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GENETIC DIVERSITY AND ECO-GEOGRAPHICAL DISTRIBUTION OF *Eleusine* SPECIES COLLECTED FROM ETHIOPIA

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ABSTRACT

Eleusine is a small grass genus with three basic chromosome numbers (x=8, 9 and 10) and comprises of eight species including *E. coracana* subsp. *coracana,* (finger millet), which is an important subsistence crop in Africa and India. Research on these species could assist the development of high yielding and multiple stress tolerant variety(s) of the cultivable species, and also guide development of strategic genetic resource management and utilisation of the genus. A total of 72 accessions, sampled from five major species, *E. coracana* (including both *E. coracana* subsp. *coracana* and *E. coracana* subsp. *africana), E. intermedia, E. indica, E. multiflora and E. floccifolia* were analysed for genetic variation and inter-relationships using 20 microsatellite markers. All the SSR markers displayed high genetic polymorphism, with polymorphic information content ranging from 0.46 (UGEP110) to 0.91 (UGEP66). A total of 286 alleles were observed with an average of 14.3 alleles per locus. Classic F-statistics revealed the highest intra-specific polymorphism recorded for *E. africana* (32.45%), followed by *E. coracana* (16.83%); implying that genetic polymorphism is higher in the cultivable subspecies and its wild relatives, than the other species. Allelic frequency based inter-species genetic distance analysis, showed wider genetic distance between *E. indica* and *E. multiflora* (0.719); a narrow genetic distance between *E. coracana sub-species africana* and *E. coracana subspecies coracana* (0.3297). The weighted neighbor joining-based clustering revealed that the majority of the accessions in a species share strong similarity and are grouped together than do accessions of inter species.

Key Words: *Eleusine,* microsatellite, polymorphism

RÉSUMÉ

Eleusine est une herbe avec trois nombres de chromosomes de base (x=8, 9 et 10) et comprend huit espèces dont *E. coracana* subsp. *coracana,* (finger millet), qui est une culture de subsistence importante en Afrique et en Inde. La recherche sur ces espèces pourrait aider dans le développement des variétés d'espèces cultivables à rendement élevé et de tolérance aux stress multiples, et guider le développement de la gestion des ressources génétiques stratégiques et l'utilisation du genus. Un total de 72 accessions échantillonnées de cinq espèces majeurs à savoir *E. coracana* (incluant *E. coracana* subsp. *coracana* et *E. coracana* subsp. *africana), E. intermedia, E. indica, E. multiflora* et *E. floccifolia* étaient analysées pour variation génétique et relations mutuelles utilisant 20 marqueurs microsatellites. Tous les marquers SSR ont manifesté un polymorphisme génétique élevé, avec un contenu d'information polymorphique allant de 0.46 (UGEP110) à 0.91 (UGEP66). Un total de 286 allèles était observé avec une moyenne de 14.3 allèles par locus. Les statistiqies classiques F ont révélé le polymorphisme intraspécifique le plus élevé enregistré pour le *E. africana* (32.45%), suivi de *E. coracana* (16.83%), ce qui implique que le polymorphisme génétique est le plus élevé dans les sous espèces cultivables et ses homologues sauvages que les autres espèces. L'analyse de la fréquence allélique de la distance génétique entre espèces a montré une plus large distance génétique entre *E. indica* et *E. multiflora* (0.719); une étroite distance génétique entre les *sous espèces Africana* de *E. coracana* et les sous espèces *coracana* de *E. coracana* (0.3297). La pondération des groupements a révélé que la majorité des accessions au sein d'une espèce partage une forte similarité et sont groupées ensemble en comparaison aux accessions des intra-espèces.

Mots Clés: *Eleusine,* microsatellite, polymorphisme

INTRODUCTION

The genus *Eleusine* Gaertn. comprises of eight species, among which the cultivable *E. coracana* subsp. *coracana* (finger millet) is the most important subsistence crop of Africa and India. On the other hand, *E. indica* (goose grass) is categorised as one of the most problematic weeds in the world (Holm *et al.*, 1977; Phillips, 1995; Devarumath *et al.*, 2005). The genus is characterised by three basic chromosome numbers of x=8, 9 and 10; whereby *E. intermdia, E. indica, E. floccifolia* and *E. tristachya* are diploids with 2n=2x=18; *E. multiflora* is a diploid with 2n= 2x=16; *E. jaegeri* is also diploid with 2x=2n=20 (Devarumath *et al.*, 2005). The tetraploid, *Eleusine* spp., comprise of *E. coracana* subsp *coracana* and subsp. *africana,* with 2n=4x=36, and *E. kigeziensis* with 2n=38, which all probably have allopolyploid origins involving two diploid species with x=9 and 10 (Bisht and Mukai, 2002; Neves, 2011).

Dida and Devos *et al.* (2006) categorised the species of the genus into a A genome group, comprising of *E. indica* and *E. tristachya* and a B genome group comprising three species, namely *E. floccifolia*, *E. intermedia* and *E. multiflora*. Since the evolution of the tetraploid species involved both A and B genome groups; *E. coracana* subspecies *coracana* (finger millet), *E. coracana* subspecies *africana* and *E. kigeziensis* belong to both genome groups (Dida and Devos, 2006). All the species are of African origin, except *E. tristachya,* which is native to South America (Devarumath *et al.*, 2005; Neves *et al.*, 2005).

The frequent and naturally occurring hybridisation between cultivated *E. coracana* subsp. *coracana* and its wild relative *E. coracana* subsp. *africana* gave rise to many morphological intermediates (De Wet *et al.*, 1984; Phillips, 1995; Neves *et al.*, 2005) and gene flow between subspecies (Dida *et al.*, 2008). This is mainly caused by co-occurrence of the two *E. coracana* subspecies in the same crop fields where they are cultivated and as weeds, for instance in Western and North Western Ethiopia (Tsehay, 2012). Several authors also confirmed that the cultivated subspp. *coracana* (finger millet) was domesticated through natural and artificial selection, from the wild type finger millet, subspp. *africana* (Hilu and De Wet, 1976; Hilu and Johnson, 1992; Dida *et al.*, 2008).

Bisht and Mukai (2002) indicated that *E. indica*, *E. tristachya*, *E. floccifolia* and *E. intermedia*, *E. coracana*, subspecies *coracana* and *E. coracana* subspecies *africana* are closely related and that there is free genetic flow between them. *Eleusine multiflora* was reported as significantly different morphologically and genetically from other species (Neves, 2011). The distribution, population, genetic variation and preferential adaptation of species can be influenced by altitude gradients that comprise of an assemblage of environmental factors such as, climatic and other edaphic factors (Korner, 2007; Ohsawa and Ide, 2008). Research on those species might bring a novel gene that can be introgrssed into the cultivable *E. coracana* subsp. *coracana* to develop good yielder and/or stress tolerant varieties, particularly against the devastating finger millet blast disease. Furthermore, studying the eco-geographical distribution of plant species is essential for efficient genetic resource collection, appropriate *in-situ* germplasm conservation/management and elucidating the taxonomy, evolution and origin of the species (Bekele, 1985; Demissie and Bjornstrand, 1996). Therefore, this study was aimed at characterising the genetic diversity, inter- and intra- species relationships and the eco-geographical patterning of the different species collected from various regions of Ethiopia as a basis for germplasms collection and conservation.

