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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Full Length Research Paper

Composition of the essential oils of three *Cyperus* species from Congo

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Essential oils from underground parts of *Cyperus articulatus* (L.), *Cyperus* sp. and *Kyllinga brevifolia* (S.) were analyzed by gas chromatography mass spectrometry (GC-MS). Thirty four (34) components were identified in the oil of *C. articulatus.* The main components were *α*-cadinol (12.07%), trans-pinocarveol (9.86%), cyperenone (7.28%), cyperene (6.29%), myrtenol (5.50%), myrtenal (4.90%) and *cis*-carveol (4.42%). Fifteen (15) components were identified in the oil of *Cyperus sp*. The main components were farnesol (28.61%), humulene epoxide (12.86%) and caryophyllene oxide (8.96%). Twenty three components were identified in the oil of *K. brevifolia* with the main components being manoyl oxide (44.08%), *β*-pinene (13.58%), cyperene (7.63%) and *γ*-terpineol (7.37%). Only *β*-pinene and limonene were present in the three species of *Cyperaceae*. The findings show broad differences in chemical compositions of the essential oils of *C. articulatus, Cyperus* sp. and *K. brevifolia*. When compared with previous studies available in literature, it was also noticed that a difference of composition existed for oils of *C. articulatus* and *K. brevifolia* of other origins, respectively.

Key words: *Cyperus articulatus, Cyperus* sp., *Kyllinga brevifolia,* Cyperaceae, essential oil, rhizome.

INTRODUCTION

Cyperus articulatus (L.), *Cyperaceae* is a rhizome-bearing herb found in Africa, Latin America, Asia and Oceania (Schultes and Raffauf, 1990). In Congo, Gabon and Benin, these rhizomes are used in traditional practice to treat many diseases such as malaria, stomach-ache, respiratory infections, toothache, migraine and epilepsy (Bouquet, 1969). In northern Congo, the powder of rhizome of *C. articulatus* is commonly used in traditional medicine as skin application, fumigant or perfume.

Chemical constituents of extract of *C. articulatus* have been reported by Nyasse et al. (1988a, b). Sedative and anticonvulsant properties were reported for decoction and methanolic extract of *C. articulatus* (Bum et al., 1996, 2001; Rakotonirina et al., 2001). Anti-plasmodial activity of extract of *C. articulatus* have been reported by Rukunga et al. (2008, 2009). The pharmacological importance of essential oil of African plant has been developed by Lawal and Ogunwande (2013). Little

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studies have been reported on chemical constituents of essential oil of rhizomes of *C. articulatus* (Couchman et al., 1964; Duarte et al., 2005; Olawore et al., 2006; Silva et al., 2014; Zoghbi et al., 2006).

Nowadays, the literature offers some data on *Kyllinga* species. According to GENEVEBOTANIC*, Kyllinga brevifolia* may be the new name of *Kyllinga erecta*. Seeing that *Cyperus brevifolius*, *Kyllinga brevifolia*, *Kyllinga brevifolius* and *Kyllinga erecta* are synonymes, we used here literature names used by the authors.

Chemical constituents of extract of rhizomes of *K. erecta* have been reported by Dolmazon et al. (1995a, b). Essential oil composition of underground and aerial parts of *Cyperus Kyllingia* Endl has been reported by Khamsan et al. (2011) and Komai and Tang (1989), respectively. While the composition of rhizomes essential oil of *K. erecta* was reported by Mahmout *et al.* (1993a, b).

Rhizomes of *C. articulatus* are difficult to find in Congo, however rhizomes of *K. brevifolia* could be easily found. *C. articulatus*, *Cyperus* sp. and *K. brevifolia* have similar odor. Literature showed that essential oil of *C. articulatus* of different origins are not similar (Duarte et al., 2005; Olawore et al., 2006; Zoghbi et al., 2006). Similar observation was described by Paudel et al. (2012) for oil of *K. brevifolia*. On the other end, to the best of our knowledge, there are no data available on essential oil of *C. articulatus* from Congo and literature researches indicated that the oil of *Cyperus* sp. has not been subject of previous studies. In this study, *C. articulatus, Cyperus* sp. and *K. brevifolia,* which have similar odor were examined to identify and compare their essential oil components for eventual substitutions in medicinal usages.

MATERIALS AND METHODS

The underground parts of *C. articulatus* (L.) were collected in the raining season (October 2005) at the full flowering stage, from Owando, Department of Cuvette-Congo. The underground parts of *Cyperus* sp. (V.) and *K.brevifolia* (S.) were collected in the raining season (November 2005) at the full flowering stage from Brazzaville in Republic of Congo. Voucher specimens were deposited in the herbarium of CERVE (Centre d'Etudes et de Recherches sur les Ressources Végétales) where they were identified by comparison with three voucher specimens Nere No. 1011 of 1963 February 2rd, P. Sita no. 400 of 1961 September and B. Descoings no. 6045 of 1960 July 1st for *C. brevifolia* and lastly *K. erecta*. With too voucher specimens B. Descoings no. 9013 of 1961 August 10th and J. Koechlin no. 1396 of 1951 October 9th for *C. articulatus*. Nowadays there are new voucher specimens, J.M. Moutsambote no. and no. 7210 of 2013 August 2rd respectively for *Cyperus* sp. and *C. articulatus*. The rhizomes and underground parts of plants were washed, dried at room temperature in the laboratory for two weeks and then grounded before use.

Extraction of essential oil

Air-dried underground parts of the plants (62.5 g, three times for each species) were subjected to hydrodistillation for 6 h using a Clevenger-type apparatus. The oil layers was separated from water layers and were collected and anhydrous sodium sulphate was added to remove any residual water. Yields of the essential oils were determined and the oils were stored at low temperature until use for analysis.

GC-MS analysis

GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d.). Oven temperature was 40-240°C at a rate of 4°C/min, transfer line temperature 260°C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, ionization energy 70 eV; scan time 1 s and mass range of 40-300 amu. The percentages of compounds were calculated by the area normalization method, without considering response factors. The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature (Adams, 1995).

RESULTS AND DISCUSSION

The oils isolated by hydrodistillation from the underground parts of *C. articulatus, Cyperus* sp. and *K. brevifolia* were obtained in yields of 1.0, 0.5 and 0.6% (v/w) respectively. The oil of *C. articulatus* was found to be dark-brown liquid and the oil of *Cyperus sp* was found to be dark-yellow liquid while the oil of *K. brevifolia* was colourless liquid.

The chemical compositions of the oils are presented in Table 1. The components are listed in order of their elution. Forty one components were detected and thirty four were identified accounting for 84.45% of components in the oil of *C. articulatus*. The main components were *α*-cadinol (12.07%), trans-pinocarveol (9.86%), cyperenone (7.28%), cyperene (6.29%), myrtenol (5.50%), myrtenal (4.90%) and cis-carveol (4.42%). Out of twenty-seven compounds detected from *Cyperus* sp. only fifteen were identified accounting for 46.79% of the oil. Farnesol (28.61%), humulene epoxide II (12.86%), caryophyllene oxide (8.96%) and an unknown compound, 4.75% were the main components. While twenty three out of twenty seven compounds detected from the oil of *K. brevifolia* were identified, accounting for 96.13% of the oil. Among these are manoyl oxide (44.08%), *β*-pinene (13.58%), cyperene (7.63%) and *γ*-terpineol (7.37%) as the main constituents.

Comparing the compositions of the oils of these *Cyperaceae*, it has been shown that only β-pinene and limonene were present in the three oils. The amount of β-pinene varied from 0.72 to 13.58% and of limonene from 0.40 to 2.41%. The difference of the essential oils is very pronounced between *Cyperus* sp. and *K. brevifolia* from where only α-pinene and limonene are common components. There are only five compounds, α-pinene, limonene, *α*-terpineol, caryophyllene oxide and humulene epoxide II, that are common to both the oils of *C. articulatus* and *Cyperus* sp. While α-pinene, β-pinene,

Table 1. Composition (%) of the essential oils isolated from rhizomes of *C. articulatus, Cyperus* sp. and *K. brevifolia.*

Compound	R.I.	C.A.	C.S.	K.B.
Diacetone alcohol	865	0.53	-	-
α-Pinene	934	3.16	-	1.86
Thuya-2,4(10)-diene	954	0.52	-	-
β-Pinene	978	2.94	0.72	13.58
β-Myrcene	982	-	-	0.54
p-Cymene	1025	-	-	0.79
Limonene	1028	0.4	1.02	2.41
1,8-Cineole	1033	0.54	-	3.15
(E)-Ocimene	1041	-	-	0.42
Cis-linalool oxide	1058	-	-	1.21
p-Cymenene	1086	-	-	1.03
Linalool	1129	-	-	0.52
Borneol	1154	-	2.46	-
Trans-Pinocarveol	1155	9.86		-
Nopinone	1157	3.63	-	-
p-Mentha-1,5-dien-8-ol	1164	0.74	-	-
α-Terpineol	1179	1.79	0.77	-
p-Cymen-8-ol	1186	2.2	-	-
Unknown	1193	-	-	1.19
Verbenone	1203	1.07	-	-
Trans-carveol	1211	-	3.64	-
γ-Terpineol	1211	-	-	7.37
Myrtenol	1212	5.5	-	-
Unknown	1214	-	2.37	-
Myrtenal	1215	4.9	-	-
Cis-carveol	1228	4.42	-	-
Methylthymol	1234	0.9	-	-
β-Selinene	1247	-	-	0.52
(-)-Carvone	1263	0.51	-	-
Unknown	1309	-	-	1.05
α-Copaene	1376	1.15	-	-
β-Maaliene	1383	2.56	-	-
β-Elemene	1394	-	-	1.11
Cyperene	1414	6.29	-	7.63
β-Caryophyllene	1427	-	-	0.65
Allo-aromadendrene	1461	-	-	0.49
Eudesma-2,4,11-triene	1469	1.08	-	0.81
γ-Muurolene	1475	-	-	0.85
Germacrene D	1478	-	-	0.67
Unknown	1489	-	1.3	-
δ-Selinene	1493	-	2.73	-
α-Selinene	1497	-	0.9	-
β-Calacorene	1540	1.11	-	-
α-Calacorene	1548	-	1.01	-
Unknown	1561	0.53	-	-
Ledol	1565	0.98	-	-
Unknown	1572	1.13	-	-
Caryophyllene oxide	1578	3.24	8.96	-
Globulol	1582	0.69	-	-
β-Copaen-4α-ol	1587	0.59	-	-

Table 1. Contd.

Guaia-5-en-11-ol	1594	-	1.18	-
Humulene epoxide II	1602	0.59	12.86	-
Unknown	1607	-	1	-
Cedrol	1610	0.68	-	-
Patchoulenone	1615	2.73	-	-
Dilapiole	1620	-	4.09	-
Unknown	1623	-	3.48	-
Eudesma-3,11-dien-5-ol	1637	-	4.17	-
Caryphylla-4(14),8(15)-dien-5α-ol	1640	1.81	-	-
Unknown	1645	1.34	-	-
Unknown	1649	-	4.75	-
Unknown	1652	1.26	-	-
α-Cadinol	1657	12.07	-	-
Unknown	1657	-	1.73	-
β-Bisabolol	1661	-	2.28	-
Unknown	1664	2.73	-	-
Unknown	1669	1.81	-	-
Palmitic acid	1673	-	-	4.24
Mustakone	1676	2.6	-	-
Unknown	1677	-	-	0.88
Cyperenone	1680	7.28	-	-
Unknown	1682	-	2.12	-
Unknown	1683	1.25	-	-
Unknown	1683	-	1.75	-
Cyperotundone	1685	0.89	-	-
Unknown	1688	-	1.16	-
Unknown	1697	-	1.89	-
Farnesol	1713	-	28.61	-
Manoyl oxide	1990	-	-	44.08
Unknown	1995	-	2.24	-
Unknown	2015	-	0.78	-
Unknown	2065	-	-	0.71
11-Oxomanoyl oxide	2065	-	-	1.05
11α-Hydroxymanoyl oxide	2110	-	-	1.15
Total detected		94.50%	71.36%	99.96%
Total identified		84.45%	46.79%	96.13%

R.I. = Retention index; K.B. = *Kyllinga brevifolia;* C.S. = *Cyperus* sp.*;* C.A. = *Cyperus articulatus.*

limonene, 1,8-cineole, cyperene and eudesma-2,4,11-triene are the six compounds found in both the oils of *C. articulatus* and *K. brevifolia*. These results showed a broad difference in the composition of these essential oils (Figures 1, 2, 3).

The essential oil of *K. brevifolia* contains 44.08% of manoyl oxide followed by 13.58% of β-pinene. Manoyl oxide, the major component in oil of *K. brevifolia*, was absent in the other oil and the concentration of β-pinene was very low, 2.94 and 0.72% respectively in oils of *C. articulatus* and *C. sp*. Farnesol was present in the amount of 28.61% in *Cyperus* sp. followed by humulene epoxide II (12.86%) and caryophyllene oxide (8.96%) which are respectively in low amount in *C. articulatus*, 0.59 and 3.24%, but absent in *K. brevifolia. α*-Cadinol and trans-pinocarveol, the two principal components of *C. articulatus* were absent in the two others species of cyperaceae. Myrtenol, myrtenal and verbenone were

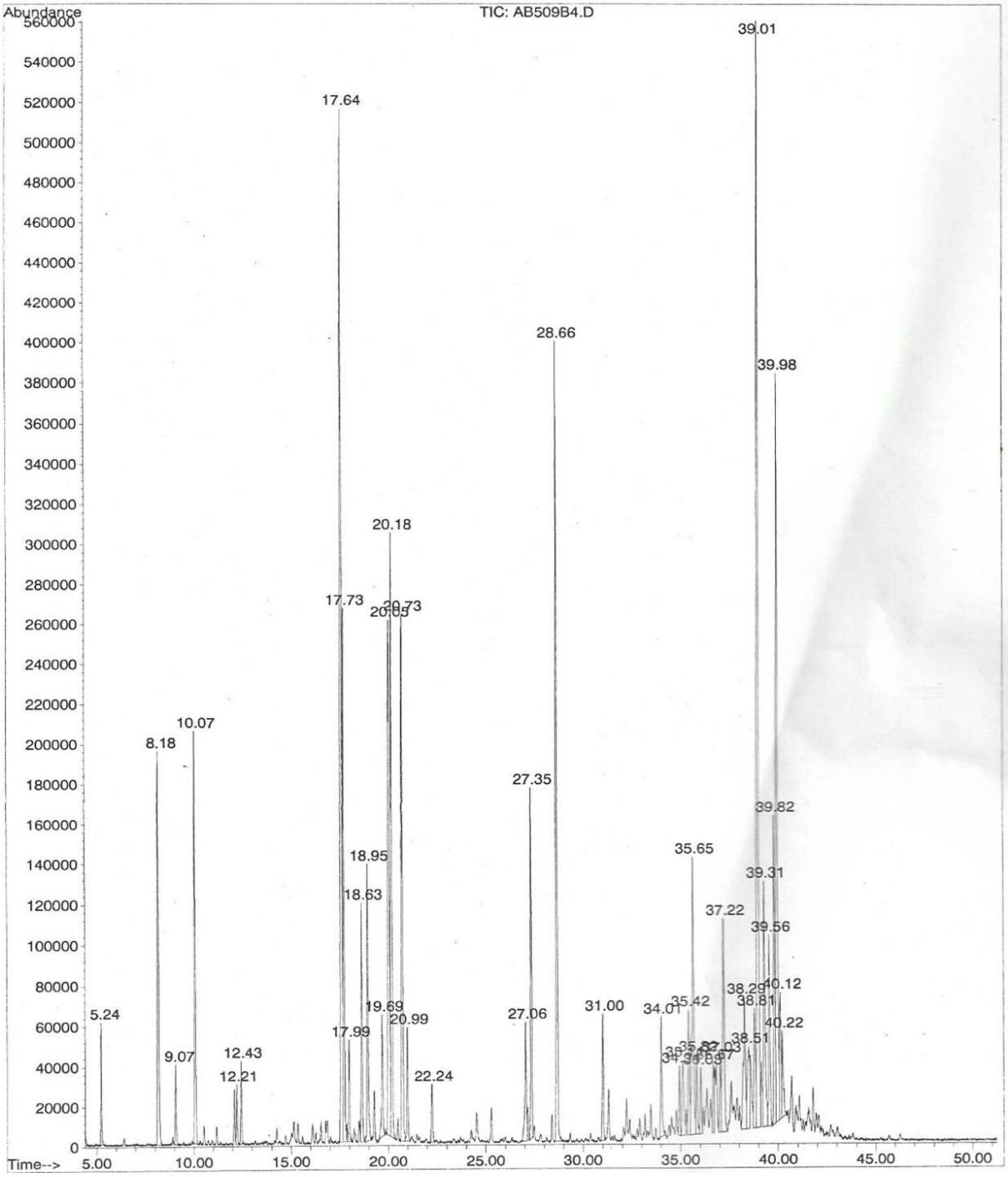

Figure 1. Spectograph of GCMS analysis of E.O. of *C. articulatus*.

absent in *Cyperus sp* and *K. brevifolia*. Cyperenone, the third major component of *C. articulatus* was found in little amount (0.88%) in *K. brevifolia*. Amounts of cyperene in *C. articulatus* (6.29%) and *K. brevifolia* (7.63%) were comparable. The results of our findings are similar to the findings of Duarte et al. (2005) and Olawore et al. (2006) where cyperotundone, cyperenone and cyperene were not simultaneously found in oil of *C. articulatus*. The lack of one of these constituents in *Cyperaceae* species can be explained by the total percentages recovered which are less than 100%. This analysis explained probably the lack of cyperotundone, cyperenone and cyperene compounds in essential oil of *C. articulatus* previously reported by Duarte et al. (2005) where only 17 components

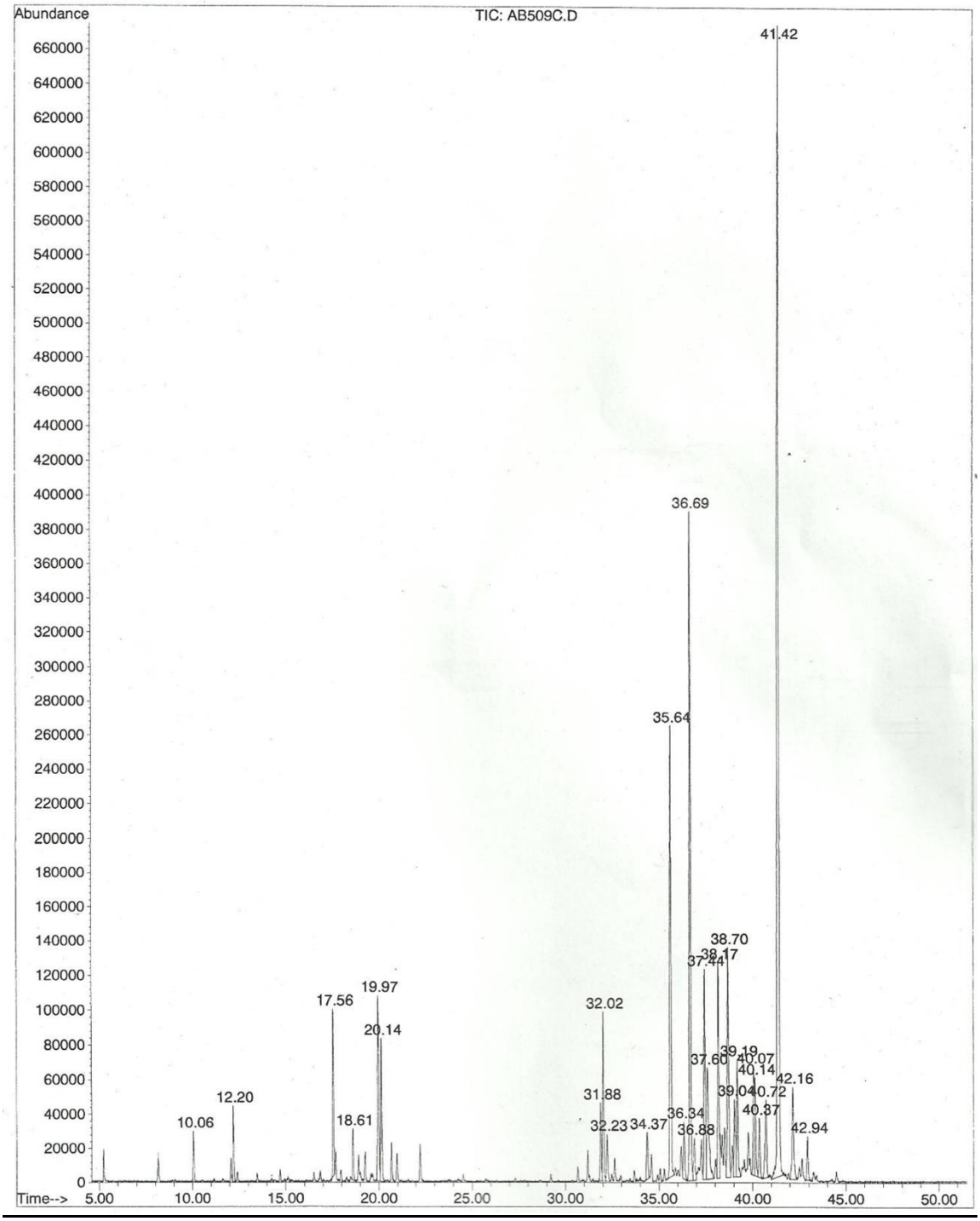

Figure 2. Spectograph of GCMS analysis of E.O. of *Cyperus* sp.

were identified accounting for 82.00%.

There are some minor components in the oil of *C. articulatus,* such as nopinone, patchoulenone, β-maaliene, *p*-cymen-8-ol, β-calacorene and others that were not present in the other two oils. However, the oils of *Cyperus* sp. and *K. brevifolia* also have minor components, such as borneol, β-bisabolol, γ-terpineol, palmitic acid and others that are not present in the *C. articulatus* oil.

