiata* (L.) Wilczek] Genotypes, in South Ethiopia

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Abstract

Understanding the nature and extent of association between seed yield and yield-related traits is important for sustainable genetic improvement. However, there is a lack of sufficient information on seed yield and yield-related trait correlation and path coefficient analysis of mung bean in Ethiopia. Therefore, there is a need to conduct correlation analysis among traits, and path coefficient analysis which splits the correlation variables into direct and indirect effects and which visualizes the relationship in a more meaningful way. To address these knowledge gaps, the present study was conducted to determine the nature and extent of phenotypic and genotypic correlation and path coefficient analysis among 17 quantitative traits. A total of 60 mung bean genotypes were tested using a 6×10 alpha lattice design at Jinka Agricultural Research Center during the 2018 cropping season. Seed yield was positively and significantly correlated with most of the traits at phenotypic and genotypic levels, indicating the presence of a strong inherent association between seed yield and other traits. Seed yield in mung bean can be improved through indirect selection for traits like plant height, the number of primary branches per plant, seeds per pod, and pod length. The information obtained from this study can be used for the genetic enhancement of mung bean thereby developing high-yielding varieties.

Key words: Direct Effect, Genotypic Association, Indirect Effect, Phenotypic Association, Seed Yield

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INTRODUCTION

Mung bean [*Vigna radiata* (L.)Wilczek] is one of the most important food legumes grown worldwide, and the most common crops in most tropical and sub-tropical regions (Allahmoradi et al., 2011). The importance of mung beans is related to desirable characteristics such as high protein content, broad adaptation, low need for agricultural inputs, and high ability to increase soil fertility. It is a valuable source of

carbohydrates 51%, protein 24-26%, minerals 4%, and vitamins 3% (Karthikeyan et al., 2011; Nair et al., 2013). Mung bean improves soil fertility by fixing atmospheric nitrogen (Yaqub et al., 2010), making it an economically and nutritionally valuable crop since it reduces the amount of nitrogen fertilizer required in the soil when grown in rotation with cereals. Perera et al. (2017) reported that mung bean improves grain and straw yields of the component crops due to its residual effects.

The existing morphological diversity of mung beans provides a great scope for genetic improvement as well as for increasing their productivity through varietal improvement. However, seed yield is a complex trait and is affected by agronomic, morphological, and physiological traits. Therefore, the genetic control of seed yield can be achieved by studying agronomic, morphological, and physiological traits (Tabasum et al., 2010). As suggested by Cruz et al. (2012), studies on correlations between characters enable one to perform an indirect selection for a quantitative trait, usually hard to be selected visually; besides, it is also able to access how a trait can interfere with another. Benti and Yohannis (2017) suggested that understanding the relationship between yield and yield contributing traits is necessary for any selection program which provides information regarding the nature and magnitude of the association between any two pairs of agromorphological traits. Similar to other crops, seed yield in mung bean is a complex character determined by various components and depends upon numerous genetic factors interacting with the environment. Thus, the knowledge of correlations between traits is useful especially for mung bean seed yield since it allows the breeder to use that additional information to discard or promote genotypes of interest. Studies on correlations between characters are indeed important to breeding programs as it enables to perform an indirect selection for a quantitative trait, usually hard to be selected, by another directly correlated trait of higher genetic gain or easy visual selection; as well, it is also able to access how a trait can interfere with another (Cruz et al., 2012). It is known that the selection for a certain trait can eventually bring effects on others with or without the interest in the intended breeding programs. Accordingly, the knowledge of correlations between traits is to use that additional information to discard or promote genotypes of interest. The correlation analysis showed the relationship between two or more series of variables (Bhanu et al., 2016). Correlation analysis between characters may be sometimes misleading due to complex interactions consequently not providing the exact impression of one character over another.

Therefore, there is a need to go further for path coefficient analysis which splits the correlation variables into direct and indirect effects and which visualizes the relationship in a more meaningful way. As suggested by (Jogdhande et al., 2017; Manisha et al., 2018), path analysis avoids the complication by measuring the direct influence of one trait on the other as well as permits the partitioning of correlation coefficients into its components of direct and indirect effects.

But information on the correlation and path coefficient analysis of seed yield and yield-related traits on mung bean genotypes in Ethiopia is limited. Therefore, the objectives of the present study were to determine the nature and extent of phenotypic and genotypic correlations among the studied quantitative traits and to identify the most important traits for indirect selection in the future mung bean breeding programs.

MATERIALS AND METHODS

Description of the Study Area

The field experiment was conducted during the main cropping season, therefore from March to June 2018 at Jinka Agricultural Research Center (JARC). Jinka Agricultural Research Center is located 729 km southwest of Addis Ababa at 36° 33' 02.7" E, 05° 46' 52.0" N, and at an altitude of 1420 m above sea level. The maximum, minimum, and average temperatures of the center for ten years (2009-2019) are 27.68°C, 16.61°C, and 22.14°C, respectively while the mean annual rainfall is 1381 mm. The soil type of the center is Cambisols (Mesfin *et al.*, 2017).

Experimental Materials

A total of 60 mung bean genotypes were used. Out of these, 44 genotypes were obtained from Melkassa Agricultural Research Center (MARC) and 16 genotypes were collected from Southern Nations, Nationalities, and People's (SNNP) region.

Experimental Design and Procedures

The experiment was conducted using a 6 × 10 alpha lattice design. The plot size was 3 m long, 0.3 m between rows, and 0.05 m between plants. Each plot consisted of five rows, accommodating 80 plants per row. The distance between plots,

blocks, and replications was 1 m, 1.5 m, and 2 m, respectively.

Data Collection

The quantitative data were collected according to the descriptor of the mung bean developed by the International Board for Plant Genetic Resources (IBPGR, 1980). The data collected on a plot basis were; days to flowering (days), days to maturity (days), hundred seed weight (g), seed yield per plot (g), biomass (g), and harvest index (%). The data collected on a plant basis were; plant height (cm), number of primary branches per plant, number of seeds per pod, pod length (cm), peduncle length (cm), number of pods per cluster,

terminal leaflet length (cm), and terminal leaflet width (cm).

Data Analyses

The collected data were subjected to analysis of variance (ANOVA) to test for the presence of variation among the genotypes for the studied traits and then based on the ANOVA result; all significant traits were promoted for correlation and path coefficient analyses. Phenotypic and genotypic correlations between seed yield and yield-related traits were estimated using the method described by Miller *et al.* (1958) as:

$$\text{The phenotypic correlation coefficient } (r_{p_{xy}}) = \frac{\text{Cov}_{pxy}}{\sqrt{(\sigma^2_{px})(\sigma^2_{py})}}$$

$$\text{The genotypic correlation coefficient } (r_{g_{xy}}) = \frac{\text{Cov}_{gxy}}{\sqrt{(\sigma^2_{gx})(\sigma^2_{gy})}}$$

Where, r_{pxy} is phenotypic correlation coefficient and Genotypic correlation coefficient ($r_{g_{xy}}$) between character x and y; Cov_{pxy} and Cov_{gxy} are phenotypic covariances and genotypic covariance between character x and y; σ^2_{gx} and σ^2_{gy} are genotypic variances traits x and y; σ^2_{px} and σ^2_{py} are phenotypic variances of traits x and y, respectively. The coefficient of correlation was tested using tabulated value at n-2 degree of freedom, at 5% and 1% probability level, where n is the number of treatments (genotypes) as described by Robertson (1959). META-R Version 6. 01 (Alvarado *et al.*, 2017) was employed for phenotypic and genotypic correlation coefficient analysis. Microsoft Excel computer program was employed for phenotypic and genotypic path coefficient analysis as well as estimation of residual effect. Path coefficient analysis was conducted as suggested by Dewey and Lu (1959) using the phenotypic as well as genotypic correlation coefficients to determine the direct and indirect effects of yield and other traits based on the following relationship.

$R_{ij} = P_{ij} + \sum_{rik} \dot{a}_{rik} P_{kj}$; Where, r_{ij} is the mutual association between the independent character (i) and dependent traits (j) as measured by correlation coefficients, p_{ij} is components of direct effects of the independent traits (i) on the dependent traits (j), $\sum_{rik} \dot{a}_{rik} P_{kj}$ = summation of components of the indirect effect of a given independent character (i) on the dependent traits (j) via all other independent traits (k).

The residual effect (R) was estimated using the formula shown below (Dewey and Lu, 1959).

$$R = \sqrt{1-R^2} \quad \text{Where, } R^2 = \sum_{ij} r_{ij} p_{ij}$$

RESULTS AND DISCUSSION

Genotypic and Phenotypic Correlations Coefficient Analysis

The analysis of variance results for correlation coefficients among seed yield and its contributing traits were computed and the values for phenotypic and genotypic correlation coefficients between each pair of traits are presented in Table 1. The present study revealed that the magnitudes of genotypic correlation coefficients (r_g) were

higher than their corresponding phenotypic correlation coefficients (r_{ph}) except in few cases, indicating the presence of strong inherent association among these traits due to the genetic factors and the dominance of genetic variance in the expression of traits. This finding is in agreement with the report of Ghosh *et al.* (2019) on 52 mung bean genotypes, who reported that the genotypic correlation was higher in magnitude than the phenotypic correlation, which coincides with the previous works (Begum *et al.*, 2012; Srivastava and Singh, 2012; Narasimhulu *et al.*, 2013; Singh and Bains, 2014; Benti and Yohannis, 2017; Abbas *et al.*, 2018; Ghosh *et al.*, 2019) on mung bean and (Bharti *et al.*, 2013; Reni *et al.*, 2013; Sarkar, 2014; Kumar *et al.*, 2015; Arya *et al.*, 2017; Partap *et al.*, 2019) on black gram genotypes. These authors reported that the magnitudes of genotypic correlation were higher as compared to their corresponding phenotypic correlation for most of the traits indicating the preponderance of genetic variance in the expression of characters. On the contrary, Tabasumet *al.* (2010) and Patel *et al.* (2014) reported that the magnitude of phenotypic correlation was higher than the genotypic correlation. Similarly; Sandhiya and Saravanan (2018) reported that the magnitude of the phenotypic coefficient of variation for 10 traits on 36 mung bean genotypes was higher than the genotypic coefficient of variation indicating the involvement of both genotype and environment for the variation. In this study, almost all the studied traits showed a significantly positive correlation with seed yield at the genotypic level.

Correlations of Seed Yield with other Traits

In this study, seed yield exhibited significant and positive correlations with hundred seed weight, biomass, and harvest index at the genotypic and phenotypic levels (Table 1). Genetic relationships between seed yield and yield-related characters are prerequisites in selecting desirable types for the target environment. Some of the yield components are highly interrelated while, seed yield is governed by many genetic as well as environmental factors that are interdependent and influenced by various components towards yield, which is associated with low heritability. Therefore, the present study suggested that the

presence of strong inherent association among the studied traits lead to direct phenotypic selection might be worthwhile for the improvement of mung bean and which implies that there is an enormous chance of exploiting the potential of these traits for effective selection in the mung bean improvement program.

This indicated that these traits are considered important for the improvement of mung bean yield through a direct selection of these traits. This finding is in agreement with the work of Al-Ballat and Al-Araby (2019) on common bean, who reported that the selection for those traits having strong and positively significant correlations with seed yield is expected to improve seed yield in common bean, suggesting that the true relationship of these traits with seed yield since these traits are seed yield predictors. Similarly, Gonçalves *et al.* (2017) indicated that the traits which have moderate to strong correlations with the desired trait are important for successful indirect selection.

Traits such as seeds per pod, seed yield per plant, and pods per cluster had a significant positive correlation with seed yield at genotypic and phenotypic levels. This finding is in agreement with the report of Partap *et al.* (2019) on the black gram genotypes, who indicated that the strong and positive association of yield-related traits might be due to the linkage of genes determining these traits which leads to the simultaneous improvement in seed yield through these traits could be achieved within a short period by simple selection procedures. Therefore; these results give some clues that simultaneous improvement in any of these traits will lead to an increase through direct selection for seed yield in mung bean. Based on these results, the genotypic and phenotypic correlations indicate that the extent to which the corresponding traits are under the control of the same set of genes having a physiological basis for their expression. Likewise, Ghosh *et al.* (2019) on mung bean genotypes, reported that seed yield exhibited a significantly ($P \leq 0.01$) positive correlation with the number of pods per plant at the genotypic (0.665) and phenotypic (0.578) levels, respectively while it had exerted significantly

($P \leq 0.05$) positive correlation with the number of seeds per pod only at the genotypic (0.261) level. Similarly, seed yield per plant showed a significantly ($P \leq 0.01$) positive correlation with the number of branches per plant at the genotypic level while it had a significantly ($P \leq 0.05$) positive correlation with the number of branches per plant at the phenotypic level. On the other hand, seed yield showed a negative and highly significant genotypic and phenotypic correlation with days to flowering (Table 1)

Path Coefficient Analysis

Whether the correlation between two characters is of phenotypic, genotypic, or environmental nature, it is the genotypic one that determines higher interest for genetic breeding as it involves an association of heritable nature (Nogueira et al., 2012). By considering this view, path analysis was made to understand the nature of correlations among yield and yield components to improve production and mitigate future demand (Kate et al., 2017). Regarding the values for direct and indirect effects, Lenka and Mishra (1973) suggested that a scale for the importance of direct and indirect effects and classified as negligible for values from (0.00 to 0.09), low, for values from (0.10 to 0.19), moderate, for values from (0.20 to 0.29), high, for values from (0.30 to 0.99), while values greater than 0.99, were considered as very high. In this study, seed yield was considered as a dependent trait while the rest of the traits that were positively correlated with seed yield were used as causal traits (Tables 2 and 3).

Phenotypic Path Coefficient Analysis of Seed Yield with other Traits

The phenotypic path coefficient analysis results of seed yield with the other 16 traits were presented (Table 2). The path coefficient analysis result revealed that days to maturity, petiole length, peduncle length, plant height, pod length, pods per cluster, pods per plant, seeds per pod, seed yield per plant, hundred seed weight, and harvest index had a positive direct effect on seed yield at the phenotypic level. Peduncle length possessed a positive direct effect (0.13) on seed yield but its negative indirect effects through petiole length (-0.26), terminal leaf length (-0.14),

terminal leaf width (-0.07), and biomass yield (-0.04), as well as its significant negative genotypic (-0.49) and phenotypic (-0.35) coefficients, contributed to a negative association with seed yield. The results showed that pods per cluster had a maximum direct positive effect on seed yield (0.41) followed by pods per plant (0.33), suggesting that the simultaneous selection of the two traits may improve genetic gain in mung bean breeding. These results showed that the selection based on these traits except the peduncle length would be highly effective for improving seed yield since the positive direct effect of the peduncle length on seed yield is diluted due to its negative indirect effects. In this study, those traits with positive direct effects should be considered as the selection criteria for yield improvement in mung bean, and it is important to reveal the effectiveness of direct selection through these traits. Direct negative effects on seed yield were attributed by days to flowering, terminal leaf length, terminal leaf width, the number of primary branches per plant, and biomass yield at the phenotypic level, indicating that improvement of these traits is essential before selecting them for high seed yield. Therefore, the results indicated that these traits are not directly associated with yield hence, selection directly through these characters results in poor selection, so indirect causal factors must be considered especially the traits contributing positively.