MATERIALS AND METHODS

Plant materials. A total of 72 accessions of five different species of the genus *Eleusine (E. coracana* (including *E. coracana* subsp. *coracana* and *E. coracana* subsp. *africana), E.intermedia, E.indica, E. multiflora and E. floccifolia)* were included in the study (Table 1). Collections were done in part of Addis Ababa administrative region, Amhara regional state, Benishangul Gumuz regional state, Oromia regional state, Southern Nations Nationalities and Peoples Regional State of Ethiopia (Table 1). Some additional accessions were originally collected from Tigray Regional State. Overall, the samples were collected from the central highlands, west, northwest, northern and southern parts of Ethiopia.

Seeds were sown in a greenhouse and fresh leaf samples were collected for SSR genotyping. The 72 accessions of the different *Eleusine* species were categorised into seven altitude classes, with relative resemblance of agro-climatic origin using the formula:

$K = 1+3.32\log_{10}n$ and $W= (L-S)/K$ (Agrawal, 1996),

where:

K= number of class intervals, W= width of class interval, L= the largest value, S= the smallest value and n= sample size (in this case the number of accessions) (Table 2).

DNA extraction. DNA was extracted from young leaves according to the modified CTAB protocol of Mace *et al*. (2003), omitting the phenol: chloroform step. Extracted DNA was visualised on a 0.8% (w/v) agarose gel and quantified spectrophotometrically, using a Nanodrop® 1000 (Thermo Scientific, USA), followed by dilution to 10 $ngìl^{-1}$ in TE buffer (10 mM Tris, 0.1 mM EDTA pH 8.0).

Polymerase Chain Reaction (PCR). DNA samples were subjected to genotyping, using 20 published SSR markers (Table 3) for finger millet (Dida *et al*., 2007). All forward primers contained an M13-tag (5’- CACGACGTTGTAAAACGAC - 3’) on the 5’ end that was fluorescently labeled to allow detection of amplification products (Shuelke, 2000). PCR amplification was performed in 10 µl in 384 well microtitre plates. Each reaction comprised of 1 x PCR buffer (20 mM Tris-HCl, pH 7.6; 100 mM KCl; and 0.1 mM EDTA. Other components included 1 mM DTT; 0.5% (w/v) Triton X-100; 50% (v/v) glycerol), 2 mM $MgCl_2$, 0.16 mM dNTPs, 0.16 µM fluorescent labeled M13-forward primer, 0.04 µM forward primer, 0.2 µM reverse primer, 0.2 units of Taq DNA polymerase (Sib Enzyme Ltd, Russia) and 30 ng of template DNA. PCR reactions were performed on a GeneAmp 9700 thermocycler (Applied Biosystems) with initial denaturation of 94 °C for 5 minutes, followed by 35 cycles of denaturation 94 °C for 30 seconds, annealing at 59 °C for 1 minute and extension at 72 °C for 2 minutes and the final elongation at 72 °C for 20 minutes.

Fragment detection and SSR data analysis. Amplification was confirmed by running 4 µl of the PCR products on a 2% (w/v) agarose gel, stained with GelRed® (Biotium, USA), and visualised under UV light. Amplification products (1.5 – 3.5 µl of each) were co-loaded in sets of 3 to 4 markers together with the internal size standard, GeneScan™ –500 LIZ® (Applied Biosystems) and Hi-Di™ Formamide (Applied Biosystems), and separated by capillary electrophoresis using an ABI Prism® 3730 Genetic analyser (Applied Biosystems). Allele calling was performed with GeneMapper 4.0 (Applied Biosystems). Allelic data such as polymorphic information content (PIC), observed heterozygosity and major allele frequency for each marker, AMOVA, genetic distance and classical F-statistics (Wright, 1965) were calculated using Power Marker ver. 3.25 software (Liu and Muse, 2005). The unbiased estimator of gene diversity at the i^{th} locus was anticipated as suggested by Weir (1996);

$$Di = (1 - \sum_{u=1}^{k} P^2_{iu}) / (1 - \frac{1+f}{n})$$

Where:

TABLE 1. List of the test accessions with their passport information

No	Accession	Species	Regional state	Admin. zone	Vernacular name	Altitude (m.a.s.l)
1	AAU-ELU-01	*E. floccifolia*	Oromia	West Shoa	Akirma	2297
2	AAU-ELU-02	*E. floccifolia*	Oromia	West Shoa	Akirma	2298
3	AAU-ELU-03	*E. africana*	Oromia	West Shoa	-	2230
4	AAU-ELU-04	*E. africana*	Oromia	West Shoa	-	2230
5	AAU-ELU-05	*E. africana*	Oromia	West Shoa	Gargara	1632
6	AAU-ELU-06	*E. coracana*	Oromia	East Wollega	-	1632
7	AAU-ELU-07	*E. coracana*	Oromia	East Wollega	-	1632
8	AAU-ELU-08	*E. africana*	Oromia	East Wollega	Gargara	1632
9	AAU-ELU-09	*E. indica*	Oromia	East Wollega	-	1632
10	AAU-ELU-10	*E. indica*	Oromia	East Wollega	-	1632
11	AAU-ELU-11	*E. africana*	Oromia	East Wollega	Gargara	1633
12	AAU-ELU-12	*E. africana*	Oromia	East Wollega	Gargara	1247
13	AAU-ELU-13	*E. africana*	Oromia	West Wollega	Gargara	1905
14	AAU-ELU-14	*E. africana*	Oromia	West Wollega	Gargara	1941
15	AAU-ELU-15	*E. africana*	Oromia	West Wollega	Gargara	1938
16	AAU-ELU-16	*E. coracana*	Oromia	West Wollega	Daguja	1938
17	AAU-EIU-17	*E. africana*	Oromia	West Wollega	-	1445
18	AAU-ELU-18	*E. intermedia*	Benishangul Gumuz	Assosa Zone	Bero Tana	1382
19	AAU-ELU-19	*E. indica*	Benishangul Gumuz	Assosa Zone	Bero Tana	1383
20	AAU-ELU-20	*E. intermedia*	Benishangul Gumuz	Assosa Zone	-	1081
21	AAU-ELU-21	*E. intermedia*	Benishangul Gumuz	Assosa Zone	-	1082
22	AAU-ELU-22	*E. intermedia*	Benishangul Gumuz	Assosa Zone	-	764
23	AAU-ELU-23	*E. intermedia*	Benishangul Gumuz	Pawe Zone	-	649
24	AAU-ELU-24	*E. coracana*	Benishangul Gumuz	Pawe Zone	-	649
25	AAU-ELU-25	*E. intermedia*	Benishangul Gumuz	Pawe Zone	-	650
26	AAU-ELU-26	*E. intermedia*	Benishangul Gumuz	Pawe Zone	-	686
27	AAU-ELU-27	*E. intermedia*	Benishangul Gumuz	Pawe Zone	-	686
28	AAU-ELU-28	*E. intermedia*	Benishangul Gumuz	Pawe Zone	-	868
29	AAU-ELU-29	*E. intermedia*	Benishangul Gumuz	Pawe Zone	-	1197
30	AAU-ELU-30	*E. coracana*	Benishangul Gumuz	Pawe Zone	-	1197
31	AAU-ELU-31	*E. coracana*	Benishangul Gumuz	Pawe Zone	Dagusa	1113
32	AAU-ELU-32	*E. africana*	Benishangul Gumuz	Pawe Zone	-	1114
33	AAU-ELU-33	*E. indica*	Benishangul Gumuz	Pawe Zone	-	1039
34	AAU-ELU-34	*E. africana*	Benishangul Gumuz	Pawe Zone	-	1040
35	AAU-ELU-35	*E. africana*	Benishangul Gumuz	Pawe Zone	-	1720
36	AAU-ELU-36	*E. africana*	Benishangul Gumuz	Pawe Zone	-	1720
37	AAU-ELU-37	*E. africana*	Amhara	Awi Zone	-	1705
38	AAU-ELU-38	*E. africana*	Amhara	Awi Zone	-	2208
39	AAU-ELU-39	*E. africana*	Amhara	Bahir Dar Sp Zone	-	1926
40	AAU-ELU-40	*E. coracana*	Amhara	Bahir Dar Sp Zone	-	1926
41	AAU-ELU-42	*E. africana*	Amhara	West Gojam	-	2540
42	AAU-ELU-43	*E. multiflora*	Amhara	West Gojam	-	2540
43	AAU-ELU-44	*E. africana*	Amhara	West Gojam	-	2402
44	AAU-ELU-46	*E. africana*	Amhara	West Gojam	-	2394
45	AAU-ELU-47	*E. floccifolia*	Amhara	East Gojam	Akirma	2415
46	AAU-ELU-48	*E. floccifolia*	Amhara	East Gojam	Chokorsa/Akirma	2548
47	AAU-ELU-49	*E. floccifolia*	Amhara	East Gojam	Chokorsa/Akirma	3100
48	AAU-ELU-51	*E. floccifolia*	Amhara	East Gojam	Chokorsa/Akirma	2636
49	AAU-ELU-52	*E. floccifolia*	Oromia	South West Shoa	Akirma	2013

TABLE 1. Contd.

No	Accession	Species	Regional state	Admin. zone	Vernacular name	Altitude (m.a.s.l)
50	AAU-ELU-53	*E. africana*	Oromia	NI	-	2015
51	AAU-ELU-54	*E. africana*	Oromia	NI	-	1669
52	AAU-ELU-56	*E. coracana*	Oromia	West Arsi	-	1950
53	AAU-ELU-57	*E. floccifolia*	Oromia	West Arsi	Akirma	1946
54	AAU-ELU-58	*E. africana*	SNNP	West Arsi	-	1922
55	AAU-ELU-59	*E. africana*	SNNP	Sidama Zone	Akirma	1805
56	AAU-ELU-60	*E. africana*	SNNP	Gedeo Zone	Qorchissa	1534
57	AAU-ELU-61	*E. africana*	SNNP	Gedeo Zone	Qorchissa	1833
58	AAU-ELU-62	*E. africana*	SNNP	Gedeo Zone	Qorchissa	2040
59	AAU-ELU-64	*E. multiflora*	Addis Ababa	Addis Ababa		2423
60	AAU-ELU-65	*E. africana*	Oromia	East Shoa	Chokorsa	1677
61	AAU-ELU-66	*E. multiflora*	Oromia	East Shoa	-	1790
62	AAU-ELU-67	*E. africana*	Oromia	East Shoa	-	1790
63	AAU-ELU-68	*E. floccifolia*	Addis Ababa	Addis Ababa	Akirma	2520
64	AAU-ELU-69	*E. coracana*	Tigray	NI	-	1568
65	AAU-ELU-70	*E. coracana*	Tigray	NI	-	1502
66	AAU-ELU-71	*E. coracana*	Tigray	NI	-	2142
67	AAU-ELU-72	*E. coracana*	Tigray	NI	-	1568
68	AAU-ELU-73	*E. coracana*	Tigray	NI	-	1800
69	AAU-ELU-75	*E. coracana*	Tigray	NI	-	2100
70	AAU-ELU-76	*E. coracana*	Tigray	NI	-	1810
71	AAU-ELU-79	*E. coracana*	Tigray	NI	-	1750
72	AAU-ELU-80	*E. coracana*	Tigray	NI	-	1820

Key: NI = not identified, m.a.s.l = meter above sea level

TABLE 2. Altitudinal distribution of *Eleusine* species used in the study in Ethiopia

Species	Altitude classes							Total
	<989	990-1331	1332-1673	1674-2015	2016-2357	2358-2699	>2700	
E. africana	0	3	6	13	4	3	0	29
E. coracana	1	2	5	7	2	0	0	17
E. floccifolia	0	0	0	2	2	4	1	9
E. indica	0	1	3	0	0	0	0	4
E. intermedia	6	3	1	0	0	0	0	10
E. multiflora	0	0	0	1	0	2	0	3
Sub total	7	9	15	23	8	9	1	72

Di is gene diversity, P_{iu} is frequency of an allele A_u in the i^{th} locus, f is inbreeding coefficient, and n the number of none missing genotypes. Allelic frequency based inter-species genetic distance were estimated as suggested by Nei and Takezaki (1983). The significance of allelic frequency for the study of accessions at locus level (population differentiation test) was calculated following the Mantel test (Mantel, 1967).