Some studies of oil of *C. articulatus* have been reported. Olawore et al. (2006) reported that the main components of oil from Nigeria were cedrol (19.0%), Guaia-5-en-11-ol (14.9%), *α*-cadinol (3.4%). Two studies of *C. articulatus* from two origins in Brazil showed

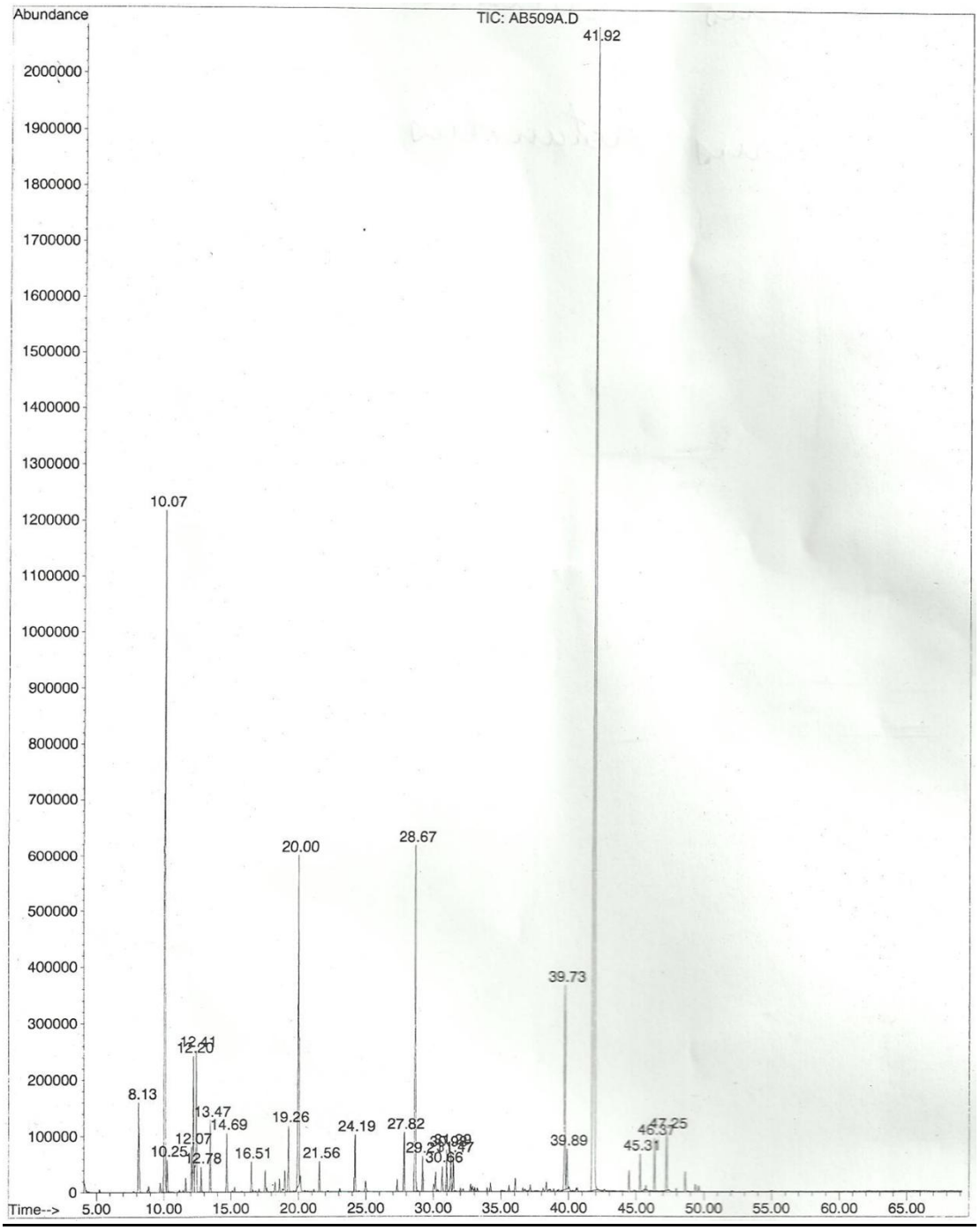

Figure 3. Spectograph of GCMS analysis of E.O. of *K. brevifolia.*

difference in oil composition. The main components of oil of rhizomes of *C. articulatus* from Campinas Brazil (Duarte et al., 2005) were reported to be verbenone (19.57%), trans-pinocarveol (17.44%), myrtenal (8.16%), *p*-mentha-1, 5-dien-8-ol (7.06%), myrtenol (4.61%) and *p*-cymen-8-ol (4.44%). The main components of oil of rhizomes of *C. articulatus* from Pará Brazil were mustakone (14.5%), caryophyllene oxide (10.1%), α-pinene (6.5%), myrtenal + myrtenol (5.8%), trans-pinocarveol (5.5%) and ledol (4.6%) (Zoghbi et al. (2006). These results showed qualitative and quantitative compositions differences in essential oils of different origins.

Paudel et al. (2014) reported composition of oil of *K. brevifolia* from Nepal which showed α-cadinol (40.3 %), -muurolol (19.5 %) and germacrene D-4-ol (12.5 %) as

major components while Guilhon et al. (2008) found mostly 13-*epi*-manoyl oxide(26.1 %) and manoyl oxide derivatives. In a previous study, Mahmout et al. (1993) reported composition of essential oil of *K. erecta* from Republic of Chad. The main components were found to be manoyl oxide (48.0%), cyperotundone (10.2%), cyperene (9.4%) and 11α-hydroxymanoyl oxide (7.5%). Similarly, for the major component (manoyl oxide), qualitative and quantitative differences in composition of oils were observed.

Comparing the present results with those previously reported in the literature, on essential oil compositions of rhizomes of *C. articulatus* and *K. brevifolia* from different countries, it is apparent that, there are many differences regarding the major and the main components of oil, which further suggest the existence of more chemical diversity within these *Cyperaceae* species. The reason for variation between the chemical compositions of oils may be depended on climatic, seasonal, geographic conditions, harvest period and isolation procedure.

Conclusion

The essential oils of *C. articulatus*, *Cyperus* sp. and *K. brevifolia* from Congo have been investigated for the first time. The yields of oil were, 1.0, 0.5 and 0.6% for *C. articulatus*, *Cyperus sp* and *K. brevifolia*, respectively. This study concluded that there is no chemical similarity between *C. articulatus*, *Cyperus sp* and *K. brevifolia* and demonstrated that *C*. *articulatus* from different origins can show difference in composition of theirs essential oils. The difference in chemical composition can also explain why *C. articulatus* have never been substituted by these other *Cyperaceae* species, in traditional practice, in spite of theirs similar odor.

Conflict of Interests

The author(s) have declared that there is no conflict of interests.

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Full Length Research Paper

Genetic variability and divergence of seed traits and seed germination of five provenances of *Faidherbia albida (Delile)* A. Chev

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Establishment of *Faidherbia albida* trees on farm is often difficult despite the plant survival adaptive mechanisms such as drought and disease resistance. Adoption of the tree to agroforestry systems is also limited due to lack of knowledge on genetic variation of its provenances. Morphological characterization of *F. albida* provenances is therefore necessary to screen for natural genetic variation in seeds traits for selection of germplasm for long term agroforestry, timber production, fodder, soil fertility increment and environmental sustainability. In this study, seed traits of five provenances of *F. albida*: Taveta Wangingombe, Lupaso, Kuiseb and Manapools were examined. Divergent studies were analyzed based on seed morphology and geo-climatic conditions of the provenances. Seed length, width, thickness and weight were analyzed to determine the extent of phenotypic and genotypic variance and heritability. This study revealed significant differences among provenances (P≤0.05) for all the studied characters indicating substantial genetic variability. Genetic variance for all seed traits was higher than environmental variance suggesting that the expressions of these traits are under genetic control. This result was supported by high heritability values for seed length (0.92), width (0.99), thickness (0.99) and weight (0.99). Seed germination test involved 4 replicates of 25 randomly selected seeds per provenance. Mean germination percentage among provenances was 83.3% with the highest being 97% and the lowest 71%, P≤0.05. Relationships among these variables were analyzed using principal component analysis and cluster analysis resulting in separation of provenances into three distinct clusters. Manapools (760 mm), Lupaso (1165 mm) and Wangingombe (628 mm) with high rainfall were placed in cluster one. Taveta (545 mm) cluster two and Kuiseb (<50 mm) cluster three. Wangingombe (1700 m a.s.l.) clustered closely to Lupaso (500 m a.s.l.) than Taveta (760 m a.s.l.). High heritability (h^2>0.5) for all traits suggests that selection based on morphological traits can be made with a high degree of confidence.

Key words: Provenances, selection, clinal distribution, geographical differentiation, genetic variation, heritability.

INTRODUCTION

Faidherbia albida (Del.) A. Chev. is a leguminous trees species belonging to *Mimosoideacea* subfamily, tribe *Acaciacea*. It is locally referred to as Apple-Ring Acacia, Ana tree, Balanzan tree and Winter Thorn (Fagg and Barnes, 2003). Due to its phytochemical properties, pollen structure and phenology, it is placed in a monotypic genus

Faidherbia (Bernard, 2002; Hyde and Wursten, 2010*). F. albida* is an important agroforestry appreciated due to it compatibility with cropping systems (Roupsard et al., 1999; Payne, 2000; Ibrahim and Tibin, 2003). The tree is used in dry lands for soil conservation (Dangasuk et al., 2001). It can grow among field crops without overshadowing them during the rainy season and provides shade during dry season (Orwa et al., 2009). Falling leaf mulch and the canopy shade creates a microclimate with better infiltration and reduced evapotranspiration which is crucial for plants (Gassama-Dia et al., 2003).

Despite the immense benefits of *F. albida*, broadening the utilization, breeding and conservation of the species still remain a challenge. *F. albida* is faced with threats to its gene pool due to drought and land use pressure; the situation is worsened by lack of natural regeneration and artificial propagation by seed or suckers among the people living in the Sahel (Weber and Hoskins, 1983; Bonkoungou, 1992; McGahuey, 1992). However, the *F. albida* trees currently growing in the Sahel regenerate naturally (Weber and Hoskins, 1983) and attempts of artificial regeneration have met failures due to poor survival and highly variable growth in early stages (McGahuey, 1985). It is therefore important to test seeds in order to screen for naturally available genetic variation for selection of germplasm for long term agroforestry, sustainability of timber production, fodder and soil fertility increment and environmental sustainability.

Seed morphology is an evolutionary trait contributing to genetic diversity (Aniszewski et al., 2001). Seed morphology influence water relation and seed dispersal, emergence, survival and seedling establishment (Milberg and Lamont, 1997); large and heavy seeds germinate rapidly with high survival and fast growth as compared to small seeds. Seed morphology is linked to fitness hence successful establishment (Zhang, 1998). Seeds traits that show high morphological variation are useful for selection of germplasm for conservation and propagation (Khurana and Singh, 2001). It has generally been reported that large seeds produce seedlings that have high survival rates (Moles and Westoby, 2004; Turnbull et al., 2008; Westoby, 1998) and trees with small seeds may produce more seeds per individual but with low seedling survival rates (Rees, 1995; Turnbull et al., 1999; Eriksson et al., 2000; Leishman and Murray, 2001). Knowledge of genetic variation among seed traits provides useful information for adaptation to heterogeneous environment. Seed traits that have high heritability values ($h^2 \geq 0.05$) are useful markers for selection (Akbar et al., 2003).

Variability studies are important in improvement of tree as it provides information relevant for selection of quality germplasm (Bhat and Chauhan, 2002). Germplasm quality affects the quality of trees propagated (IFSP, 2000) and should be considered during selection. Ibrahim (1996) and Dangasuk et al. (1997) examined the variation in seed and seedling traits of *F. albida* but did not provide any information on genetic and phenotypic variance, phenotypic and genotypic coefficient of variation and heritability of *F. albida* provenances. The current study is novel and aims at testing the reliability of selection of *F. albida* provenances based on seed morphological traits and to determine the genetic relationships among the provenances under study based on seed morphology and geoclimatic conditions.

MATERIALS AND METHODS

Study site

Morphological characterization of the seeds was conducted in April 2014 at the seed laboratory of the World Agroforestry Centre (ICRAF) in Nairobi, while the seeds were germinated in the ICRAF nursery greenhouse. ICRAF is located about 20 km south east of Nairobi, Kenya at latitude of 1° 33' S longitude 37° 14' E and an altitude of 1580 m above sea level. The mean annual rainfall ranges between 500 and 1370 mm and the mean temperature of 21°C.

Seed sources

The seeds of five provenances, obtained from ICRAF seed bank were used for the study. The seeds were collected by Oxford Forest Institute (OFI) in 1990 for international provenance trials. The seeds were then processed and sent to ICRAF seed bank. For each provenance, OFI selected trees that had geographical discontinuity from other populations. The selected trees were obtained from sites with varying climate, soil, altitude and ecology. Undisturbed natural populations with distinct morphological and phenological traits were selected.

OFI collected 25 mother trees to represent a provenance. The mother trees were spaced 100 m apart to avoid collections from related individuals and to capture a large proportion of the natural genetic variation within the provenance (Ofori, 2001). The geographical range of the seeds varied from 3°24'S, 37°42'E to 23°34'S, 15°02'E longitude and altitude of 360 to 1700 m above the sea level. Taveta and Wangingombe represented eastern Africa provenances while Lupaso, Kuiseb and Manapools represented southern Africa provenances (Figure 1 and Table1).

Characterization of seed morphology

To investigate variation in seed length, width, weight and thickness, 100 seeds per provenance were randomly selected from the seed bulk. The seed were organized in a completely randomized experimental design with 25 seeds replicated four times per provenance (25 x 4 x 5) = 500 experimental units (Table 2). A Vernier caliper calibrated to two decimal places was used to measure seed length, width and thickness. Seed length was measured over the seed coat along the longest axis of the seed;

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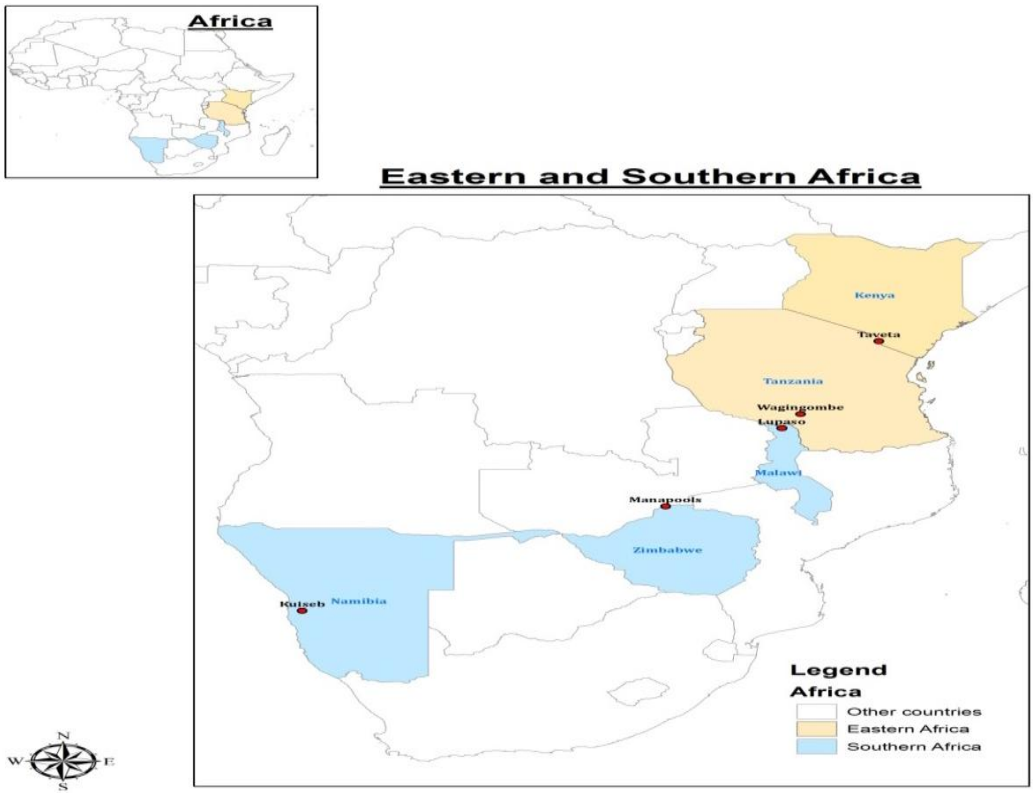

Figure 1. Map of the collection site for *Faidherbia albida* provenances under study.

Table 1. Geo-climatic (latitude, longitude, altitude, rainfall and temperature) of the different provenances of *F. albida* under study.

Country	Region	Provenance	Location	Altitude (m asl)	Rainfall (mm)	Temperatures (°C)
Kenya	Eastern Africa	Taveta	3°24'S, 37°42"E	760	545	28.1
Malawi	Southern Africa	Lupaso	9°55'S, 33°53'E	500	1165	24.8
Namibia	Southern Africa	Kuiseb	23°34'S, 15°02'E	400	<50	15.2
Tanzania	Eastern Africa	Wangingombe	8°51'S, 34°38'E	1700	760	27.1
Zimbabwe	Southern Africa	Manapools	15°45'S, 29°20'E	360	628	25.1

Table 2. Completely randomized experimental design for characterization of seed morphology and seed germination test

Provenance	Number of seeds per replicate				Total
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	
Taveta	25	25	25	25	100
Wangingombe	25	25	25	25	100
Lupaso	25	25	25	25	100
Kuiseb	25	25	25	25	100
Manapools	25	25	25	25	100
Total	100	100	100	100	500

seed width measurements were taken on one of the widest faces at the middle of the seed; seed coat thickness was measured without the removal of seed coat from the seed. An electronic top pan model was used to weigh each seed for all replicates.

Table 3. Morphological variation in seed length, width, thickness and seed weight among the five provenances of *F. albida*

Provenance	Seed length(mm)	Seed width(mm)	Seed thickness (mm)	Seed weight (mg)
Taveta	6.73c	4.98d	2.96c	83.83d
Lupaso	8.86b	5.18c	3.05a	126.43c
Kuiseb	10.21a	5.89ab	3.01ab	160.62a
Manapools	10.44a	6.00a	3.03ab	167.15a
Wagingombe	9.30b	5.75b	3.04ab	143.20b
CV	12.26%	6.80%	0.16%	30.41%
SD	1.74	0.55	0.014	50.9

CV = coefficient of variation, SD = standard deviation. Means followed by a different letter within a column are significant different according to LSD post-hoc test for all the traits.

Seed germination test

After recording the variation in seed morphology, the seeds were germinated. The seeds were nicked (Bewley and Black, 1994) at the distal end near the microphyle (Manz et al., 2005; Wojtyla et al., 2006) using a nicking caliper. The seeds were then soaked in water for 24 h before sowing. Rapid influx of water was observed during the first twelve hours due to low water potential of dry seeds (Obroucheva and Antipora, 1997). Polythene tubes measuring 10 x 20 cm, filled with sterilized sand were used to germinate the seeds. The sand was sterilized using the oven method. Prior to sterilization, the sand was cleaned in tap water then placed in metal baking pans up to four inches in depth. The metal pans were then tightly covered with aluminum foil and placed in the oven at temperatures between 180 to 200°F; the temperatures were maintained for 30 min. After heating, the oven was cooled and metal pans removed. The aluminum foil covering the metal pans was left intact to prevent the sand from contamination. The sand was watered to field capacity before sowing the seeds. The polythene tubes containing the seeds were kept under a temperature range of 25-30°C and photoperiod of 12 h light and 12 h dark. Seed germination was monitored for thirty days and mean germination percentage was calculated following ISTA (1993).

Statistical analysis

Seed morphological data and germination percentage were subjected to one-way analysis of variance after testing for homogeneity of variance and normality (Zar, 1996). The genotype means were further separated and compared using least significant difference test at a 0.05 significance level. The genetic, phenotypic and environmental variance, genotypic, environmental and phenotypic coefficient of variation and heritability for each trait were calculated from one way analysis of variance using formulas formulated by Zobel and Talbert (1991) as shown below:

$$\sigma^2_g = \frac{(MSG - MSE)}{r}$$

$$\sigma^2_p = \frac{(MSG)}{r}$$

$$\sigma^2_e = \left(\frac{MSE}{r}\right)$$

$$h^2 = \frac{\sigma^2_g}{\sigma^2_p}$$

$$PCV = \sqrt{\frac{\sigma^2_p}{\bar{x}}} \times 100$$

$$GCV = \sqrt{\frac{\sigma^2_g}{\bar{x}}} \times 100$$

Where

σ^2_g= Genotypic variance, σ^2_p = phenotypic variance, σ^2_e = environmental variance MSG = mean sum square for genotype, MSE = mean sum square for error and r, is the number of replications, h^2 = narrow sense heritability, PCV = phenotypic coefficient of variation, GCV = genotypic coefficient of variation and $\bar{x}$ = grand mean.

Pearson correlation coefficient for seed traits and among seed traits with geo-climatic conditions of seed source was determined. In order to examine differences and relationship among seed traits and geo-climatic conditions, principal component analysis and cluster analysis were used in Multivariate Statistical Package (MVSP). PCA solutions were accepted when Eigen values were greater than one (Kaisers criterion) and compatible with Cat tells scree rule. Component scores and factor loading were calculated after varimax rotation. Factors equal or greater than 0.7 were considered as defining part of PCA. Hierarchical cluster analysis was used to group provenances. The nearest neighbour method was utilized for classification and Square Euclidean method used as dissimilarity. Discriminant analysis was used to determine the variables responsible for the cluster formation. Dendograms were plotted to determine phylogenetic relationships among the five provenance of *F. albida*.

RESULTS

Morphological variation in seed traits

There were significant differences ($p \leq 0.05$) among provenances in all seed traits measured except seed coat thickness (Table 3). The coefficient of variation (CV) was 30.41 % for seed weight and 12.26% for seed length. Seed thickness and seed width (CV 1.2 and 6.8% respectively) recorded a lower coefficient of variation suggesting minimal environmental effect on expression of these traits. On average, seed length among provenances ranged from 6.73 ± 0.01 (Taveta) to 1.04 ±

Table 4. Genetic variance components (σ^2_g, σ^2_p and σ^2_e), genotypic (GCV) and phenotypic (PCV) coefficient of variation along with heritability (h^2) for seed traits of the five provenances of *F. albida*

Seed traits	σ^2_g	σ^2_p	σ^2_e	GCV	PCV	h^2
Length	0.53	0.57	0.04	75.7	79.1	0.92
Width	0.03	0.03	0	75.72	35.71	0.99
Thickness	0.0003	0.0002	0.0001	4.69	4.54	0.99
Weight	0.02	0.02	0	121.2	122.31	0.99

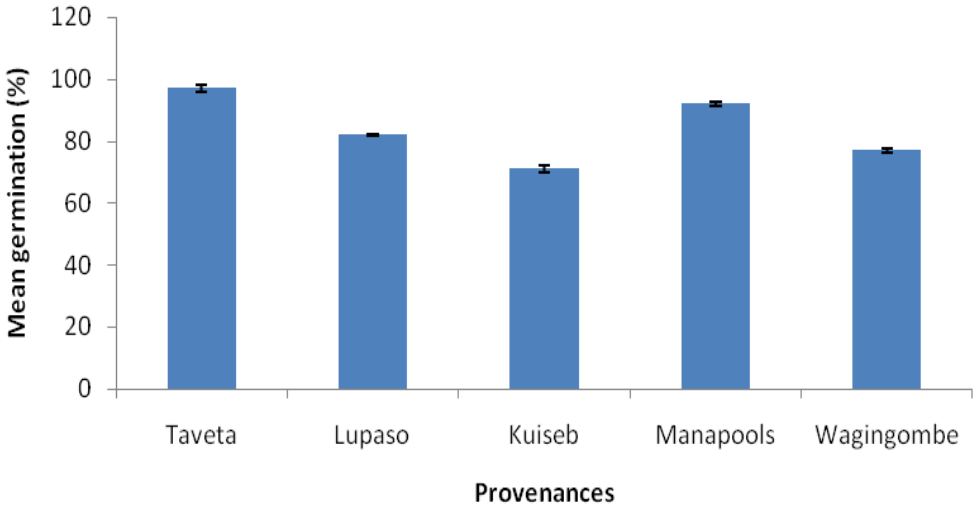

Figure 2. Mean germination percentage of five provenances of *F. albida* at four weeks after sowing.