Days to flowering had a negative direct effect (-0.16) on seed yield because of its negative indirect effect through terminal leaf length, terminal leaf width, plant height, number of primary branches per plant, pods per cluster, pods per plant, seeds per pod, seed yield per plant, biomass yield and harvest index at the phenotypic level. This finding complies with the results of Bhanu *et al.* (2016) who reported that days to flowering showed a negative direct effect on seed yield at the phenotypic level. Terminal leaf length had exerted a negative direct effect (-0.15) on seed yield due to its negative indirect effects through days to flowering, days to maturity, terminal leaf width, seed yield per plant, hundred seed weight, and biomass yield. Terminal leaf width had exerted a negative direct effect (-0.02) on seed yield due to its negative indirect effects

through days to flowering, days to maturity, terminal leaf length, the number of primary branches per plant, pods per plant, seeds per pod, and seed yield per plant. The number of primary branches per plant exerted a negative direct effect (-0.23) on seed yield due to its negative indirect effects through days to flowering, days to maturity, terminal leaf width, plant height, pods per plant, hundred seed weight, and biomass yield. The negative direct influence (-0.27) of biomass yield on seed yield was nullified by its positive indirect effects via petiole length, terminal leaf length, terminal leaf width, plant height, pod length, pods per plant, seeds per pod, and seed yield per plant.

Days to maturity had affected seed yield positively and indirectly through days to flowering, petiole length, peduncle length, the number of primary branches per plant, pod length, and 100-seed weight at the phenotypic level. Whereas, the negative indirect effect of plant height on seed yield was observed through days to flowering, days to maturity, petiole length, the number of primary branches per plant, and pods per cluster at the phenotypic level. Similarly, a negative indirect effect of harvest index on seed yield was noted through days to flowering, days to maturity, and biomass yield at the phenotypic level. The considerable indirect effect of pods per cluster through petiole length (-0.28) and hundred seed weight (-0.19) were counter-balanced by the positive direct effect of pods per cluster (0.41) on seed yield and reduced the correlation coefficient to 0.25. Pods per plant are affected positively and indirectly via petiole length, terminal leaf length, peduncle length, plant height, pod length, pods per cluster, biomass yield, and harvest index at the phenotypic level. Hundred seed weight had influenced positively and indirectly seed yield via days to flowering, days to maturity, and terminal leaf width, peduncle length, and plant height, seeds per plant, and harvest index at the phenotypic level. Likewise, a positive indirect effect of biomass yield on seed yield was noted through terminal leaf width, pods per cluster, and pods per plant at the phenotypic level. Harvest index affected seed yield negatively and indirectly through days to flowering and days to

maturity at the phenotypic level. The result of phenotypic path analysis showed that due importance should be given for plant height, pods per plant, pods per cluster, hundred seed weight, and harvest index because of their significant correlation and high direct effects. This indicates that there is always scope for enhancement of grain yield by the selection of these traits at the phenotypic level.

The residual effect at the phenotypic level ($h = 0.2047$) was relatively low indicating that the traits considered in this study are enough to adequately explain the variation in seed yield. About 79.53% of the total variation on seed yield was contributed by 16 independent traits that were included in this study. The remaining 20.47% is explained by other traits not considered in the study. This further clarified that seed yield attributing traits included in this study were good enough. It is also suggested that further study should be made with more characters to find out other traits that contribute to the rest of the proportion of the yield. Generally, the present investigation suggested that maximum emphasis should be given to the phenotypic traits studied in selecting mung bean with higher seed yields. The contribution of residual effects that influenced seed yield was low at the phenotypic levels, reflected that the traits in the study were sufficient enough to account for the variability in the dependent character. This finding was supported by the previous reports on black gram genotypes (Mohammad *et al.*, 2016; Arya *et al.*, 2017; Partap *et al.*, 2019). Similarly, Khan *et al.* (2016), observed the phenotypic residual effect ($h = 0.0925$), indicating that about 91% of the phenotypic total variation was explained by the included traits and how best the causal factors account for the variability of the dependent factor on snake gourd genotypes.

Therefore, path analysis indicated that petiole length, plant height, pods per plant, pods per cluster, hundred seed weight, and harvest index were the main determinants of seed yield. Thus, more emphasis should be given during the selection of these traits for yield improvement in mung beans. Generally, the seed yield is an important parameter among all the morphological

as well as yield traits. Improvement in seed yield in mung bean could be brought through the selection of component characters like pods per plant, pod length, 100 seed weight, and harvest index which are directly related to final yield in mung bean and exhibited positive direct effects at the phenotypic level.

Genotypic Path Coefficient Analysis of Seed Yield with other Traits

The genotypic path coefficient analysis results of seed yield with the other 16 traits were presented (Table 3). Days to maturity, terminal leaf width, peduncle length, plant height, number of primary branches per plant, pod length, pods per cluster, pods per plant, seeds per pod, seed yield per plant, hundred seed weight, biomass yield, and harvest index had a positive direct effect on seed yield. Plant height possessed a high positive direct effect (0.54) on seed yield but its negative indirect effects were also detected through days to maturity (-0.17), petiole length (-0.01), pods per plant (-0.11), seeds per pod (-0.08), and biomass yield (-0.76). Biomass yield had exerted a positive direct effect (0.21) on seed yield but its negative indirect effects through days to maturity (-0.07), terminal leaf length (-0.33), peduncle length (-0.05), pods per cluster (-0.05), seeds per pod (-0.16), seed yield per plant (-0.12) and harvest index (-0.10). The results showed that plant height had a maximum direct positive effect on seed yield (0.54) followed by the number of primary branches per plant and seeds per pod (0.36), days to maturity (0.34), and biomass yield (0.21), respectively suggesting that the simultaneous selection of these traits may improve genetic gain in mung bean breeding. The strong indirect effect of plant height through biomass yield (-0.76), days to maturity (-0.17), the number of pods per cluster (-0.11), petiole length (-0.01), and the number of seeds per pod (-0.08) were counterbalanced by the positive direct effect of plant height (0.54) on seed yield and reduced the correlation coefficient to -0.01. Similarly, the indirect effect of biomass yield through terminal leaf length (-0.33), days to maturity (-0.07), peduncle length (-0.05), pods per cluster (-0.05), the number of seeds per pod (-0.16), seed yield per plant (-0.12), and harvest index (-0.1) were counter-balanced by the direct

effect of biomass yield (0.21) on seed yield and reduced the correlation coefficient to (-0.1). The positive direct effects of plant height and biomass yield on seed yield were diluted due to their negative indirect effects. The selection of these traits except plant height and biomass yield would be highly effective for improving seed yield. Direct negative effects on seed yield were attributed to days to flowering, petiole length, terminal leaf length, pod length, and pods per cluster, indicating that improvement of these traits is essential before selecting them for high seed yield. The negative direct effect of days to flowering on seed yield was nullified by its positive indirect effects via petiole length, terminal leaf length, terminal leaf width, peduncle length, the number of primary branches per plant, plant height, seeds per pod, seed yield per plant, hundred seed weight, biomass yield, and harvest index. Petiole length had exerted a negative direct effect (-0.17) on seed yield due to its negative indirect effects through days to flowering, days to maturity, pods per plant, seed yield per plant, and hundred seed weight. The negative direct influence of terminal leaf length on seed yield was nullified by its positive indirect effects via days to flowering, days to maturity, petiole length, peduncle length, plant height, number of primary branches per plant, pods per plant, seeds per pod, seed yield per plant and harvest index. Terminal leaf width had exerted a positive direct effect (0.09) on seed yield due to its positive indirect effects through days to maturity, terminal leaf length, the number of primary branches per plant, plant height, pod length, pods per cluster, seeds per pod, hundred seed weight and harvest index. Pod length had exerted a negative direct effect (-0.11) on seed yield due to its negative indirect effects through days to maturity, terminal leaf length, peduncle length, seeds per pod, seed yield per plant, and hundred seed weight. Pods per cluster exerted a negative direct effect (-0.17) on seed yield because of its negative indirect effects through terminal leaf width, seed yield per plant, hundred seed weight, and harvest index.

Days to maturity had exerted positive and indirect effects on seed yield through terminal leaf length, terminal leaf width, peduncle length, number of primary branches per plant, pods per cluster, and

Pods per plant at the genotypic level. The number of pods per cluster exhibited positive and indirect effects on seed yield through days to maturity, petiole length, terminal leaf width, peduncle length, plant height, pod length, seeds per pod, seed yield per plant, hundred seed weight, and harvest index at the genotypic level. This result is in agreement with other reports (Gadakh *et al.*, 2013; Itafa *et al.*, 2014; Muthuswamy *et al.*, 2019). Selection based on the number of pods per cluster would increase the seed yield indirectly via the number of pods per plant. Path coefficient analysis also revealed that the number of pods per plant had a positive direct effect on seed yield. This trait also recorded a strong positive genotypic correlation with seed yield per plant. This indicated that the selection of the genotypes having more pods per plant along with optimum seed weight would be rewarding for isolating high-yielding genotypes in this crop. This is in agreement with Ghosh *et al.* (2019) on mung bean, who reported that pods per plant have been exerted maximum positive direct effect (0.717) on seed yield per plant, which coincides with the similar works (Katiyar *et al.*, 2012; Manggoel *et al.*, 2012) who reported that positive direct effect of pods per plant on seed yield of mung bean. Pods per cluster had exerted a negative direct effect on seed yield. This result is controversial with the previous reports (Anand *et al.*, 2016) who described that pods per cluster had exerted a positive direct effect on seed yield of mung bean.

There were positive indirect effects of pods per plant through terminal leaf length, terminal leaf width, peduncle length, pod length, pods per cluster, seed yield per plant, and biomass yield at the genotypic level. Likewise, a positive indirect effect of pods per plant on seed yield was observed through terminal leaf length, terminal leaf width, peduncle length, pod length, pods per cluster, seed yield per plant, and biomass yield at the genotypic level. Positive indirect effects of 100-seed weight on seed yield were observed through days to flowering, terminal leaf width, plant height, seeds per pod, seed yield per plant, and biomass yield at the genotypic level. Biomass yield affected seed yield indirectly and negatively through the traits like days to maturity, terminal leaf length, terminal leaf width, plant height, the

number of primary branches per plant, seeds per pod, pods per plant, seed yield per plant, and hundred seed weight at genotypic level; while it affected positively and indirectly seed yield through days to flowering, petiole length, peduncle length, pod length, pods per cluster and harvest index at the genotypic level. Harvest index affected seed yield negatively and indirectly through days to maturity, pods per cluster, seeds per pod, and biomass yield at the genotypic level. This result confirmed the previous findings (Lalinia and Khameneh, 2014; Abbas *et al.*, 2018). The result of genotypic path analysis showed that due importance should be given for days to maturity, the number of primary branches per plant height, and seed yield per plant because of their significant correlation and high direct effects. This indicates that there is always scope for enhancement of grain yield by the selection of these traits at the genotypic level. Days to maturity, the number of primary branches per plant, seed yield per plant, and plant height are the most important yield contributing components as they recorded high direct effects towards seed yield in mung bean.

The residual effect at the genotypic level ($h = 0.0943$) was low, signifying that the traits considered in this study are enough to adequately explain the variation in seed yield. The estimation of direct and indirect effects was more pronounced in the genotypic path than the phenotypic path. About 90.57% of the total variation on seed yield was contributed by 16 independent traits that were included in this study, while other factors not included in the study might explain 9.43%. Therefore, the traits included in this study were good enough to explain the variability in seed yield. Generally, the low residual effect indicated the independent traits explained the dependent trait (seed yield) more than two-thirds (2/3); "therefore, selection based on genotypic path coefficient analysis for mung bean improvement is very appropriate. . This finding is supported by the report of Asari *et al.* (2019) who observed a low genotypic residual effect ($h = 0.0324$). Likewise, Anand *et al.* (2014) observed a low residual effect and stated that the appropriateness of the traits chosen to explain the variability. Similar results were also supported on

black gram genotypes (Mohammad *et al.*, 2016; Arya *et al.*, 2017; Partap *et al.*, 2019). Similarly, Khan *et al.* (2016), observed the genotypic residual effect ($h= 0.024$), indicating that about 98% of the genotypic total variation was explained by the included traits and how best the causal factors account for the variability of the dependent factor on snake gourd genotypes.

This result is contradictory with the reports of Kumar (2014) and; Bhanu *et al.* (2016) who reported that the high residual effect observed at the genotypic level is due to the warm and dry weather condition indicating that some of the characters influencing the seed yield of mung bean have not been included in this study.

Table 1. Genotypic (Above Diagonal) and Phenotypic (Below Diagonal) Correlation Coefficients among 17 Traits on Mung Bean.