Thirteen SSR markers (UGEP024, UGEP053, UGEP084, UGEP027, UGEP095, UGEP064, UGEP033, UGEP106, UGEP110, UGEP046, UGEP079, UGEP020 and UGEP073) that amplified well, were used for weighted neighbor joining

TABLE 3. List of SSR markers used in this study with repeat motifs and primer sequences

Primer	Forward primer sequence	Reverse primer sequence	Repeat motif	Mapped
UGEP05	TGTACACAACACCACACTGAT	TTGTTTGGACGTTGGATGTG	(TC)12AC(TC)4	9B
UGEP20	GGGGAAGGCAATGATATGTG	TTGGGGAGTGCCAACAATAC	$(GA)_{20}$	ND
UGEP27	TTGCTCTGAGGTTGTGTGTTGC	TCAAGCATAGTGCCCTCCTC	$(GA)_{19}$	ND
UGEP24	GCCTTTTGATTGTTCAACTCT	CGTGATCCTCTCCTCTCTG	(GA)26	3B
UGEP12	ATCCCCACCTACGAGATGC	TCAAAGTGATGCGTCAGGTC	(CT)22	8B
UGEP84	GGAACTTCCGTCAGTCCTT	TGGGGAAGGTGTTGAATC	$(CT)_{24}$	ND
UGEP96	TAATGGGCCTAATGGCAATG	CAAAATCCGAGCCAAGATTC	$(CT)_{10}$	ND
UGEP98	GTCTTCCATTTGCAGCAACC	ACGCGTACTGACGTGCTTG	$(GCC)_{8}$	ND
UGEP67	CTCCTGATGCAAGCAAGGAC	AGGTGCCGTAGTTTGTGCTC	$(TC)_{22}TT(GT)_{5}$	ND
UGEP79	CCACTTTGCCGCTTGATTAG	TGACATGAGAAGTGCCTTGC	$(CT)_{12}$	ND
UGEP33	TAGCCGTTTGCTTGTTGTTTTG	AAGGCCCTAGAACGTCAAGC	$(TC)_{18}$	ND
UGEP46	CAAGTCAAAACATTCAGATGG	CCACTCCATTGTAGCGAAAC	$(GA)_{14}$	ND
UGEP53	TGCCACAACTGTCAACAAAAG	CCTCGATGGCCATTATCAAG	(AG)26	2A
UGEP57	CCATGGGTTCATCAAACACC	ACATGAGCTCGCGTATTGC	$(AG)_{16}$	ND
UGEP64	GTCACGTCGATTGGAGTGTG	TCTCACGTGCATTTAGTCAT	$(CT)_{23}$	ND
UGEP66	CAGATCTGGGTAGGGCTGTC	GATGGTGGTTCATGCCAAC	$(AG)_{29}$	ND
UGEP95	AGGGGACGCTTGGAGTTTG	GCCTCTACCTGTCTCCGTTG	$(TC)_{14}$	ND
UGEP73	GGTCAAAGAGCTGGCTATCG	ACCAGAACCGAATCATGAGG	$(CT)_{4}CC(CT)_{10}$	ND
UGEP106	AATTCCATTCTCTCGCATCG	TGCTGTGCTCCTCTGTTGAC	(AC)12	9B
UGEP110	AAATTCGCATCCTTGCTGAC	TGACAAGAGCACACCGACTC	(CT)12	7AB

Key: ND = not done, B = B genome, A = A genome, AB = both A and B genome of *Eleusine coracana* subsp *coracana* (Dida *et al.*, 2007)

and analysis of molecular variance (AMOVA). Weighted neighbor joining and the relative positions of accessions on the principal coordinate axis (PCoA) were analysed using DARwin-5 (Perrier and Jacquemoud, 2006).

RESULTS AND DISCUSSION

Genetic polymorphism and gene diversity. All the SSR markers used in the study exhibited high polymorphism with PIC ranging from 0.46 for UGEP110 to 0.91 for UGEP66 (Table 4). UGEP066, UGEP046 and UGEP024 revealed the highest PIC (0.91, 0.90 and 0.90) and most abundant gene diversity (0.92, 0.91 and 0.91, respectively), amplified larger numbers of fragments with different allele sizes and had low major allele frequencies. Relatively lower gene diversity and minimal PIC were recorded for UGEP098 and UGEP110. The number of alleles per locus varied from 6 (UGEP098) to 22 (UGEP024) and a total of 286 alleles were produced with an average of 14.30 alleles per locus. Eighteen markers detected highly significant allelic differences ($P<0.01$) and two other markers (UGEP64 and UGEP96) detected significant allelic differences ($P<0.05$) among the study accessions (Table 4).

The highest intra-specific polymorphisms were recorded for *E. africana* (32.45%), followed by *E. coracana* (16.83%); implying that genetic polymorphism is higher in the cultivable subspecies and its wild relatives than the other species. This could be due to the compatibility of these sub species for ease and likelihood of gene flow, resulting in diversity of intermediate races (Neves *et al*., 2005; Dida and Devos, 2006; Dida *et al*., 2008), which does not exhibit distinct groups but are considered as either cultivable *(E. coracana)* or wild type *(E. africana)*. The other possibility could be due to natural and artificial selection of the cultivable *(E. coracana)* based on its adaptability and traits of interest in different agro-ecologies and parallel evolution. Moreover, the co-occurrence of cultivated and wild species in the same field; and seed admixtures of cultivated and wild could partly be the reason for observed patterns of diversity. The lowest polymorphism were exhibited by *E. indica*

TABLE 4. Summary of genetic parameters for the different accessions of *Eleusine* species of Ethiopia

Marker	Major Allele frequency	Allele No.	Availability	Gene diversity	Heterozygosity	PIC	P-value
UGEP024	0.19	22.00	0.84	0.91	0.13	0.90	0.000
UGEP053	0.31	16.00	0.88	0.83	0.23	0.81	0.000
UGEP084	0.42	13.00	0.89	0.77	0.41	0.75	0.000
UGEP027	0.19	17.00	0.69	0.90	0.02	0.89	0.000
UGEP098	0.69	6.00	0.49	0.49	0.00	0.47	0.002
UGEP095	0.16	16.00	0.66	0.90	0.10	0.89	0.000
UGEP064	0.46	14.00	0.81	0.74	0.47	0.71	0.034
UGEP033	0.22	15.00	0.64	0.89	0.62	0.88	0.000
UGEP067	0.25	9.00	0.22	0.84	0.13	0.83	0.000
UGEP106	0.30	11.00	0.96	0.83	0.17	0.81	0.000
UGEP110	0.71	8.00	0.91	0.48	0.09	0.46	0.005
UGEP057	0.16	15.00	0.46	0.90	0.09	0.89	0.000
UGEP096	0.35	10.00	0.32	0.81	0.38	0.79	0.029
UGEP066	0.17	21.00	0.61	0.92	0.07	0.91	0.000
UGEP046	0.13	17.00	0.73	0.91	0.26	0.90	0.000
UGEP079	0.24	12.00	0.99	0.84	0.58	0.83	0.000
UGEP020	0.23	20.00	0.91	0.90	0.22	0.89	0.000
UGEP012	0.31	13.00	0.61	0.84	0.02	0.82	0.000
UGEP073	0.44	15.00	0.64	0.78	0.02	0.77	0.000
UGEP005	0.27	16.00	0.55	0.87	0.20	0.86	0.000
Mean	0.31	14.30	0.69	0.82	0.21	0.80	0.000

TABLE 5. AMOVA showing genetic diversity among and within species of genus *Eleusine*

Source	df	SS	MS	% variance contribution	Expected variance	st.dev	st.error
Within *E. africana* accessions	26	154.898	6.20**	32.450	5.889	2.427	0.093
Within *E. floccifolia*	4	16.460	4.12**	3.450	2.098	1.449	0.290
Within *E. indica*	3	15.417	5.14**	3.230	2.184	1.478	0.369
Within *E. intermedia*	6	70.971	14.19**	14.870	0.006	0.078	0.013
Within *E.coracana*	12	80.331	6.69**	16.830	0.115	0.339	0.026
Among species	5	138.954	27.79**	29.110	3.922	1.980	0.035

Key: df = degree of freedom, SS = sum of squares, MS = mean square, st.dev = standard deviation, ** = highly significant ($P<0.01$)

(3.23%), followed by *E. flocifolia* (3.45%) (Table 5).