0.008 mm (Manapools) with a grand mean of 9.11 ± 0.003 mm. The mean seed length for Wangingombe, and Eastern African provenance was higher than Lupaso (southern Africa provenance) though the difference was not statistically significant (P ≤0.05). Southern African provenances recorded higher mean seed length as compared to East Africa provenances. Manapools recorded the maximum seed width 0.60 ± 0.003 mm followed by Kuiseb with 0.58 ± 0.004 mm. Taveta recorded lowest mean seed width as compared to the other provenances; seed width ranged from 0.49 ± 0.003 to 0.60 ± 0.003 mm. Seed weight was smaller in eastern Africa provenances than in southern African provenances. One way analysis of variance of seed thickness separated *F. albida* seeds into two thickness groups ranging from 0.29 to 0.305 mm. Seed thickness did not follow the same pattern as for seed length and seed width.

Genetic variance components

Genetic variance components are represented in Table 4. Seed length recorded the highest variation but the lowest heritability index. Nonetheless, heritability indices ranging from 0.92 to 0.99 suggest that environmental influence on seed characteristics is minimal and hence genetic factors have a lot of influence on the seed traits analyzed. This suggests that selection based on morphological traits can be made with a high degree of confidence.

Seed germination

There were significant differences ($p \leq 0.05$) among provenances in seed germination percentage (Figure 2). Seed germination percentage was highest for Taveta (97%) followed by Manapools (95%) and was lowest in Kuiseb (71%). Mean germination in nursery averaged 83.3% varying from 97 to 71% and was significantly different among provenances ($p \leq 0.05$). Taveta with the smallest seeds recorded the highest seed germination. No significant correlation ($r=0.15$ $p>0.05$) was observed between seed size or seed weight with mean germination.

Correlation among seed traits

Correlation of seed traits showed that seed weight correlated with seed length ($r = 0.494$, $p \leq 0.05$) and seed width ($r = 0.433$, $p \leq 0.05$) (Table 5). Seed length and

Table 5. Mean correlation coefficient (r) between seed traits and germination percentage of *F. albida* provenances

Seed traits	Length	Width	Thickness	Weight
Width	0.597**			
Thickness	0.133**	0.104*		
Weight	0.494**	0.433**	0.094*	
Germination percentage	0.12	0.09	0.04	0.15

*, **Significance at 5 and 1% probability level, respectively.

Table 6. Pearson correlation coefficient (r) for seed trait and geo-climatic condition of the seed origin of the five provenances of *F. albida.*

Variables	Length	Width	Thickness	Weight	Altitude	Rainfall
Width	0.562**					
thickness	-0.054	-0.095				
weight	0.545**	0.527**	-0.053			
altitude	-0.306**	-0.165	0.064	-0.117		
Rainfall	-0.262**	-0.353**	0.139	-0.121	0.219*	
Temperature	-0.435**	-0.371**	0.023	-0.264**	0.248*	0.659**

*, **Significance at 5 and 1% probability level, respectively.

Table 7. Factor loading for each variable on the component of PCA analysis.

Variables	PC1	PC2
Length	**0.964**	-0.21
Width	**0.93**	-0.247
Thickness	**0.921**	0.372
Weight	**0.966**	-0.222
Altitude	0.077	0.618
Rainfall	-0.127	**0.864**
Temperatures	-0.37	**0.756**
Eigen values	3.74	1.99
Percentage of variance	53.35	28.48
Cumulative variance (%)	53.35	81.83

Values in bold (>0.7) are the principal components that explain most of the observed variation.

seed width also showed a significant positive correlation (r=0.597 p≤0.05). Seed thickness showed a weak correlation with seed weight, width and length (r = 0.094, 0.104, and 0.133 p≤ 0.05). No significant correlation was recorded between seed size and seed weight with mean germination percentage.

Correlation between seed traits and geo-climatic conditions of the seed origin

Correlation among seed data and geo-climatic data showed that seed length, width and weight were significantly negatively correlated to altitude, rainfall and temperature of the seed collection zone. Correlations among seed traits with geo-climatic condition of the provenances suggest the possibility of ecoclinal distribution of *F. albida* provenances (Table 6).

Genetic divergence

Genetic divergence of seed traits were analyzed using principal component analysis and cluster analysis. An acceptable solution of component analysis was reached when two dimension models were found to be significant and explained 81.83% of the total variance observed. The total variance was partitioned into the two principal components as: 53.35% of the total variance/variation for the first component (PC1) which was dominated by seed weight, seed width and seed length and seed thickness, 28.48% for the second component (PC 2) defined by rainfall and temperature (Table 7). PCA dendrogram analysis based on squared Euclidean distance for dissimilarity from these three principal components revealed three clusters (Figure 3). Cluster 1 composes of Lupaso, Manapools and Wangingombe which receive high rainfall and temperatures; their seeds have large to moderate sizes and are heavy. Kuiseb and Taveta were placed in separate clusters. Taveta (cluster 2) on the other hand has the smallest and lightest seeds and receive higher rainfall and temperature as compared to Kuiseb (545 mm, 28.1°C annually). Kuiseb in cluster

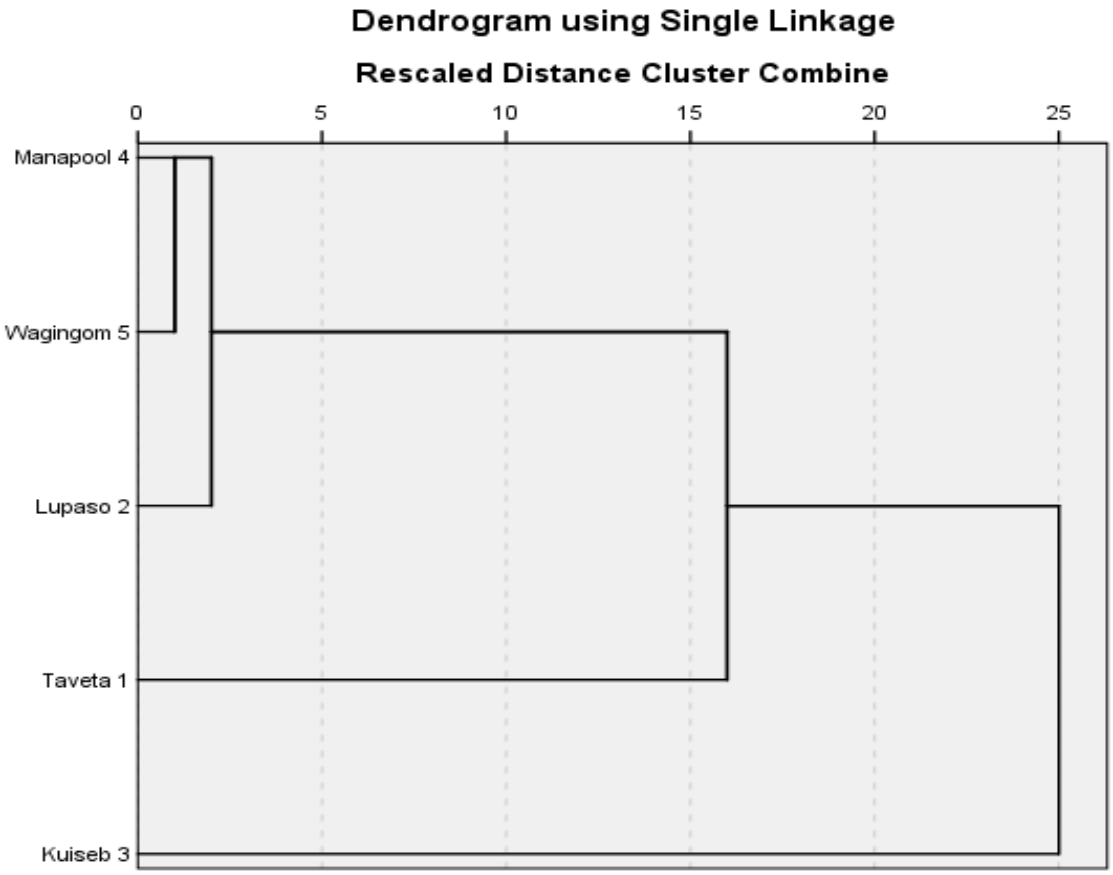

Figure 3. Dendogram for classification of five provenances of *F. albida* based on seed and geo-climatic conditions components from PCA.

three receives extremely low rainfall and temperatures as compared to other provenances under study (<50 mm, 15.2°C annually). Kuiseb has large and heavy seeds.

DISCUSSION

Variation in seed morphology

F. albida exhibited considerable amount of genetic variation in seed morphology, which could be due to its occurrence over a wide range of geo-climatic condition (Joly et al., 1992). Analysis of variance of seed traits showed significant differences, $p<0.05$ indicating high genetic variation among the five provenances of *F. albida* obtained from eastern and southern African provenances. Provenances were not regionally structured, and also showed a higher variability between eastern Africa provenances (Taveta and Wangingombe) than southern African provenances (Manapools, Kuiseb and Lupaso) as revealed by Euclidian distances of the cluster analysis. This could be due to the high variability in environmental conditions within Eastern and Southern Africa regions which is supported by PC2, pointing out that rainfall and temperature have great influence on the phenotypic variation. Ibrahim (1996), Harris et al. (1997) and Dangasuk and Gudu (2000) reported similar results in earlier studies. The variation in seed morphology could be due to interplay of both genetic and varying environmental conditions (interaction among rainfall, temperatures and altitude), exposing the trees to different selective pres-sures, resulting to different degrees of adaptation to climatic conditions (Mathur et al., 1984). The role of genetic and environment in seed morphology is also supported by the correlation between seed trait with geo-climatic conditions of the seed origin. Among all the traits studied, seed weight had the highest coefficient of variation indicating its sensitivity to environmental conditions. In the previous studies of tree species (Gera et al., 2000; Sivakumar et al., 2002; Mkonda et al., 2003), genetic control of seed morphology was observed which is consistent with our finding.

Analysis of genetic components of seed traits

Genetic analysis of seed trait revealed the genetic control of seed traits. The phenotypic variance was greater than

genotypic variance for seed length and seed width which agrees with earlier findings of Jonah et al. (2013), Tanimu and Aliyu (1997) and Tanimu et al. (1990). Genotypic variance was higher than environmental variance except for seed thickness indicating the role of the environment in determining the extent of the seed thickness. The genotypic coefficient of variation was either equal or greater than phenotypic coefficient of variation which shows the contribution of the environment in expression of seed traits. Jonah et al. (2013) and Agbo and Obi (2005) in their study of Bambara groundnut reported similar finding; indicating that the interaction of genetics and environment plays an important role in selection and tree improvement programs. The 100% heritability for seed width, weight and thickness indicates their high response to selection which is supported by the findings of Vanaja and Luckins (2006).

Seed germination

Seed germination percentage varied significantly among provenances, $p<0.05$ and did not follow a regional pattern as was also shown by the morphological traits. Generally, large seeds are expected to have higher seed germination ability than small seeds (Isik, 1986). Seeds from Manapools provenance, having largest seeds had the second highest seed germination percentage after Taveta provenance which had the smallest sized seeds. This deviates from most findings (Vakshasya et al., 1992; Ginwal et al., 1994), except for Fenner (1991) who observed no significant correlation between seed size or seed weight with the seed germination hence caution should be taken in making such decisions based on seed morphology alone. Seed coat thickness was measured without separating it from the seed which may be another factor; however scarification and soaking of scarified seeds in water for 24 h before sowing might address the physical barriers imposed by seed coat thickness. Hence genotypic factors should not be overlooked and must always be taken into consideration (Ibrahim, 1996; Gera et al., 2000; Jayasankar et al., 1999; Mkonda et al., 2003; Sivakumar et al*.,* 2002). This is supported by the high heritability for the various traits studied (low environmental variance), and thus suggest that most of these traits are under genetic control.

Correlations of seed among seed traits and with geo-climatic conditions of seed sources

Seed characters were highly correlated. Correlated traits are of interest to tree breeding because change of one trait leads to simultaneous improvement of the correlated trait. Correlation among seed traits have been documented in tree species for example, Barracosa et al. (2007) and Dangasuk et al. (1997). The significant correlation among seed traits and geo-climatic conditions of the seed source shows that the environments affect the expression of seed traits and a possibility of clinal distribution of *F. albida* provenance under study.

Seed divergence

High rate of environmental variability within Eastern and Southern Africa regions show that species distribution is discontinuous, with barriers to gene flow among populations, leading to isolation of populations. It is there-fore not expected to observe regional based population structure but rather habitat based structure for instance, Lupaso (Malawi), Manapools (Zimbabwe) and Wangingombe (Tanzania) that clustered together from regions receiving high rainfall and temperatures. Comparing the Eastern Africa provenances, Wangingombe has a higher rainfall (760 mm) and also located in a high altitude (1700 m above sea level) as compared to Taveta that is located in low rainfall area (545 mm) and in a lower altitude (760 m asl). Similarly, Kuiseb from the southern Africa region is located in extremely low rainfall area (<50 mm annually), very low temperature (15.2°C) as well as low altitude (400 m asl) as compared to the other two provenances hence its separation into a different cluster. The results of principal component analysis and cluster analysis indicated the role of interaction among different factors in genetic differen-tiation of *F. albida*.

Conclusions

The high heritability for the traits under study show minimal environmental influence on seed characteristics hence genetic factors have a lot of influence on the seed traits analyzed. This suggests that selection based on morphological traits can be made with a high degree of confidence. The variation in the total germination percentage at the nursery level emphasizes the need for wide-range trials to enhance selection of the best provenances for breeding and conservation of *F. albida* genetic resources. The low germination percentage of Kuiseb could be due to absence of ripen pods during collection or poor handling and processing of the seeds, there is need therefore to recollect the seeds with proper handling for fairer assessments.

The current work is a baseline study aimed at identifying useful traits for selection of the best provenances of *F. albida* for tree improvement, breeding and conservation of its genetic resources. More research is needed to provide more information on the identified traits before a general conclusion is made.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Review

Advance research on *Striga* control: A review

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The parasitic weed–(Striga spp.), is a major biotic constraint and a serious threat to subsistence cereal crop (sorghum, maize, pearl millet, finger millet and upland rice) production in sub-Saharan Africa. Severity of the parasitic weed in this area is aggravated by the inherent low soil fertility, recurrent drought and overall natural resource degradation. *Striga hermonthica* (Del.) Benth. and *S. asiatica* (L.) Kuntze are the major biotic constrains to crop production, especially in the non-fertile semi-arid region of Africa, whereas *S. aspera* (Willd.) Benth. and *S. forbesii* Benth. are of lower economic importance. Striga produces numerous minute seeds, which can remain dormant in the soil for as long as up to 20 years. Yield losses due to Striga damage range between 20-80% in Africa but total crop failure is possible in the worst situations. A review of these findings has been discussed for the benefit of poor-resource farmers. Based from these findings, different control measures has been recommended in tackling the negative effects of this weed. Striga can be managed using one or more methods: use of cultural and mechanical control practices, nitrogen fertilizers, push pull technology, biological control practices, resistant host crops, use of herbicides and integrated Striga control methods. However, an integrated Striga management strategies suitable approach, a combined use of two or more control measures, is required to achieve success against this pernicious weed.

Key words: *Striga* spp., host crops, crop losses, control methods.

INTRODUCTION

Striga spp. (witch weed), a root parasitic flowering plant, is common in sub Saharan Africa (SSA) causing severe constraints to crop production. It survives by diverting essential nutrients, which are otherwise taken up by cereal crops such as sorghum (*Sorghum bicolor* [L.]), pearl millet (*Pennisetum glaucum* [L.]), finger millet (*Eleusine coracana* [L.] Gaertn), maize (*Zea mays* [L.]) and upland rice (both *Oryza glaberrima* [Steudel] and *O. sativa* [L.]) (Rodenburg et al., 2006; Atera et al., 2011). These cereals are of utmost significance to African farmers for their home consumption. Underground the weed siphons water and nutrients for its growth, while above the ground, the crop withers and grain yield is reduced (Khan et al., 2007).

'*Striga*' is the Latin word for 'witch'. *Striga* is known as witch weed because plants diseased by *Striga* display stunted growth and an overall drought-like pheno type long before *Striga* plants appear. Some local names to Striga are; in west Kenya, farmers' refer to it as Kayongo (Luo), Oluyongo (Luhya), and Imoto (Teso). In Tanzania it

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is known as Kiduha in Kiswahili and in Ethiopia such as, 'Akenchira', 'Metselem' and others (Fischer, 2006).

Striga species are obligate hemi-parasite plants that attach to the root of their host to obtain water, nutrients and carbohydrates (Van Ast, 2006). Crop yield loss due to *Striga* attacks can vary depending on *Striga* seed density, soil fertility, rainfall distribution, the cereal host species and variety grown.

In total, 25 African countries reported *Striga* infestations in 2005 (De Groote et al., 2008). *Striga* affects the life of more than 100 million people in Africa and causes economic damage equivalent to approximately 1 billion $US per year (Labrada, 2008; Waruru, 2013). It infects important cereal crops such as maize, sorghum, pearl millet, finger millet and upland rice, causing devastating losses in yields in sub-Saharan Africa, thereby limiting food supply in many developing countries (Joel 2000; Scholes and Press 2008). Farmers have reported losses between 20% and 80%, and are eventually forced to abandon highly infested fields (Atera and Itoh, 2011). Grain yield losses even can reach 100% in susceptible cultivars under a high infestation level and drought conditions (Haussmann et al., 2000). According to estimates by Gressel et al. (2004), 17.2 million ha (64% of the total area) of sorghum and pearl millet production in west African are infested with *Striga*.

The infestation area and level are expected to increase in the future, because of continued cereal monoculture in combination with low organic and mineral fertilizer input rates. *Striga hermonthica* (Del.) Benth. and *S. asiatica* (L.) Kuntze are the major biotic constraints to crop production, especially in the non-fertile semi-arid region of Africa, whereas *S. aspera* (Willd.) Benth. and *S. forbesii* Benth are of lower economic importance (Haussmann et al., 2000). Controlling *Striga* has therefore become an huge task considering the seed production rate of 10,000 - 100,000 seed/plant which remain viable in the soil for up to 20 years (Ikie et al., 2006). This can lead to seed shed rates of over 1,000,000 seeds per square meter per year (Kroschel and Müller-Stöver, 2004). This can lead to a rapid buildup of the seed bank in the soil, once fields have been contaminated (Van Mourik et al. 2008).

Research on *Striga* control has been carried for a long time and a wide range of technologies have been developed (Atera et al., 2011). Despite efforts made to control the *Striga* problem, it has persisted and increased in magnitude. Although research on the parasitic weed has a long history, adoption of the control options is limited (Emechebe et al., 2004). This is one of the greatest tests to be addressed by researchers as to why farmers are not embracing the control mechanisms.

There is so need to adopt a farming systems approach for the development and implementation of integrated *Striga* management strategies. The main objective of this review paper is to document recent and alternative options in research findings to *Striga* control methods.

ORIGIN, OCCURRENCE AND DISTRIBUTION OF *STRIGA*

Plants belonging to genus *Striga* (Scrophulariaceae) comprise obligate root parasites of cereal crops that inhibit normal host growth via three processes, competition for nutrients, impairment of photosynthesis (Joel, 2000) and a phytotoxic effect within days of attachment to its hosts (Gurney et al., 2006).

Striga are generally native to semi-arid, tropical areas of Africa, but have been recorded in more than 40 countries (Ejeta, 2007; Vasey et al., 2005). *Striga* possibly originates from a region between the Semien Mountains of Ethiopia and the Nubian Hills of Sudan (Atera and Itoh, 2011). This region is also the birthplace of domesticated sorghum (*Sorghum bicolor* L.).

Approximately 30 *Striga* species have been described and most parasitize grass species (Poaceae). *Striga gesnerioides* (Willd.) Vatke is the only *Striga* species that is virulent to dicots (Mohamed and Musselman, 2008). Among the 23 species of *Striga* prevalent in Africa, *Striga hermonthica* is the most socio-economically important weed in eastern Africa (Gressel et al., 2004; Gethi et al., 2005). Occurrences of *S. hermonthica* have also been reported from south-east Africa. *S. hermonthica* is particularly harmful to sorghum, maize and millet, but is also increasingly being found in sugarcane and rice fields (Atera and Itoh, 2011). Upland rice is becoming more and more important for African agriculture, not least because it can sustain more people per crop area than can maize or sorghum (Atera and Itoh, 2011).

Crops previously unaffected by *Striga* are now showing serious infestation. *Striga* is, therefore, fast becoming a pandemic of serious proportions in Africa because of its vast geographic spread and its economic impact on millions. The enzyme systems of the parasite thrive under low soil fertility and moisture stress conditions, where most soils have been depleted of fertility through removal of organic matter and limited use of manure. It's low fertility in combination with drought induced stress and susceptible host cropping that predisposes the area to *Striga* (Fasil, 2002).

STRIGA CONTROL METHODS

The most and the recent control methods of *Striga* seem as follows:

Cultural and mechanical control methods

A number of cultural practices have been recommended for *Striga* control such as crop rotation (Oswald and Ransom, 2001); intercropping (Udom et al., 2007); transplanting (Oswald et al., 2001); soil and water management (Fasil and Verkleij, 2007); use of fertilizers (Jamil et al., 2011); and hand weeding (Ransom 2000) to

reduce the production of further *Striga* seed. These methods should also reduce the density of *Striga* seeds already in the soil seed bank (Fasil and Verkleij, 2007). Some of these practices improve soil fertility, which will stimulate the growth of the host but also adversely affects germination, attachment and subsequent development of the juvenile *Striga* plants (Fasil and Verkleij, 2007). However, this approach has only limited success for small-scale farmers, largely due to socio-economic and financial constraints.

