

Assessing the impact of continued regeneration on accession's genetic integrity

Alemayehu Teressa Negawo¹, Meki S. Muktar¹, Chris S. Jones^{1,2} and Alice Muchugi¹

¹Feed and Forage Development Program, International Livestock Research Institute, Addis Ababa, Ethiopia;

A.teressa@cgiar.org (A.T.N.); M.Shehabu@cgiar.org (M.S.M.); a.muchugi@cgiar.org (A.M.); c.s.jones@cgiar.org (C.S.J)

²Feed and Forage Development Program, International Livestock Research Institute, Nairobi, 00100, Kenya

Introduction

ILRI holds over 18,000 accessions of tropical and subtropical grass and legume forages in its 'in trust' collection. Annually hundreds of samples are requested and distributed to researchers and forage seed producers. To ensure the continuous availability of seeds, accessions are regenerated in the field to replenish exhausted inventory. Thus, a rationalized strategy is required to ensure the integrity of the germplasm over continuous generations to maintain stocks of seeds for distribution¹⁻³. In such effort, it is important to know the implication of continuous regeneration on the genetic integrity of accessions. Variability in seed color was noticed among lots of accession of the different forage species (Figure 1). Therefore, the aim of this project was to assess the effect of continued regeneration on genetic integrity of germplasm in the genebank using molecular markers.

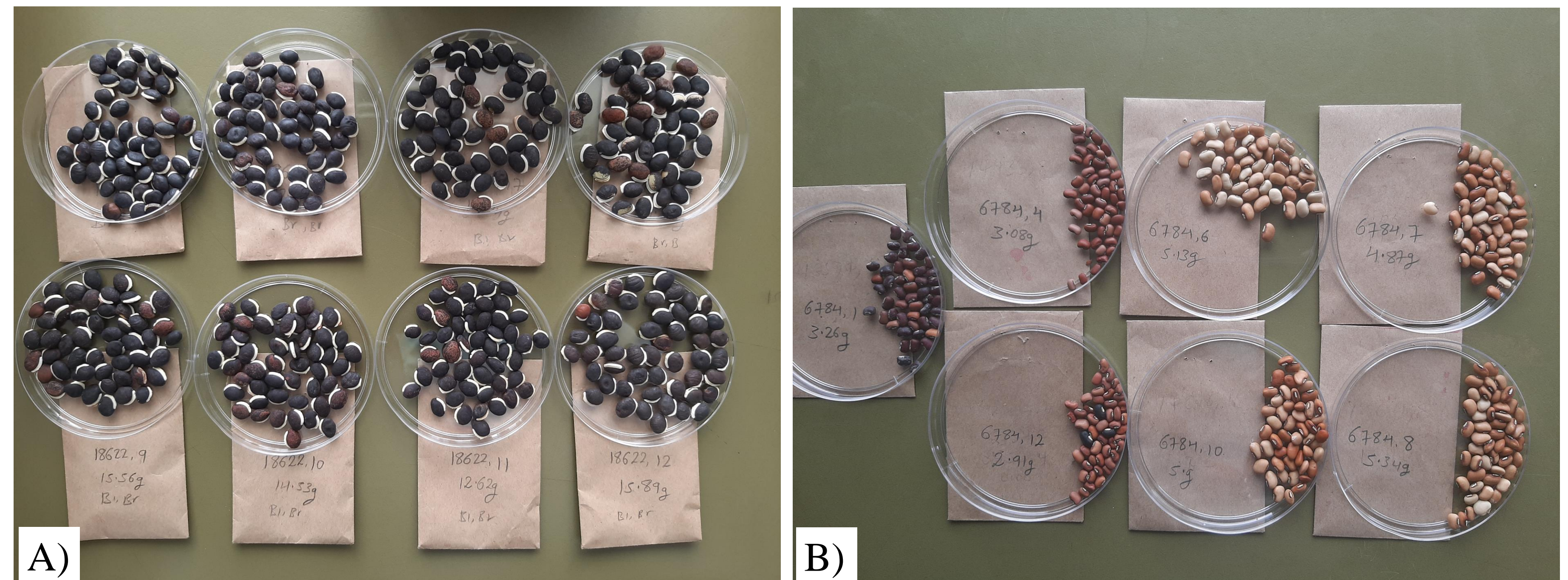


Figure 1. Variability in seed color among lots of (A) lablab and (B) cowpea accession

Materials and methods

- ❖ Three forage species *Lablab purpureus*, *Vigna unguiculata* and *Stylosanthes capitata* were used for the study.
- ❖ Accessions with large number of lots were selected.
- ❖ Genomic DNA was extracted from young leaves collected from actively growing seedlings in the greenhouse.
- ❖ DArTSeq markers were generated on DArTSeq platform and used for genetic integrity assessment.
- ❖ The generated markers were subjected to different analyses such hierarchical clustering, principal component analysis (PCA), genetic relationship matrix (GRM), probability of identity by descent (IBD) and genetic distance.