Tsehay (2012) reported that ISSR analysis conducted on 65 accessions of the different *Eleusine* species using 6 markers, revealed 68 clearly amplified bands, out of which 59 (86.76%) were polymorphic and the number of polymorphic loci ranged from six for marker UBC-880 to eleven for marker UBC-834. The highest gene diversity was observed for *E. africana* (0.32) and the lowest for *E. floccifolia* (0.16). Other previous studies revealed that the degree of polymorphism depended on sample size (Sharma *et al*., 2010), sampling strategy (Kong *et al*., 2011) and types of test material (He *et al*., 2011). Contradicting the postulation of sample size as a factor of polymorphism, Salimath *et al.* (1995) reported higher polymorphism within species exhibited by two accessions of *E. flocciifolia* than the 16 accessions of *E. coracana* considered in the study conducted using different DNA markers.

Panwar *et al.* (2010) found an average of 50.2% polymorphism and mean PIC of 0. 505 using 10 SSR markers from 18 RAPD markers for 52 finger millet genotypes collected from different districts of Uttarakhand (India). PIC values ranging from 0 to 0.50 were reported by Bezawuletaw (2011), using 15 RAPD markers for 66 finger millet genotypes. Das and Misra (2010) found a range of PIC value between 0.17 to 0.38 using 25 RAPD markers for 15 finger millet accessions. Gupta *et al.* (2010) assessed the genetic relatedness of three finger millet genotypes with different seed coat color using 10 RAPD and 10 ISSR markers and found an average of 8.5 alleles per locus for RADP and 5.7 for ISSR. In general, polymorphism, gene diversity, the number of alleles per locus and the total number of alleles detected in the different *Eleusine* species investigated in this study were diverse and much higher than previously reported in cultivable, *E.coracana* subsp. *coracana.*

Analysis of Molecular Variance (AMOVA). AMOVA showed 29.11% of the total SSR allelic variation among the species and 70.9% within the different species, which could implies the presence of gene flow among different species of *Eleusine* (Table 5). Similarly, Tsehay (2012) reported higher variation (90.59%) attributed to the within species variation; while the remaining variation was due to the among species variation (9.41%).

Genetic distance and inter-species relationship. Allelic frequency-based inter-species genetic distance measure revealed narrow genetic diversity between *E. coracana* sub-species *africana* and *E. coracana* sub-species *coracana* (0.3297), confirming the ancestral relationship between the wild and cultivated finger millet, respectively (Table 6). This is in agreement with several research findings reported on those two subspecies (Hilu and Johnson, 1992; Neves *et al*., 2005; Dida *et al*., 2008). It appeared that *E. coracana* sub-species *africana* also shows relatively narrow genetic distance with *E. intermedia* (0.354). The ease and likelihood of free genetic flow between *E. coracana* and *E. intermedia* as reported by Bisht and Mukai (2002) might be the possible factor.

A relatively wider genetic distance was observed between *E. indica* and *E. multiflora* (0.719) (Table 6). Previous findings reported that *E. multiflora* were significantly different morphologically and genetically from other species (Neves *et al*., 2005; Neves, 2011) and an improbable genome donor or ancestry relationship with *E.coracana* (Clayton and Renvoize 1986; Mysore and Baird, 1997; Hilu and Johnson, 1992). Maxted and Kell (2009) categorised *E. multiflora* as the last set in groupings of species of the genus *Eleusine* as the possible wild relative to the cultivated finger millet.

Eleusine indica also showed wider genetic distance with *E. floccifolia* (0.6609) (Table 6). On the contrary, based on the result of genomic in situ hybridisation (GISH) conducted, Bisht and Mukai (2001) suggested that *E. indica* and *E. floccifolia* were the two A genome donors to *E.*

TABLE 6. The genetic distances between different species of the genus *Eleusine* of Ethiopia

Species	*E. africana*	*E. floccifolia*	*E. indica*	*E. intermedia*	*E. multiflora*	*E.coracana*
E. africana	0.0000	0.4661	0.4210	0.3540	0.4967	0.3297
E. floccifolia		0.0000	0.6609	0.6217	0.6618	0.5051
E. indica			0.0000	0.5000	0.7190	0.6333
E. intermedia				0.0000	0.5841	0.4207
E. multiflora					0.0000	0.4998
E.coracana						0.0000

coracana. However, this suggestion was latter contradicted by Neves *et al*. (2005) showing that GISH can be useful for assessment of chromosome genetic similarity; but such results cannot be reliably used for phylogenetic inference, particularly among closely related species that naturally have some degree of genomic similarity (Neves *et al*., 2005). Salimath (1995) also found that *E. floccifolia* was the most distinct among the other *Eleusine* species examined. Recent findings by Tsehay (2012), using 6 ISSR markers, revealed that the different species of genus *Eleusine* showed an intermingled similarity in the neighbor joining tree and unweighted per group method for arithmetic average (UPGMA) based clustering. The continual contradictory result from the different findings and the argument in the taxonomy of the genus *Eleusine* needs further studies.