Hand-weeding and Sanitation

Today the most used control method against *Striga* is hand weeding. It is recommended to prevent seed set and seed dispersal. Weeding the small *Striga* plants is a tedious task and may not increase the yield of already infected plants, it is necessary to prevent seed production and reinfestation of the soil. Due to high labour costs in repeated hand-pulling of *Striga*, it is recommended that hand pulling should not begin until 2-3 weeks after *S. hermonthica* begins to flower to prevent seeding (Parker and Riches, 1993). New shoots may sprout out below the soil from infected plants requiring a second weeding before crop maturity. Sanitation consists of taking care to note infested areas and to isolate them. Seeds in the soil can be spread by wind, rainwater, plowing, and soil on tools or root crops. Seed pods on *Striga* plants attached to maize or sorghum plants pulled for forage will infest manure and feeding areas (Parker and Riches, 1993). Crop stubble should also be uprooted or burned to prevent the continued growth and seeding of the parasite (Ramalah, Parker, Vasudeva, and Musselman, 1983). This weed competes for water and nutrients as a root parasite. In so doing, crop growth is stunted and yields are generally reduced (Ayongwa et al., 2010).

It is not practical to hand weed dense infestations, and weeding is often ineffective, particularly since it is time consuming and labor-intensive (Parker and Riches, 1993). It is practical, at a low level of infestation before *Striga* flowers and in combination with herbicides or fertilizer.

Crop rotation

Crop rotation of infested land with non-susceptible crops or fallowing is theoretically the simplest solution. Rotation with non-host crops interrupts further production of *Striga* seed and leads to decline in the seed population in the soil. The practical limitations of this technique is required more than three years for rotation. The choice of rotational crop should therefore be based 1st on its suitability to the local conditions and only secondarily on its potential as a trap crop (Parker and Riches, 1993).

Rotating the infested maize or sorghum areas to wheat/barley, pulses, or groundnuts are viable and effective options in Ethiopia. In Ethiopia two years of cropping to a non-host was reported to reduce *Striga* infestation by 50% (Shank, 2002). In the Sahel, the results of a four year experiment in bush fields indicated that one season cowpea in 1998, had a positive effect on subsequent millet grain yields, soil organic carbon and nitrogen, and reduced *Striga* infestation. The increase in yields due to millet-cowpea rotation was 37% in 1999 compared to three to five years continuous millet cropping (Samake, 2003). However small-holder farmers desiring to maximize the grain production potential of their land may be difficult to be persuaded to grow other crops. Practical control measures are effective when a combined program of crop rotation, weeding, sanitation and, resistant varieties is included.

Trap crops and catch crops

Trap crops: Trap-crops cause suicidal germination of the weed, which reduces the seed bank in the soil. Some varieties of cowpea, groundnut and soybean have potential to cause suicidal germination of *S. hermonthica* and improve soil fertility (Carsky et al; 2000; Schulz et al., 2003).

The use of trap crops such as soybean causes suicidal germination of the *Striga* seedlings which do not attack the soybean consequently; the *Striga* is ploughed off before flowering thereby reducing the seed density of *Striga* in the soil (Umba et al., 1999). In IITA, about 40 lines of soybean were screened for their ability to induce *Striga hermonthica* seeds to germinate using the cut roots of soybean plants. The results showed variability among the soybean lines in their ability to stimulate seed germination. Hess and Dodo (2003) also found that the use of leguminous trap crops that include varieties of groundnut (*Arachis hypogaea*), soybean (*Glycine max*), cowpea (*Vigna unguiculata*), and sesame (*Sesamum indicum*) stimulate the suicidal germination of *Striga* is another technology to control *Striga*. De Groote et al. (2010) found that soybean triggers suicidal germination of *Striga* and reduces the *Striga* seed bank in the soil when intercropped with maize.

Catch crops: Catch crops are planted to stimulate a high percentage of the parasite seeds to germinate but are destroyed or harvested before the parasite can reproduce. A thick planting of Sudan grass at 20-25 kg seed per hectare should be sown and either ploughed in or harvested for forage at 6-8 weeks before *Striga* seeds. The main crop could then be planted during the main rains (Parker and Riches, 1993). From the available studies, it can be concluded that trap crops should be cultivated for at least three consecutive years in order

to reduce parasite seeds (Esilaba and Ransom, 1997).

Pasture legumes; *Mucuna gigantica, Stylosanthes guyanensis* and *Desmodium* spp. were investigated for their ability to induce germination of conditioned *S. hermonthica* seed, for their effect on *Striga* attachment and on *Striga* shoot emergence. Laboratory experiments showed that the root exudates of the legumes stimulated up to 70% more *Striga* seeds to germinate than exudates of maize. Maize-Mucuna combination had the highest number of attachments while all other combinations and maize planted in pure stand had lower numbers of attached *Striga*. Cowpea varieties, cv. Blackeye bean and cv. TVU 1977 OD, produced potent exudates, which were highly compatible with sorghum as intercrops in field trials (Fasil, 2002).

In other research findings also reported the effectiveness of the combined use of trap-cropping, fertilization and host plant resistance to control *S. hermonthica* (IITA, 2002; Tesso, et al., 2007).

Intercropping

Intercropping cereals with legumes and other crops is a common practice in most areas of Africa, and has been reported as influencing *Striga* infestation. Intercropping is a potentially viable, low-cost technology, which would enable to address the two important and interrelated problems of low soil fertility and *Striga* (Fasil, 2002). Growing of sorghum in association with cowpea and haricot bean was effective against *S. hermonthica* and produced significantly improved yield per unit area in preliminary trials in Ethiopia. Intercropping had rather detrimental effect on yield performance of sorghum and showed two cowpea varieties - cv. TVU 1977 OD and cv. Blackeye bean produced the highest supplemental yield of up to 329 and 623 kg ha^{-1} grain and 608 and 1173 kg ha^{-1} biomass at Adibakel and Sheraro (Tigray, Ethiopia) in 1999 and 2000, respectively (Fasil, 2002).

Recent result shows that intercropping maize with cowpea and sweet potato can significantly reduce the emergence of *Striga* in Kenya (Oswald et al., 2002). In Kenya, more recently, it was discovered that inhibition of *Striga hermonthica*, was significantly greater in maize-silver leaf [*Desmodium uncinatum* (Jacq.) DC.] intercrop than that observed with other legumes, for example, sun hemp (*Crotolaria* spp.), soybean or cowpea (Khan *et al.* 2000). Consequently, the yield of maize was significantly increased by two tons per ha. *Disodium* species are legumes that can easily be controlled by regular cutting in order to avoid or minimize the competition with the crop if any.

The mechanisms by which *D. uncinatum* reduce *Striga* infestation in intercropping was found to be the allelopathic effect inhibiting the development of haustoria of *Striga* (Khan *et al.* 2001). Identification of the compounds released from *D. uncinatum* involved in the suppression of the parasite may give more exploitation for developing reliable intercropping strategies, as well as new approaches for molecular biology in *S. hermonthica* (Gressel, 2000).

According to Khan et al. (2007), intercropping different legumes with maize and sorghum helps reduce *Striga* but does not eliminate the weed. This explains why, in spite of most farmers intercropping cereals with legumes as the dominant cropping system in western Kenya, *Striga* infestation is still high in most fields. A variant of intercropping system dubbed “push-pull” where Desmodium spp. is intercropped with cereals with an edge of fodder crops is effective in *Striga* management. There is therefore need to combine more than one strategy to improve the effectiveness of existing control strategies (Ejeta and Gressel, 2007).

Soil fertility

Nitrogen and phosphorus deficiency as well as water stress accentuate the severity of *Striga* damage to the hosts. *Striga* is particularly a pest of low fertile soil and usually the infection decreases if mineral nutrients, especially nitrogen and phosphorus, are applied in sufficient quantities (Adagba et al., 2002).

Fertilizer application had significant effect on height, vigour score, reaction score of sorghum as well as shoot count, days to emergence, dry matter of production and dry weight of *Striga*. The application of high nitrogen (N) increases the performance of cereal crops under *Striga* infestation. This is due to the fact of that nitrogen reduced the severity of *Striga* attack while simultaneously increasingly the host performance (Lagoke and Isah, 2010).

Results of an experiment, designed to develop integrated nutrient management strategy, confirmed that the combined use of 41 kg N/ha and 30 t/ha of manure could lead to significant reduction in infestation and considerable increase in sorghum yield (Esilaba et al., 2000). Esilaba et al. (2000) and Gacheru and Rao (2001) also found that increasing soil fertility not only stimulates the growth of the host but also adversely affects longevity of the seeds in the soil, germination and attachment.

Shank (2002), has been noted in western countries that host plant shading can restrict *Striga* growth when generous soil fertilizer is applied Table 1.

Application of high dosage of nitrogen fertilizer is generally beneficial in delaying *Striga* emergence and obtaining stronger crop growth (Dugje et al., 2008). Also other advantageous effects of fertilizers include increasing soil nitrogen and other nutrients, replenishing the organic matter of the soil and increasing soil moisture holding capacity (Ikie et al., 2006).

‘Push–pull’ technology

The ‘push-pull’, as a tool in integrated pest management,

Table 1. Effect of soil fertility level on *Striga* growth and plant characters of 4 maize hybrids in Nigeria.

NPK % of recommended fertilizer	No of Striga plants/m of row[1]	No of Striga seed capsules /plant[1]	Maize plant height (cm) Res/Sus[1]	Grain Yield g/plant[1]
0	150	12	102/53	10
30	102	54	103/65	17
50	85	33	124/75	13
100	23	6	146/119	36

Source: Shank (2002).

first conceived by Pyke et al. (1987), and later formalized by Miller and Cowles (1990), involves use of behaviour-modifying stimuli to manipulate the distribution and abundance of a pest and/or beneficial insects for management of the pest (Cook et al., 2007). This technology was first developed to control stem borers but was later found to also suppress *Striga* weed in the field depending on which push component the main crop has been intercropped. In a 'push–pull' strategy, pests are repelled or deterred away from the target crop (push) by stimuli that mask host appearancy. The pests are simultaneously attracted (pull) to a trap crop where they are concentrated, leaving the target crop protected (Cook et al., 2007; Hassanali et al., 2008).

Desmodium is extremely effective in controlling *Striga*, resulting in significant yield increases in maize from 1 to 3.5 ton/ha per cropping season (Khan et al., 2008a) and improving farm productivity (Khan et. al. 2008b). In addition to benefits derived from increased availability of nitrogen, an allelopathic effect of the root exudates of desmodium is responsible for the dramatic reduction in *Striga* infestation (Khan et al., 2002). Secondary metabolites with *Striga* seed germination stimulatory and post-germination inhibitory activities are present in the root exudates of *D. uncinatum*, which directly interferes with parasitism (Khan et al., 2008c). This combination thus provides a novel means of *in situ* reduction of the *Striga* seed bank in the soil through efficient suicidal germination even in the presence of cereal hosts in the proximity (Khan et al., 2008c; Hooper et al., 2009). Other Desmodium spp. have also been evaluated and demonstrated similar effects on *Striga* (Khan et al., 2006a) and have been incorporated as intercrops in maize (Khan et al., 2007), sorghum (Khan et al., 2006b), millet (Midega et al., 2010) and rice (Pickett et al., 2010).

Desmodium also fixes atmospheric nitrogen (110 kg N/ha), adds organic matter to the soil, conserves soil moisture and enhances soil biodiversity, thereby improving soil health and fertility, which directly contribute to *Striga* control. Additionally, it provides ground cover and, together with surrounding Napier grass, protects the soil against erosion (Khan et al., 2006a).

It therefore improves agro-ecosystem sustainability, resilience, and has great potential to mitigate the effects of climate change. Both Desmodium and Napier grass provide valuable year-round quality animal forage whilst the sale of Desmodium seeds generates additional income for the farmers (Khan et al., 2008b). There are significantly higher returns to land and labor and overall gross benefits from this technology than from conventional farmer practices (Khan et al., 2008b) and other soil and *Striga* management practices (De Groote et al., 2009).

Desmodium has also been reported to have additional soil improvements such as; increasing of soil nitrogen, organic matter and conserving moisture (Khan et al. 2006). The 'push-pull', technology described involves intercropping maize with a repellent plant such as desmodium, *Desmodium uncinatum* Jacq., and planting an attractive trap plant such as *Napier grass*, *Pennisetum purpureum* Schumach, as a border crop around this intercrop. Gravid stem borer females are repelled from the main crop and are simultaneously attracted to the trap crop (Khan et al., 2000, 2001; Cook et al., 2007).

The technology, so far the most effective and indeed the only 'push–pull' strategy in practice by farmers (Cook et al., 2007; Hassanali et al., 2008), also enhances productivity of maize-based farming systems through *in situ* suppression and elimination of *Striga, S. hermonthica* (Khan et al., 2000, 2001, 2002). According to a study done by Khan (2010), push-pull technology helps controlling both *Striga* and stem borers with at least 2 tons per hectare higher grain yield. The technology is currently being disseminated among smallholder farmers in eastern Africa and adoption rates are rising.

Biological control method

The objective of weed biological control is not the eradication of weeds but the reduction and establishment of a weed population to a level below the economic threshold (Rajni and Mukerji, 2000). Means of biological control of weeds comprise herbivorous insects, microorganisms (especially fungi), and smother plants (Sauerborn and Kroschel, 1996). The method, involves importation, colonization, and establishment of exotic natural enemies, which include predators and parasitoids.

Efforts to manage weeds using biological control have been gaining momentum throughout the world, especially in the recent past (Delfosse, 2004). Biological control is

considered as a potential cost-effective, safe and environmentally beneficial alternative mean of reducing weed populations in crops, forests or rangelands (Charudattan, 2001). Disadvantages of weed biological controls include it will usually require a long period (5 to 10) years of research and a high initial investment of capital and human resources (Culliney, 2005). Biological control is unattractive as a private entrepreneurial effort (Hill and Greathead, 2000; Coombs et al., 2004).

This is because the intensive use of chemical herbicides came under scrutiny due to several areas of concern, which include the development of herbicide resistant or tolerant weeds and environmental contaminations, comprehending effects on non-target organisms as well as the pollution of soil, underground water and food. Strong public criticism due to health concerns arose from such contaminations (Green et al., 1998). These limitations of chemical herbicides encouraged researchers to look for alternative systems of weed control.

Biological control using insects

The insects that attack *Striga* can be classified according to their damage as defoliators such as *Junonia spp*., gall forming as *Smicronyx spp*. (Coleoptera: Curculionidae) in India and Africa; shoot borers as *Apanteles sp*., miners as *Ophiomyia strigalis*, Spencer (Diptera: Agromyzidae) in East Africa; inflorescence feeders as *Stenoptilodes taprobanes* and fruit feeders as *Eulocastra spp*. (Lepidoptera: Noctuiidae) in India; (Kroschel et al., 1999).

In the 1990s, studies in Burkina Faso and Northern Ghana have been carried-out by Jost et al. (1996) and Traoré et al. (1996) to investigate the potential of the weevils *Smicronyx guineanus* and *Smicronyx umbrinus* and the butterfly *Junonia orithya* as biocontrol agents for *Striga*. As a result of Smicronyx infestation the *Striga* seed production was reduced by 17.4% on the average (Kroschel et al., 1999).

Kroschel et al. (1999) have been concluded that the use of herbivorous insects could play a role in an integrated control package, lowering the *Striga* population by reducing its reproduction capabilities and spread. However, the augmentation of native insect populations through inundative releases is not applicable in the third world, mainly due to the infeasibility of mass rearing.

Biological control using pathogens

Most organisms have natural enemies that balance their populations, avoiding excessive abundance (Templeton, 1982). Biological control of *S. hermonthica* using *Fusarium oxysporum* is considered as one of the novel management strategies (Sauerborn et al., 2007). Fungi are preferred to other microorganisms as bio-herbicides because they are usually host specific, highly aggressive, and easy to mass produce and are genetically diverse (Ciotola et al., 2000). Field and laboratory tests showed that *F. oxysporum* is highly effective in hindering germination, growth and development of Striga and thus may lead to reduction of Striga seed bank in the soil (Ciotola et al., 2003).

Extensive surveys in Burkina Faso, Mali and Niger also demonstrated the occurrence of highly pathogenic and *Striga* specific isolates of *F. oxysporum* (Ciotola et al., 2000). Among this isolate virulent isolate of *F. oxysproum* M12-4A provided more than 90% control of *Striga*, and a three-fold increase in sorghum biomass (Ciotola et al., 1996). The use of a myco-herbicide, that is *F. oxysporum* coated seeds and host plant resistance reportedly reduced *Striga* emergence by 95% and increased sorghum yield by 50% (Franke et al., 2006).

Recent findings indicated the effectiveness of integrated use of *F. oxysporum* compatible and *Striga* resistant sorghum genotypes to control *Striga* in Ethiopia (Rebeka et al., 2013). To realize the full potential of this approach it is important to recombine traits of Fusarium compatible and *Striga* resistant sorghum lines. This would allow continued selection of targeted progenies with combined resistance and Fusarium compatibility and for subsequent seed treatment of suitable hybrid(s) for direct use. Thus effective *Striga* control would be possible through synergistic effect of biocontrol and host resistance.

Recently, the combined application of two or more control measures has been promoted for effective *Striga* management. The use of bio-control agent such as virulent isolate of *F. oxysporum* f.sp. strigae as a component of integrated *Striga* management was identified to have several advantages (Ciotola et al., 2000; Fen et al., 2007). Marley et al. (2004) and Schaub et al. (2006) also found that the application of integrated *Striga* management package combining a mycoherbicide based on *F. oxysporum* isolate and host plant resistance has been demonstrated on farmers fields as effective *Striga* control approach. There is other agreed combined use of resistant varieties with the application of *Fusarium oxysporum* as pest granules or as a seed coating was reported to be effective to controlling *Striga* (Marley et al., 2004; Julien et al., 2009).

Various *Fusarium spp*. and *vesicular arbuscular mycorrhizal* (VAM) fungi have been found which can reduce *Striga* infestations significantly on sorghum and maize when used together with resistant host (Ciotola et al., 2000; Lendzemo et al., 2005; Franke et al., 2006). These control options when applied individually are not effective and sometimes affected by environmental conditions. Therefore the use of *F. oxysporum* in combination with other cost effective control methods may provide an effective and sustainable control option for subsistence farmers.

However, integrated *Striga* management approach relies

on the use of resistant host genotypes and *Striga* pathogenic *F. oxysporum* application to control *S. hermonthica* emergence and growth lead to effective results (Hearne, 2009; Julien et al., 2009).

Chemical control method

Germination stimulants

Certain chemicals such as ethylene, ethephon, strigol and strigol analogues can induce germination of *Striga* seeds in the absence of a suitable host and therefore seed reserves in the soil (Esilaba and Ransom, 1997). In dicotyledonous plant species there is evidence that the production of strigolactone by the host plant could be reduced if sufficient minerals are available (Lopez-Raez et al. 2008).

Pre emergence herbicides

Technology currently being deployed as a complement to *Striga* resistance in maize involves use of herbicide as a seed coating. The parasite competes with its host for resources; changes host plant architecture and reduce the photosynthetic rate and the water use efficiency of the host (Watling and Press, 2001). This has led to the emergence of a new technology known as imazapyr-resistant maize (IRM) which has proven to be efficient for *Striga* control (Kanampiu et al., 2006; De Groote et al., 2006). The International Maize and Wheat Improvement Center (CIMMYT), Badische Anlin and Soda Fabrik (BASF), African Agricultural Technology Foundation (AATF) and other stakeholders have made efforts in bringing imazapyr-resistant maize (IRM) technology to farmers as assistance for *Striga* control.

Result of experiments also proved that herbicide seed treatment using imazapyr appears to be a promising approach for the control of *Striga* in maize or sorghum (Dembele et al., 2005). Ndung'u (2009) has also reported coating sorghum seed with herbicide reduced *Striga* infestation, *Striga* flowering and *Striga* seed set, and it is considered as the most effective approach as it does not affect sorghum biomass.

Research on-farm trials in Kenya and Tanzania indicate that seed dressing with Imazapyr and Pyrithiobac offers good *Striga* control and increased maize yields (Kanampiu et al., 2004). IR maize has been used in controlling *Striga* but is toxic to all other crops that do not have resistance to imazapyr herbicide, therefore not very suitable in mixed cropping systems. Many herbicides are useful in preventing the build-up of *Striga* seeds in the soil but may not prevent damage prior to their emergence (Kanampiu et al., 2003). The sustainability of many technologies will only be maintained when integrated with other technologies.

Post emergence herbicides

Herbicides tested for the selective control of *Striga* mostly acts through the foliage, although some have soil residual effects. Among the herbicides tested, 2, 4-D has been the most selective and is the cheapest. 2-methyl-4-chlorophenoxyacetic acid (MCPA), a compound closely related to 2, 4-D, has also been effective especially when mixed with bromoxynil (Ejeta et al., 1996). Post emergence application of 2,4-D (1 L product/ha), Glufosinate (2 L product/ha) and Oxyflourfen (1 L product/ha) was effective in preventing the top growth of *Striga.* Unfortunately, most of those products had narrow window of application and the only safe treatment for the crop was targeted spray of 2,4-D (Fasil, 2004). Babiker et al. (1996) reported that a combination of urea and dicamba effectively controlled *Striga* between 62-92% on sorghum, while chlorsulfuron in combination with dicamba controlled *Striga* as much as 77-100% on sorghum. However, results of the experiments showed that pre and post emergence herbicides do not prevent crop yield loss, because they cause their impact after *Striga* has already attached and damaged the host.

Research efforts on the identification of systemic herbicides, which could ideally translocate through the host crop to prevent initial stages of parasite development, were not successful. So Research efforts should therefore be directed towards identifying herbicides that persist in the soil, allowing the germination of *Striga* seeds but killing the seedlings before attachment to the host. Herbicides must also be compatible with the mixed cropping systems practiced by farmers and be profitable to use with low initial capital outlay.

Host plant resistance

Host plant resistance would probably be the most feasible and potential method for parasitic weed control. Using biotechnological approaches (including biochemistry, tissue culture, plant genetics and breeding, and molecular biology) significant progress has been made in developing screening methodologies and new laboratory assays, leading to the identification of better sources of parasitic weed host resistance (Ejeta et al., 2000; Haussmann et al., 2000; Omanya, 2001). It is potentially an acceptable *Striga* control option to resource-poor farmers (Gurney et al., 2003; Rich et al., 2004). However, reliance on host resistance alone is not ideal because so far complete resistance against *Striga* cannot be attained through breeding (Gurney et al., 2002), and usually the newly developed varieties may not fulfill farmers preference traits (Adugna, 2007).

Reports of genetic resistance to *Striga* have been documented in rice (Bennetzen et al., 2000; Gurney et al., 2006), sorghum (*Sorghum bicolor*) (Haussmann et

Table 2. Striga tolerant and resistant maize varieties developed and released in Nigeria.