| | DTF | DTM | PTL | TLL | TLW | PDCL | PHT | BRN | PODL | PPC | PPP | SPP | SYPP | HSW | SYLD | BM | HI |
|------|--------|--------|---------|--------|---------|--------|---------|--------|---------|--------|---------|--------|---------|---------|---------|---------|---------|
| DTF | | 0.08* | -0.39ns | -0.38* | 0.26* | 0.06ns | 0.03** | 0.02* | 0.23** | 0.06* | -0.26** | -0.01* | 0.07** | 0.17** | -0.22** | 0.16* | 0.01* |
| DTM | 0.06* | | 0.92ns | -0.06* | 0.18* | 0.06* | -0.31** | 0.03* | 0.08ns | 0.26ns | 0.29* | 0.21* | 0.24* | 0.15** | 0.43** | 0.33** | 0.15* |
| PTL | 0.31ns | 0.03ns | | -0.18* | 0.17* | 0.16ns | -0.25** | 0.11ns | 0.19** | 0.16ns | -0.31** | 0.23ns | -0.29** | -0.13** | -0.45** | 0.41** | -0.01* |
| TLL | 0.04* | -0.11* | 0.12ns | | -0.06ns | -0.13* | 0.04** | 0.05* | -0.24** | -0.13* | 0.51ns | 0.19ns | 0.06* | -0.48* | 0.23** | -0.43ns | -0.01* |
| TLW | 0.17* | 0.14* | 0.06** | 0.04* | | 0.09* | 0.15** | 0.19ns | 0.11* | 0.09ns | 0.12* | 0.21* | -0.43* | 0.20* | 0.11* | 0.11* | -0.01ns |
| PDCL | -0.08* | 0.03* | 0.10** | 0.02* | 0.05* | | 0.17** | 0.08* | -0.08ns | 0.04* | 0.03ns | 0.09ns | 0.48** | 0.02* | -0.49** | -0.07* | -0.19* |
| PHT | 0.18* | 0.05* | 0.06* | -0.03* | 0.09* | 0.10* | | 0.12* | 0.03* | 0.17* | -0.23* | -0.08* | -0.11** | 0.09* | 0.24** | 0.20** | -0.01* |
| BRN | 0.04ns | -0.11* | 0.12* | -0.29* | 0.04* | 0.02* | -0.03** | | 0.05* | 0.08* | -0.07* | 0.02* | 0.04** | 0.10** | 0.14** | 0.03** | -0.09* |
| PODL | -0.12* | -0.25* | 0.06* | 0.13* | -0.16* | -0.15* | 0.19** | 0.13* | | -0.08* | -0.03ns | -0.10* | 0.03ns | 0.25ns | 0.11ns | 0.28ns | 0.16ns |
| PPC | -0.03* | 0.02* | 0.07* | 0.18* | -0.05* | 0.01* | -0.04** | 0.18* | 0.10* | | 0.02** | -0.11* | 0.08** | -0.40* | 0.05* | 0.55** | -0.01* |
| PPP | -0.08* | 0.09* | -0.10* | 0.46* | 0.08* | 0.02* | 0.03* | 0.46* | -0.16* | -0.15* | | -0.04* | -0.20* | -0.14* | 0.17* | 0.24* | 0.12* |
| SPP | -0.04* | -0.19* | 0.26* | -0.02* | 0.08* | 0.18* | 0.14* | -0.02* | 0.13* | -0.12* | 0.11* | | 0.05* | 0.07** | 0.65** | 0.15* | 0.03** |
| SYPP | 0.08* | 0.12* | 0.03* | 0.02* | 0.15* | 0.17* | 0.21* | 0.02* | -0.43* | -0.09* | 0.26* | 0.13* | | 0.68** | 0.93** | -0.03* | -0.01* |
| HSW | 0.12* | -0.06* | -0.16* | -0.35* | -0.10* | -0.09* | 0.23* | -0.35* | -0.02* | -0.08* | -0.29* | -0.24* | 0.02* | | 0.43** | 0.33* | 0.21** |
| SYLD | -0.20* | 0.06* | 0.25* | 0.10* | 0.13* | -0.35* | 0.16* | 0.10* | 0.31* | 0.17* | 0.22* | 0.56* | 0.48* | -0.15* | | 0.70* | 0.01* |
| BM | -0.23* | -0.02* | 0.27* | 0.21* | 0.15* | 0.45* | 0.07* | 0.21* | -0.02* | 0.21* | 0.29* | 0.51* | 0.43* | 0.45* | 0.55* | | 0.67* |
| HI | -0.23* | -0.23* | -0.02* | 0.27* | 0.21* | 0.15* | 0.35* | 0.07* | 0.21* | -0.02* | 0.21* | 0.29* | 0.51* | 0.43* | 0.44* | 0.65* | |

DTF=days to flowering, DM= days to maturity, PTL = petiole length (cm), TLL = terminal leaf length (cm), TLW = terminal leaf width (cm), PDL = peduncle length (cm), PHT= plant height (cm), BRN=number of primary branches per plant, PODL = pod length (cm), PPC=number of pods per cluster, PPP=number of pods per plant, SPP= number of seeds per pod, SYPP= seed yield per plant (g), HSW= hundred seed weight (g), SYLD= seed yield (kg ha⁻¹), BM= biomass yield (kg ha⁻¹), HI=harvest index.

Table 2. Phenotypic Path Coefficients, Direct and Indirect Effects.

| | DTF | DTM | PTL | TLL | TLW | PDCL | PHT | BRN | PODL | PPC | PPP | SPP | SYPP | HSW | BM | HI | r_{ph} |
|------|---------------------|--------------------|--------------------|---------------------|---------------------|--------------------|-------------------|---------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|---------------------|--------------------|----------|
| DTF | <u>-0.16</u> | 0.07 | 0.02 | -0.14 | -0.11 | 0.02 | -0.01 | -0.04 | 0.06 | -0.07 | -0.01 | -0.06 | -0.01 | 0.05 | -0.03 | -0.03 | -0.45 |
| DTM | 0.07 | <u>0.07</u> | 0.02 | -0.14 | -0.07 | 0.02 | -0.02 | 0.2 | 0.02 | -0.04 | -0.02 | -0.02 | -0.02 | 0.04 | -0.03 | -0.03 | 0.05 |
| PTL | 0.02 | 0.02 | <u>0.21</u> | -0.02 | 0.2 | 0.02 | -0.01 | 0.03 | -0.03 | -0.17 | 0.1 | 0.05 | 0.12 | -0.07 | -0.03 | 0.01 | 0.45 |
| TLL | -0.14 | -0.14 | 0.01 | <u>-0.15</u> | -0.23 | 0.08 | 0.12 | 0.02 | 0.08 | 0.01 | 0.04 | 0.06 | -0.07 | -0.01 | -0.02 | 0.03 | -0.31 |
| TLW | -0.11 | -0.07 | 0.05 | -0.05 | <u>-0.02</u> | 0 | 0.01 | -0.02 | 0 | 0.08 | -0.11 | -0.12 | -0.01 | 0.01 | 0.09 | 0.03 | -0.24 |
| PDCL | 0.02 | 0.02 | -0.26 | -0.14 | -0.07 | <u>0.13</u> | 0.02 | 0.02 | 0.02 | 0.1 | 0.01 | 0.04 | 0.01 | 0.02 | -0.04 | 0.02 | -0.08 |
| PHT | -0.01 | -0.02 | 0 | 0.01 | 0.1 | 0.01 | <u>0.2</u> | 0.06 | -0.01 | 0.04 | 0.01 | 0 | 0.04 | 0.1 | -0.01 | 0.05 | 0.57 |
| BRN | -0.04 | -0.04 | 0.01 | 0.01 | -0.01 | 0.01 | -0.02 | <u>-0.23</u> | 0.01 | 0.09 | -0.01 | 0.02 | 0.08 | -0.12 | -0.06 | 0.01 | -0.29 |
| PODL | 0.06 | 0.04 | 0.02 | -0.03 | -0.23 | 0.01 | 0.02 | -0.03 | <u>0.11</u> | 0.02 | 0.06 | 0.31 | 0.01 | 0.01 | -0.16 | 0.03 | 0.25 |
| PPC | -0.07 | -0.07 | -0.28 | 0.07 | 0.14 | 0.04 | -0.09 | 0.12 | 0.08 | <u>0.41</u> | 0.03 | -0.11 | -0.04 | -0.19 | 0.2 | 0.01 | 0.25 |
| PPP | -0.01 | -0.01 | 0 | 0.05 | 0.11 | -0.06 | 0.2 | -0.01 | 0.06 | 0.03 | <u>0.33</u> | -0.1 | -0.06 | -0.01 | 0.02 | 0.03 | 0.57 |
| SPP | -0.06 | -0.06 | 0.03 | -0.03 | 0.15 | 0.05 | 0.01 | 0.02 | 0.31 | -0.11 | -0.1 | <u>0.07</u> | 0.01 | 0.09 | -0.13 | 0.04 | 0.29 |
| SYPP | -0.01 | -0.01 | -0.13 | 0.17 | -0.01 | 0.02 | 0.04 | 0.03 | 0 | 0.03 | -0.03 | 0.03 | <u>0.23</u> | -0.08 | -0.03 | 0.07 | 0.32 |
| HSW | 0.05 | 0.04 | 0.03 | 0.01 | 0 | 0.1 | 0.1 | 0.12 | 0.01 | -0.25 | -0.05 | 0.15 | 0.08 | <u>0.19</u> | -0.02 | 0.06 | 0.62 |
| BM | -0.03 | -0.03 | 0.04 | 0.1 | 0.21 | -0.06 | 0.03 | -0.06 | 0.09 | 0 | 0.02 | 0.08 | 0.06 | -0.02 | <u>-0.27</u> | -0.06 | 0.1 |
| HI | -0.03 | -0.03 | -0.01 | 0.12 | 0.09 | -0.04 | 0.05 | 0.01 | 0.03 | 0.01 | 0.03 | 0.04 | 0.07 | 0.06 | -0.04 | <u>0.13</u> | 0.49 |

Residual = 0.2047

DTF=days to flowering, DM= days to maturity, PTL = petiole length (cm), TLL = terminal leaf length (cm), TLW = terminal leaf width (cm), PDL = peduncle length (cm), PHT= plant height (cm), BRN=number of primary branches per plant, PODL = pod length (cm), PPC=number of pods per cluster, PPP=number of pods per plant, SPP= number of seeds per pod, SYPP= seed yield per plant (g), HSW= hundred seed weight (g), SYLD= seed yield (kg ha⁻¹), BM= biomass yield (kg ha⁻¹), HI=harvest index, r_{ph} =phenotypic correlation

Table 3. Genotypic Path Coefficients, Direct and Indirect Effects.

| | DTF | DTM | PTL | TLL | TLW | PDCL | PHT | BRN | PODL | PPC | PPP | SPP | SYPP | HSW | BM | HI | rg |
|------|--------------|-------------|--------------|--------------|-------------|-------------|-------------|-------------|--------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|-------|
| DTF | -0.81 | -0.12 | 0.2 | 0.03 | 0.02 | 0 | 0.2 | 0.08 | -0.03 | -0.01 | -0.12 | 0.01 | 0.07 | 0.09 | 0.19 | 0.21 | 0.01 |
| DTM | 0.26 | 0.34 | -0.07 | -0.01 | 0.01 | 0.03 | -0.11 | 0 | -0.01 | 0.03 | -0.11 | 0.11 | -0.03 | -0.11 | -0.02 | -0.04 | 0.27 |
| PTL | -0.17 | -0.02 | -0.17 | 0 | 0.09 | 0.02 | 0.03 | 0 | 0.04 | 0.02 | -0.31 | 0.02 | -0.2 | -0.04 | 0.13 | 0 | -0.56 |
| TLL | 0.82 | 0.11 | 0.18 | -0.08 | -0.02 | 0.01 | 0 | 0.02 | -0.01 | -0.13 | 0.01 | 0.19 | 0.08 | -0.37 | -0.04 | 0.2 | 0.97 |
| TLW | -0.01 | 0.02 | -0.01 | 0 | 0.09 | -0.02 | 0.07 | 0.03 | 0 | 0.07 | 0.06 | 0.01 | -0.03 | 0.05 | -0.13 | 0.21 | 0.41 |
| PDCL | 0.01 | 0.12 | 0.01 | -0.01 | 0.01 | 0.06 | 0.06 | 0.08 | -0.06 | 0.01 | 0.04 | 0.03 | 0.08 | -0.05 | 0.02 | 0.15 | 0.56 |
| PHT | 0.02 | -0.17 | -0.01 | 0 | 0.01 | 0.01 | 0.54 | 0.1 | 0.02 | 0.22 | -0.11 | -0.08 | 0.04 | 0.16 | -0.76 | 0 | -0.01 |
| BRN | 0.12 | 0.01 | 0 | 0.03 | 0.05 | 0.04 | 0.07 | 0.36 | 0.07 | -0.01 | -0.03 | -0.06 | 0.08 | -0.23 | -0.01 | 0 | 0.49 |
| PODL | 0.08 | -0.01 | 0.03 | -0.01 | 0 | -0.03 | 0 | 0.02 | -0.11 | 0.01 | 0.09 | -0.19 | -0.06 | -0.02 | 0.02 | 0.02 | -0.16 |
| PPC | 0.03 | 0.05 | 0.04 | 0 | -0.01 | 0 | 0.03 | 0.01 | 0 | -0.17 | 0.01 | 0.25 | -0.01 | -0.04 | 0.01 | -0.3 | -0.1 |
| PPP | 0 | 0.05 | 0 | -0.01 | 0.1 | -0.02 | 0.2 | 0.03 | 0.02 | -0.02 | 0.07 | 0.01 | -0.02 | -0.01 | -0.11 | 0.09 | 0.38 |
| SPP | 0.03 | -0.01 | -0.04 | 0.03 | -0.02 | 0.22 | -0.01 | 0.02 | 0.01 | 0.22 | -0.05 | 0.36 | 0.12 | 0.14 | -0.06 | -0.02 | 0.94 |
| SYPP | 0.28 | -0.05 | -0.01 | -0.17 | 0.07 | 0.01 | 0.03 | 0.4 | 0.09 | 0.1 | 0.03 | -0.07 | 0.06 | 0.4 | -0.41 | 0 | 0.76 |
| HSW | 0.04 | -0.01 | 0.06 | -0.02 | 0.05 | 0.01 | 0.53 | 0.11 | 0.17 | 0.01 | -0.4 | -0.14 | 0.17 | 0.18 | -0.05 | 0.15 | 0.86 |
| BM | 0.02 | -0.07 | 0 | -0.33 | 0.09 | -0.05 | 0 | 0.03 | 0.21 | -0.05 | 0.06 | -0.16 | -0.12 | 0.16 | 0.21 | -0.1 | -0.1 |
| HI | 0.07 | -0.06 | -0.26 | -0.01 | 0.16 | 0.21 | 0.09 | 0.01 | 0.05 | 0.21 | -0.21 | 0.03 | 0.33 | -0.06 | 0.12 | 0.09 | 0.77 |

Residual = 0.0943

DTF=days to flowering, DM= days to maturity, PTL = petiole length (cm), TLL = terminal leaf length (cm), TLW = terminal leaf width (cm), PDL = peduncle length (cm), PHT= plant height (cm), BRN=number of primary branches per plant, PODL = pod length (cm), PPC=number of pods per cluster, PPP=number of pods per plant, SPP= number of seeds per pod, SYPP= seed yield per plant (g), HSW= hundred seed weight (g), SYLD= seed yield (kg ha⁻¹), BM= biomass yield (kg ha).

CONCLUSIONS

Assessment of genotypic correlation between different traits exhibited close parallelism with their corresponding phenotypic correlation. The result depicted that for most of the trait pairs, the genotypic and phenotypic associations were in the same direction and the genotypic estimates were higher than the phenotypic ones, demonstrating that these traits had an inherited association between the studied characters. All the studied parameters showed a significant correlation with days to flowering except petiole and the number of primary branches per plant at the phenotypic level.

In this study, all traits had significantly correlated with the seed yield both at the genotypic and phenotypic levels.

The present investigation showed that days to maturity, plant height, seeds per plant, and pods per plant had exerted a positive direct effect on seed yield of mung bean both at the genotypic and phenotypic levels. Additionally, a hundred seed weight and harvest index had exerted a positive direct effect on seed yield both at the genotypic and phenotypic levels. Therefore, direct selection for these traits may prove effective for the improvement of seed yield in the germplasm under study. The residual effect ($h = 0.2047$), shows traits that are included in the phenotypic path coefficient analysis explained by 79.53% of the total variation in seed yield. The genotypic residual effect was low ($h = 0.0943$), indicating that the traits which were included in the genotypic path analysis explained 90.57% of the total variation by seed yield showing that the independent traits were explained the dependent trait more than two-third, therefore, the genotypic path coefficient analysis based selection for mung bean improvement is very appropriate. This analysis confirmed that day to maturity, plant height, the number of primary branches per plant, and seeds per pod produced a high positive direct effect on seed yield, which appeared to be the prominent traits when selecting for seed yield in mung bean genotypes. Therefore, the present study put forward that days to maturity, plant height, number of primary branches per plant, and seeds per pod exerted the highest direct effect on seed yield and it should be given maximum consideration for total yield improvement as the appropriate selection indices. The selection procedure should be formulated so that the advance in one component is not risked by the deterioration effect of the other. Therefore, the results of the path coefficient analysis

indicated that days to maturity, plant height, number of primary branches per plant, and seeds per pod were the main determinants of seed yield in mung bean.