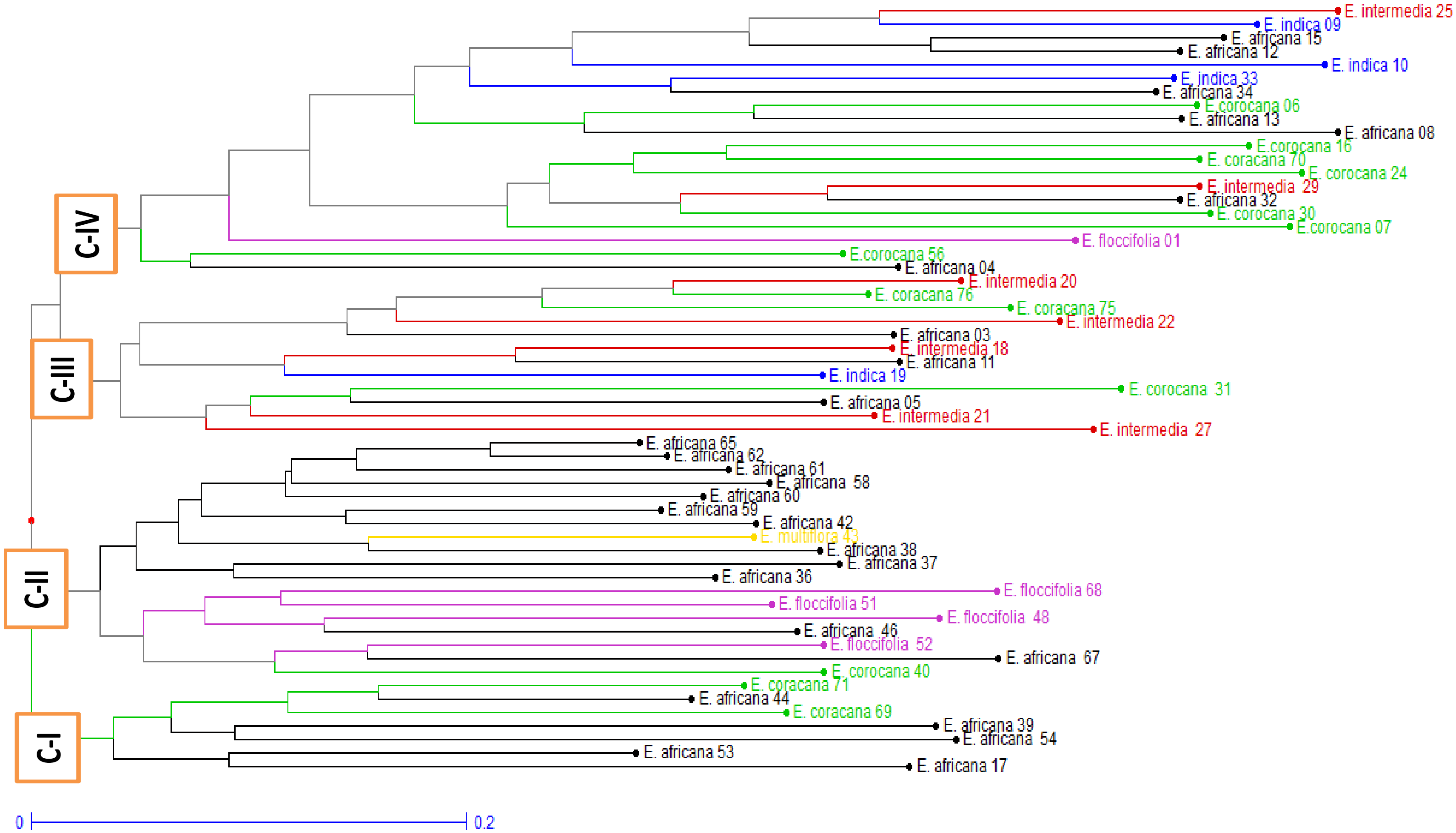
Cluster analysis. Weighted neighbor-joining based clustering was done for 57 accessions that were sufficiently discriminated by the selected markers (Fig. 1). Those accessions were grouped into four major clusters comprising of 7, 18, 12 and 20 accessions in the first, second, third and fourth cluster, respectively. As expected, the majority of accessions belonging to the same species grouped in the same clusters. For instance, four of the five accessions of *E. floccifolia* grouped in the second cluster, five of the seven accessions of *E. intermedia* grouped in third cluster; and three of the four accessions of *E. indica* grouped in the fourth cluster. But *E. coracana* subsp. *coracana* and its wild type *E. coracana* subsp. *africana*, which had relatively larger numbers of accessions, were distributed across all clusters to different degrees. The first cluster was assembled solely from the cultivated and wild species of *E. coracana,* implying an ancestral relationship. The genetic similarity and differences among accessions, were also confirmed using Principal Coordinate Analysis (PcoA) in such a way that the relative position and distribution of accessions among quadrants corroborated the clustering pattern (Fig. 2).

Previous studies also revealed that a simplified phylogenetic tree of the combined sequences of the nuclear ITS ribosomal DNA and plastid *trnT-trnF* regions, indicated close similarities between the A genome sequences of *E. coracana* (for both sub-species) and *E. indica* (Neves *et al*., 2005). The author also indicated a strong genetic similarity between the B genome sequences of *E. coracana* sub-species *coracana* and *E. coracana* subspecies *africana.* Relatively higher genetic relatedness was reported between *E. multiflora* and *E. floccifolia* by Neves *et al*. (2005), while Lui *et al*. (2007) reported that *E. coracana* and *E. indica* were clustered together in phylogenetic analysis of finger millet clade.

Eco-geographical distribution of the species. Although sample sizes were not proportional for all species, there were clear indications of the favourable ecological adaptation zones for the different species. All accessions of *E. floccifolia* were collected from three different administrative regions between altitudes of 2000 - 3100 m.a.s.l, except for accession AAU-ELU-57, which was sampled from the high rainfall region of West Arsi zone (Arsi Negele Agricultural Research compound, 1946 m.a.s.l) (Table 1). Similarly, all three accession of *E. multiflora* were collected from mid to high altitude regions (Tables 1 and 2). Phillips (1972) also reported that *E. floccifolia* and *E. multiflora* were adapted to upland habitats (grassland and open forest or bush land) in altitudes above 1,000 m.a.s.l.

Contrary to the findings of Phillips (1972), about 64% of accessions of *E. intermedia* used in the current study were found in below 1000 m.a.s.l. Cultivated subsp. *E. coracana* and its wild relative *E. africana* shared similar agro ecologies, particularly mid-to-high altitude regions (Table 2), thus confirming co-evolution, likelihood of cross-fertility and the ancestral relationship (Hilu and De Wet, 1976; Neves *et al*., 2005; Dida *et al*., 2008). The National Research Council (1996) of the National Academy Press, USA, also suggested that *E. coracana, E. indica,* and *E. tristachya* can grow in a wider range of open habitats; but that the most favourable altitude regions was between 1000 – 2000 m a.s.l. in Africa; and up to at least 2400 m.a.s.l in Nepal.

Overall, the current study provided base line information for the likelihood of the center of diversity and eco-geographical distribution of the



Key: The serial number after the species name refers to the accession number/code where the "AAU-ELU-" prefix has been omitted. Eg. *E.africana* 12 means, the species is *E. africana* and the accession name is AAU-ELU-12.