Release name	Year of release	Hybrid/ OPV	Maturity range	Suitable agro-ecologies	Grain yield	Additional traits/remarks
Oba Super 7	2009	Hybrid	Medium-late	Moist savannas	High	Striga-resistant
Oba Super 9	2009	Hybrid	Medium-late	Moist savannas	High	Striga-resistant
Sammaz 15	2008	OPV	Medium-late	Moist savannas	High	Striga-tolerant with good nitrogen use efficiency
Sammaz 18	2009	OPV	Early	Guinea and Sudan Savanna	High	Striga-tolerant
Sammaz 19	2009	OPV	Medium-late	Moist savannas	High	Striga-tolerant
Sammaz 20	2009	OPV	Early	Guinea and Sudan Savanna	High	Striga-tolerant
Sammaz 26	2009	OPV	Medium-late	Moist savannas	High	Striga-tolerant
Sammaz 27	2009	OPV	Early	Guinea and Sudan Savanna	High	Striga-tolerant
Sammaz 28	2009	OPV	Extra-early	Guinea and Sudan Savanna	Medium	Striga-tolerant
Sammaz 29	2009	OPV	Extra-early	Guinea and Sudan Savanna	Medium	Striga-tolerant
Sammaz 32	2011	OPV	Extra-early	Guinea and Sudan Savanna	Medium	Striga-tolerant, drought escaping and QPM
Sammaz 33	2011	OPV	Extra-early	Guinea and Sudan Savanna	Medium	Striga-tolerant, drought escaping and QPM
Sammaz 34	2011	OPV	Early	Guinea and Sudan Savanna	High	Multiple cob bearing
Sammaz 35	2011	OPV	Early	Guinea and Sudan Savanna	High	Striga-tolerant
Sammaz 38	2011	OPV	Extra-early	Guinea and Sudan Savanna	Medium	Striga-resistant and QPM
Ifehybrid 5	2013	hybrid	Extra-early	Guinea and Sudan Savanna	High	Low soil nitrogen-tolerant, Striga-resistant, single-cross
Ifehybrid 6	2013	hybrid	Extra-early	Guinea and Sudan Savanna	High	Low soil nitrogen-tolerant, Striga-resistant, top-cross

Source: Prof. S. G. Ado shehuga@gmail.com, shehuado@hotmail.com

al., 2004; Mohamed et al., 2003; Rich et al., 2004), cowpea (Riopel and Timko, 1995) and maize (Adetimirin et al., 2000; Menkir, 2006). Identifying source germplasm with different resistance mechanisms can facilitate combining several resistance genes to obtain more durable and stable polygenic resistance to Striga in cereals (Ejeta et al., 2000; Menkir, 2006). Various molecular markers are also available for genetic analysis such as restriction fragment length polymorphisms (RFLPs) (Perumal et al., 2007), random amplification of polymorphic DNAs (RAPD) (Agrama and Tuinstra, 2003), amplified fragment length polymorphisms (AFLP) (Perumal et al., 2007), microsatellites or simple sequence repeats (SSRs) (Ganapathy et al., 2012) and single nucleotide polymorphisms (SNPs) (Arai-kichise et al., 2011). Various studies have reported combined use of phenotypic and molecular markers in genetic analyses of cereals such as ryegrass (Jianyang, 2005), rice (Ogunbayo et al., 2005), maize (Beyene et al., 2005; Wende et al., 2012), and sorghum (Agrama and Tuinstra, 2003; Anas and Tomohiko, 2004; Bucheyeki et al., 2009).

The use of resistant varieties has been highlighted as the most effective and environmentally sound method for the control of *Striga*. This has been demonstrated in multi-location field tests conducted in Ethiopia and Tanzania (Mbuwaga et al., 2007; Tesso et al., 2007). The International Institute for Tropical Agriculture (IITA) has released *Striga* resistant, drought-tolerant, and low soil nitrogen-tolerant extra-early maturing white maize varieties in Nigeria (Table 2).

There is also *Striga* resistant/tolerant maize hybrids and varieties released in West Africa are shown in Table 3.

Recognizing that improved cultivars of cowpea for West Africa incorporate resistance to both parasites (*S. gesnerioides* and *A. vogelii*), IITA developed cultivars with individual and dual parasite resistance. Several *Striga* and Alectra resistant varieties have been released in Africa. The variety, IT97K-499-35, has been adopted by

Table 3. Striga tolerant and resistant maize varieties developed and released in W. Africa.

Variety name	IITA designation	Types of cultivars	Country	Year of release	Adaptation zone
SAMMAZ11	Aer 97 TZL Comp 1-W	Striga tolerant late maturing OPV	Nigeria	2001	Moist savannas
SAMMAZ28	99TZEE-Y-STR	Extra-early Striga tolerant OPV	Nigeria	2001	Sudan Savannas
SAMMAZ29	2000SynEE-W-STR	Extra-early Striga tolerant OPV	Nigeria	2001	Sudan Savannas
SAMMAZ21	TZE Comp 5-W	Striga tolerant early maturing OPV	Nigeria	2001	Moist savannas and Sudan Savannas
SAMMAZ27	EV99DT-W-STR	Early drought and Striga tolerant OPV	Nigeria	2001	Moist savannas and Sudan Savannas
EV97DT-W- STR	TZE-W Pop DT STR C3	Early drought and Striga tolerant OPV	Benin Mali	2006 2008	Moist savannas and Sudan Savannas
SAMMAZ15	IWDC2SynF2	Striga tolerant medium maturing OPV	Nigeria	2008	Moist savannas
SAMMAZ16	TZLComp1SynW-1	Striga resistant late maturing OPV	Nigeria	2008	Moist savannas
Oba Super 7	H05-01STR	Stirga resistant hybrid	Nigeria	2009	Moist savannas
Oba Super 9	H05-02STR	Striga resistant hybrid	Nigeria	2009	Moist savannas

Source: Menkir, et.al. (2009). IITA.

approximately 600,000 farmers in northeastern Nigeria (Amaza et al., 2009). Improved varieties have better yields (1-2 ton/ha) than local farmers control (0.3-0.5 ton/ha). In rice, *Oryza glaberrima* lines 'ACC102196', 'Makassa', and 'IG 10', as well as *O. sativa* lines 'IR49255-BB-5-2' and IR47255-BB-5-4' showed partial resistance to *S. aspera* and *S. hermonthica* under field conditions in Cote d'Ivoire (Johnson *et al,* 2000).

More than 80 resistant sorghum lines have been selected by the International Center for Dryland Research (ICRSAT) in India. Recently, of these, some high yielding *Striga* resistant sorghum and millets varieties have been made by the Ethiopia Institute of Agriculture Research at Nazreth, and introduced and registered in the country Ethiopia (Table 4) (Adugna, 2007; Ejeta, 2007). These varieties when deployed along with moisture conservation practices and soil amendment inputs can dramatically reduce *Striga* infestation and increased sorghum yield by up to 400%. However, adoption of these varieties has been slow primarily due to the introduced germplasm do not fulfill farmers preferred traits (Adugna, 2007), and lack of effective seed production and delivery mechanism. Purdue University in USA also identified two sorghum varieties: P9401 and P9403 have been recommended for full commercial production. These varieties combine excellent grain quality and drought tolerance. They have been highly preferred by Ethiopian farmers. They were named Gubiye (P9401) and "Abshir (P9403) that are resistant or tolerant to *Striga*.

Hiriray, Higretay and Korokora are Ethiopian maize varieties that are resistant due to their early maturing characters, which is an escape mechanism against the infestation of *Striga* (Kidane et al., 2004). Promising results were also obtained in sorghum when both traits, *Striga* and drought resistance, were combined by classical breeding.

Basically the resistant varieties were low yielding and not desirable in other agronomic characteristics. However, integrating genetic resistance with other control measures is the smartest option possible both for effectiveness of control as well as for increasing durability of resistance genes (Ejeta, 2007).

Integrated Striga management

Striga has a high fecundity, it uses the host plants nutrients and the seed is asynchronous. These characteristics make the weed difficult to control (Andrianjaka et al., 2007). It is also difficult to control effectively because most of its damage to the host plant occurs underground before the parasitic plant emerges (Rich et al., 2004). The rate of infestation needs therefore to be managed through different control methods. Today there are several control options have been recommended to reduce *Striga* damage such as the use of resistant cultivars, crop rotation, intercropping with

Table 4. Introduced exotic sorghum and millets varieties released/registered in Ethiopia.

Crop	Variety name	Original name	Year of release/ registration	Source	Specific character
Sorghum	Dinkmash 86	ICSV 1	1986	ICRISAT	Early
Sorghum	Melkamash 79	Diallel Pop 7-682	1979	ICRISAT	
Sorghum	Kobomash 76	NES-830x705	1976	ICRISAT	
Sorghum	Seredo	Seredo	1986	ICRISAT	
Sorghum	76T1#14	76T1#14	1979	ICRISAT	
Sorghum	76T1#19	76T1#14	1976	ICRISAT	
Sorghum	76T1#23	76T1#23	1976	ICRISAT	Early
Sorghum	76T4#416	76T4#416	1976	ICRISAT	
Sorghum	Meko	M36121	2000	ICRISAT	Good food making quality
Sorghum	Teshale	3443-2-OP	2002	ICRISAT	
Sorghum	Gubiye	P9401	2000	Purdue University	Striga resistant
Sorghum	Abshir	P9403	2000	Purdue University	Striga resistant
Sorghum	Birhan	PSL5061	2002	Purdue University	Striga resistant
Sorghum	IS9302	IS9302	1986	ICRISAT	Adapted to mid altitude areas
Sorghum	IS9323	IS9323	1986	ICRISAT	
Sorghum	Red Swazi	Red Swazi	2007	ICRISAT	Early, malt sorghum variety
Sorghum	Macia	Macia	2007	ICRISAT	Malt sorghum variety
Sorghum	Yeju	ICSV 111Inc	2002	ICRISAT	
Sorghum	Hormat	ICSV 1112BF	2005	ICRISAT	Striga resistant
Finger millet	Tadesse	KNE#1098	1998	EARSAM	Good threshing quality and wide adaptation
Finger millet	Padet	KNE#409	1998	EARSAM	
Finger millet	Boneya	KNE#4011	2002	EARSAM	
Finger millet	Kola-1	ICMV 221	2007	ICRISAT	

Adugna, 2007.

pulse crops, late planting, deep planting, using trap crops, use of organic and inorganic fertilizers, herbicides, and biological control (Hearne, 2008). Although the level of *Striga* infestation and damage is increasing, farmers rarely adopt *Striga* control methods either due to limitations associated with the technology itself, access and costs of the technology or due to lack of information about available technology options (Oswald, 2005; Hearne, 2008). Furthermore, available options when applied individually are not effective and sometimes affected by environmental conditions.

Integration of weeding with high urea application, appropriate sowing date, and effective control of weeds which may serve as alternative hosts, will further enhance the long-term control of *Striga* (Fasil, 2002). Combined use of row planting, fertilizers and hand pulling (during flowering) registered 48% higher grain yield and over 50% reduction in *Striga* shoot counts compared to the farmer's practice at Adibakel (Table 5), in Tigray, Ethiopia (East Africa). However, from this result of research experiment showed that the best solution in the control of *Striga* is an integrated approach that includes a combination of methods that are affordable and acceptable by farmers.

Striga. According to the research findings, the integration of multiple control options is suggested as a better approach to combat *Striga* problem (Kuchinda et al., 2003; Schulz et al., 2003, Aliyu et al., 2004; Temam, 2006; Tesso et al., 2007). Schulz et al. (2003) and Hearne (2009) also proved that the best options for successful *Striga* control lies in an integrated *Striga* management (ISM) approach.

Table 5. Improved management practices on *Striga* infestation and sorghum yield (Adibakel)

Treatment	*Striga* count (Shoots/plot)	Grain yield (Kg/ha)	Biomass yield (Kg/ha)
Variety (V)			
Local check	262	307	4793
ICSV 1006	42	621	2440
ICSV 1007	166	549	2527
SRN 39	80	453	2840
Management (M)			
BC –F +HP	198	381	2767
BC +F +HP	193	532	3042
RP +F +HP	92	564	3483
RP –F +HP	141	393	2642
RP +F +2,4-D	73	541	3817
LSD (0.05) (V)	105	162	1149
LSD (0.05) (M)	117	181	NS
LSD (0.05) (V X M)	235	362	2569
CV (%)	80	35	39

BC, broadcasting; RP, row planting; HP, hand pulling; F – with (+) and without (-) fertilizer. Source: Fasil, 2002.

DISCUSSION

The seriousness of the *Striga* problem was repeatedly reaffirmed in many national and international workshop and research works. In many areas it is becoming steadily more serious, as in many Africa countries including other regions, there is considerable alarm resulting from the acute susceptibility of many of the new high-yielding sorghum and maize hybrids.

Available control measures were reviewed in detail. Most various control options (cultural, chemical, biological, and use of resistant varieties) are either impracticable for the majority of small farmers or too expensive or unavailable due to different reasons to reduce *Striga* damage. In the development of resistant varieties, there has been some notable progress as a result of IITA, CIMMYT, International Sorghum and Millets (INTSORMIL), ICRISAT's and other govenments and non governments efforts, but progress against the more virulent *S. hermonthica* has been less rapid. Variability in farming systems, literacy level, ecological peculiarities and farmers' resources will go a long way in the choice and use of method to apply. The important thing is to control this devastating parasitic weed, so as to enhance higher crop yield per hectare and to better the standard of living of poor resource farmers.

Considering the constraints to a successful control of parasitic weeds so far, it is well recognized that no single method of control can provide an effective and economically acceptable solution. Therefore, an integrated control approach is essential, ideal and useful to small-scale farmers, in order to achieve sustainable crop production. Therefore it needs to be adjusted to individual cropping systems, local needs and preferences may be helpful in adapting and optimizing control strategies to different agro-ecosystems.

RECOMMENDATIONS

Short term

Some of the points that should receive an immediate attention include:

i) Identify and mark the farms classified as to level of infestation and develop treatment plans according to cost and return potential

ii) Generate information from which farmers can make optimum decisions on choice of cereal species and variety, time and method of planting, mixed cropping, herbicide and hand pulling as relevant to the farming system.

iii) Use clean crop seeds to avoid *Striga.*

iv) Improve soil fertility by using fertilizers.

v) Crop rotation with non host crops or crops that induce suicidal germination.

Long term

To alleviate the alarming problem of *Striga* in the long-run emphasis should be placed on:

i) Research efforts should be focus on controlling the production of new *Striga* seeds and reducing the number of seeds in the soil.

ii) Demonstration of existing improved technologies that are effective and feasible for the small scale farmers.

iii) *Striga* control approaches, namely cultural, chemical, genetic, and biological options should be widely investigated and developed.

iv) Practices and measures should be easily affordable, economical, and practicable to poor farmers.

v) Finding suitable companion and trap crops that fit into

the farming systems of target communities.
vi) The use of trap crops as an intercrop with susceptible hosts to reduce the seed bank needs prolonged investigations.
vii) Effective preventive measures need to be taken through seed quarantine and *Striga* free equipment.
viii) Developing and use of resistant crop varieties.
ix) Demonstration and training should be strongly focus in integrated *Striga* control
x) Need to launch an action program for the control of *Striga*. This program should cover all aspects of the problem.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Observations on anatomical aspects of the fruit, leaf and stem tissues of four *Citrullus* spp.

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Morphological characteristics of the fruit, stem and leaf tissues of four species of *Citrullus* (L.) Schrad. were examined using standard histological methods. Plant materials included the cultivated watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) and three of its related species (*Citrullus colocynthis* (L.) Schrad., *Citrullus ecirrhosus* Cogn. and *Citrullus rehmii* de Winter). Variation among the species was observed in the thickness of the subepidermal layer(s) of the fruit, this layer being thicker in fruits of *C. ecirrhosus* (~ 200 uM) than in fruits of *C. lanatus* (~100 uM), *C. colocynthis* (~100 uM) or *C. rehmii*.(~100 µM). Variation was also observed in the extent and organization of the subtending sclerenchymatous cells which were up to 10 layers thick in fruits of *C. colocynthis*, 1 or 2 cell layers thick in the fruits of *C. lanatus*., and an intermediate number of layers (3 to 6) in fruits of *C. ecirrhosus* and *C. rehmii*. A greater degree of lignifications was observed in the stem tissue of *C. colocynthis* and *C. ecirrhosus,* in comparison with that of *C. lanatus* and *C. rehmii*. Leaf thickness (~250 µM) was similar among three of the four species examined, but was reduced in *C. rehmii* (~150 µM). Further study is required to assess the possible contribution of these, and other, morphological attributes to drought tolerance in members the genus *Citrullus*.

Key words: Watermelon, *Citrullus lantatus*, *Citrullus colocynthis*, *Citrullus rehmii*, *Citrullus ecirrhosus*, histology, anatomy, structure, drought tolerance, caudex, pollen viability.

INTRODUCTION

The sweet-fleshed form of the cultivated watermelon (*Citrullus lanatus*) is an important crop in the USA and internationally (Levi et al., 2012). US PRODUCTION of watermelon ranked 5th globally at ~50 x 10^3 hectares with an estimated value of more than a half-billion dollars in 2012(http://www.agmrc.org/commodities__products/vegetables/watermelon/). In 2014, watermelon production in China and the USA exceeded 68.8 x 10^6 and 1.69 x 10^6 m/t, respectively (Food and Agriculture Organization of the United Nations, 2014). These data do not include the production of non-sweet citrons, egusi types and the bitter apple. In order to maintain (and improve) the current high levels of production of this crop, breeding objectives have typically included disease and insect tolerance/resistance, (Duke, 1978). in addition to fruit quality attributes. However, recent concerns associated with the increasing costs of irrigation (and water scarcity), and anticipated changes to the environment (including

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increased risk of environmental stress) have provided an additional area of study for the improvement of watermelon and other crops -drought tolerance (Condon et al., 2004; Liuigi et al., 2008). Fortunately, drought tolerance is a characteristic associated with members of this genus that are native to southern and western Africa (Meeuse, 1961, 1962; Jeffrey, 1975; Russell, 1985; Singh, 1990, Chomicki and Renner, 2014).

The *Citrullus* genepool is relatively small in comparison with that of other major vegetable crops (Whitaker and Davis, 1962; Bates and Robinson, 1995). Four diploid (2n=22) species are generally recognized; *Citrullus lanatus* (Thunb.) Matsum. & Nakai - the cultivated watermelon, *Citrullus colocynthis* (L.) Schrad., *Citrullus ecirrhosus* Cogn. and *Citrullus rehmii* De Winter. Recent taxonomic changes suggest that additional species be recognized (Chomicki and Renner, 2014). *C. ecirrhosus* is a desert perennial (Jeffrey, 1975; Russell, 1985). Fruits mature in February to March in the Namib where the leaves and stems die back each year and regrow from a caudex. This is a hardy species surviving on minimal ground water and morning fogs where it serves as an important source of water for desert fauna. In contrast to other members of the genus, C. *ecirrhosus* lacks tendrils. Its distribution appears to be restricted to Namibia and the Richtersveld of the Northern Cape (South Africa). *C. colocynthis* (a perennial in warmer climates) is also highly xerophytic and thrives on sandy loams, subdesert soils and along sandy seacoasts (Jeffrey, 1961; Duke, 1983). This species is distributed from northwestern Africa and the Mediterranean eastwards to Pakistan, India and Afghanistan (Sarafis, 1999; Renner and Pandey, 2013). In addition to being a source of seed-extractable oil, it is also used for medicinal purposes and has been cultivated in the Mediterranean region for centuries (Hussain et al., 2014). The annual C. *rehmii* is a more recently described species (De Winter, 1990) endemic to the desert regions of western and central Nambia. Possessing a central taproot, it inhabits gravelly to sandy-gravelly flats (De Winter 1990). *C. lanatus*, also an annual, is the most morphologically variable member of the group and has been cultivated in Central Africa for at least 5000 years (Whitaker and Davis, 1962). Though perhaps not generally considered to be as drought tolerant as its desert-dwelling related species, specific forms of *C. lanatus* [egusi types also referred to as *C. lanatus* var. (or subsp.) *mucospermus*] are common in the extremely arid regions of Northwestern Africa (National Research Council, 2006).

All species in this genus are regarded as xerophytes (Arnold and De Wet, 1993). However, relatively little information is available in the scientific literature regarding the mechanisms that affect their drought tolerance. Akashi et al. (2001) attributed elevated levels of citrulline in leaves to providing drought tolerance in *Citrullus* spp. Akashi et el. (2004) later demonstrated that drought-resistance in wild watermelon was correlated with the expression CLMT2, a gene sharing significant homology with a type-2 metallothionein (MT). Liu et al. (2008) noted that thicker leaves and stronger roots were associated with drought tolerance in mature *C. lanatus* plants, and that drought tolerance of *C. lanatus* genotypes at the seedling stage was not always correlated with tolerance at a more advanced stage of plant maturity. Zhang et al. (2011) screened 820 genebank accessions of *C. lanatus* for drought tolerance and assigned these to four groups, including tolerant, intermediate tolerant, moderately sensitive, and sensitive, respectively. The most drought-tolerant *Citrullus* germplasm, including 13 *Citrullus lanatus* var. *lanatus* and 12 *C. lanatus* var. *citroides* accessions, originated from Africa. It was suggested that these genetic materials could be used for rootstock breeding or for developing drought-tolerant watermelon cultivars, though no specific attribute conferring drought tolerance to the tolerant genotypes was identified.

The surface and other anatomical characteristics of stems, leaves and fruits are important factors capable of adaptations conferring drought tolerance (Baker and Procopiou, 2000). Thus, this study was undertaken to anatomically characterize stem, leaf and fruit pericarp tissue of the *Citrullus* spp. to attempt to identify unique anatomical features associated with individual taxa, and perhaps with drought tolerance.

MATERIALS AND METHODS

Plant materials for this study were obtained from the USDA/ARS genebank in Griffin, GA. Seeds of *C. lanatus* cv. Sugar Baby (PI 665007) and *C. colocynthis* (PI 652254) were treated with fungicide (Arasan) and planted in a commercial potting mix (Metro-Mix 360) in 2.5" x 2.5" x 3.5" peat pots. Previous efforts to germinate seed of *C. ecirrhosus* and *C. rehmii* in soil following the previously described procedure, proved unsuccessful. Seeds of *C. ecirrhosus* - (GRIF 15029) and *C. rehmii* GRIF 16946) were surface sterilized in 10% bleach and rinsed with tap water. The seed coats were then cracked with a pair of pliers and the seeds subsequently germinated in a germination chamber in darkness at a constant 30°C. Germinated seeds were transferred to peat pots containing the soil mix noted previously. When plants (3/spp.) reached the four to five-leaf stage, they were transplanted into plastic pots (~24 cm diameter x 18 cm depth) containing a 1:1:1 (v/v) mixture of the previously mentioned potting mix, pearlite and course sand. All plants received periodic fertilization with macro and micronutrients. Mature plants were established and maintained in the greenhouse from January 2012 through June 2013 without supplemental lighting at an average temperatures of 21 (days) and 16°C (nights). Fruit (1/plant; 3/spp.) were produced by manually pollinating (sib-mating) flowers. Efforts to establish plants of *C. rehmii* and *C. ecirrhosus* in the field in Griffin, GA were unsuccessful. Hence, only greenhouse-grown plants and fruits were included in the analyses.