CONFLICTS of INTEREST

The authors declare that there is no conflict of interest.

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Prelacteal feeding practice and associated factors among children under 24 months old in Degahbour town, Somali region, Ethiopia

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Abstract

Prelacteal foods might not be safe due to high risk of contaminating them with biological entities, which can cause infection in the newborn and might affect the colostrum intake. However, in a nation like Ethiopia where diverse cultural practices are prevalent, this might vary depending on the context. Therefore, the aim of this study was to assess prelacteal feeding practice and its associated factors among children under 24 months old in Degahbour town, Somali region, Ethiopia. Methods: A community-based cross-sectional study was conducted from June 5 to July 30, 2021. Data were collected from 300 mothers with children under 24 months of age in Degahbour town by following systematic random sampling method. Bivariable and multivariable logistic regression analyses were performed in order to determine the factors associated with prelacteal feeding practices. Result: 47.3%, 95% CI (42.0%, 53.3%) of the mothers had given prelacteal foods to their newborn baby in the first three days of their life. Being a mother living in semi-urban area [AOR: 4.49 (95% CI 2.39,8.42)], delivered at home [AOR=2.15, 95% CI: (1.21,3.82)], lately initiated breastfeeding for indexed child after the first hour of delivery [AOR=2.34, 95% CI: (1.28,4.28)], not receiving counseling about breastfeeding [AOR = 2.23, 95% CI: (1.28,3.88)], not received ANC follow-up [AOR:2.11; 95%CI (1.19,3.74)], had poor maternal knowledge about breastfeeding [AOR:5.51; 95%CI (3.16,9.62)] were identified as factors associated with practicing prelacteal feeding. Conclusion: In comparison to the regional level figure of 38.8 % and nationally (7.9%) reported in the 2016 EDHS, the prevalence of prelacteal feeding practice is high in Degahbour town. Therefore, to reduce prelacteal feeding practice focus should be placed on initiatives that can enhance institutional delivery, ANC follow-up, and improve mothers' understanding of nutrition throughout their pregnancies, particularly the significance of colostrum feeding right away after delivery.

Keywords: Children, Colostrum, Prelacteal feeding, Early Initiation of breastfeeding Somali region

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INTRODUCTION

Prelacteal feeding is defined as the administration of any substance to newborn babies other than breast milk during the first three days after birth (CSA and ICF, 2016). Although prelacteal feeding is a barrier to the implementation of exclusive breastfeeding practice and raises the risk of mortality and neonatal illness, it is still practiced as a deep-rooted nutritional malpractice in

developing countries (Khanal et al., 2013). Among the commonest prelacteal foods provided to infants in many low and middle-income countries (LMICs), plain water, water-based products (rice water, herbal mixture and juices), and milk based products (milk and infant formula) are mentionable (Khanal et al., 2013). Thus, it contributes to the increased risk of illnesses such as infection related diarrhea and also diseases related to allergic reactions, once it is given before

the onset of feeding colostrum to the newborn (Koukou et al. 2023). Furthermore, if it is practiced, the suckling of the breast, intake of breast milk and the expected attachment between the baby and his/her mother might also be affected, resulting in inappropriate feeding practices of the newborn baby in general (Koukou et al. 2023).

Globally, prelacteal feeding has been practiced by more than half of mothers, which varies from region to region. For instance, it is higher in the middle East, Asia (59.0%) (Oakley et al., 2018), followed by middle east 46.3% and sub-Saharan Africa 32.2% (Berde & Ozcebe, 2017). However, of the estimated 3 million neonatal deaths has been registered every year, two-thirds are shared by South-East Asia and sub-Saharan Africa countries (UNICEF, WHO, World Bank, 2012). More specifically, the highest neonatal mortality rates have been registered in sub-Saharan African countries among different regions globally (UNICEF, WHO, World Bank, 2012).

Among the sub-Saharan African countries, plain water, raw butter, milk, and glucose solution are among the most popular prelacteal foods given to children in Ethiopian communities (Chea & Asefa, 2018; Legesse et al., 2014). They are given to newborn children, due to the deep rooted tradition or culture of the communities to give them since the mothers give birth, or sometimes related to insufficient amount of breast milk to feed the newborn baby (Belachew et al., 2016; Bililign et al., 2016). According to the Ethiopian Demographic and Health Survey (EDHS 2016),

25.9% of children were given prelacteal feeds during the first three days of their lives, and it was found to be more practiced and widespread in rural areas than in urban areas (CSA and ICF, 2016). Despite a lot has been improved after the endorsement of the National Infant and Young Child Feeding (IYCF) guidelines in Ethiopia and endorsed as one of the a major component of the primary health care activities, yet significant proportion of the society has been practicing prelacteal feeding practice (Federal Ministry of Health Family Health Department Ethiopia, 2004). However, the problem is more dominant in rural than urban communities, as the rural communities are cultural bounded, in which prelacteal feeding practice is among the recognized cultural practices. Unlike this evidence, CSA (2016) reported prelacteal feeding in urban (12.7%) and 7.3% for rural settings of the country. It also varied from region to region, of them Somali region is the 2nd most prevalent, where more than a third (38.8%) of children below the age of two year were fed with non-breast milk immediately after they were born. These variations in the prevalence of prelacteal feeding could be linked to inconsistencies of prelacteal feeding practices (Temesgen et al. 2018).

Therefore, the objective of this study was to determine the prevalence of prelacteal feeding practice and its associated factors among children less than 24 months old in Degahbour town of Somali region, Ethiopia. In order to achieve this objective, exhaustive literature review was conducted and the following conceptual framework developed (Figure 1).

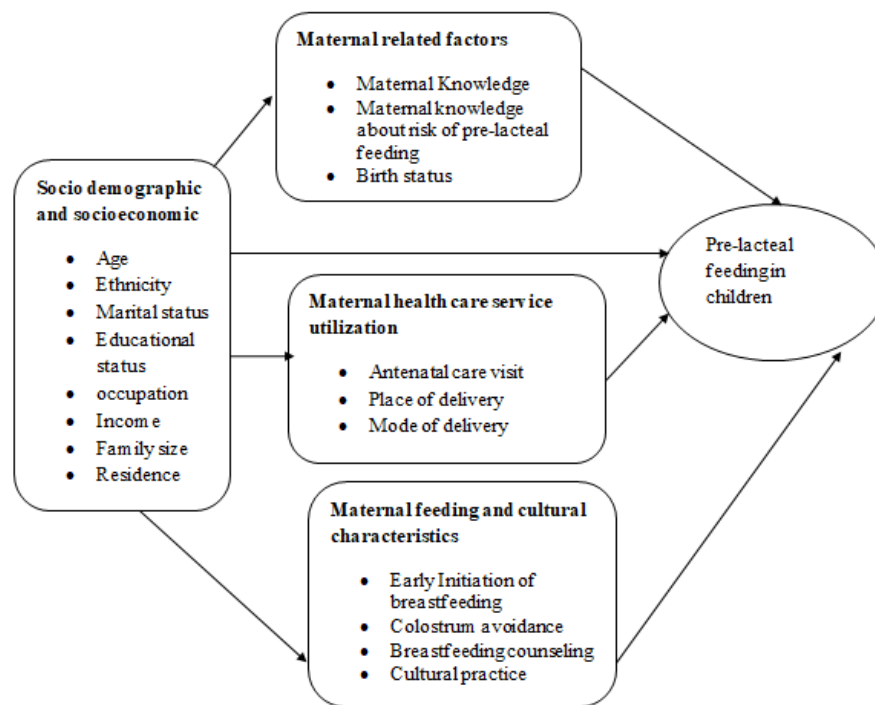


Figure 1. Conceptual framework adapted after reviewing different literatures

MATERIALS AND METHODS

Study Area and Period

The study was conducted in Degahbour town located in Jarar zone, Somali regional state of Ethiopia, located in the southeast part of the country. It is situated at a distance of 785 km away from Addis Ababa and 165 km away from Jigjiga, the regional capital. Degahbour has a hot semi-arid climate; the mean annual temperature of Degahbour is about 25.9 °C or 78.6 °F. The maximum and minimum annual temperatures are 32.8 °C (91.0 °F) and 19.0 °C (66.2 °F) respectively. There are two rain seasons in the region; a short rainy season from March to April, and a long rainy season from July to August. The region's average annual rainfall from these two seasons is around 583 millimeters or 23.0 inches (Deghabour town administration, 2018).

The total population of the city was 92,272, who are living in urban and semi-urban areas of the city. Out of the total population in the city, 526397 are male and female (39633). Almost all (99.7%) participants were Muslim. The total number of under-five aged children is estimated to be 7372.

The city has one type B health center, which provides a service for more than 35,000 people. The Degahbour zonal referral hospital is the main facility in the town, serving 1,000,000 people; on the other hand there are four health posts available in the town (Deghabour town administration, 2018). The study was conducted from June 5 to July 30 2021.

Study Design

A community-based cross-sectional study design was used to assess the prevalence of prelacteal feeding practice and associated factors among children under 24 months old in Degahbour town.

Source and Study Population

Mothers of children aged under 24 months living in the Degahbour town were the source population, while mothers of children aged less than 24 months living in the selected three kebeles of Degahbour town were the study population.

Inclusion and Exclusion Criteria

The mother of a child less than 24 months old who had lived for at least six months in the selected kebeles of the Degahbour town was the inclusion

criteria. Mothers of children who had unable to communicate due to disability or any other health problem were excluded from the study.

Sample Size Determination
Sample Size Determination for the First Objective

Sample size was determined based on the formula used to estimate a single population proportion. Estimated proportion of prelacteal feeding (72.5%) taken from the previous study conducted in Ethiopia (CSA, 2011) and considering the assumptions; a 95% confidence level, margin of error (0.05) and 10% non-response rate. The first sample size was 306. Therefore, the number of children less than 24 months in selected kebeles of Degahbour town was 2550, which is under 10, 000 there for a correction formula used. Then, the sample size was adjusted using the finite population correction formula, which was 273. Then, adding 10% of non-response rate, which was 27, the final sample size for the first objective was 300. Whereas, for the second specific, considering the associated factors such as breastfeeding initiation and known risks of prelacteal feeding practices for prelacteal; feeding

practices, the sample sizes were 169 and 213, respectively (Legesse et al., 2014; Sorrie et al., 2020). Then, considering the largest sample size from the three calculated sample size, the final sample size was 300.

Sampling Technique and Procedure

Out of the ten kebeles in Degahbour town, three were chosen through the lottery method using a basic random sample technique. The chosen Kebeles received a proportionate share of the computed sample size of 300, determined by their average number. A systematic random sampling technique was used to choose the number of study participants; the study population (2550) should be divided by the sample size (300),

$$K=N/n= 2550/300=8$$

Then, based on the k value, it was identified. Then, randomly select one number between 1 and k value which is 8 for the 1st selection. Then, the next participants were selected by adding the k value (8) until the whole sample size was maintained (Figure 2).

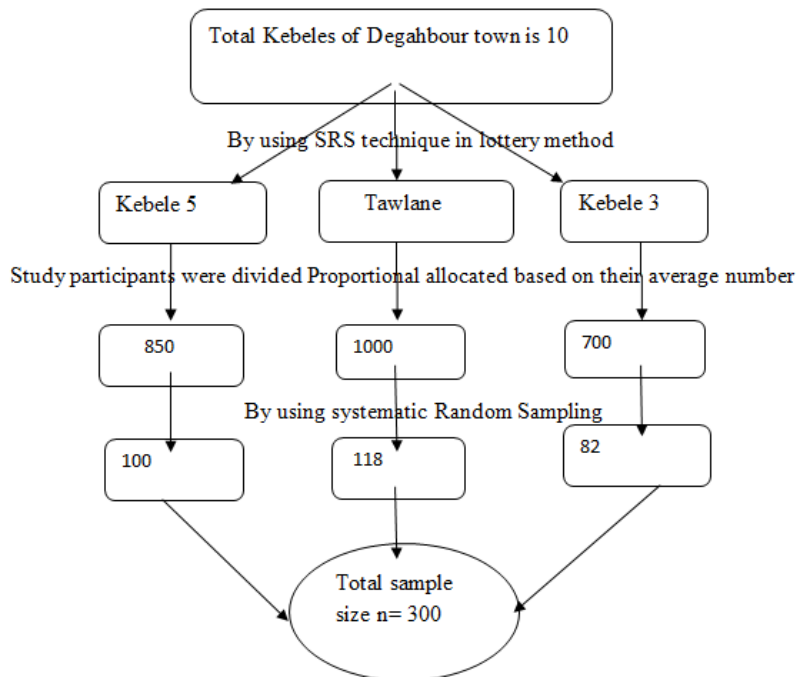


Figure 2. Schematic presentation of sampling procedure

Study Variables

The dependent variable of this study was prelacteal feeding practice whereas, socio-

demographic and economic characteristics (age, ethnicity, marital status, educational status, Occupation, income, family size and residence), maternal feeding and cultural characteristics (early Initiation of breastfeeding, colostrum avoiding, breastfeeding counseling and cultural practice) maternal health care service utilization (antenatal care visit place of delivery and mode of delivery) and maternal related factors (maternal knowledge maternal knowledge about risk of prelacteal feeding and birth status) were the independent variables considered in this study.

Data Collection Tool and Procedure

Data was collected with face-to-face interviews using a pre-tested and structured questionnaire which was adapted from the Ethiopian National Nutrition Survey questionnaire and Ethiopian Demographic and Health Survey (CSA and ICF, 2016). The adapted questionnaire was modified according to the research objective and the actual setup. The questionnaire was written in English first and then translated into Somali (the local language) and back into English by fluent speakers of both languages to check its consistency. Two diploma midwives and additional one who had Bachelor of Science degree holder were hired as supervisors to collect the data. The data collectors and the supervisors' received an intensive training for two days and they were attentively followed during the practical training.

Data Quality Control

To ensure data quality, the following steps were taken: data collectors and supervisors were trained on the study's goals, validity, confidentiality of information, respondent rights, informed consent principles, and techniques of interviewing. The questionnaire was initially written in English and then translated into the Somali version (local language) by nutrition experts and then back translated to English to ensure accuracy. The pre-testing was taken place in 5% of the total sample size in the kebeles of Degahbour town not to be included in the study. Modifications were made based on feedback from the pretest to enhance consistency, understandability, and simplicity of the messages contained in the questionnaire to make them clearer. Throughout the data collection process, the supervisor and principal investigator

checked and reviewed completed questionnaires for their completeness, precision and continuity on a regular basis.

Data Processing and Analysis

The data was cleaned, coded and entered into Epi Data version 3.1 and exported to SPSS version 20 for analysis. Descriptive statistics was used to determine the results in frequencies, proportion, cross tabulation, and measures of central tendency. The results were presented using tables, graphs and texts. A bivariate logistic regression was used to identify candidate variables for the final multivariable binary logistic regression at p-value <0.25. Finally, multivariate binary logistic regressions were used to identify the independent predictors or variables that have a significant association with prelacteal feeding practice. The Hosmer-Lemeshow test was used to check the model fitness. The cut point to announce the existence of an association between the dependent and independent variable was p-value <0.05 with 95% confidence interval. Multicollinearity was checked using Variance Inflation Factor (VIF) and Standard Error (SE), which were less than 10 and 2, respectively, indicating that there was multicollinearity in the final regression model.