Figure 1. Tree constructed based on 13 polymorphic SSR markers for 57 accessions of the different *Eleusine species.*

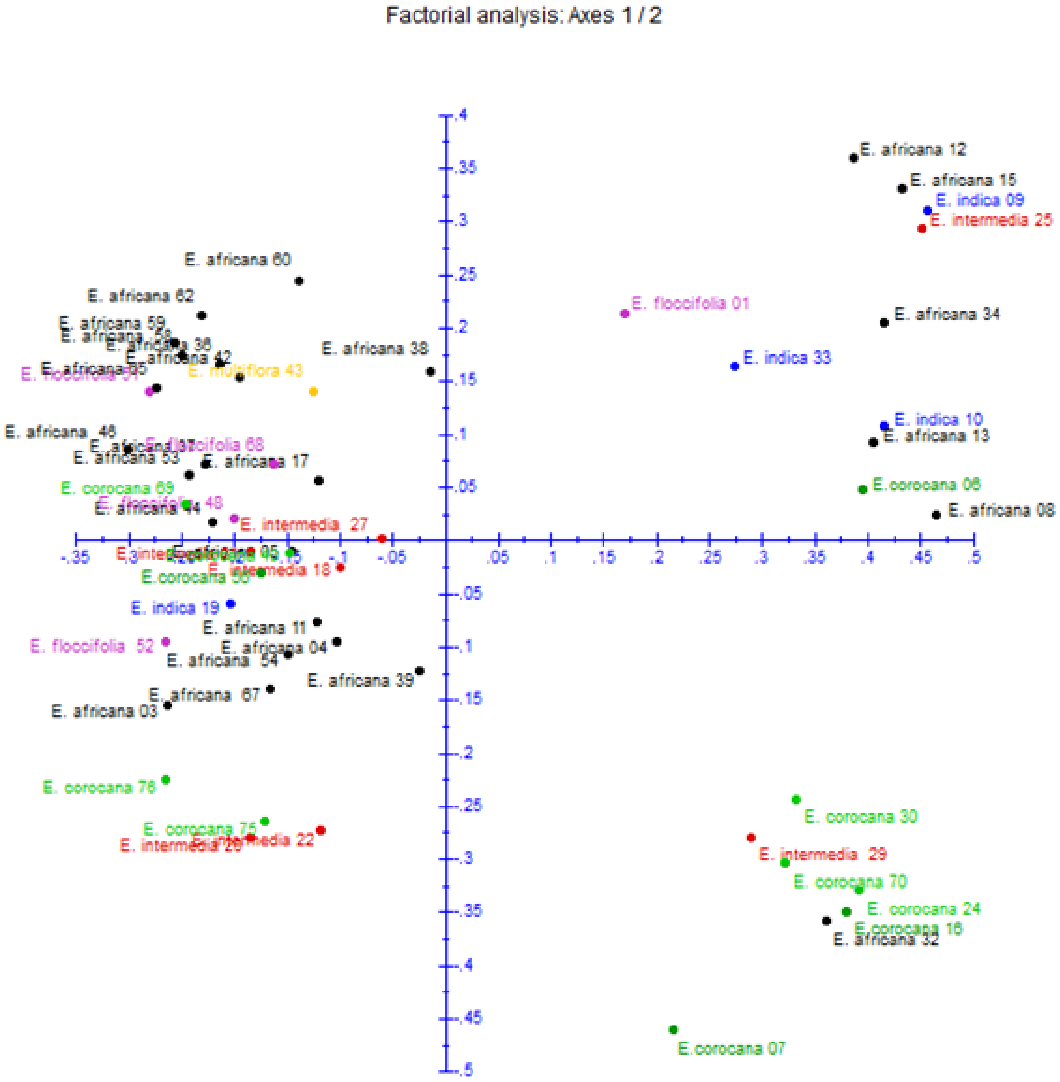


Figure 2. The relative position of 57 accessions of different *Eleusine* species on Principal Coordinates axis. The serial number in front of the species name refers to the accession number/code by adding "AAU-ELU-" as prefix for all of the accessions. Eg. *E.africana*- 12 means, the species is *E. africana* & the accession name is AAU-ELU-12.

different species of genus *Eleusine* for germplasms collection and conservation in Ethiopia.

CONCLUSION

The analysis of genetic polymorphism and molecular analysis of variance have confirmed the presence of genetic variability among and within *Eleusine* species. AMOVA and neighbor joining cluster analysis reveal substantial intra-species variation for cultivated finger millet and its wild relatives, implying that gene flow occurred between the two subspecies that resulted in several races with intermediate characters that can be considered as either of the two. Another reason could be purposeful and natural selection of the cultivated sub species in the different agro-ecologies that resulted in wider diversity. Cluster analysis also reveal that the majority of accessions of a given species tend to group together. The current collection included an altitude range from 649 up to 2636 m.a.s.l with an interval of at most 100 m in the subsequence classes except for one outlier at 3100 m (accession AAU-ELU-49 of *E. floccifolia*). However, the distribution of most of the species is well determined by the altitude classes. Therefore, this urges further confirmation and it could be a basis for quick core–collection assembly of the germplasm to capture maximum diversity from the potential agro-ecological zones and strategic approach for genetic conservation and utilisation.

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REFERENCE

Agrawal, B. 1996. Basic Statistics, 3rd Ed. New Age International (P) Limited, New Delhi, India pp. 713.

Bekele, E. 1985. The biology of cereal landrace populations. Problem of gene conservation, plant breeding schemes and sample size requirement. *Hereditas* 103: 119-134.

Bezaweletaw, K. 2011. Genetic diversity of finger millet [*Eleusine coracana* (L.) Gaertn] landraces characterised by random amplified polymorphic DNA analysis. *Innovative Systems Design and Engineering* 2: 207-218.

Bisht, M.S. and Mukai, Y. 2002. Genome organisation and polyploid evolution in the genus *Eleusine* (Poaceae). *Plant Systematics and Evolution* 233: 243-258.

Bisht, M.S. and Mukai, Y. 2001. Genomic in situ hybridization identifies genome donor of finger millet (*Eleusine coracana*). *Theory and Applied Genetics* 102:825-832.

Clayton, W.D. and Renvoize, S.A. 1986. Genera graminum: grasses of the world, vol 13, Kew Bulletin Additional Series. Her Majesty's Stationery Office, London, UK.

Das, S. and Misra, R.C. 2010. Assessment of genetic diversity among finger millet genotypes using RAPD markers. *Indian Journal of Agricultural Research* 44 (2):112 - 118.

Demissie, A. and Bjonstrand, A. 1996. Phenotypic diversity of Ethiopian barley in relation to geographic regions, altitudinal range and agro ecological zones as an aid to germplasm collection and conservation strategy. *Hereditas* 124:17-29.

Devarumath Rachayya Mallikharjun, Subhash, C. Hiremath, Satyawada Rama Rao, Arun Kumar and Suman Shivamurti Sheelavanthmath. 2005. Genome interrelationship in the genus *Eleusine* (Poaceae) as revealed through heteroploid crosses. *Caryologia* 58: 300-307.