Results

- ❖ **PCA and hierarchical clustering of lots within accession:** The result showed that in most cases the lots from the same accession clustered together with genetic similarity close to 1 (Figure 2 and 3). In a few cases, the lots from accessions clustered in two groups.
- ❖ **IBD analysis.** In most cases, the lots within accession showed a genetic relationship of sibling and above (Table 1). In few cases, a lot showed a distant (cousin) or unrelated relationship with the rest of the lots in the accession.
 - *Vigna unguiculata*: In one accession (Acc 7310), lots showed cousin relationship. In 11 accessions, the lots had a relationship of sibling and above. Unrelated lot was not detected.
 - *Lablab purpureus*: In three accessions, lots with cousin and/or unrelated relationship was observed while in the rest of the accessions, the lots had half-sib and above relationships.
 - *Stylosanthes capitata*: One lot showed unrelated relationship with the rest of lots within the accession (Acc 12086). For accessions 159, 12086, 12087 and 12090, lot with cousin relationship was observed. Majority of the lots had at least a sibling relationship among themselves.
- ❖ On average the results show that despite continued regenerations, the genetic integrity of the selected accessions has not been comprised. Only three accessions showed unrelated individuals.
- ❖ This could be as a result of the species being predominantly selfing and/or the results of good practice observed during seed production and processing. Quality management system should ensure the sustain of the good practice measures.
- ❖ Further study is required to determine the extent of variability among lots of other species with multiple lots.