Ethical Considerations

Ethical clearance was obtained from the Institutional Review Board of Jigjiga University. Each study participant was adequately informed about the objective and purpose of the study and they were interviewed when they agreed to give verbal consent to participate individually. Participants were also told they had the right not to respond to the questions if they didn't want to respond or to withdraw from the interview at any time. Furthermore, both data collectors and supervisors ensured the confidentiality of information by using code numbers rather than personal identifiers and locking the questionnaire.

RESULTS AND DISCUSSION

Socio-Demographic Characteristics

A total of 300 mothers were participated in this study with a response rate of 100%.

Table 1. Socio-demographic Characteristics of the respondent Mothers of Children Aged <24 months in Degahbour town, Eastern Ethiopia, 2021

| Variable | Categorical | Frequency | Percent |
|---------------------------------|----------------------------------|-----------|---------|
| Sex of index child | Male | 170 | 56.7 |
| | Female | 130 | 43.3 |
| Age of child | 0-6 Months | 93 | 31.0 |
| | 7-12 Months | 106 | 35.3 |
| | 13-23 Months | 101 | 33.7 |
| Birth order of the child | 1 | 107 | 35.7 |
| | 2-3 | 111 | 37.0 |
| | 4 and above | 82 | 27.3 |
| Age of mother | 15-24 years | 114 | 38.0 |
| | 25-34 years | 152 | 50.7 |
| | 35 and above | 34 | 11.3 |
| Marital status | Married | 220 | 73.3 |
| | Divorced | 58 | 19.3 |
| | Widowed | 22 | 7.3 |
| Ethnicity | Somali | 260 | 86.7 |
| | Oromo | 22 | 7.3 |
| | Amhara | 14 | 4.7 |
| | Others | 4 | 1.3 |
| Maternal religion | Muslim | 287 | 95.7 |
| | Christian | 13 | 4.3 |
| Residence | Urban | 154 | 51.3 |
| | Semi-urban | 146 | 48.7 |
| Maternal education level | Unable to read and write | 137 | 45.7 |
| | Completed primary | 89 | 29.7 |
| | Secondary level education | 54 | 18.0 |
| | Diploma and above | 20 | 6.7 |
| Maternal Occupation | House wife | 127 | 42.3 |
| | Merchant | 98 | 32.7 |
| | Farmer | 10 | 3.3 |
| | Student | 16 | 5.3 |
| | Government /private organization | 49 | 16.3 |
| Family monthly income | 1000-2000 ETB | 87 | 29.0 |
| | 2000-4000 ETB | 111 | 37.0 |
| | More than 4000 ETB | 102 | 34.0 |
| Family size | 1-3 | 177 | 59.0 |
| | 4 and above | 123 | 41.0 |

Maternal Health Care Service Utilization

Of them, 152 (50.7%) mothers were in the age group between 25 and 34 years. Regarding marital status, the majority of women 220 (73.3%) were married and belongs to Somali ethnic group, 260 (86.7%). more than half, 154 (51.3%) of the

women were living in urban areas based on the selected kebele of Degahbour town, had male children, 170 (56.7%) and living in a family sized 1-3, 240 (55.4%). Almost all mothers, 287 (95.7%) were followers of the Muslim religion, About half of the mothers, 127 (47.3%) were housewives, while more than one-thirds of the

mothers included in this study had 106 (35.3%) children in the age group of 7-12 months (Table 1). Of the mothers who took part, 186 (62.0%) had their babies at home. The majority of the mothers 292 (97.3%) were delivered naturally, and 151-50.3% of them had used ANC services. Of the moms who visited ANC services, 94 (31.3%) used

one to three times. Among mothers who attended an antenatal care visit, 143 (47.7%) mothers had received counseling on breastfeeding, more than 153 (51.0%) had initiated breastfeeding within the first hour after birth (Table 2).

Table 2. Maternal health care service utilization

| Variable | Category | Frequency | Percent |
|---------------------------------|---|-----------|---------|
| Place of birth | Home | 186 | 62.0 |
| | Health facility | 114 | 38.0 |
| Mode delivery | Normal | 292 | 97.3 |
| | Cesarean | 8 | 2.7 |
| ANC Visit | No | 149 | 49.7 |
| | Yes | 151 | 50.3 |
| Time of ANC visit | 1-3 | 94 | 31.3 |
| | 4 and above | 55 | 18.3 |
| Breastfeeding initiation | Immediately/within first hour after birth | 153 | 51.0 |
| | After the first hour | 147 | 49.0 |
| Breastfeeding Counseling | No | 157 | 52.3 |
| | Yes | 143 | 47.7 |

Prevalence of Prelacteal Feeding Practice and Type of Prelacteal Food Given to Children

The prevalence of prelacteal feeding practice in this study was 47.3% (95% CI 42.0%, 53.3%) (Figure 3). Of them, 28.2% provided plain water, 33.1% gave sugar or glucose water, 19.7% fed milk based, 16.2% tea or infusions and 2.8% used butter. More than half, 165 (55.0%) mothers fed Colostrum to their children.

Among the main reasons for prelacteal feeding practices, 62.7% complained of insufficient breast milk, 20.4% cultural practice, 14.8% maternal sickness and 2.1% Infant illness, (Figure 4).

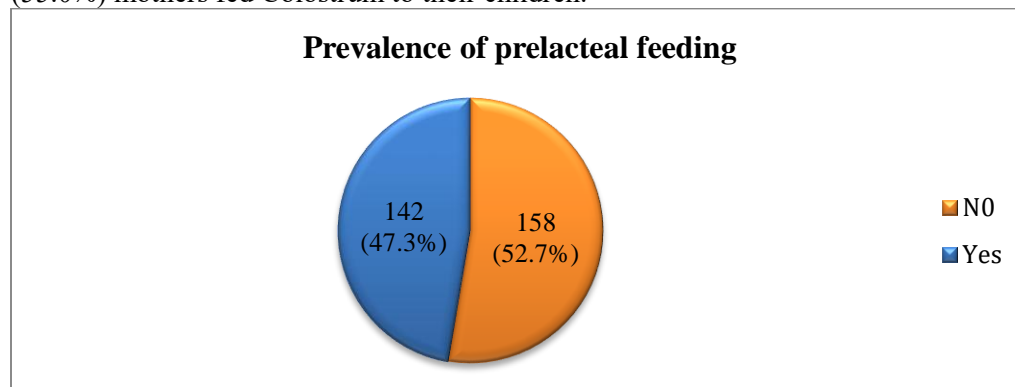


Figure 3. Prevalence of prelacteal feeding

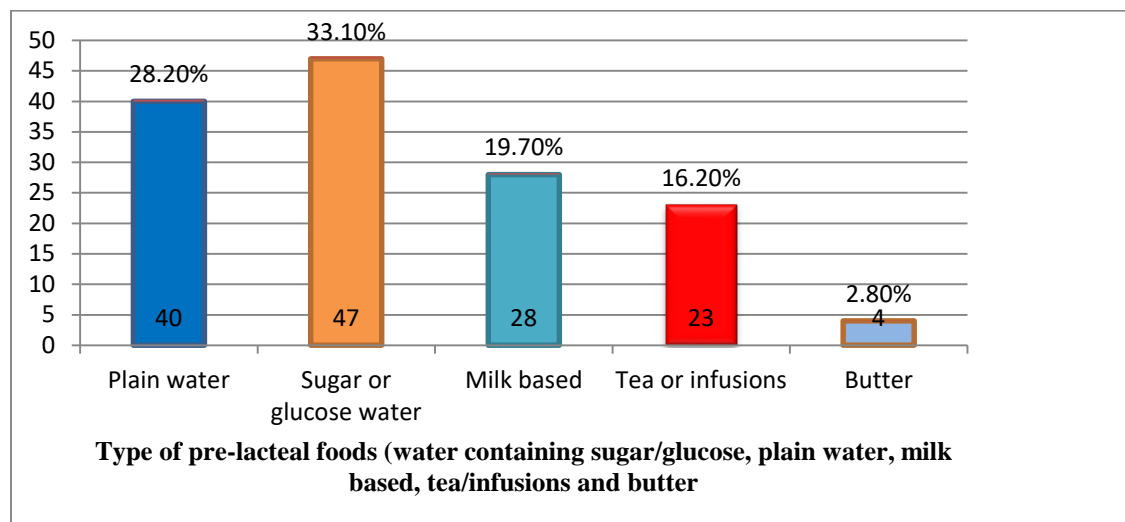


Figure 4. Types of pre-lacteal foods given by mothers of Degahbour town

Maternal Information and Knowledge

More than half, 182 (60.7%) of respondents mothers were told that they knew the risk of pre-lacteal feeding. According to maternal information related to PLF, 95 (31.7%) of the mothers knew the PLF practice causes poor growth for infants, followed by vomiting (18%)

and causes infant illness (16.7%). Whereas, 17.7% of the mothers said that PLF practice has nutritional benefits, while comparable proportion of mothers also revealed that PLF practice supported the growth of children (16%) (Figure 5).

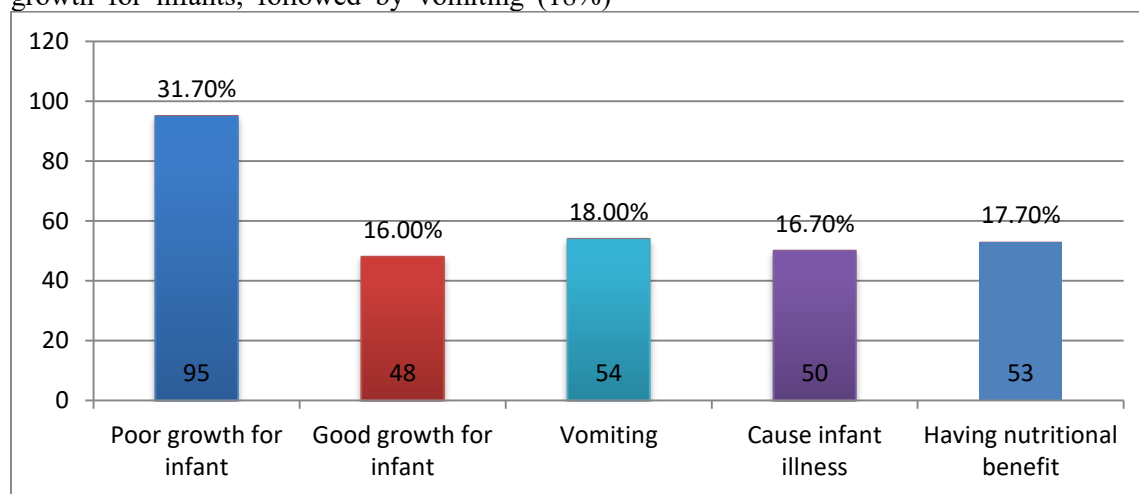


Figure 5. Maternal information about pre-lacteal feeding practice

Table 3. Maternal information and knowledge of prelacteal feeding practice

| Variable | Category | Frequency | Percent |
|---|---|-----------|---------|
| Prelacteal feeding practice | No | 158 | 52.7 |
| | Yes | 142 | 47.3 |
| Colostrum feeding | No | 135 | 45.0 |
| | Yes | 165 | 55.0 |
| Type of liquid | Plain water | 40 | 28.2 |
| | Sugar or glucose water | 47 | 33.1 |
| | Milk based | 28 | 19.7 |
| | Tea or infusions | 23 | 16.2 |
| | Butter | 4 | 2.8 |
| Time of introduced | within the first hours after giving birth | 63 | 44.4 |
| | After the first hours | 79 | 55.6 |
| Reason of practicing | Insufficient/breast milk secretion | 89 | 62.7 |
| | Cultural practice | 29 | 20.4 |
| | Maternal sickness | 21 | 14.8 |
| | Infant illness | 3 | 2.1 |
| Risk of PLF | No | 118 | 39.3 |
| | Yes | 182 | 60.7 |
| Maternal Knowledge on breastfeeding category | Poor | 151 | 50.3 |
| | Good | 149 | 49.7 |

Factors Associated with Prelacteal Feeding Practice

Results of Bivariable Logistic Regression

Place of residence, place of delivery, breastfeeding counseling, colostrum feeding, ANC visits, known risk of PLF, and maternal knowledge of PLF were found to be statistically associated with prelacteal feeding in the bi-variable logistic regression analyses. Variables that demonstrated association in the bivariate analysis, defined as ≤ 0.25 , were included in the multivariate logistic regression analysis during the binary logistic regression analysis (Table 4).

Result of Multivariable Logistic Regression Analyses

The multivariable analysis showed that place of residence, place of birth, breast feeding initiation time, counseling related to breast feeding, ANC visit and maternal knowledge on breastfeeding were found to be statistically significant and identified as determinant factors for prelacteal feeding practices among mothers of children aged less than 24 months in Degahbour town. Mothers who were living in the semi-urban areas of Degahbour town were 4 times more likely to practice prelacteal feeding compared to those mothers who were living in urban areas of the town [AOR: 4.49 (95% CI 2.39,8.42)].

Those mothers who delivered their indexed child at home had 2.15 times higher odds of practicing prelacteal feeding compared to their counterparts [AOR=2.15, 95% CI: (1.21, 3.82)]. Mothers who lately initiated breastfeeding after the first hour of delivery had 2.34 odds for practicing prelacteal feeding to their children compared to mothers who started breastfeeding within an hour period immediately after delivery [AOR=2.34, 95% CI: (1.28,4.28)]. Mothers who were not counseled about breastfeeding had an odd ratio of 2.23 for providing prelacteal food to their children compared to the mothers counseled about breastfeeding [AOR = 2.23, 95% CI: (1.28, 3.88)]. Mothers who didn't attend ANC follow up were about 2.11 times more likely to practice prelacteal feeding than mothers who attended ANC follow up during the pregnancy period of the indexed children [AOR: 2.11; 95%CI (1.19, 3.74)]. Mothers who had poor nutrition knowledge on breastfeeding had an odd of 5.51 for practicing prelacteal feeding than mothers who had good maternal knowledge [AOR: 5.51; 95%CI (3.16, 9.62)] (Table 4).

DISCUSSION**Prevalence of Prelacteal Feeding**

The purpose of this study was to determine the prevalence of prelacteal feeding among mothers in the Somali region of the Ethiopian town of Degahbour and to identify potential risk factors related to these practices. Accordingly, the prevalence of prelacteal feeding by the mothers was 47.3% (95% CI 42.0%, 53.3%) of the moms in Degahbour town reported prelacteal feeding. Similar findings have also been reported in various regions of Ethiopia, including the Amhara region (47.8%) (CSA and ICF, 2016), 46.4% for Kersa District (Adem et al., 2021), and 45.4% for Harari regions (Bekele et al., 2014).