Fixing, embedding and thin-sectioning of plant tissues

Procedures for the fixing, paraffin embedding and staining of tissues generally followed the procedures of Sass (1958) as described here. Sections of mature fruit pericarp (~1.5 cm in depth), mature leaf tissue and stem tissue (from the terminal 10 cm of

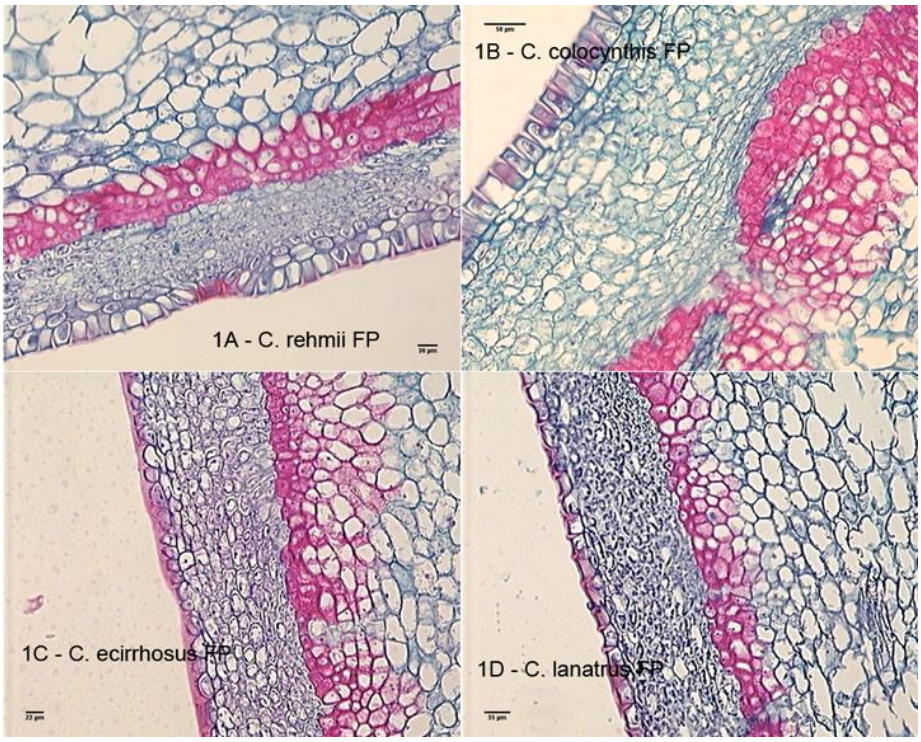

Figure 1. Cross-sections of pericarp of mature (greenhouse-grown) fruit of: A) *C. rehmii*, B) *C. colocynthis*, C) *C. ecirrhosus* and D) *C. lanatus*. Layer of lignified cells in red.

rapidly growing vines) were harvested and placed in glass vials. FAA (50% EtOH, 5% acetic acid, 10% (37-40%) formaldehyde, and 35% ddH_2O, v/v) was added to completely cover the tissue samples which were then vacuum infiltrated for 15 min. The vials were subsequently drained, fresh FAA added, and the tissues allowed to fix for 48 h at RT. Tissues were then transferred to 70% EtOH for a maximum of 2 weeks before dehydration and embedding.

Tissues were dehydrated in an alcohol series (60 min in each) of 70, 80, 95 and 100%, and then cleared in ethylene:xylene (30 min each in 3:1 ethanol:xylene, 1:1 ethanol:xylene, 1:3 ethanol:xylene and 100% xylene) (v/v). Fully dehydrated tissues were placed in molten Tissue Prep II (Fisher Scientific) at 60°C. The Tissue Prep was replaced 3 times at 12 h intervals followed by a final immersion for 48 h. Thin-sections (10 µ) were cut on a Ventana model 100 rotary microtome (Ventana Medical Systems, Tucson, AZ) and placed on glass slides.

Staining of plant tissues and microscopy

Paraffin was removed and tissues prepared for staining by successive immersions of 2 min each in 100% xylene, 100% EtOH, 90% EtOH, 70% EtOH, 50% EtOH, 30% EtOH and distilled H_2O. Rehydrated tissues were stained in an acquoeous solution (1%, w/v) of safranin (Sigma Chemical) for 8 h. Excess safranin was removed by immersion in ddH_2O (several water changes) and then dehydrated in an alcohol series (5 min each) of 30, 50, 70 and 95% EtOH. Tissues were counter-stained for ~5 s in 0.1% fast green (in 95% EtOH). Stained tissues were briefly immersed in 100% EtOH (3X) - 5 min each, 100% xylene (3X) - 5 min each, drained and the cover slip mounted with Permount (Fisher Scientific). Pollen grains were collected at ~8AM and immediately stained with 3.3. diaminobenzidine (Sigma Cat. # D-4168) as described by Dafni et al. (2005).

Microscopy was accomplished on an Olympus Model CX31 bright field microscope equipped with a digital camera.

RESULTS AND DISCUSSION

Cucurbitaceaous fruits possess a rind which serves to confine the fleshy tissues, and that is typified by the presence of a uniserate epidermis with a cuticle (containing stomata), and a subepidermal tissue that varies in width among species. This subepidernal tissue may consist of parenchyma (colored or colorless) and sometime collenchymatic (non-lignified) cells. At a greater depth, a layer of sclerids (sclerenchyma) occurs beneath which parenchmous tissue extends to the center of the fruits (Esau, 1977).

Variation among *Citrullus* species was observed in the thickness of the subepidermal tissue layer (Figure 1A - 1D) being less than 100 µM thick in fruit of *C. ecirrhosus* and approximately 175 to 225 µM in fruit of *C. colocynthis* and *C. rehmii.* Fruit of *C. lanatus* were intermediate to these values for this characteristic (125 to 150 µM). Variation among species was also seen in the extent and degree of organization of the subtending sclerenchymous cells being most extensive (up to 10 cell layers thick) in *C. colocynthis,* only a single or perhaps two cell layers thick in *C. lanatus*, and an intermediate layer of cells (3 to 6) in *C. rehmii* and *C. ecirrhosus*. Observations of this sclerenchymous layer suggested that is more highly

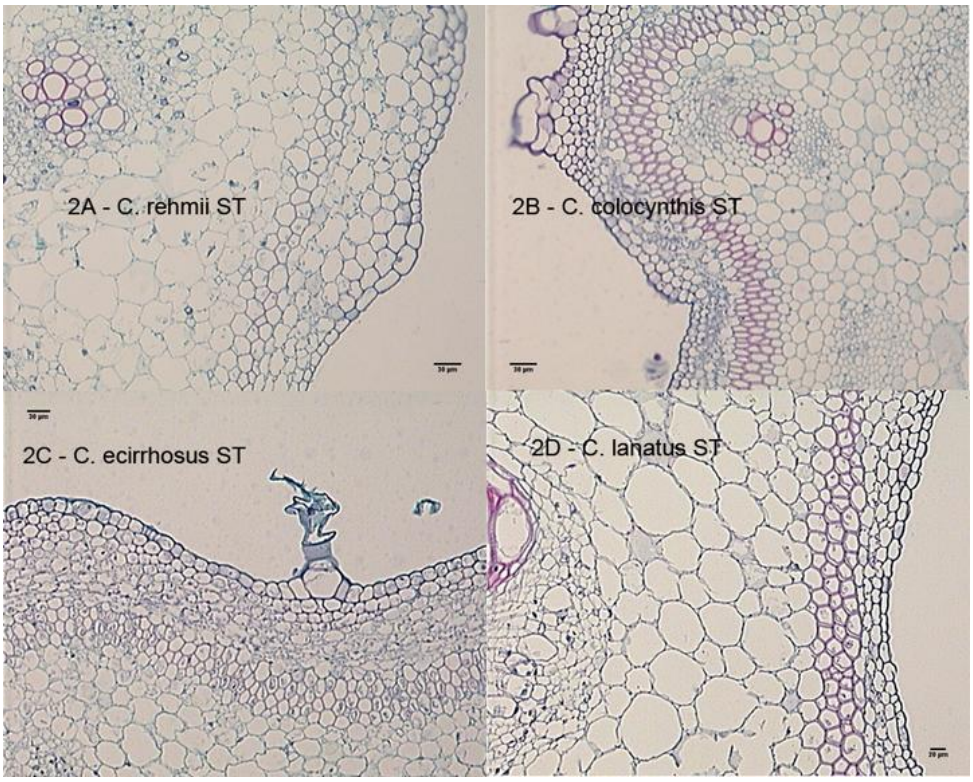

Figure 2. Cross-sections of stem tissue of: A) *C. rehmii*, B) *C. colocynthis*, C) *C. ecirrhosus* and D) *C. lanatus*.

organized in the fruits of *C. rehmii* than in the other species examined (Figure 1A to D).

The occurrence of a greater degree of lignification within the exocarp of fruits of *C. colocynthis* is in keeping with the noticeably firmer fruit of this species when compared with *C. ecirrhosus*, *C. lanatus* or *C. rehmii*. Fruits of *C. colocynthis* were globose, 6 to 10 cm in diameter with green striping and intensely bitter flesh. In contrast to the other members of this genus that were examined in this study, the fruits of *C. rehmii* had neither a smooth surface nor were noticeably firm. The fruits of *C. rehmii* had a semi-flexible rind with a rugose-surface resembling, in that respect, fruit of certain *Cucumis* spp. The surface of mature *C. rehmii* fruits was mottled in appearance being generally dark green to tan in color with salmon-pink to orange-pink splotches as described by De Winter (1990). The fruit flesh of *C. rehmii* is known to be bitter and contain cucurbitacin E, B and I (Enslin and Rehm, 1958). Mature fruit of *C. lanatus* and *C. ecirrhosus* remained green at maturity, whereas mature fruit of *C. colocyhthis* developed a yellowish overcast as noted by Sarafis (1999).

Cross-sections of stem (vine) tissue revealed a somewhat greater degree of lignification in the sections of *C. colocynthis* and *C. ecirrhosus* as illustrated in Figure 2B and C, and a reduced and more organized cell layer in *C. colocynthis*, as compared to other species. In contrast to *C. lanatus* and *C. rehmii*, both *C. colocynthis* and *C. ecirrhosus* develop swollen stems (caudices) from which multiple shoots arise. A greater degree of caudex development was observed in *C. ecirrhosus* as compared to *C. colocynthis.* Caudices averaged 2.5 cm in diameter in *C. ecirrhosus* and 1 cm or less in *C. colocynthis* - over the time span of these experiments. Histological examination of the caudices of these two species was not undertaken. A determination of the extent to which, if any, a caudex contributes to drought tolerance may prove useful.

Adaptation to arid conditions is partially determined by the ability of the plant to regulate water loss from the aerial tissues (Ali and Grace, 1986; Baker and Procopiou, 2000; Benesova et al., 2012). Increased leaf (Karaba et al., 2007) and cuticle thickness (Martin and Juniper, 1974) have been shown to be associated with drought tolerance and leaf thickness has been suggested as a characteristic to be used in the selection of drought tolerant breeding lines (Hameed et al., 2002, 2012). We found leaf thickness to be variable among the four *Citrullus* species: *C. lanatus* - 80 to 100 µM; *C. colcynthis* - 200 to 250 µM; *C. rehmii* 100 to 150 µM; *C. ecirrhosus* - 250 to 350 µM (Figure 3A to D). General leaf morphology of *C. rehmii* was most similar to *C. lanatus* (broadly cordate and lobed) though its leaf size is reduced as compared to *C. lanatus*. The leaf surface area of *C.*

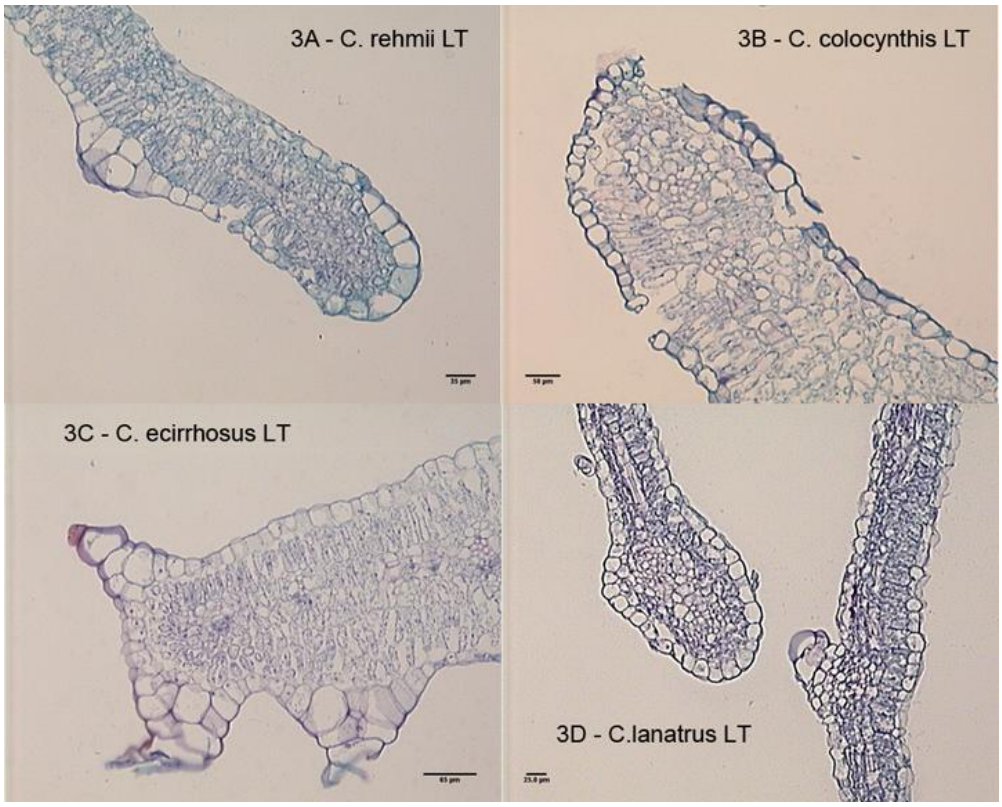

Figure 3. Transverse sections of leaf blade tissue harvested from greenhouse-grown plants of: A) *C. rehmii*, B) *C. colocynthis*, C) *C. ecirrhosus* and D) *C. lanatus*.

colocynthis is also reduced as compared to both *C. lanatus* and *C. rehmii,* its leaves being triangular in shape and very deeply lobed. The leaves of *C. ecirrhosus* have the most distinctive morphology of the 4 species. The lamina of its leaves is curved over the mid-rib and the lateral veins so that when viewed from above the top surface is only visible in the vein regions and the leaves have a greenish white appearance due to the lower epidermis being reflected up as the upper surface of the leaf. This curving of the lamina is less evident when plants are grown in the greenhouse. The lower epidermis is covered with warts and hairs which account for the whitening effect. Both lower and upper epidermis contain similar amounts of stomata (Sarafis, 1999).

The extent to which the drought tolerance inherent in the *Citrullus* genepool can be characterized and harnessed to substantially improve the drought tolerance of the crop, remains to be determined. Drought tolerance is a complex trait involving multiple mechanisms that typically co-exist and that together contribute to whole-plant drought resistance (Xiong et al., 2012). The potential contribution of specific anatomical, morphological, physiological and other unique characteristics present in this genus towards improving drought tolerance in watermelon (and perhaps other crops as well) requires additional study. *C. rehmii* was successfully hybridized with *C. lanatus* by Rehm and Neethling (Unpublished Progress Report, 1967 as cited by De Winter, 1990). *C. rehmii* was also successfully hybridized with *C. lanatus*, *C. ecirrhosus* and *C. colocynthis* and the F_1 hybrids found to be fertile. The F_2 progeny of the crosses *C. lanatus* x *C. colocynthis* and *C. lanatus* x *C. ecirrhosus* showed a high degree of sterility whereas progeny of the cross *C. colocynthis* x C*. ecirrhosus* proved to be fertile (R.P. Elis as noted by De Winter, 1990). Navot and Zamir (1987) suggested the use of *C. ecirrhosus* as a source of drought tolerance genes for watermelon improvement and successfully hybridized *C. ecirrhosus* and *C. lanatus* (Navot et al., 1990). This has been viewed as demonstrating the potential for breeding *C. lanatus* containing genes from *C. ecirrhosus* (Sarafis, 1999).

We have found (Jarret - unpublished) that F_1 hybrids of *C. ecirrhosus* x *C. lanatus*, *C. rehmii* x *C. lanatus* and *C. colocynthis* x *C. lanatus* (and their reciprocal hybrids) have significantly reduced pollen viability (Figure 4). Although hybrid F_1 seed may be readily produced, F_2 seed produced from a selfed F_1 *C. lanatus* x *C. ecirrhosus* had a very low rate of germination (< 5%). Fertility of the F_2 plants is yet to be examined. Reduced pollen viability in hybrids does not preclude the use of the wild *Citrullus* species for the improvement of *C. lanatus.*

Intraspecific variation for the characteristics examined in this study is to be expected. However, only a limited

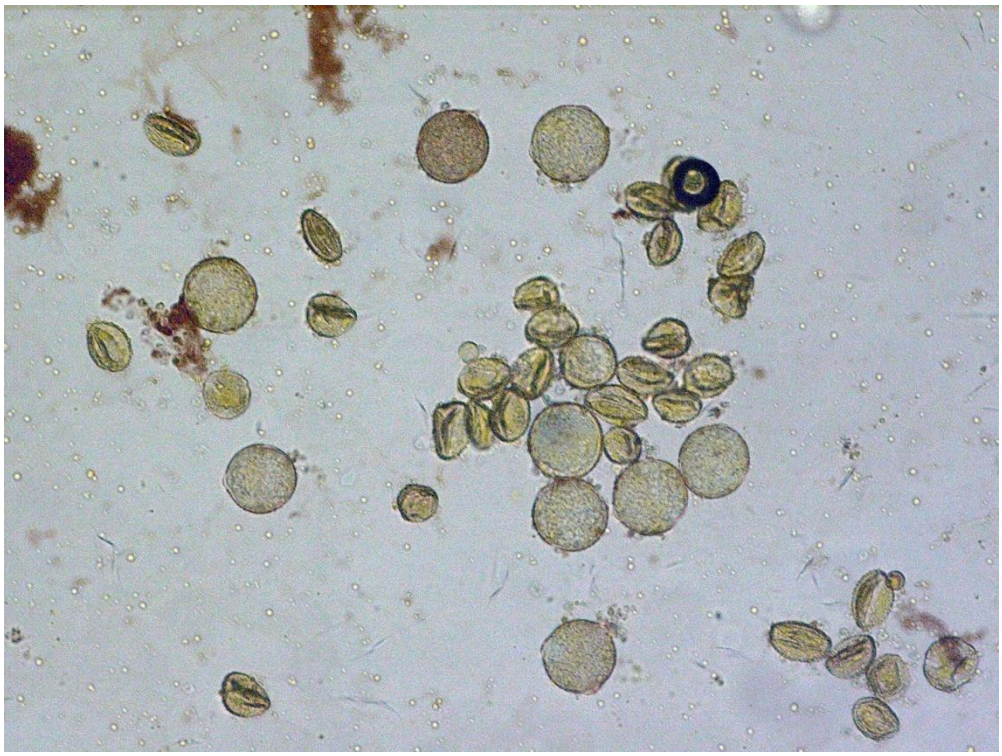

Figure 4. DAPI-stained pollen grains from a *C. ecirrhosus* x *C. lanatus* F_1 hybrid.

amount of plant material of each species was available for examination. No commercial source of seed of either *C. rehmii* or *C. ecirrhosus* was identified. In as much as these two species could not be established in the field in Griffin, GA, an evaluation of the extent to which environmental variables affect the characteristics examined, was not attempted. Nevertheless, a further investment in support of efforts to identify and fully characterize the mechanisms endowing *Citrullus* spp. with drought tolerance could result in the identification of a trait or traits or gene(s) capable of enhancing drought tolerance in the cultivated crop. Such information might also be expected to increase our understanding of the fundamental processes involved in stress/drought tolerance in other cucurbitaceous crops.

Conflict of Interests

The author(s) have declared that there is no conflict of interests.

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Full Length Research Paper

Correlation and path coefficient analysis of seed yield and yield components in lentil (*Lens culinaris* Medik.) genotype in Ethiopia

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Knowledge of correlation among different traits and further partitioning of the correlation coefficients into direct and indirect effects on yield is one of the approaches to understand the nature and extent of the relationship among characters. The objective of this study was to determine the degree of associations between seed yield and yield components of lentil. In this study, 228 genotypes from diversified origin were considered. The experiment was conducted at three locations using randomized complete block design and augmented design. Significant strong positive phenotypic and genotype correlations were observed between seed yield and biomass, seed weight per plant, number of seed per plant and number of pods per plant. These four yield-contributing characters had also strong positive correlations with each other. The path coefficient analysis is in harmony, with the phenotypic and genotypic correlation, that is, seed weight per plant, followed by number of pods per plant, biomass yield and 100 seed weight have a considerable positive direct effect on lentil seed yield. However, days to 50% flowering, days to 90% maturity and rust disease severity score had negative phenotypic correlation and negative direct effect on seed yield.

Key words: Genotype correlation coefficient, lentil, path analysis, phenotype correlation coefficient.

INTRODUCTION

Lentil is a short and slender annual cool-season food legume, which was, domesticated early in the Fertile Crescent of the Near East (Sarker et al., 2010). Lentil provides sufficient amounts of the most essential amino acids to meet the nutrient requirements of humans. It is also a cash crop fetching a lot of money in domestic

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markets as compared to all other food legumes and major cereals (Geletu et al., 1996). Because of its significant economic roles and social contribution, lentil production has recently been expanding both in stressed and non-stressed environments (Asnake and Geletu, 2006). The global lentil productivity is about 0.9 t/ha, while in Africa it is about 0.6 t/ha (FAO, 2013) and in Ethiopia about 1.2 t/ha (CSA, 2012). In many traditional lentil-producing countries, most of the lentil production area is covered by the local landraces that are vulnerable to a range of biotic and abiotic factors and produced lower yield and seed quality (Sarker et al., 2003).