Figure 2. Hierarchical clustering of the accessions

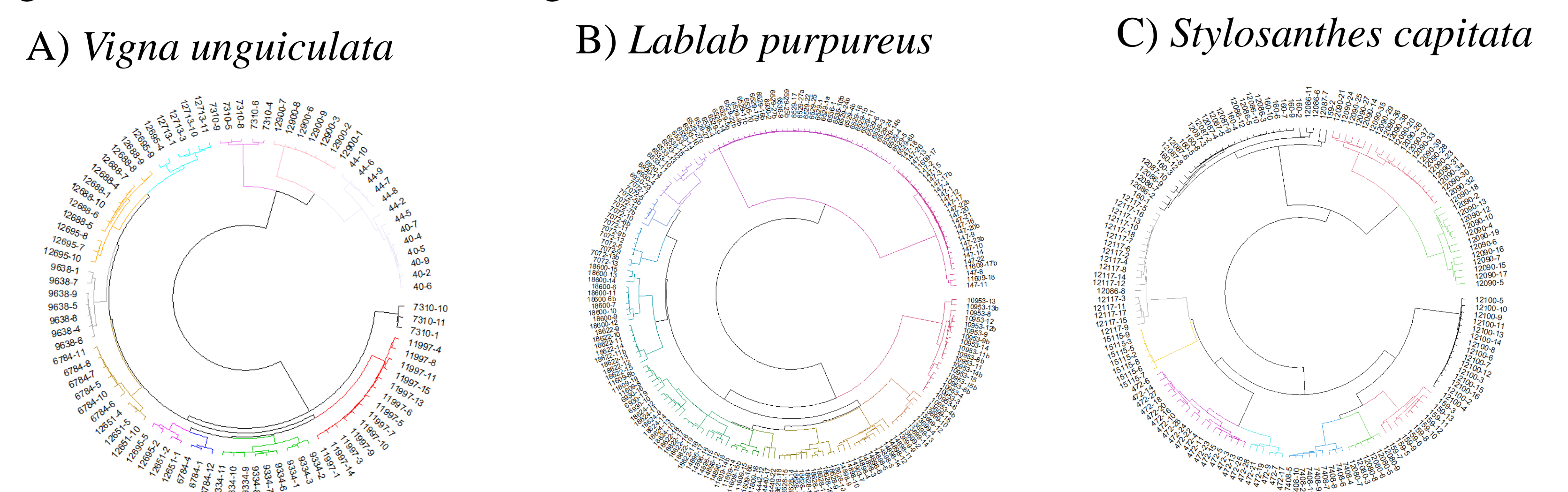


Figure 3. PCA of the accessions

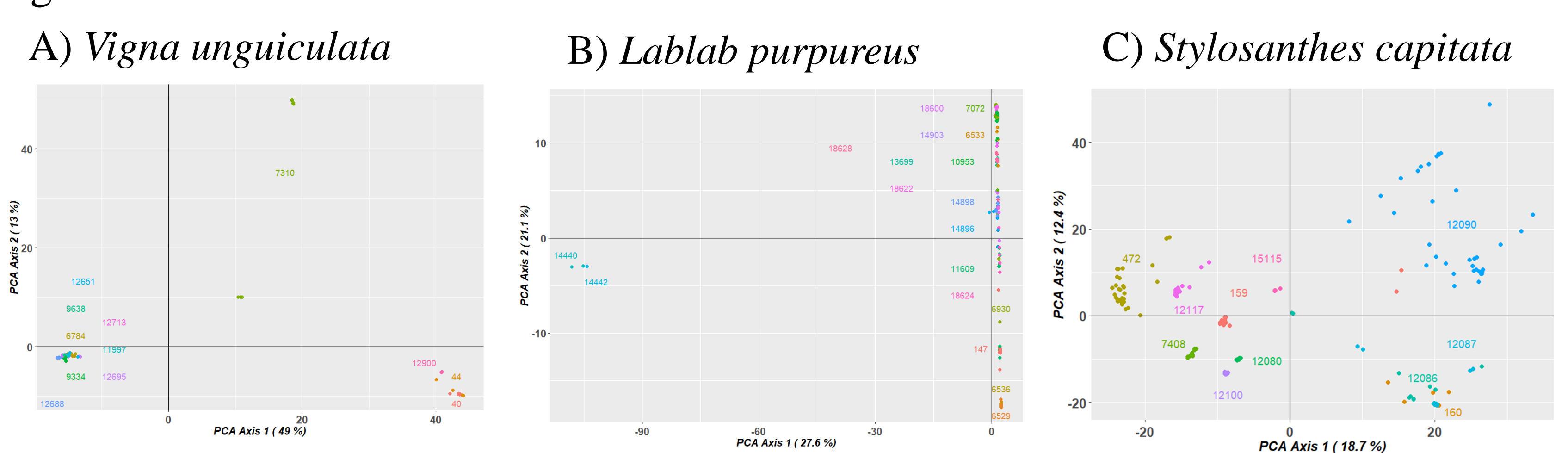


Table 1. Summary of IBD analysis

A) <i>Vigna unguiculata</i>								B) <i>Lablab purpureus</i>								C) <i>Stylosanthes capitata</i>									
Acc	# of Lots	Fullsib	Half-sibs	Parent o/spring	Self/dupli cate/Twin	Cousin	Unrelated	Acc	# of Lots	Fullsib	Half-sibs	Parent o/spring	Self/dupli cate/Twin	Cousin	Unrelated	Acc	# of Lots	Fullsib	Half-sibs	Parent o/spring	Self/dupli cate/Twin	Cousin	Unrelated		
40	6	0	0	0	15	0	0	147	21	43	0	0	310	0	0	159	13	0	4	25	12	36	0	0	
44	7	0	0	5	16	0	0	6579	16	0	0	0	378	0	0	160	11	0	3	13	53	0	0	0	
6784	9	14	2	8	12	0	0	6533	8	12	5	0	11	0	0	472	24	0	3	249	51	0	0	0	
7310	8	0	5	0	13	10	0	6536	6	0	0	0	15	0	0	7408	9	0	0	16	20	0	0	0	
9334	9	0	0	18	18	0	0	6930	8	0	5	2	6	5	10	12080	6	0	0	0	21	0	0	0	
9638	7	0	0	11	10	0	0	7072	9	47	6	0	26	12	0	12086	21	0	0	28	27	4	6	0	
11997	13	0	0	10	68	0	0	10953	10	34	0	0	137	0	0	12087	9	0	0	0	37	18	0	0	
12651	5	4	0	2	4	0	0	11609	7	17	10	2	10	0	27	12090	33	0	30	268	83	7	0	0	
12688	8	0	0	3	25	0	0	13699	8	1	6	0	21	0	0	12100	15	0	0	0	105	0	0	0	
12695	7	8	6	3	4	0	0	14440	2	0	0	0	1	0	0	12117	17	0	0	59	77	0	0	0	
12713	4	0	0	2	4	0	0	14896	5	4	5	0	1	0	0	15115	6	0	0	0	21	0	0	0	
12900	7	0	0	0	21	0	0	14898	10	26	3	0	16	0	0										
								18600	10	17	7	0	21	0	0										
								18622	13	12	35	0	31	0	0										
								18624	6	11	3	0	1	0	0										
								18628	11	25	13	0	15	0	0										

➤ Acc 7310 had lots with distant relationship.

➤ Acc 6930 and 11609 had unrelated lots while Acc 7072 had distantly related lots

➤ Acc 12086 had distantly related or/and unrelated lots.

➤ Acc 159, 12087 and 12090 had lot with cousin relationship with other lots.

References

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Alice Muchugi:
a.muchugi@cgiar.org, Genebank, Feed and Forage Development program, International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia. ilri.org



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