However, the finding of this study was higher than reported for the Somali region, 38.8% in EDHS 2016 (CSA and ICF, 2016), Motta town, which was 20.2% (Tewabe, 2018), and also 15.9% for the Benishangul-gumuz region (Ayana et al., 2017). The difference might be due to variation in socio-demographic, cultural, lifestyle and maternal beliefs and attitudes towards breastfeeding. Furthermore, it might be related to the inclusion of mothers, both from urban and semi-urban areas of Degahbour, unlike that of the study in Motta town, and the better access to maternal and child health services, nearby to health institutions and would have more information those who are living in rural areas.

Table 4. Bivariable and multivariate logistic regression factors associated with prelacteal feeding practice among mothers of children aged less than 24 months in Degahbour town

| Independent variables | Category | Prelacteal feeding | | COR (95%CI) | AOR (95%CI) |
|---|----------------------|--------------------|------------|-------------------|---------------------|
| | | No, n (%) | Yes, n (%) | | |
| Residence | Urban | 96 (62.3%) | 58 (37.7) | 1 | |
| | Semi-urban | 62 (42.5%) | 84 (57.5) | 2.24 (1.41, 3.56) | 4.49 (2.39,8.42)** |
| Place of birth | Home | 86 (46.2%) | 100 (53.8) | 1.99 (1.24,3.21) | 2.15 (1.21,3.82)* |
| | Healthy facility | 72 (63.2%) | 42 (36.8) | 1 | |
| Breastfeeding initiated | Within first hour | 88 (57.5%) | 65 (42.5) | 1 | |
| | After the first hour | 70 (47.6%) | 77 (52.4) | 1.49 (0.94,2.35) | 2.34 (1.28,4.28)** |
| Counseling on breastfeeding practice | No | 71 (45.2%) | 86 (54.8) | 1.88 (1.19,2.98) | 2.23 (1.28,3.88)** |
| | Yes | 87 (60.8%) | 56 (39.2) | 1 | |
| Colostrums feeding | No | 64 (47.4%) | 71 (52.6) | 1.47 (0.93,2.32) | 1.31 (0.76,2.26) |
| | Yes | 94 (57.0%) | 71 (43.0) | 1 | |
| ANC visit | No | 70 (47.0%) | 79 (53.0) | 1.58 (0.99,2.49) | 2.11 (1.19,3.74)* |
| | Yes | 88 (58.3%) | 63 (41.7) | 1 | |
| Knowledge on risk of prelacteal feeding practice | NO | 55 (46.6%) | 63 (53.4) | 1.49 (0.94,2.38) | 1.34 (0.76, 2.36) |
| | Yes | 103 (56.6%) | 79 (43.4) | 1 | |
| Maternal Knowledge about breastfeeding | Poor | 52 (34.4%) | 99 (65.6) | 4.69 (2.88,7.65) | 5.51 (3.16, 9.62)** |
| | Good | 106 (71.1%) | 43 (28.9) | 1 | |

* P < 0.05, **P<0.001, AOR: Adjusted Odds Ratio, COR: Crud Odds Ratio, CI: Confidence Interval, 1: Reference group

The finding of this study is also lower than what was reported for the Somali region (72.5 %) in EDHS 2011 (CSA and ICF, 2011), for Mansoura district in Egypt 58% (El-Gilany & Abdel-Hady, 2014) and Jubek State, South Sudan 53% (Tongun et al., 2018). The variation could be due to the difference in study setting, year of the study, sample size, study selection and maternal health service utilization between study populations, for the former study. Furthermore, the differences from the other studies might be related to differences in culture, local beliefs and socio-demographic, lifestyle.

Factors Associated with Prolactal Feeding

The current study also showed that prolactal feeding of newborns in the first three days following delivery was associated with mothers residing in semi-urban areas of Degahbour town. Being mothers from semi-urban areas had 4.49 times higher odds of practicing prolactal feeding compared to their counterparts. A study conducted in Debre Tabor town reported consistent results (Bayih et al., 2020). This could be due to semi-urban residence mothers having less access, low awareness about optimal breastfeeding and risks of prolactal feeding, and also low BF counseling. Furthermore, access to ANC and PNC programs that incorporate optimal breastfeeding was difficult for mothers living in rural or semi-urban areas. Urban environments may offer better access to and the caliber of health services, and urban women tend to be better educated than their rural counterparts, which may enhance mothers' understanding of mental and psychological awareness (Temesgen et al., 2018).

Mothers who gave birth at home had more than two times higher odds of practicing prolactal feeding compared to those mothers who gave birth at health institution. This is consistent with the finding of a study conducted in Kersa District, Eastern Ethiopia (Adem et al., 2021). This could be because mothers who gave birth at home were frequently influenced by traditional birth attendants and used prolactal feeding, whereas mothers who gave birth at a health facility might have received better ANC visits and breastfeeding counseling, which includes proper breastfeeding practices and improves optimal breastfeeding practices (Bayih et al., 2020). Alternatively, health professionals may advise mothers who gave birth in a medical facility to refrain from prolactal feeding and

the associated risks that come with it for the children whose mothers or caregivers feed them before they are ready. This study showed that mothers who initiated breastfeeding after the first hour of delivery were nearly two and half times more likely to practice prolactal feeding compared to those initiated within the first hour. This is consistent with what was reported for Raya Kobo district, North-eastern Ethiopia (Legesse et al., 2014). There is a close relationship between early initiation and avoiding prolactal feeding because prolactal feeding might also be the reason for the late initiation of breastfeeding (Temesgen et al., 2018).

In the present study, mothers who didn't get breastfeeding counseling were 2.23 times more likely to practice prolactal feeding when compared to those mothers who had it during their pregnancy period. A study conducted on the mothers of Jinka town also reported similar findings (Sorrie et al., 2020). This could be breastfeeding counseling is important for improving maternal knowledge, change attitude and also practice, including awareness about the risk of prolactal feeding practice and the importance of optimal breastfeeding practices that might decrease PLF practice.

Prolactal feeding was found to be 2.11 times more common in mothers who did not receive ANC services during the indexed child's pregnancy than in mothers who did receive ANC services during that same period. The findings of a study carried out in the Eastern Ethiopian region of Kersa District are in line with this outcome (Adem et al., 2021). Actually, this may have something to do with regular counseling that is provided at the health facility during the ANC follow-up. Additionally, our study revealed a 5.51-fold increase in the likelihood of prolactal feeding practiced by counterparts who had inadequate breastfeeding knowledge. A study conducted in Hawella Tula areas of Sidama region also reported the direct association of poor knowledge with prolactal feeding practice (Chea & Asefa, 2018).

Finally, like other studies, this study has its own strength and limitations. Accordingly, conducting it at the community level by including the semi-urban and urban setting is the strengths of this study as most often such kinds of studies are conducted in

rural and also facility-based settings. However, it also has two limitations. The first one is, as the data was collected from the mother self-report, the data could be affected by recall bias. The second one is related to the cross-sectional nature of the study design, which couldn't support to identify the causality.

CONCLUSIONS and RECOMMENDATIONS

In this study area, the prevalence of PLF was 47.3%, which is relatively lower than reported for the Somali region in EDHS 2011, but higher than reported in EDHS 2016. However, this prevalence is higher than the national level of 26% and remains as a challenge for optimal breastfeeding in the town, and the region at large. The most popular prelacteal food types offered to newborns were plain water, milk-based beverages, tea/infusions, butter, and water with sugar or glucose in it. The main justifications for feeding these prelacteal foods were the custom of feeding them as part of a cultural practice and the mothers' belief that nursing alone does not meet the needs of a newborn baby. Furthermore, place of residence, place of delivery, late initiation of breastfeeding after delivery, not receiving ANC during pregnancy period and poor maternal knowledge on breastfeeding were identified as determinants for prelacteal feeding practice by mothers of Degahbour town. Therefore, activities aiming to reduce the high prevalence of prelacteal feeding practice in Degahbour town and similar settings of Somali and other regions should consider the factors associated with practice feeding practice by mothers in this study.

CONFLICTS of INTEREST

The authors declare that they have no conflicts of interest.

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Dairy Cattle Production under Changing Crop-Livestock Production Systems: Performance in Milk Production, Reproduction and Quality of Milk in Selected Districts of West Arsi Zone, Oromia Region, Ethiopia

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Abstract

This study was conducted in the central highlands of Ethiopia, which has a long history of improved dairy cattle management. The study area is found in one of the most developed milk sheds as a potential area to develop dairying in the country. However, the current performance of dairy cattle needs to be investigated and documented. This study evaluated the production and reproductive performance of cows, and microbial quality of milk. A total of 124 smallholder farmers were randomly selected from lists of households who at least keep one lactating crossbred cow in 12 kebeles of Kofele, Shashemene and Dodola districts. For microbial analysis, 60 milk samples were collected from producers, milk collectors and consumers. Farmers usually keep mixed herds of local breed cows, HF and a few Jersey crosses. Farmers in Kofele district have the highest HF crossbred cows. The overall production and reproduction performance of dairy cows show the positive legacies/experience of improved dairy cattle management in the area, with an overall mean daily milk yield of 7.24±0.217 litres, at first calving of 33.54±0.82 months, calving intervals of 16.70±0.61 months, and a lactation length of 9.70±0.54 months for crossbred cows. Significant differences were observed between breeds and districts. The overall total bacterial, coliform and yeast and mould counts were 6.51±0.31, 4.74±0.24 and 3.55±0.20 log₁₀cfu/ml, respectively. The microbial quality of the milk was satisfactory and it has acceptable ranges of Ethiopian standards. Farmers perceived feed shortage, the declining trends in crossbreeding programs and lack of sufficient services as market as important barriers to improve milk production. Farmers are switching from typical dairy farmers to cash crop producers. Maintaining the extension systems, good cattle feeding systems, breed improvements practices as well as connecting farmers to the market would stimulate dairy farmers to regain their position as typical dairy production/milk sheds.

Keywords: Production and reproduction traits, microbiological quality of milk, Arsi cattle breed, handling practices, crossbred cows

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INTRODUCTION

Ethiopia has the largest cattle population in Africa with over 70 million heads (CSA, 2021). However, the total milk production from cattle is

below its potential, that is estimated at 4.9 billion litres per year (without considering the amount which is produced from urban dairy cattle production systems) (CSA, 2021). This is due to the low milk production from the dominant (over

97%) local cattle breeds, with an estimated 1.48 litres milk production per cow/day. Despite modest increases in recent years, Ethiopia has one of the lowest per capita consumption of milk in Africa. However, milk is a vital component of the diet and serves as a significant source of income for many peri-urban and rural households in the country. A recent report by FAO (2018) showed that per capita milk consumption in Ethiopia is more than 40 litres.

Although smallholder dairy production is a viable economic sector in Ethiopia, it is constrained, among others, by poor access to good nutrition, improved breeds, and output markets i.e. dominated by inadequate processing and informal milk markets. Crossbreeding indigenous cattle with exotic breeds has been shown to substantially contribute to increases in milk production, and to reproduction performances of cattle (Osei-Amponsah et al., 2020).

In addition to the issues of milk production and productivities, quality and safety of dairy products are issues of concern to smallholder systems, including Ethiopia. High microbial counts and the occurrence of pathogens in milk are likely to affect the safety of consumers and keeping quality and shelf life of products derived from it (Abebe et al., 2018). Minten et al. (2021) showed that with the growth in dairy products consumption and rapidly increasing dairy processing companies in the country, it is high time for smallholder dairy farmers to satisfy this growing market for high volume and high quality milk.

This study was conducted in the West Arsi zone, which had several decades of smallholder dairy interventions by successive governments and programs in the country. It is an area where crossbreeding programs on Arsi cattle breeds were heavily implemented for several decades (Aboagye, 2014; Teshome et al., 2019). Arsi cattle are among the tropical zebu cattle breeds not genetically advantaged in terms of milk production, but are well adapted to the area. Arsi Rural Development Unit (ARDU) program, which was established in the 60s, and Gobe Cattle Breeding Ranch (GCBR) played an important role in dairy development in the area, where over

10,000 crossbreed dairy cows were distributed to smallholder farmers in the area (Ulfina et al., 2013). The GCBR, which was in service for over eight decades, was a public institution, which provided several services to smallholder farmers particularly in the neighbouring districts of Kofele, Kore, Dodola, Gedeb, Adaba and Shashemene districts in West Arsi Zone. For several years, the GCBR, before it was privatized in 2018, gave technical trainings to farmers on improved dairy cattle management and milk production. Oghaiki et al. (2021), when mapping Ethiopian milk sheds, identified key biophysical and socio-economic variables that describe the milk shed and concluded that the current study area/milk shed is the second most important region, after North Shoa, with the highest potential to develop dairying in Ethiopia. Even though the study area was famous in terms of its historical dairy development practices, and mentioned as one of the most developed milk sheds in the country, the current performance of dairy cattle and farms needs to be investigated and documented. Thus, this study was conducted to evaluate the current performance of crossbred cows in terms of milk production, reproductive performance, as well as the microbial quality of milk produced by smallholder farmers and compared three different districts which are located at different distances from GCBR.

MATERIALS AND METHODS

Study Sites

The research was conducted in three purposively selected districts namely: Kofele, Shashemene and Dodola districts of the West Arsi zone, Oromia Region, Ethiopia. The West Arsi zone has large number of cattle with 2,095,572 heads, out of which 74, 913 are crossbreds (CSA, 2020). The study area has the highest number of total cattle population as well as crossbred cattle among Ethiopian highlands (Oghaiki et al., 2021) and is amongst the few milk sheds with huge potential for dairying. This is attributed to the long years of dairy interventions in the area, due to the presence of a crossbreeding ranch called Gobe Ranch and several dairy

development programs. Gobe Ranch is found in Kofele district, and had a total of 1,800 hectares of land and served the community for over eight decades. Gobe Ranch, which was later named Gobe Cattle Breeding Ranch since 1960s, played key roles in the development of the dairy sector in the area. This ranch was established in 1928-1930 by Swedish government aid under Swedish International Development Agency (Sida) for the selection of Arsi livestock species, and since 1960s it has been used for improving dairy cattle production by distributing crossbred heifers of Arsi cattle and Holstein Friesian to the farmers.

Kofele, Shashemene and Dodola are districts which are known for their high crossbreds Holstein Friesian and Jersey among the districts in the West Arsi zone. These districts were selected by considering its distance from GCBR.

Kofele district's main town-Kofele is located at 7° 00' 0.00" N and 38° 44' 59.99" E, respectively. The district receives an annual average rainfall of 1,232 ml. The mean annual temperature ranges between 11 and 25°C (KLDO, 2021). Shashemene district's main town is Shashemene which is located at 7° 11' 50.2692" N and 38° 36' 1.9188" E. The area has an annual average temperature ranging from 12 to 28°C and annual rainfall in the ranges of 1500 to 2000mm (ShLDO, 2021). Dodola district's main town is Dodola which is located at 06°54'20"N and 39°13'50"E. It is located at an elevation ranging between 2490 to 3218 m.a.s.l. The mean annual rainfall is 964 mm. The mean monthly minimum and maximum temperatures are about 5°C and 27°C, respectively (DLDO, 2021).