Characterizing crop genotypes for agronomic morphological traits have immense importance in crops breeding. Some of the traits are easy to select due to its visual nature but its scientific understanding is important for its manipulation. To facilitate selection in breeding for high yield, it is important to examine the association of various yield components and give more attention to those having greatest influence on yield. Gomez and Gomez (1984) showed that in order to determine important components of crop performance among their variables, it is essential to examine association between primary and secondary traits. Falconer and Mackey (1996) also indicated correlation coefficients measures the degree of association between characters and relative influence of various characters on yield and amongst themselves. Since breeding is commonly aimed at multiple traits, it is essential to know the correlated responses in primary traits like seed yield through selection of secondary traits (Borojevic, 1990).

Knowledge of correlation among different traits and further partitioning of the correlation coefficients into direct and indirect effects is one of the approaches to understand the nature and extent of such relationship among the characters in both local and introduced genotypes. As more traits are considered for correlation study, the indirect associations between traits become more complex, less obvious and somewhat perplexing. The path coefficient analysis, a method developed by Wright (1921) and later elaborated by Dewey and Lu (1959), provides an effective means of partitioning direct and indirect effect of association. Path coefficient is simply a standardized partial regression coefficient, measures the direct influence of one variable upon another, and permits the separation of correlation coefficient into components of direct and indirect effects This technique allows identifying major yield contributing characters, and specific traits producing a given correlation (Rao et al., 1997).

Researchers in Ethiopia and elsewhere have reported association yield and yield components traits of lentil germplasm, and the information was not comprehensive and exhaustive (Erskine et al., 1989; Ramgiry et al., 1989; Geletu et al., 1996; Abebe et al., 2001; Tigest, 2003; Edossa et al., 2010; Roy, et al., 2013). Besides, the value of correlation coefficients and the contribution of different components vary in different environments and, in different population and the magnitude of correlation coefficient can often be influenced by the choice of individuals upon which the observations are made (Jatasra and Paroda 1978; Dabholkar, 1992). It is therefore, advisable to consider large number of genotypes and use the correlation to establish an index in deciding the direction of selection. Hence, understanding of such an important association is necessary to conduct effective selection activities. An understanding of the genetic relationships of different yield components and their direct and indirect effect is imperative for Ethiopian lentil, in relation to lentil from exotic origin. The objective of this study was to determine the degree and nature of associations among seed yield and yield components in local and exotic lentil genotypes under the Ethiopia conditions.

MATERIALS AND METHODS

Description of the study site

The field experiment was conducted on selected hot spot locations for rust. The locations included Sirinka Agricultural Research Center (SRARC) in the northeastern part of Ethiopia for two seasons (2011 and 2012), Chefe Donsa in the central part of Ethiopia and Sinana Agricultural Research Center (SARC) in southeastern parts of Ethiopia during the 2011/12 cropping season (Table 1 and Figure 1).

Plant materials

The experiments consist of 228 genotypes collected from Ethiopian Biodiversity Institute (EBI) and DebreZeit Agricultural Research Station (DZARC), Ethiopia, and the International Center for Agricultural Research in Dry Areas (ICARDA). Name of genotype number of genotype, source of origin, and breeding status of the genotypes were presented in Table 1. In 2010/2011 cropping season, 158 genotypes were planted for morphological evaluation at SRARC. In the 2011/12 cropping season, 228 genotypes including recombinant inbreed lines (RIL) were included (Table 1) in the study across three locations: Sirinka, Chefe Donsa and Sinana.

Experimental layout and design

A randomized complete block design (RCBD) with three replications, at SRARC in the 2010/11 cropping season, and an augmented design with five blocks were used in 2011/12 cropping season over the three locations. The genotypes were planted in July in a two rows plot size of 0.8 m^2. The row to row distance was 20 cm. The distance between two plots was 50 cm and the distance between two blocks was 100 cm. Eight checks were replicated within each block. Planting was done in the first week of July at SRARC, in August at Chefe Donsa and in mid-September at SARC. The recommended agronomic packages at each location were applied for raising a successful crop.

Data collection

Data were recorded on 10 important phonological, yield and yield

Table 1. List of genotypes used in this study and their origin.

Source of origin	No. of genotypes	Name of genotype	Breeding status of the genotypes
Tigray	8	Acc. no. 219957,235383, 237503,237504, 241785, 242604, 243447	Landrace
Amhara	54	Acc. no. 36003, 36025, 36028, 36039, 36041, 36061, 36071, 36085, 36088, 36089, 36097, 36103, 36104, 36105, 36137, 36139, 36150, 36162, 36165, 36168, 207258, 207274, 207287, 207309, 212745, 215248, 215249, 223221, 228242, 229179, 229182, 229183, 231247, 235013, 235015, 235016, 235017, 236484, 236486, 236487, 237502, 238978, 238979, 241784, 241786, 243433, 243436, 243440, 243443, 244606, 244610, 244615, 244619, 244623	Landrace
Oromya	29	Acc. no. 36001, 36007, 36009, 36013, 36015, 36019, 36023, 36029, 36033, 36042, 36048, 36058, 36110, 36120, 36131, 203141, 215806, 216877, 228809, 230521, 230833, 230834, 230837, 231248, 235698, 236438, 236892, 237027, 238971	Landrace
SNNP	2	Acc. no. 36147 and Acc. No.,228243	Landrace
Somali	1	Acc. no. 230832	Landrace
DZARC	6	/ILL4225 x ILL4605/ /ILL 6821/ Alemaya, /ILL 1 x ILL 1169//ILL 6027/ ADAA, /ILL 7978/ Teshale, / Alemaya x FLIP88-41L/ DERASH, /ILL 7981/ *Aleme Tena* and P160/ILL 2704/ / Chekol/	Commercial varieties
Unknown	10	Acc. no. 36134, 207260, 211062,211078, 211110, 220120, 211131, 233349, 233973, 241782	Landrace
ICARDA	22	X2003S 222/ILL 213/, X2003S 238 /ILL 4605/, X2006S 128/ILL 5480/, L-9-12, X2002S 219 /ILL 6821/, X2006S 129 /F2/, X2005S 215 /ILL 6002/, X2006S 133/FLIP87-21L/ /ILL 4349 x ILL4605//ILL 6211/, X2006S 130/FLIP 93-46L/ /ILL 7547/, FLIP-2004-7L, X2003S 223, X2003S 195/ILL 7115/, X2006S 130/FLIP 96-46 L//ILL 7978/, X2002S 221/FLIP 96-47 L//7979/, X2002S 221 /7980/, X2003S 233 /ILL 8009/, X2006S 134/ILL8174/, 2006S 122 /FLIP 2003-43L/ /ILL 7010 x ILL 1939/ /ILL 9932/, /FLIP 2003-56L/ /ILL 9945/X2006S 127, /ILL 2573 x ILL 7537/ /FLIP 2003-62 L/ /ILL 9951/X2006S 122, X2002S 219 /shehor-74/ /ILL 7554/, 2003S 236	Parent
DZARC	11	EL-142 /ILL 5071/, EXOTIC #DZ/2008 AK, R-186XFLIP-86-38L-24, ILL-358 X ILL-2573-2-2000, 87S-93549XEL-1O3-4, 87s-93549XEL-03-5, Chekol X R-186-1, R-186X FLIP-86-38L, Chekol x R-186-2, EL-142 X R-186-2, EL-142 X R-186-3	Breeding line
ICARDA	15	ILL-590/NEL 590/, FLIP-2006-60L, FLIP-97-68L, FLIP-04-26L, ILL-28501, FLIP-2006-20L, FLIP-87-68L, /ILL 6037 x ILX 87062/ FLIP2005-24L/ ILL-10045, FLIP-97-16L/ILL 8078, ILL-10680, FLIP-2004-37L, FLIP-84-95L /ILL 5722, FLIP-97-61L, L-830, Precoze/ILL 4605/	Breeding line
ICARDA	70	RIL1- RIL70	RIL

Unknown: Originated from Ethiopia but site of collection not mentioned RIL: Recombinant inbred line.

components and reaction to rust disease. The data were recorded in randomly selected plants on plant and plot basis (Table 2).

Data analysis

The data were subjected to statistical analysis using GenStat Release 15.1 statistical software (VSN International Ltd., 2012). The correlation and path coefficient was analyzed based on the row data value for each agro-morphological trait. The phenotypic and genotypic correlation coefficients were calculated for data from RCBD by the following formula as suggested by Miller et al. (1959). Only the phenotypic correlation coefficients were calculated for augmented design using the formula adopted by Singh and Chudhary (1977) on Genstat 15 Release 4.2 software. Path coefficient analysis was carried out to partition the phenotypic and genotypic correlation coefficients into direct and indirect effects of yield attributing traits (independent characters) on grain yield (dependent character) using the general formula suggested by Wright (1921) and worked out by Dewey and Lu (1959). For augmented design, path coefficient analysis was calculated using the formula and syntax developed by Singh and Chudhary (1977) on Genstat 15 Release 4.2 software.

RESULTS AND DISCUSSION

Phenotypic and genotypic correlation

Significant positive phenotypic correlation was observed between number of pods per plant and seed weight per plant (r_p = 0.9), seed yield and above ground biomass (r_p = 0.7), seed weight per plant (r_p = 0.6), number of seed per plant (r_p = 0.5) and number of pods per plant (r_p = 0.5). In addition, numbers of seeds per pod, plant height and 100 seed weight have significant (*P≤ 0.05*) positive association with seed yield at Sirinka (Table 3).

Highly significant strong positive correlations were observed between number of pods per plant and seed weight per plant (r_p = 0.9), seed weight per plant and

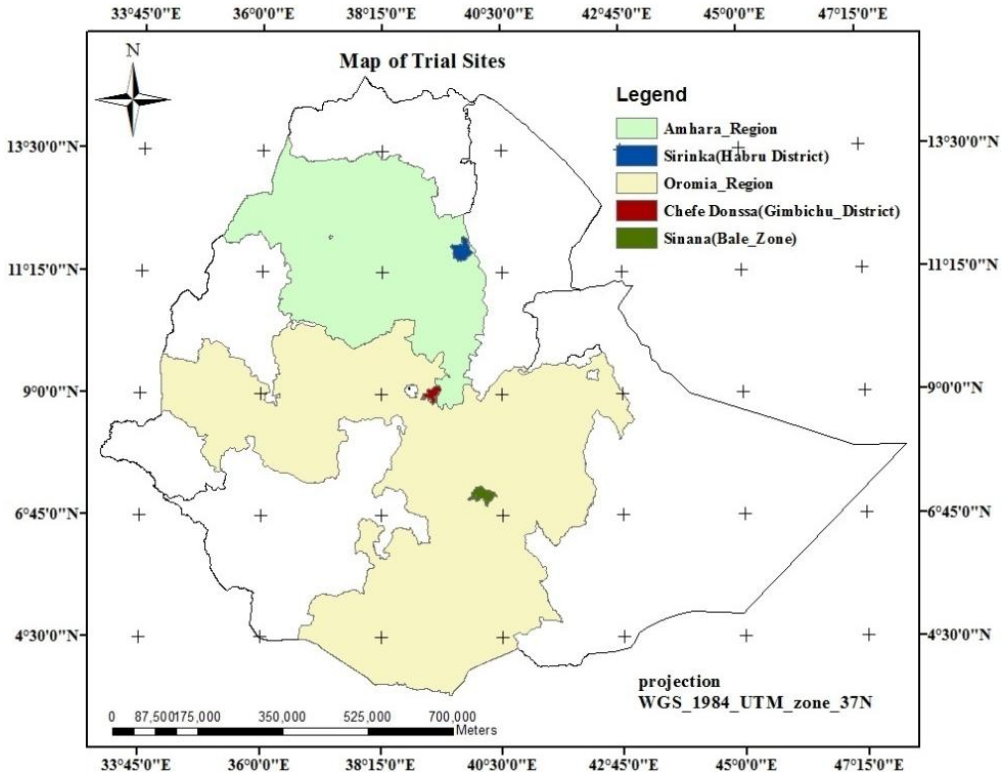

Figure 1. Geographical position of the experimental sites.

Table 2. List of agro-morphological, phonological traits and rust severity score collected for the study.

Traits	Description
Number of pods/plant (NPPP)	Mean of pods from ten randomly selected plants
Number of seeds/ Pod (NS)	Average number of seeds per pods counted from 10 randomly sampled pods of 10 different plants.
Seed weight per plant (SWPP)	The average matured seed weight from 10 randomly selected plants.
Number of seeds per plant (NSPP)	Mean of number of seeds counted from 10 randomly selected plants
100-seed weight (SW)	Weight of 100 seeds taken from the bulk harvest in gram (g)
Plant height (PH)	The height from the ground surface to the top of the main stem at physiological maturity in centimeter (cm)
Days to flowering (DFF)	Days from planting to flowering of 50% plant
Days to maturity (DM)	Days from planting to physiological maturity of 75% plant
Biomass per plant (BI)	Above ground biomass of the mean yield of two harvestable rows in gram
Seed yield per plant (SY)	Mean seed yield in gram of two harvestable rows in gram
Rust disease severity score after flowering (RDSAF)	The RILs and the accession were scored for rust severity quantitative scale developed by Chen (2007) had 1–9 scale, whereby 1 = 0–10% leaf area infected, 3 = 11–30% leaf area infected, 5 = 31–50% leaf area infected, 7 = 51–70% leaf area infected, 9 = more than 70% leaf area infected.

number of seeds per plant (r_p = 0.8), number of pods per plant and number of seeds per plant (r_p = 0.8). Whereas intermediate significant positive correlations were observed between days to 50% flowering and days to 90% maturity (r_p = 0.6), seed weight per plant and above ground biomass (r_p = 0.6), plant height and above ground biomass (r_p = 0.6) and number of seeds per plant and above ground biomass (r_p = 0.5) at Sirinka in 2010/11 (Table 3).

Significant ($P \leq 0.05$) negative phenotypic correlation

Table 3. Genotypic correlation in above diagonal and phenotypic correlation in below diagonal of yield and yield component characters in lentil genotypes at Sirinka in 20110/11.

Trait	DFF	DM	NPPP	NS	SWPP	NSPP	PH	SW	BI	AY
DFF		0.8**	0.1	-0.3**	-0.1	0.3*	0.3*	-0.1	0.5**	0.2
DM	0.6**		-0.1	-0.8**	-0.3**	0.1	-0.1	0.1	0.3**	0.04
NPPP	0.05	-0.2*		0.1	0.8*	0.8**	0.3**	-0.3**	0.4**	0.4**
NS	-0.2**	-0.5**	0.1*		0.4**	0.5**	0.1	-0.07	0.2**	0.3**
SWPP	-0.1	-0.2**	0.9**	0.2**		0.8**	0.2	0.20*	0.6**	0.6**
NSPP	0.06	-0.04	0.8**	0.4**	0.8**		0.3**	-0.3**	0.4**	0.**
PH	0.2*	-0.2*	0.4**	0.001	0.3**	0.3**		-0.14	0.6**	0.3**
SW	-0.06	0.2*	-0.07	-0.03	0.2**	-0.2**	-0.1*		0.2*	0.2*
BI	0.3**	0.2*	0.5**	0.13*	0.6**	0.5**	0.6**	0.2**		0.7**
AY	0.02	-0.07	0.5**	0.2**	0.5**	0.5**	0.3**	0.2**	0.66**	

DFF = days to 50% flowering, DM = days to 90% maturity, NPPP = number of pods per plant, NS= number of seeds per pod, SWPP = Seed weight per Plant, NSPP = Number of Seeds per Plant, PH = Plant height in cm, SW = 100-seed weight in gram, BI = above ground biomass, SY = seed yield,
** and * =Significant at 0.01and 0.05 probability levels.

Table 4. Pearson's phenotype correlations of yield and yield component characters in lentil genotypes at Sirinka 2011/12.

Trait	DF	DM	NPPP	NS	NSPP	SW	SWPP	PH	BI	SY
DF	-									
DM	0.70**	-								
NPPP	0.002	-0.16**	-							
NS	0.20**	0.17	0.03	-						
NSPP	0.03	-0.15*	0.62**	0.32**	-					
SW	-0.02	0.22	-0.19**	-0.02	-0.22**	-				
SWPP	0.07	-0.01	0.51	0.19**	0.83**	0.11	-			
PHH	0.20**	-0.06	0.31	0.10	0.47**	-0.11	0.41**	-		
BI	0.26**	0.14*	0.39**	0.19**	0.57**	0.03	0.65**	0.57**	-	
SY	0.05	-0.10	0.43**	0.13*	0.64**	0.04	0.72**	0.41**	0.77**	-

DFF = days to 50% flowering, DM = days to 90% maturity, NPPP = number of pods per plant, NS= number of seeds per pod, SWPP = Seed weight per Plant, NSPP = Number of Seeds per Plant, PH = Plant height in cm, SW = 100-seed weight in gram, BI = above ground biomass, SY = seed yield,
** and * =Significant at 0.01and 0.05 probability levels.

coefficients were observed between days to 90% maturity and number of seeds per pods (r_p = -0.5), plant height and number of pods per plant and 100 seed weight and number of seeds per plant (Table 3).

Similarly, significant phenotypic correlation was observed between seed yield and number of pods per plant, number of seeds per pods, number of seeds per plant, seed weight per plant and plant height at Sirinka, Chefe Donsa and Sinana in 2011/12 cropping season (Tables 4, 5 and 6). However, negative significant correlations were observed between seed yield and days to 50% flowering, and rust disease score with seed yield at Chefedonsa and Sinana locations (Tables 5 and 6). Above ground biomass showed significant positive phenotypic correlation with all characters except rust disease severity score (Tables 4, 5 and 6). Significant positive correlations were observed between days to 50% flowering and days to 90% maturity, and between days to 90% maturity and 100 seed weight in all the three environments; significant phenotypic correlations were observed between number of pods per plant and seed weight per plant at Sirinka (r_p = 0.51) and Chefe Donsa (r_p = 0.85), and number of pods per plant and number of seeds per plant at Sirinka (r_p = 0.62), Chefe Donsa (r_p = 0.94) and Sinana (r_p = 0.92). Seed weight per plant and number of seeds per plant (r_p = 0.83) at Sirinka, seed weight per plant with plant height (r_p = 0.41) at Sirinka and number of seeds per plant with plant height (r_p = 0.47) at Sirinka. Highly significant phenotypic correlation was observed between seed weight per plant and above

Table 5. Pearson's phenotype correlations of yield and yield component characters in lentil genotypes at Chefe Donsa 2011/12.

Traits	DF	DM	NPPP	NS	NSPP	SW	SWPP	PH	BI	SY
DF	-									
DM	0.54**	-								
NPPP	-0.15*	-0.19**	-							
NS	-0.26**	-0.38**	0.10	-						
NSPP	-0.21**	-0.26**	0.94**	0.40**	-					
SW	0.15*	0.45**	-0.22**	-0.25**	-0.26**	-				
SWPP	-0.12	-0.06	0.85**	0.18**	0.83**	0.08	-			
PH	0.27**	0.21**	0.02	-0.05	0.01	-0.02	0.01	-		
BI	0.30**	0.39**	0.07	0.05	0.06	0.14*	0.17**	0.44**	-	
SY	-0.15*	-0.18*	0.45**	0.22**	0.47**	-0.09	0.54**	0.25**	0.51**	-

DFF = days to 50% flowering, DM = days to 90% maturity, NPPP = number of pods per plant, NS= number of seeds per pod, SWPP = Seed weight per Plant, NSPP = Number of Seeds per Plant, PH = Plant height in cm, SW = 100-seed weight in gram, BI = above ground biomass, SY = seed yield,
** and * =Significant at 0.01and 0.05 probability levels.

Table 6. Pearson's phenotype correlations of yield and yield component characters in lentil genotypes at Sinana in 2011/12.

Trait	DF	DM	NPPP	NS	NSPP	SW	PHH	BI	SY	RDSBF	RDSAF
DF	-										
DM	0.2**	-									
NPPP	-0.14*	0.2**	-								
NS	-0.03	0.07	0.14*	-							
NSPP	-0.10	0.2**	0.9**	0.5**	-						
SW	0.15*	0.3**	0.2**	0.01	0.2**	-					
PH	0.09	-0.01	-0.02	0.11	0.05	0.09	-				
BI	0.02	0.11	0.03	0.06	0.07	0.13*	0.2**	-			
SY	-0.09	0.2**	0.5**	0.03	0.4**	0.5**	0.2**	0.3**	-		
RDSBF	-0.2**	-0.3**	-0.2**	0.01	-0.2**	-0.3**	0.04	0.06	-0.3**	-	
RDSAF	-0.06	-0.4**	-0.3**	0.02	-0.2**	-0.5**	0.00	-0.12*	-0.5**	0.40	-

RDSBF= rust disease severity score before flower, RDSAF= rust disease severity score after flower, DFF = days to 50% flowering, DM = days to 90% maturity, NPPP = number of pods per plant, NS= number of seeds per pod, SWPP = Seed weight per Plant, NSPP = Number of Seeds per Plant, PH = Plant height in cm, SW = 100-seed weight in gram, BI = above ground biomass, SY = seed yield, ** and * =Significant at 0.01and 0.05 probability levels.

ground biomass (r_p = 0.65) at Sirinka (Table 4). Negative phenotypic correlations were observed between days to 90% maturity and rust disease score before and after flowering (r_p = 0.3 and 0.4) at Sinana (Table 6). Negative phenotypic correlations were observed between days to 90% maturity and number of pods per plant, number of seed per plant and seed weight per plant at Sirinka and ChefeDonsa (Tables 4 and 5). Hundred seed weight also showed significant negative phenotypic correlation with number of seeds per plant at Sirinka and ChefeDonsa but not at Sinana (Tables 4, 5 and 6).

Seed yield per plant positively and significantly correlated with biomass (r_g = 0.7), seed weight per plant (r_g = 0.6), number of seeds per plant (r_g = 0.5), number of pods per plant (r_g = 0.4) (Table 3). Significant positive genotypic correlation coefficient were also observed among yield component traits including number of pods per plant with seed weight per plant (r_g = 0.8) and number of seeds per plant (r_g = 0.8), seed weight per plant with number of seeds per plant (r_g = 0.8), days to 50% flowering with days to 90% maturity (r_g = 0.8). However, negative and significant genotypic correlation coefficients were observed between days to 90% maturity and number of seeds per pod (r_g = -0.8), number of pods per plant with 100 seed weight (r_g = - 0.3), number of seeds per plant and 100 seed weight (r_g = -0.3), days to 50% flowering and number of seeds per pod (r_g = -0.3).

Estimates of direct and indirect effects of yield component traits on seed yield

In the present study, seed yield (SY) was considered as

Table 7. Phenotypic direct effects on main diagonal (bold) and indirect effects of different agronomic traits on seed yield of lentil genotypes at Sirinka in 2010/11.