De Wet, J.M.J., Prasada Rao, K.E., Brink, D.E. and Mengesha, M.H. 1984. Systematics and evolution of *Eleusine coracana* (Gramineae). *American Journal of Botany* 71:550-557.

Dida, M., Srinivasachary, M., Ramakrishnan, S., Bennetzen, J.L., Gale, M.D. and Devos, K.M. 2007. The genetic map of finger millet, *Eleusine coracana*. *Theory and Applied Genetics* 114:321-332.

Dida, M., Wanyera, N., Dunn, M., Bennetzen, J. and Devos, K.M. 2008. Population structure and diversity in finger millet *Eleusine coracana* germplasm. *Tropical Plant Biology* 1: 131-141.

Dida, M. and Devos, K.M. 2006. Finger millet. In: Kole, C. (Ed.). Genome mapping and molecular breeding in plants, vol 1, Cereals and millets. Springer, Heidelberg. pp. 333-343.

Gupta, R., Krishan V., Joshi D.C., Dinesh, Y. and Munna, S. 2010. Assessment of genetic relatedness among three varieties of finger millet with variable seed coat color using RAPD and ISSR markers. *Genetic Engineering and Bio*technology *Journal* 2:1-9.

He, Q., Li, X.W., Liang, G.L., Ji K, Guo, Q.G., Yuan, W.M., Zhou, G.Z., Chen, K.S., van de Weg, W.E. and Gao, Z.S. 2011. Genetic diversity and identity of Chinese loquat cultivars/ accessions (*Eriobotrya japonica*) using apple SSR markers. *Plant Molecular Biology Reporter*. 29:197-208. doi:10.1007/s11105-010-0218-9

Hilu, K.W. and De Wet, J.M.J. 1976. Domestication of *E. coracana*. *Economic Botany* 30:199-208.

Hilu, K.W. and Johnson, J.L. 1992. Ribosomal DNA variation in finger millet and wild species of *Eleusine* (Poaceae). *Theory and Applied Genetics* 83:895-902.

Holm, L.G., Plucknet, D.L., Pancho, J.V. and Herberger, J.P. 1977. *Eleusine indica* (L.) Gaortn. In: The World's Worst Weeds: Distribution and biology. University Press of Hawaii, Honolulu, USA.

Kong, Q., Li, X., Xiang, C., Wang, H., Song, J. and Zhi, H. 2011. Genetic diversity of radish (*Raphanus sativus* L.) germplasm resources revealed by AFLP and RAPD markers. *Plant Molecular Biology Reporter* 29:217-223. doi: 10.1007/s11105-010-0228-7.

Korner, C. 2007. The use of altitude in ecological research. *Trends in Ecology & Evolution* 22: 569-574. doi: 10.1016/j.tree.2007.09.006.

Liu, K. and Muse, S.V. 2005. PowerMarker: Integrated analysis environment for genetic molecular data. *Bioinformatics* 21*:* 2128-2129.

Liu Qing, Paul Peterson, Travis Columbus, Nanxian Zhao, Gang Hao and Dlanxiang Zhang. 2007. Inflorescence diversification in the "finger millet clade" (Chloridoideae, Poaceae): A comparison of molecular phylogeny and developmental morphology. *American Journal of Botany* 94(7): 1230-1247.

Mace, E.S., Buhariwalla, H.K. and Crouch, J.H. 2003. A high-throughput DNA extraction protocol for tropical molecular breeding programs. *Plant Molecular Biology Reporter* 21:459a-459h

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27:209-220.

Maxted, N. and Kell, S.P. 2009. Establishment of global network for the in Situ conservation of crop wild relatives: Status and needs. FAO commission on genetic resources for food and agriculture. Rome, Italy.

Mysore, K.S. and Baird, V. 1997. Nuclear DNA content in species of *Eleusine* (Gramineae): A critical re-evaluation using laser flow cytometry. *Plant System Evolution* 207:1-11

National Research Council. 1996. Lost crops of Africa, vol 1, Grains. National Academy Press, Washington, DC, USA.

Neves, S.S. 2011. *Eleusine.* In: Kole, C. (Ed.), Wild crop relatives: Genomic and breeding resources, millets and grasses, DOI 10.1007/978-3-642-14255-0_7, # Springer-Verlag Berlin Heidelberg 2011.

Neves, S.S., Swire-Clark, G., Hilu, K.W. and Baird, W.V. 2005. Phylogeny of *Eleusine* (Poaceae: Chloridoideae) based on nuclear ITS and plastid trnT-trnF sequences. *Molecular Phylogenetic Evolution* 35:395-419

Nei, M. and Takezaki, N. 1983. Estimation of genetic distances and phylogenetic trees from DNA anlysis. Proceedings of the 5th World Congress on Genetics and applied. *Livestock Production* 21: 405-412.

Ohsawa, T. and Ide, Y. 2008. Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. *Global Ecology and Biogeography* 17: 152-163. doi: 10.1111/j.1466-8238.2007.00357.

Panwar, P., Nath, M., Kumar, V. and Kumar, A. 2010. Comparative evaluation of genetic diversity using RAPD, SSR and cytochrome P450 gene based markers with respect to calcium content in finger millet (*E. coracana* L. Gaertn.) *Journal of Genetics* 89:121-13.

Perrier, X. and Jacquemoud, J. 2006. DARwin software.

Phillips, S. 1995. Poaceae (Gramineae) flora of Ethiopia and Eritrea, vol 7. In: Hedberg, I. and Edwards, S. (Eds.). The National Herbarium, Addis Ababa University/Department of Systematic Botany, Uppsala University, Addis Ababa/Uppsala, Sweden.

Phillips, S.M. 1972. A survey of the genus Eleusine Gaertn. (Gramineae) in Africa. *Kew Bulletin* 27: 251-270. Royal Botanic Gardens, Kew.

Sharma, R., Deshpande, S.P., Senthilvel, S., Rao, V.P., Rajaram, V., Hash, C.T. and Thakur, R.P. 2010. SSR allelic diversity in relation to morphological traits and resistance to grain mould in sorghum. *Crop and Pasture Science* 61:230-240.

Salimath, S., Olivera, A., Godwin, I. and Bennetzen, J. 1995. Assesment of genome origins and diversity in the genus *Eleusine* with DNA markers. *Genome* 38: 757-763.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments © 2000 Nature America Inc.

Tsehay, S. 2012. Genetic diversity and relationships among cultivated and wild *Eleusine* species collected from Ethiopia as revealed by ISSR Marker. MSc thesis, Addis Ababa University, Ethiopia.

Weir, B.S. 1996. Genetic data analysis II, Sunderland, UK, Sinauer Associates, Inc.

Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems mating. *Evolution* 19:395-420.