Study Designs

Sampling and Data Collection for Household Survey

Data were gathered from milk producers using semi-structured questionnaire. After purposive sampling of the three districts, a total of 12 rural kebeles which are located within 10 km radius from urban center were selected. It means 4

kebeles were selected from each district, and at this stage randomization was applied. The required number of samples of farmers was determined by Yamane formula (1967).

$$n = \frac{N}{1+N(e^2)} \dots \dots \dots (1)$$

Where n = sample size, N = population size and e = level of precision (at 5%).

The sampling framework was narrowed down by listing the names of farmers who keep at least one crossbred lactating cows in each of the selected 12 kebeles. Accordingly, 124 households were randomly selected. It was then proportionally distributed to each district, and hence 43 farmers from Kofele, 41 from Shashemene, 40 farmers from Dodola were selected.

A pre-tested questionnaire was used to collect farm household data including, socioeconomic variables, herd sources, current herd compositions, as well as, on production reproduction performance of cows. A progeny history survey approach, which consider cows as research units rather than the household itself. Accordingly data calving intervals (CI), Age at first calving (AFC), lactation length (LL), daily milk yield (DMY), and number of services per conception (NSC) were generated.

Sampling and Data Collection for Laboratory Analysis

A total of 60 raw cow milk samples were randomly collected for laboratory analysis from 30 milk producers, 12 milk cooperatives (pooled samples) and 18 consumers from the three study districts. Raw milk samples were collected into a sterilized labelled sample bottles in an icebox and transferred into a refrigerator adjusted at a temperature of 4°C. Then samples were transported to Hawassa University Dairy Technology Laboratory for microbial analysis, which was completed within 24 hours.

Total Bacteria Count (TBC), Coliform Count (CC) and Yeast and Mould counts (YMC) were conducted using standard procedures (Richardson, 1985). One ml of milk sample was added into sterilized test tube having 9ml autoclaved at 120°C for 15 minutes peptone water. Standard serial dilutions were made using the sterilized multiple

test tubes. The right diluted milk samples were pour-plated onto 20ml autoclaved culture media prepared for TBC, CC, and YMC. Nutrient Agar was prepared for TBC and YMC, whereas non-autoclaved Violet Red Bile Agar was prepared for CC, which was mixed thoroughly. The plates of samples were allowed to solidify and then inverted to incubate at 32°C for 48hrs for TBC, at 32°C for 24hrs for CC and at 25°C for 5 days for YMC. Each analysis was made in duplicate and precision of the analysis was determined at 5% level.

Bacterial and yeast/mould colonies were counted using colony counter (Richardson, 1985). The following formula was used to calculate the counts for total bacterial coliform and yeast and mould counts.

$$N = \frac{\sum c}{[(1 \times n_1) + (0.1 \times n_2)] \times d} \dots \dots \dots (2)$$

Where N = number of colonies per ml of milk; $\sum c$ = sum of all colonies on all plates counted; n1 = number of plates in first dilution counted; n2 = number of plates in second dilution counted; d = dilution from which the first counts were obtained.

Statistical Analysis

The household survey data were analyzed using descriptive as well as General Linear Model (GLM) procedures, as one way and two-way ANOVA depending on the variables using by the Statistical Package for Social Sciences Software (SPSS, version 25). The effect of district was

taken for socio economic variables, while both district and breeds of cattle were used for production and reproduction traits analysis. Data of microbial counts were log transformed (log10cfu/ml) before analysis. The transformed microbial count value was analyzed using SPSS software General Linear Model (GLM) procedure. Mean separation was carried out using the Tukey technique when analysis of variance shows significant differences between means and differences were considered significant at $P < 0.05$.

The statistical model used for this study was $Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_k + e_{ijk} \dots \dots \dots (3)$

Where, Y_{ij} : microbial count (TBC, CC, YMC);

μ : the overall mean;

α_i : effect of districts;

β_j : effect of milk sources

$\alpha\beta_k$: interaction effect

e_{ijk} : random error term.

Ranking Methods

Parameters which need ranking were expressed by calculating an index. Index mean was calculated in Microsoft Excel as shown below and multiplied by hundred to get the aggregate value for the ranking of needed parameters compared in the study areas. Index mean = $\frac{\sum [(n \times \text{no of R for 1st rank}) + (n-1 \times \text{no of R for 2nd rank}) + \dots + (1 \times \text{no of R last})]}{\sum [(n \times \text{total R for 1st rank}) + (n-1 \times \text{total R for 2nd rank}) + \dots + 1 \times \text{total R for last}]}$

Where: R=number of response, n=value given for the factor, no=number

RESULTS AND DISCUSSION

Household Characteristics

The overall mean of male and female headed households of the study districts were 66% and 34%, respectively (Table 1). There were large numbers of female headed households in Kofele district than Dodola and Shashemene districts. The respondents had different educational status, 46% were able to read and write; 34.7% did not have any kind of formal education and 10.5% of those surveyed had attended high school and 8.9% college education (Table 1).

Table 1. Socio-economic characteristics of respondents in the study areas

| Variables | Districts | | | Overall mean n=124 |
|--------------------------------|----------------|----------------|--------------------|-----------------------|
| | Kofele n=43 | Dodola n=40 | Shashemene n=41 | |
| Sex of household head (%) | N (%) | N (%) | N (%) | N (%) |
| • Male | 23 (53.5) | 33 (82.50) | 26 (63.41) | 82 (66) |
| • Female | 20 (46.5) | 7 (16.28) | 15 (34.88) | 42 (34) |
| Education level of hh head (%) | | | | |
| • None literate | 13 (30.2) | 18 (45.0) | 12 (29.3) | 43 (34.7) |
| • Read and write | 22 (51.2) | 17 (42.5) | 18 (43.9) | 57 (46.0) |
| • High school level education | 4 (9.3) | 2 (5.0) | 7 (17.1) | 13 (10.5) |
| • College level education | 4 (9.3) | 3 (7.5) | 4 (9.8) | 11 (8.9) |
| Family size in ranges (%) | | | | |
| • 0-5 | 14 (32.6) | 27 (67.5) | 29 (70.7) | 70 (56.5) |
| • 6-10 | 24 (55.8) | 8 (20) | 9 (22) | 41 (33) |
| • Above 10 | 5 (11.6) | 5 (12.5) | 3 (7.3) | 13 (10.5) |

N= number of respondents, hhs = households

Dairy Cattle Herd Structures, Sources and Contributions of Gobe Ranch

As shown in Table 2, the overall mean cattle holding and proportion of HF crossbred cows in the Kofele district was significantly ($p < 0.05$) higher than Dodola and Shashemene districts. This study revealed that the overall mean cattle holding per household with 16.95 ± 0.72 TLU, is

one of the highest among the highlands of Ethiopia. Belete (2006) reported that the average cattle holding per household level was 7.3 in the northern Fogera districts. Table 3, further shows the original sources of cattle herds, showing that Gobe Ranch has contributed to these differences

Table 2. Herd sizes (TLU) and composition by breeds of cattle owned by smallholder farmers in the study areas (M \pm SE)

| Variable | Study districts | | | | P - value |
|------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------|-----------|
| | Kofele N=43 | Shashemene N=41 | Dodola N=40 | Over all N=124 | |
| Total Cattle herd size | 18.84 \pm 1.18 ^b | 15.44 \pm 1.05 ^a | 16.63 \pm 1.23 ^a | 16.95 \pm 0.72 | 0.001 |
| Local breed | 5.76 \pm 0.36 | 5.58 \pm 0.40 | 6.63 \pm 0.46 | 6.01 \pm 0.25 | 0.11 |
| HF crosses | 9.36 \pm 0.80 ^b | 6.16 \pm 0.65 ^a | 6.30 \pm 0.76 ^a | 7.23 \pm 0.46 | 0.02 |
| Jersey crosses | 3.72 \pm 0.02 | 3.70 \pm 0.0 | 3.70 \pm 0.0 | 3.71 \pm 0.72 | 0.534 |

Means with different superscripts in the same row shows significant difference between districts ($P < 0.05$). TLU=tropical livestock unit, was calculated with standard reference of 1.2 for crossbred cows and bulls, 0.8 for local breed cows and bulls, 0.5 for heifers and 0.25 for calves)

Farmers in the Kofele district seem to benefit more from Gobe ranch due to its proximity. Interestingly, the number of farmers and crossbred cow populations decreases with increasing distance of farm households from Gobe Ranch, even within the district Kofele. Farmers in all the

three districts know the Gobe ranch and benefitted in different forms. However, the majority of farmers in Kofele got their first crossbred herds from Gobe ranch, while in Shashemene and Dodola they mainly got their first crossbred cows via AI services from proximate service providers.

Table 3. Sources of cattle herd and experience of farmers in keeping crossbred cattle in the study districts

| Variables | Districts | | | |
|-----------------------------------|----------------|----------------|--------------------|-------------------|
| | Kofele N=43 | Dodola N=40 | Shashemene N=41 | Over all N=124 |
| Original source of crossbred cows | | | | |
| • Gobe Ranch | 15 (34.9) | 4 (10.0) | 11 (26.8) | 30 (24.2) |
| • From market | 10 (23.3) | 8 (20.0) | 10 (24.4) | 28 (22.6) |
| • AI with own cow | 9 (20.9) | 21 (52.5) | 14 (34.1) | 44 (35.5) |
| • From neighbour | 9 (20.9) | 7 (17.5) | 6 (14.6) | 22 (17.7) |
| Years since linked to Gobe Ranch | | | | |
| • 5-10 | 5 (11.6) | 4 (10) | 3 (7.3) | 12 (9.7) |
| • 10-30 | 22 (51.2) | 25 (62.5) | 18 (43.9) | 65 (52.4) |
| • Above 30 | 16 (37.2) | 11 (27.5) | 20 (48.8) | 47 (37.9) |
| Benefits from Gobe Ranch | | | | |
| • Heifer | 20 (46.5) | 11 (27.5) | 17 (41.5) | 48 (39) |
| • Sire | 6 (14) | 5 (12.5) | 7 (17.1) | 18 (14.5) |
| • Cows | 10 (23.2) | 5 (12.5) | 9 (22.0) | 24 (19) |
| • Job | 5 (11.6) | 0 (0.0) | 1 (2.4) | 6 (5) |
| • Other services | 2 (4.7) | 19 (47.5) | 7 (17.1) | 28 (22.5) |

Reproductive and Productive Performances of Cows Calving Interval (CI): the overall mean calving interval in the study areas was 16.70±0.61 months which is significantly different ($p<0.05$) between study districts, and breeds of cows (Table 4). The calving interval was longer for local cows than crosses of Jersey and HF. The overall mean calving interval of the study area was less than that reported by Mitiku et al. (2019) who documented 18.82 months for local breeds of cattle in Meta district of the East Hararghe zone and greater than the report of Megersa (2016) which shows 14.3 months for HF crosses in West Shoa and Dessalegn et al. (2016) which is 13 months for Bishoftu & Akaki towns. CI is affected mainly by management, the longer it takes during open days before cows are inseminated for the next calving, it would result in longer CI. The longer calving interval reported for local and crossbred cows in various parts of the country mainly attributes to the poor reproduction management practices (such as poor heat detection, untimely bull and AI

service provisions) as well as poor access to all season feeding management. Poor nutrition leads to poor body condition that prevents cows from showing true estrus (Law et al., 2009).

Lactation Length (LL): the overall mean lactation length was 9.70±0.54 months, and it is significantly different ($p<0.05$) between breeds of cows and districts (Table 4). This overall mean result is similar to the finding of most studies in similar highlands of Ethiopia. For example, Dessalegn *et al.* (2016) and Lemma (2004) reported 9.2 and 9.5 months, respectively for Bishoftu & Akaki towns in the East Shoa zone, respectively. However, the study area has a better lactation length than the national average of 7 months (CSA, 2021). The national average usually reflects the LL of local cows, as 97% of cattle in Ethiopia are local breeds. The lactation length was reported in this study was similar between local and crosses of Jersey cows, but differs from HF crossbred cows. It means, Holstein Friesians are in lactation for more months than local and crossbred

Jersey cows. This result was similar to the report of Megersa (2016) who reported 11.8 months for crossbred cows in the West Shoa zone of Oromia Region, Ethiopia. Like the CI, LL is also affected also by the plane of nutrition. Lactation physiology demands continuous supply of nutrients to the mammary gland. Udder and reproduction management practices also play pivotal roles to lactation persistency. Most hormones that affect reproduction cycle also affects lactation physiology (Law et al., 2009). Longer lactation length beyond optimal and recommended level indicates that farmers in the study area milk cows until cows naturally stop milking, and there is not lactation length control/management in the area. However, this practice may have negative effect on calving interval, next season milk and overall productive efficiency of the cow.

Daily Milk Yield: The overall mean daily milk yield in the study districts was 7.24 ± 0.22 litres per cow per day, which was significantly different ($p < 0.05$) between breeds cows and also districts. The present result indicates that crossbred cows were better performing than Jersey crosses and local cows. The overall average milk yield of cross bred cows was 10.30 ± 0.31 litres per day, which is less than reported by Abebe *et al.* (2014) with 14.8 litres average value of daily milk yield in Ezha Districts of the Gurage Zone under improved urban dairy management conditions. The quantity and quality of feed resources available to dairy animals is likely the primarily factor for such low performance. Crossbreeding has led to higher milk production per animal, higher income for the families and provision of high-value food and is thus an important livestock improvement tool in the tropics (Richard et al., 2020).