Traits	DF	DM	NPPP	NS	SWPP	NSPP	PHH	SW	PSS
DF	**-0.06**	-0.12	0.03	0.03	0.11	-0.04	0.05	0.03	-0.02
DM	0.04	**-0.19**	-0.03	0.03	0.12	-0.01	-0.01	0.11	-0.02
NPPP	0.01	0.02	**0.31**	0.00	0.36	-0.22	0.05	-0.04	0.00
NS	0.01	-0.02	0.00	**0.21**	0.15	-0.13	0.01	-0.01	-0.01
SWPP	0.01	-0.04	0.23	0.07	**0.48**	-0.22	0.04	0.05	-0.003
NSPP	0.01	-0.003	0.25	0.10	0.39	**-0.27**	0.05	-0.03	-0.002
PHH	0.02	0.01	0.10	0.02	0.10	-0.08	**0.17**	-0.03	0.002
SW	0.01	-0.09	-0.05	-0.01	0.10	0.04	-0.02	**0.22**	-0.001
PSS	-0.02	0.06	0.00	-0.03	-0.03	0.01	0.01	-0.01	**0.05**

Contribution of residuals in the variability = 0.57. DFF = days to 50% flowering, DM = days to 90% maturity, NPPP = number of pods per plant, NS = number of seeds per pod, SWPP = seed weight per plant, NSPP = number of seeds per plant, PH = plant height in cm, SW = 100-seed weight in gram, BI = above ground biomass, SY = seed yield, **, * = Significant at 0.01 and 0.05 probability levels.

effect dependent on nine independent variables, which were considered as causes. The independent characters were: days to 50% flowering (DFF), days to 90% maturity (DM), number of pods per plant (NPPP), number of seeds per pods (NS), number of seeds per plant (NSPP), seed weight per plant (SWPP), plant height (PH), 100 seed weight (SW) and rust disease severity score.

The path analysis revealed that seed weight per plant (0.48) followed by number of pods per plant (0.31) and 100 seed weight (0.22) had exerted positive direct effects on seed yield at Sirinka in 2010/11 (Table 7). In addition, numbers of seeds per pod and plant height have positive direct effects on seed yield. However, number of seeds per plant (-0.27) and days to 90% maturity (-0.19) had negative direct effects on seed yield. With the exception of seed weight per plant and number of seeds per plant, the indirect effects of all traits on seed yield via other traits were small and negligible. The contribution of all traits in explaining the variability of seed yield was lower than the residual effect (0.57). More than 50% of the variability of seed yield at Sirinka 2011/12 was accounted for by seed weight per plant and number of pods per plant followed by the interaction of seed weight per plant and number of pods per plant, the rest of the traits had very little effect on the variability of seed yield.

Result of the path analysis are consistent with the phenotypic and genotype correlation coefficients which showed that seed weight per plant and number of pods per plant had highly significant genotypic and phenotypic correlation coefficients with seed yield (Table 3). Number of seeds per plant had significant positive correlation with seed yield and had a substantially high negative direct effect (-0.27). The positive indirect effects of number of seeds per plant via seed weight per plant, number of pods per plant and number of seeds per pod, might counter-balance the final positive correlation with seed yield (Table 7). Path coefficient analysis at Sirinka in 2011/12 cropping season, gave essentially similar pattern of relationship between yield and yield components with that of Sirinka in 2010/11. Seed weight per plant, plant height and numbers of pods per plant had strong positive direct effect on seed yield. These characters had positive indirect influence via seed weight per plant, days to 90% maturity, number of pods per plant, number of seeds per plant and 100 seed weight on seed yield (Table 8).

At Chefe Donsa, seed weight per plant (0.62), plant height (0.32), and number of pods per plant (0.28) had positive direct effect on seed yield. Number of seeds per pod, number of seeds per plant, and 100 seed weight had significant positive indirect effect on seed yield via seed weight per plant and plant height. The strong direct effects of these three characters on seed yield is consistent with the phenotypic correlation coefficient of these traits with seed yield (r_p = 0.54, 0.25 and 0.45, respectively) (Table 5). Number of seeds per plant had negative direct effect on seed yield but it had a significant phenotypic correlation coefficient (r_p= 0.47) with seed yield. This may be due to the indirect positive effect of this character on seed yield via seed weight per plant, number of pods per plant, number of seeds per pod, 100 seed weight and days to 50% flowering and 90% maturity that counter balanced the final correlation in the positive direction (Table 9). Rust disease score, days to 50% flowering and 90% maturity and 100 seed weight in gram had considerable negative direct and indirect effects on seed yield via days to 50% flowering, days to 90% maturity, number of pods per plant, number of seeds per pod, plant height and 100 seed weight (Table 9). The direct and indirect effect of the casual variables considered in this study were higher than the residual effects

Table 8. Phenotypic direct effects on main diagonal (bold) and indirect effects of different agronomic traits on seed yield of lentil genotypes at Sirinka in 2011/12.

Trait	DF	DM	NPPP	NS	SWPP	NSPP	PHH	SW
DF	**-0.04**	-0.023	-0.001	-0.001	0.027	-0.001	0.018	-0.0001
DM	-0.021	**-0.045**	-0.006	-0.001	-0.01	-0.01	-0.004	-0.002
NPPP	0.001	0.005	**0.056**	0.001	0.303	0.027	0.033	0.001
NS	-0.006	-0.008	0.002	**-0.01**	0.116	0.014	0.012	0.0002
SWPP	-0.002	0.0004	0.026	-0.001	**0.641**	0.038	0.044	-0.001
NSPP	0.001	0.005	0.033	-0.002	0.533	**-0.046**	0.051	0.002
PHH	-0.007	0.002	0.017	-0.001	0.256	0.021	**0.11**	0.001
SW	-0.001	-0.01	-0.011	0.001	0.072	-0.01	-0.01	**-0.007**

Contribution of residuals in the variability = 0.4195. DFF = days to 50% flowering, DM = days to 90% maturity, NPPP = number of pods per plant, NS = number of seeds per pod, SWPP = seed weight per plant, NSPP = number of seeds per plant, PH = plant height in cm, SW = 100-seed weight in gram, BI = above ground biomass, SY = seed yield, **, *Significance at 0.01 and 0.05 probability levels.

Table 9. Phenotypic direct effects on main diagonal (bold) and indirect effects of different agronomic traits on seed yield of lentil genotypes at Chefe Donsa in 2011/12.

Trait	DF	DM	NPPP	NS	SWPP	NSPP	PHH	SW	RDSAF
DF	**-0.09**	-0.1	-0.01	-0.02	-0.08	0.04	0.08	-0.02	0.03
DM	-0.05	**-0.19**	-0.02	-0.03	-0.05	0.05	0.06	-0.05	0.06
NPPP	0.02	0.04	**0.28**	0.01	0.52	-0.16	0	0.03	-0.01
NS	0.02	0.08	0.01	**0.07**	0.11	-0.06	-0.01	0.03	-0.03
SWPP	0.01	0.02	0.07	0.01	**0.62**	-0.14	0.006	-0.01	0.01
NSPP	0.02	0.06	0.07	0.03	0.51	**-0.17**	0.002	0.03	-0.02
PHH	-0.02	-0.04	0.001	0.003	0.01	0	**0.32**	0.01	0.01
SW	-0.02	-0.09	-0.02	-0.02	0.03	0.05	-0.01	**-0.11**	0.06
RDS	0.02	0.08	0.01	0.02	-0.04	-0.03	-0.03	0.05	**-0.13**

Contribution of residuals in the variability = 0.4905. DFF = days to 50% flowering, DM = days to 90% maturity, NPPP = number of pods per plant, NS= number of seeds per pod, SWPP = Seed weight per Plant, NSPP = Number of Seeds per Plant, PH = Plant height in cm, SW = 100-seed weight in gram, BI = above ground biomass, SY = seed yield, ** and * =Significant at 0.01 and 0.05 probability levels.

(0.49) which explain its effect on seed yield. More than 50% of seed yield response was explained by seed weight per plant followed by plant height at Chefe Donsa in 2011/12.

At Sinana in the 2011/12 cropping season, the highest positive direct effect were shown by number of pods per plant (0.49), followed by 100 seed weight in gram (0.28) (Table 10). Plant height (0.18) showed the third largest positive direct effect on seed yield. In addition to its direct effect, the indirect influence of number of pods per plant via number of seeds per plant was substantially important for final contribution to seed yield (P_r=0.4). However, rust disease severity score (-0.25), number of seeds per plant (-0.171) and days to 50% flowering (-0.12) showed negative direct effects on seed yield.

In general, from path analysis result of the four environments, we can deduce that seed weight per plant, number of pods per plant and plant height had consistently strong direct effects on seed yield. These traits had also average strong phenotypic correlation coefficient values (r_p = 0.64, 0.47 and 0.29, respectively) with seed yield. However, days to 50% flowering, days to 90% maturity, rust disease severity score and number of seeds per plant had negative direct effect on seed yield at the four environments. Phenotypic correlation coefficients also revealed that these traits had negative association with seed yield of lentil except for number of seeds per plant with an average value of r_p = -0.023, -0.26 and -0.055, respectively. Despite, negative direct effect of number of seeds per plant on seed yield, it had an average strong phenotypic correlation coefficient (r_p= 0.54) with seed yield. This may be due to the significant positive indirect effect of this character via number of pods per plant, seed weight per plant, number of seeds per pod, 100 seed weight in gram, days to 50% flowering and 90% maturity, and plant height that substantially step

Table 10. Phenotypic direct effects on main diagonal (bold) and indirect effects of different agronomic traits on seed yield of lentil genotypes at Sinana in 2011/12.

Trait	DF	DM	NPPP	NSPP	PHH	SW	RDSAF
DF	**-0.12**	-0.012	-0.094	0.023	0.014	0.05	0.025
DM	-0.03	**0.042**	0.093	-0.04	0.009	0.09	0.121
NPPP	0.02	-0.008	**0.493**	-0.16	-0.002	0.06	0.07
NSPP	0.02	-0.009	0.451	**-0.17**	0.014	0.06	0.061
PHH	-0.01	-0.002	-0.007	-0.01	**0.18**	0.03	0.011
SW	-0.02	-0.014	0.104	-0.03	0.017	**0.28**	0.125
RDSAF	0.01	0.02	-0.14	0.042	-0.008	-0.14	**-0.25**

Contribution of residuals in the variability = 0.5398. DFF = days to 50% flowering, DM = days to 90% maturity, NPPP = number of pods per plant, NS= number of seeds per pod, SWPP = Seed weight per Plant, NSPP = Number of Seeds per Plant, PH = Plant height in cm, SW = 100-seed weight in gram, BI = above ground biomass, SY = seed yield, ** and * = Significant at 0.01and 0.05 probability levels.

down the final correlation in the positive direction (Tables 8, 9 and 10).

DISCUSSION

Seed yield is a complex quantitative character governed by polygene, and is highly influenced by the environment. To improve yield, study of association of yield and yield components is of paramount importance. To increase yield, the breeder has to give sufficient attention to yield components that are favorably correlated with seed yield. Studies on correlation provide an opportunity for critically assessing the relationship of the component characters with seed yield. At Sirinka in 2010/11 cropping season, the magnitudes of genotypic correlation coefficients between all traits were higher than their phenotype correlation coefficients except for number of pods per plant, signifying that the association among various characters were of genetic causes rather than environmental effects. A similar trends were reported by Singh et al. (1999) and Tyagi and Khan (2011), genotypic correlation were of higher magnitude than their phenotypic counter parts in lentil.

Significant positive correlations were observed between seed yield and biomass, number of pods per plant, seed weight per plant, number of seed per plant and plant height at the four environments (Tables 3, 4 and 5). Other investigators also reported that seed yield was positively correlated with number of pods per plant, number of seeds per pods, seed weight per plant and biological yield in lentil (Dixit and Dubey, 1984; Balayan and Singh, 1986; Esmail et al., 1994; Bhattacharya, 1999; Naji et al., 2003; Singh et al., 2003). In another study, Abo-Shetaia et al. (1997) showed similar report both with highly significant phenotypic and genotypic association between lentil seed yield and number of pods per plant, and number of seeds per plant, the genotype correlation coefficient of seed yield with number of seeds per pod being negative in their study.

Number of pods per plant, number of seed per plant, seed weight per plant and above ground biomass had strong positive correlations among each other. Kumar and Sapra (1984) and Singh et al. (2003) reported that seed weight per plant was directly linked with number of pods per plant. Sinha and Singh (2002), Tigest (2003) and Ketema (2007) reported that number of seeds per plant was strongly and positively associated with number of pods per plant in lentil. This implies that genotype with larger number pods per plant produce higher number of seeds per plant. The relationship between seed yield and number of pods per plant, number of seeds per plant, seed weight per plant and above ground biomass suggested that selection based on these component characters would result in maximum yield in lentil. From this, we can infer that selection for either character increases the other traits and has to be considered as selection index for seed yield of lentil.

In support of our recommendation, Hamdi et al. (1991) and Khattab (1999) observed that pods per plant, seeds per plant and seed weight and biomass yield were the most important characters that contributed to grain yield. In addition to these, number of seeds per pods, plant height and 100 seed weight had significant ($p \leq 0.05$) positive phenotypic association with seed yield in all location except with 100 seed weight at Chefe Donsa. Vir et al. (2001) and Tyagi and Khan (2011) had reported such positive correlation between seed yield with plant height and 100-seed weight in lentil. Sinha and Singh (2002) and Sing et al. (2003) also reported in agreement with our findings that seed yield was significantly correlated with plant height, number of seeds per pods and seed weight. Hundred seed weight has negative phenotypic association with number of seeds per plant,

number of pods per plant and number of seeds per pods at all the environments except at Sinana. Such observation was also made by others (Tyagi and Sharma, 1985; Sharma et al., 1993; Tyagi and Khan, 2011). However, as opposed to our finding, Sinha and Singh (2002) reported that 100 seed weight was strongly and positively associated with number of seeds per plant and number of pods per plant in lentil.

Days to 50% flowering and 90% maturity and rust disease severity score showed significant negative phenotypic correlation with seed yield at all locations, except at Sinana where positive association was observed between days to 90% maturity and seed yield. At Sirinka and Chefe Donsa where terminal drought is a common phenomena, earliness is favorable characters for better performance in such location however, at Sinana, incidence of terminal drought is not common, late maturing genotype may better perform for seed yield. Others also reported that seed yield had negative correlation with time to flowering, days to 90% maturity and seed weight in moisture deficit environments (Kumar and Sapra, 1984; Mia et al., 1986; Esmail et al., 1994; Tyagi and Khan, 2011). In another high potential environment, Manara and Manara (1988) found positive correlation of seed yield with days to 90% maturity, suggesting that seed yield could be increased by selecting late maturing genotypes with greater number of pods per plant whenever there is no terminal drought. These findings are also strengthened by Tyagi and Sharma (1985) and Dutta et al. (1993) who reported that above ground biomass and seed yield were negatively correlated with earliness.

Two characters may show correlation just because they are correlated with a common third one. In such cases, it becomes necessary to study a method that takes into account the causal relationship between the variables in addition to the degree of such relationship. The phenotypic and genotypic correlations were further subjected to path coefficient analysis, which involves partitioning of the correlation coefficients into direct and indirect effects via alternative characters (Falconer and Mackay, 1996). Since correlations provide only limited information ignoring complex interrelationships among traits, further partitioning of genetic correlations into direct and indirect effects using the path-coefficient analysis provides better picture of the relationship of predictor variables with the response variable (Rao et al., 1997). Seed yield being a complex outcome contributed by different component traits, it was considered to be the resultant variable, while days to 50% flowering, days to maturity, number of pods per plant, number of seeds per pods, pod weight per plant, number of seeds per plant, seed weight per plant, plant height and 100 seed weight were causal variables.

Path coefficient analysis for seed yield over the two seasons at Sirinka revealed that seed weight per plant, number of pods per plant, and plant height had consistently strong direct effects on seed yield. Therefore, for selecting high yielding genotypes, the breeder should give more emphasis to plants with higher seed weight per plant, and more number of pods per plant. Phenotypic and genotype correlation coefficients of seed weight per plant and number of pods per plant were also high with seed yield at Sirinka 2010/11. Dixit and Dubey (1984), Balayan and Singh (1986), Tikka et al. (1997), Hamdi et al. (2003), Verma et al. (2004) and Tyagi and Khan (2011) also reported that number of pods per plant, plant height and seed weight per plant were the highest direct contributors towards better seed yield in their respective studies. In addition, Jain et al. (1991) and Begum and Begum (1996) reported that a combination of two or three variables, viz. plant height, number of branches per plant and pods per plant were found to be better than other combination of characters for the improvement of seed yield in lentil.

However, days to 50% flowering and days to 90% maturity, which had negative phenotypic correlation coefficients with seed yield, had also substantial negative direct effects on seed yield. In spite of the negative direct effect of number of seeds per plant it had high genotypic and phenotype correlation coefficient with seed yield, and this association was due to positive indirect effect of this character via seed weight per plant, number of pods per plant, days to 50% flowering and 90% maturity, plant height and 100 seed weight that step down the final association in the positive direction (Table 7). Verma et al. (2004) also reported that number of seeds per plant has significant correlation with seed yield but it had a negative direct effect on seed yield under rain fed condition. The path analysis residual values, at Sirinka in 2010/11 was high indicating that some other factors, which have not been considered need to be included in the analysis to fully account for the variation in seed yield (Annex 1). The contribution of all traits in the variability of seed yield was higher than the residual effects (0.42) in 2011/1 (Table 8). More than 50% of seed yield variability was contributed by the effect of seed weight per plant (Annex 1, 2 and 3).

At Chefe Donsa and Sinana 2012, almost similar results were obtained in terms of both magnitude and direction. The highest positive direct effects were shown by number of pods per plant (0.49), followed by 100 seed weight (0.281) (Table 9). Plant height (0.18) showed the third largest positive direct effect on seed yield. These results were consistent with results from other studies (Khattab, 1999; Bhattacharya, 1999; Singh et al., 2003; Çokkizgin, 2007).

Days to 90% maturity, rust disease score and days to 50% flowering had negative direct effects on seed yield at the four environments, except positive direct effects of days to 90% maturity at Sirinka in 2010/11. The same traits had also negative phenotypic correlation coefficients

with seed yield of lentil. This was obvious for a dry land site like Sirinka where terminal drought is a common phenomena, genotypes which had long flowering period and late maturing genotypes with their physiological seed sinking process would be disturbed and fail to bring better seed yields. Whereas genotypes possessing early flowering and maturity attributes do well in these kinds of environments by escaping the terminal drought.

However, at Sinana where we do not have the incidence of terminal drought, late maturing genotype perform better in seed yield. Similar observation were reported by Kumar and Sapra (1984) and Tyagi and Khan (2011). However, Dutta et al. (1993) and Kumar et al. (2004) observed that days to 50% flowering and 90% maturity had the highest direct effects on seed yield, and could justify that increased seed yield were due to a long crop growing season that may affect an increase sink sites, not to impaired grain development during high temperature in the latter part of the season.

Conclusion

This study clearly showed that for selecting high yielding genotypes, the breeder should give more emphasis to plants with higher seed weight per plant, more number of pods per plant and higher number of seeds per plant, and plants with higher above ground biomass. The path coefficients were consistent with phenotypic and genotype correlation coefficients in that seed weight per plant and number of pods per plant showed not only significant positive genotypic and phenotypic correlation coefficients with seed yield but also higher direct effects. On the other hand, days to 50% flowering and 90% maturity and rust disease severity score showed both negative phenotypic correlation coefficient and negative direct effects on seed yield except for the positive direct effect of days to 90% maturity with seed yield at Sinana. From phenotypic, genotypic and path coefficients it could be conclude that traits like seed weight per plant, number of pods per plant, above ground biomass, number of seeds per plant and plant height had a considerable role as selection criteria to improve lentil seed yield.

Conflict of Interests

The author(s) have declared that there is no conflict of interests.

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Annex 1. Contribution of direct and indirect effects of agro-morphological traits on seed yield variability of lentil at Sirinka 2011 cropping season.

Trait	DFF	DM	NP	NSS	SWPP	TNSPP	PH	SW
DFF	0.003							
DM	-0.014	0.035						
NP	0.003	0.011	0.095					
NSS	0.004	-0.009	0	0.046				
SWPP	0.012	-0.043	0.221	0.062	0.233			
TNSPP	-0.005	0.002	-0.135	-0.054	-0.214	0.074		
PH	0.005	0.004	0.032	0.006	0.035	-0.028	0.028	
SW	0.003	-0.039	-0.024	-0.005	0.045	0.017	-0.01	0.048

Contribution of residuals in the variability = 0.5713.

Annex 2. Contribution of direct and indirect effects of agro-morphological traits on seed yield variability of lentil at Sirinka 2012 cropping season.

Trait	DFF	DM	NP	SWPP	TNSPP	PH	SW
DFF	0.00163						
DM	0.00188	0.00206					
NP	0.00011	0.00055	0.00309				
SWPP	-0.00221	0.00054	0.03364	0.41112			
TNSPP	0.00008	0.00049	0.00304	0.04878	0.00209		
PH	-0.00144	0.00036	0.00365	0.0565	0.00463	0.01215	
SW	0.00001	0.00014	0.00015	-0.00099	0.00014	0.00017	0.00005

Contribution of residuals in the variability = 0.4195.

Annex 3. Contribution of direct and indirect effects of agro-morphological traits on seed yield variability of lentil at Chefe Donsa 2012 cropping season.

Trait	DFF	DM	NP	NS	SWPP	TNSPP	PH	SW	RDSAF
DFF	0.009								
DM	0.019	0.037							
NP	0.002	0.006	0.006						
NS	0.003	0.011	0.001	0.005					
SWPP	0.014	0.019	0.081	0.015	0.385				
TNSPP	-0.007	-0.018	-0.024	-0.009	-0.169	0.027			
PH	-0.015	-0.024	0.001	-0.001	0.004	-0.001	0.105		
SW	0.003	0.020	0.004	0.004	-0.006	-0.010	0.002	0.013	
RDSAF	-0.006	-0.023	-0.002	-0.004	0.012	0.007	0.008	-0.013	0.018

Contribution of residuals in the variability = 0.4905.

Annex 4. Contribution of direct and indirect effects of agro-morphological traits on seed yield variability of lentil at Sinana 2012 cropping season.

Trait	DFF	DM	NP	NSPP	PH	SW	RSBF	RSAF
DFF	0.015							
DM	0.003	0.002						
NP	0.023	-0.008	0.243					
NSPP	-0.006	0.003	-0.154	0.029				
PH	-0.003	-0.001	-0.002	-0.005	0.032			

Annex 4. Contd.

SW	-0.011	-0.008	0.059	-0.019	0.009	0.079		
RSBF	-0.001	-0.001	0.005	-0.002	0.000	0.004	0.001	
RSAF	-0.006	-0.010	0.069	-0.021	0.004	0.070	0.005	0.061

Contribution of residuals in the variability = 0.4905.

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