Table 4. Mean (\pm SE) Reproductive and Productive Performances of Cows owned by smallholder farmers

| Variable | Breeds | Districts | | | Overall mean | P-value |
|----------|--------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|---------|
| | | Kofele n=43 | Shashemene n=41 | Dodola n=40 | | |
| CI | Local | 16.46 \pm 1.09 ^a | 22.24 \pm 0.99 ^a | 16.94 \pm 1.06 | 18.54 \pm 0.55 ^a | 0.01 |
| | Jersey | 14.71 \pm 1.36 ^{ab} | 17.00 \pm 1.66 ^{ab} | 16.89 \pm 1.45 | 16.2 \pm 0.78 ^{ab} | |
| | crosses | | | | | |
| | HF cross | 13.36 \pm 0.72 ^{ab} | 15.03 \pm 0.96 ^{ab} | 17.71 \pm 1.16 | 15.38 \pm 0.49 ^{ab} | |
| | Overall mean | 14.84 \pm 0.63 ^A | 18.09 \pm 0.72 ^{AB} | 17.18 \pm 0.71 ^{AB} | 16.70 \pm 0.61 | |
| | P-value | 0.01 | 0.00 | 0.86 | 0.000 | |
| LL | Local | 7.55 \pm 0.95 ^a | 7.32 \pm 0.76 ^a | 8.94 \pm 0.71 | 7.94 \pm 0.48 ^{ab} | 0.009 |
| | Jersey | 11.86 \pm 1.1 ^{ab} | 9.00 \pm 1.28 ^{ab} | 7.78 \pm 0.8 | 9.55 \pm 0.68 ^{ab} | |
| | crosses | | | | | |
| | HF cross | 13.84 \pm 0.63 ^{ab} | 10.75 \pm 0.74 ^b | 10.21 \pm 0.78 | 11.60 \pm 0.43 ^a | |
| | Overall mean | 11.08 \pm 0.55 ^B | 9.25 \pm 0.56 ^A | 8.98 \pm 0.52 ^A | 9.70 \pm 0.54 | |
| | P-value | 0.00 | 0.012 | 0.16 | 0.000 | |
| DMY | Local | 3.59 \pm 0.72 ^a | 3.14 \pm 0.63 ^a | 2.60 \pm 0.48 ^a | 3.11 \pm 0.356 ^a | 0.017 |
| | Jersey | 7.07 \pm 0.10 ^b | 7.00 \pm 1.07 ^b | 7.86 \pm 0.66 ^{ab} | 7.31 \pm 0.505 ^b | |
| | crosses | | | | | |
| | HF cross | 11.14 \pm 0.47 ^c | 10.83 \pm 0.62 ^c | 8.93 \pm 0.53 ^{ab} | 10.30 \pm 0.313 ^c | |
| | Overall mean | 7.26 \pm 0.41 ^B | 6.99 \pm 0.41 ^{AB} | 6.46 \pm 0.38 ^A | 7.24 \pm 0.217 | |
| | P-value | 0.00 | 0.00 | 0.00 | 0.000 | |
| AFC | Local | 38.23 \pm 2.48 ^b | 39.53 \pm 1.99 ^a | 36.76 \pm 1.99 | 38.17 \pm 1.25 ^b | 0.089 |
| | Jersey | 28.57 \pm 3.11 ^a | 37.17 \pm 3.36 ^b | 32.56 \pm 2.74 | 32.77 \pm 1.78 ^a | |
| | crosses | | | | | |
| | HF cross | 26.08 \pm 1.64 ^a | 28.33 \pm 1.94 ^b | 34.64 \pm 2.20 | 29.69 \pm 1.12 ^a | |
| | Overall mean | 30.96 \pm 1.14 | 35.01 \pm 1.47 | 35.65 \pm 1.58 | 33.54 \pm 0.82 | |
| | P-value | 0.000 | 0.001 | 0.560 | 0.000 | |
| NSC | Local | 2.64 \pm 0.81 ^{ab} | 2.71 \pm 1.10 | 2.65 \pm 1.00 | 2.68 \pm 0.14 ^b | 0.261 |
| | Jersey | 2.29 \pm 0.95 ^{ab} | 2.00 \pm 0.89 | 2.11 \pm 0.78 | 2.24 \pm 0.20 ^{ab} | |
| | crosses | | | | | |
| | HF cross | 1.76 \pm 0.78 ^a | 1.89 \pm 0.83 | 2.21 \pm 10.97 | 2.00 \pm 0.13 ^a | |
| | Overall mean | 2.23 \pm 0.16 | 2.20 \pm 0.16 | 2.53 \pm 0.152 | 2.32 \pm 0.92 | |
| | P-value | 0.014 | 0.060 | 0.700 | 0.003 | |

N=number of respondents. CI = Calving Interval (in months), LL = lactation length (in months), DMY = Daily milk yield (litres/day/cow), NSC=number of services per conception (counts). Means with different lower superscripts that compares breeds of cows in the column, shows significant difference (p<0.05), , whereas upper superscripts compares districts for overall values of the respective production and reproduction traits (p<0.05).

Age at First Calving (AFC): The overall mean age at first calving in the study districts was 33.54 \pm 0.82 months. The mean age at first calving for local, Jersey and HF crossbred cows were 38.17 \pm 1.25, 32.77 \pm 1.78 and 29.69 \pm 1.12 months, respectively and is significantly different (p<0.05) between breeds of cows (Table 4). This result was higher than that reported by Megersa (2016) who

found AFC of 31.2 months for crossbred dairy cows in the West Shoa zone and less than that reported by Belay *et al.* (2012) with 36.6 months for Zebu \times Holstein-Friesian crossbred dairy cows in Jimma.

Number of Services per Conception (NSPC): the overall average mean number of services per

conception in the study districts was 2.32 ± 0.92 . The means number of services per conception for local, jersey and crossbred cows were 2.68 ± 0.14 , 2.24 ± 0.20 and 2.0 ± 0.13 , respectively (Table 4). The finding in the present study agrees with the 2.0 services per conception reported for cows at Asella (Negussie *et al.*, 1998), but higher than that reported by Megersa (2016) with 1.8. As explained above, this is an important factor that determines the reproduction cycles of cows, notably the CI.

Major Milk Production Constraints of the Study Area

The majority of the respondents ranked feed shortage as the first constraint which contributes to the low performance of dairy cows in the area (Table 5). Feed shortage ranked as the most significant production constraint likely due to declining communal and private grazing lands due to the rapid expansion of crop fields, such as malt barley, pasta wheat and vegetables for cash income. The second most important challenge perceived by farmers affecting the performance of dairy cattle in the area was poor management practices. This was followed by lack of improved breeds of cattle

and AI services. This is in line with a previous qualitative study conducted by Oghaiki *et al.* (2021), showing that currently farmers could not get sufficient services for breed improvement or heifers. With the growing attention to other cash crops, less land is dedicated to feed production for cattle. As typical small holder mixed crop-livestock systems, more land is dedicated for crop production and hence feed shortage is among the top ranked problems of farmers. Land use change have been shown to affect the dairy potential of milk sheds/clusters (Oghaiki *et al.*, 2021). Especially getting pasture land is one of the key challenges for dairy producers. This study area in particular is unique in that in the past three decades there was a huge/wide expansion of land for malt barely, pasta wheat and various types of vegetable production as alternate income source for smallholder farmers. When dairy development programs, such as breed improvement programs are introduced, they have to be holistically designed in such a way that farmers get sustainable and stable market for their products.

Table 5. Major Milk production constraints of the study area ranked by farm households

| Constraints | Districts | | | | | | | |
|---|-----------------|-----------------|---------------------|-----------------|-----------------|-----------------|------------------|-----------------|
| | Kofele N= 43 | | Shashemene N= 41 | | Dodola N= 40 | | Overall N=124 | |
| | Index | Rank | Index | Rank | Index | Rank | Index | Rank |
| Shortage of feed | 0.3 | 1 st | 0.23 | 1 st | 0.25 | 2 nd | 0.25 | 1 st |
| Lack of improved breed | 0.2 | 3 rd | 0.19 | 3 rd | 0.22 | 3 rd | 0.20 | 3 rd |
| Poor herd management | 0.3 | 2 nd | 0.17 | 5 th | 0.28 | 1 st | 0.23 | 2 nd |
| Limited services like AI and vet health | 0.1 | 5 th | 0.22 | 2 nd | 0.14 | 4 th | 0.16 | 4 th |
| Low access to market | 0.2 | 4 th | 0.19 | 4 th | 0.11 | 5 th | 0.15 | 5 th |

N= number of respondents, Index =the sum of (5 times 1st order + 4 times 2nd order +3 times 3rd order+ 2 times 4th order + 1 times 5th order) given for individual variables divided by the sum of (5 times 1st order + 4 times 2nd order +3 times 3rd order+ 2 times 4th order + 1 times 5th order) for all preferred respondents; AI=Artificial insemination

Microbial Quality of Raw Cow Milk in the Study Districts Total Bacterial Count (TBC)

Table 6 summarizes the bacterial loads of milk samples collected from producers, cooperatives and consumers found in the study districts. The overall mean TBC was $6.51 \pm 0.31 \log_{10} \text{cfu/ml}$. A

significant difference was observed between districts and milk sources (Table 6). The contamination of milk might be due to initial contamination originating from the udder surface, quality of water, milking utensils, and also during transportation. The overall mean of TBC reported for the Meta district of the Eastern Hararghe zone by Mitiku *et al.* (2019) was $6.21 \pm 0.05 \log_{10} \text{cfu/ml}$ and a report from Shashemene by Teshome and Tesfaye (2017), was $6.62 \pm 0.05 \log_{10} \text{cfu/ml}$. The lower TBC in this study could attribute to a strong traditional smoking practices for all type of milk containers. Abebe *et al.* (2018) has reported similar practices in Ethiopia showing how smoking practice reduces microbial loads in milk. Since 2021, the Ethiopian Standard Authority has declared compulsory standards for four dairy products including raw cow milk (ESA, 2021). According to this standard, the acceptable TBC for a raw cow milk in Ethiopian market is 10^6 cfu/ml . Therefore, the study area has acceptable ranges of TBC established for raw cow milk.

Coliform count (CC)

The overall mean of coliform count/ml of raw milk from different milk sources was $4.74 \pm 0.24 \log_{10} \text{cfu/ml}$ (Table 6). The mean coliform count was significantly different ($P < 0.05$) between districts and milk sources. The overall mean coliform count (CC) obtained from raw milk in the current study was comparable with the findings of Mitiku *et al.* (2019) who reported $4.82 \pm 0.082 \log_{10} \text{cfu/ml}$. In another study by Fufa *et al.* (2019) higher CC with $5.56 \log_{10} \text{cfu/ml}$ from sub-cities of Addis Ababa has been reported. Overall our study has CC below the acceptable value 5×10^4 set by the Ethiopian Standard Authority (ESA, 2021). In the current study area, still it was observed that dairy cos are managed in muddy barn and a poor hygienic conditions are practiced, which might expose the milk for more contamination, increases the microbial count. The presence of coliform in milk indicates that the milk has been contaminated with fecal materials and this could be attributed to insufficient pre-milking udder hygienic practices. Bulk milk coliform bacteria are used as a good indicator for presence of pathogens in milk.

Table 6. Microbial counts ($\log_{10} \text{cfu/ml}$) of fresh cow milk from different milk sources

| Variables | Sampling unit | Districts | | | Overall mean | P-value |
|-----------|---------------|--|--|---|-------------------|---------|
| | | Kofele N=22 (P =12 C = 4 Cs = 6) | Shashemene N = 20 (P =10 C = 4 Cs = 6) | Dodola N = 18 (P =8 C = 4 Cs = 6) | | |
| TBC | Producer | 6.20 ± 0.21^A | 6.62 ± 0.23^A | 6.50 ± 0.23^A | 6.42 ± 0.29^A | 0.014 |
| | Cooperative | 6.79 ± 0.11^C | 7.01 ± 0.04^B | 6.86 ± 0.05^B | 6.88 ± 0.11^B | |
| | Consumer | 6.49 ± 0.19^B | 6.48 ± 0.32^A | 6.30 ± 0.19^A | 6.42 ± 0.24^A | |
| | Overall mean | 6.38 ± 0.30^a | 6.66 ± 0.30^b | 6.51 ± 0.28^{ab} | 6.51 ± 0.31 | |
| | P-value | 0.000 | 0.010 | 0.002 | 0.000 | |
| CC | Producer | 4.63 ± 0.19 | 4.91 ± 0.06^{AB} | 4.53 ± 0.23 | 4.70 ± 0.23^A | 0.000 |
| | Cooperative | 4.85 ± 0.14 | 5.04 ± 0.06^B | 4.82 ± 0.14 | 4.90 ± 0.15^B | |
| | Consumer | 4.76 ± 0.13 | 4.89 ± 0.14^A | 4.43 ± 0.29 | 4.69 ± 0.28^A | |
| | Overall mean | 4.71 ± 0.18^b | 4.93 ± 0.10^c | 4.56 ± 0.27^a | 4.74 ± 0.24 | |
| | P-value | 0.081 | 0.050 | 0.064 | 0.002 | |
| YMC | Producer | 3.39 ± 0.16 | 3.61 ± 0.12 | 3.52 ± 0.18^{AB} | 3.50 ± 0.18^A | 0.000 |
| | Cooperative | 3.49 ± 0.09 | 3.79 ± 0.14 | 3.78 ± 0.08^B | 3.69 ± 0.17^B | |
| | Consumer | 3.51 ± 0.07 | 3.71 ± 0.18 | 3.41 ± 0.23^A | 3.55 ± 0.21^A | |
| | Overall mean | 3.44 ± 0.14^a | 3.67 ± 0.15^b | 3.54 ± 0.22^a | 3.55 ± 0.20 | |
| | P-value | 0.173 | 0.110 | 0.022 | 0.005 | |

Means followed by different lower superscript within rows (compare districts) and different superscript upper superscripts within columns (for milk sources) are significantly different at $P < 0.05$, N= number of sample. P=producers, C = Cooperative, Cs = consumers

Yeast and Mould Count (YMC)

Yeast and mould are considered to be spoilage microorganisms. The overall mean of 3.55 ± 0.20 \log_{10} cfu/ml showed a fairly good quality milk. The mean value of yeast and mould counts was significantly different ($P < 0.05$) between districts as well as milk sources (Table 6). This yeast and mould result is also related to the finding of Mitiku *et al.*, (2019) who reported 3.9 ± 0.08 \log_{10} cfu/ml for raw cow's milk from Meta district, Hararge Zone and the report of Teshome and Tesfaye (2017) shows 3.902 ± 0.477 \log_{10} cfu/ml for raw cow's milk from Bench Maji-Zone, Ethiopia. According to Ethiopian Standard Authority, YMC in raw milk samples should be lower than 2.1 \log_{10} cfu/ml, but this result was higher than that set for maximum YMC. The presence of yeasts and moulds in milk from the producers, cooperative and consumers is higher than the acceptance levels of yeast and moulds in raw milk. The high YMC observed in milk obtained from milk collector and cooperative might be attributed to contamination from dust, air, containers, water used, poor personal hygiene, and poor hygiene of milk selling environment along the value chains. The YMC is not listed under compulsory standards of ESA.

CONCLUSIONS

The study area has been shown to exhibit a positive legacy of previous dairy development programs and the presence of institutions like the Gobe crossbreeding ranch has contributed to the relatively high crossbred HF crosses in the study area. It was also observed that closer a district to the Gobe ranch, in this case Kofele district, had more numbers of crossbred dairy herds it had, notably HF crosses. On the other hand the production and reproduction traits shows that the study area is among the few milk sheds with good production and reproduction performance of crossbred dairy cows under rural smallholder management conditions. Even the microbial quality of raw milk collected from farm households and milk collection units (of cooperatives) are fairly good compared to the national standards. These are some of the legacies of previous dairy development programs and such institutions like the Gobe cattle cross breeding institutions. However, farmers perceived that the attention given to the sector in recent years have exposed them to

problems such as feed shortage, breed improvement and access to services such as veterinary and AI services. Overall, the study area is known for long years of dairy development initiatives, showing a great potential for further development as there exist large number of crossbred dairy cows and well experienced smallholder dairy farmers. However, with the growing attention to other cash crops in the study area, less land is dedicated to feed production, whereas the market is not well developed for milk and milk products. This will have less incentive for dairy farmers in order to still dedicate themselves for improving the reproductive and production performance of dairy cows as well as for further improvements on the quality of milk. Therefore, should farmers and the sector benefit from the dairy development in the study area and the whole milk shed, re-strengthening the extension systems, availing services for improved feeding management, breed improvement and health management as well as availing stable and sustained markets for milk and milk products is necessary.

CONFLICTS of INTEREST

The authors declare that they have no conflicts of interest.

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Journal of Science and Development

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