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Understanding the Phenotypic Diversity and Ecological Distribution of Noug (Guizotia abyssinica) for Its Improvement and Conservation

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Abstract
Noug *(Guizotia abyssinica)* is an important oil crop to Ethiopia where it has been cultivated as source of edible oil since antiquity. Despite the country harbors ample genetic resources of noug, diversity and ecological information useful for its improvement and conservation is very limited. The aim of this study was to assess noug phenotypic diversity along with its ecological distribution based on morphological characters and environmental data. During 2009/10 cropping season, one hundred noug accessions which were collected from different parts of Ethiopia were planted augmented with four standard checks at two testing sites namely Holetta and Ginchi. Diversity and correlation analysis followed by principal component analysis were conducted for agro-morphological traits using statistical software package *R v. 2.10.0/1.17-3*. Non-metric multidimensional scaling (NMDS) analysis was made to understand which environmental variable that drives phenotypic diversity. Ecological niche modeling using *Maxent* software programme was also employed to comprehend the agro-ecological niche of noug. According to diversity analysis of phenotypic data, most traits showed considerable diversity within and among populations. The correlation analysis revealed that the number of seeds per plant and number of heads was positively correlated with the number of primary or secondary branches. A significant positive correlation was also obtained between the number of heads and number of seed per plant, and 1000 seed weight and the number of seed per plant. Seven noug populations (N07027, N07028, N07001, N08009, N08002, N08048 and N07009) were found to be unique populations. NMDS analysis showed precipitation was the most important environmental factor that drives noug phenotypic diversity. Ecological niche modeling indicates that the northern and western parts of Ethiopia were found to be the dominant noug niches which accounts > 80% of the total noug growing areas. Kenya followed by Eritrea were other African countries where the crop was grown in some pocket area. A number of noug populations useful for breeding efforts were also identified. In conclusion, noug populations variable for many traits have been identified and such variability is useful for its improvement and *ex situ* conservation effort of the crop.
Keywords: - Noug (*Guizotia abyssinica*), genetic diversity, noug improvement and conservation.
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1. Introduction

Noug (*Guizotia abyssinica*) is an oil-seed crop, indigenous to Ethiopia, where it is the major source of edible oil and holds significant promise for improving rural livelihoods. Noug oil is the most preferred cooking oil and thus most oil mills located in the major cities or small towns of Ethiopia extract noug oil. In addition to its oil, noug is an important source of seed proteins, carbohydrates, minerals, vitamins and fiber that significantly contributes to the human dietary intake of resource-poor farmers (Tesfaye Deme et.al. 2017). Noug is used in intercropping systems and can grow on waterlogged, marginal and poor soils where most other crops fail to grow. The noug seed is consumed after being processed in various forms for its unique nutritional, medicinal and cultural values (Geleta et al. 2002). Despite its enormous importance to Ethiopian farmers, the national average seed yield of noug landraces is not more than 9Q/ha (CSA 2016), which is significantly lower than that of improved varieties of other edible oil crops, such as sunflower and oilseed brassicas. The main aim of noug improvement efforts in Ethiopia is, therefore, provision of high yielding noug cultivars to farmers through breeding using the available genetic resources along with suitable crop management practices.

Ethiopia is considered the center of origin and domestication of noug (Murthy, Hiremath & Salimath , 1993). It is believed that the country possess wide genetic resources of noug which are conserved in gene banks or maintained by farmers. Utilization of the existing gene pool of any crop though evaluation of its germplasm is the base for its improvement (Rauf et.al., 2010).The first major characterization and evaluation scheme of noug were conducted in 1982 and 1983 using 243 and 127 noug accessions, respectively which were collected from major regions of Ethiopia, Gojam Gondor, Shewa Wellega and Wello in 1981. These accessions were evaluated at Holetta Agricultural Research Center for various agro-morphological and quality traits (Getinet and Sherma, 1996). It was also possible to group these accessions in to different maturity groups such as early flowering type (75 -90 days), medium groups (105-120 cm) and late groups (105 -120 days). Based on these accessions some diversity studies were conducted by Genet and Ketema (2000) that indicated more diverse noug population found in major noug growing regions of Ethiopia namely Wellega, Gojjam, Shewa and Gondor. Recent studies on Ethiopian noug populations revealed high levels of genetic variation for different agro morphological traits (Geleta et al. 2007, 2008). According to the recommendations of the above authors, additional collections missions are necessary to exploit the gene pool of noug so that the wealth genetic diversity of the crop will be exploited for its improvement and conservation. Therefore, the objective of this study was to comprehend the genetic diversity of noug germplasms based on morphological characters and its ecological distribution.

2. Materials and methods

Noug collections were made in the central, western and northern part of Ethiopia during the noug harvest seasons of 2007 and 2008. The collection targeted to areas where noug has either not been collected or has never been collected before (Table 1 and Fig 1). A GPS device was used to record the precise geographical location of each collection. A total of 116 accessions were planted on the 15^{th} and 17^{th} of July 2010 at Holetta and Ginchi, respectively augmented with four checks in four blocks each containing 29 accessions. Each accession sown with 0.3 m distance between two rows of the same accessions and 0.6m distance between plots of different accessions. Each plot was phenotyped using 10 randomly selected plants in terms of plant height, number of primary and secondary branch, leaf width, and number of seed per plant, head size, number of head per plant, number of seed per head and 1000 seed weight. Environmental data were taken for all collecting locations using the publicly available Worldclim/Bioclim dataset (http://www.worldclim.org/bioclim), which contains a set of 19 environmental variables, including precipitation and temperature data. Data were transformed using square (height), square root (leaf width, number of secondary branches and seeds per head) or logarithmic (number of heads, number of primary branches, head size and seeds per plant) to meet the assumptions of normality.

Phenotypic diversity for morphological traits was analyzed considering both testing sites. Pearson's partial correlation analysis was used to measure phenotypic correlations while the level of correlations of traits with different environmental variables was investigated using a non-metric multidimensional scaling (NMDS). Principal component analysis (PCA) was also performed to identify unique populations from the noug populations under study. Statistical package *R,* version *2.10.0* (*R* Development Core Team 2011) and version *1.17-3* (Oksanen et al. 2010) were used for the above analysis. Ecological niche modeling technique was employed using the software *Maxent* to model noug's agro-ecological niche both for Ethiopia and Africa.

3. Results and discussion

3.1 Phenotypic diversity and correlation

According to diversity analysis of phenotypic data, most traits showed considerable diversity within and among populations as shown in Fig.1, which displays most of the traits assessed in both locations (Holetta and Ginchi). Conducting phenotypic characterization at two different sites allowed us to assess the level of phenotypic plasticity which was found to be high for most traits.

In addition to the diversity study, phenotypic correlation among agronomically important traits was also analyzed using Pearson's Partial Correlation Analysis. This result showed that significant positive correlation between the number of primary/secondary branches and the number of heads and seed per plant at both sites (Holetta and Ginchi). A significant positive correlation was also observed between the number of head and number of seed per plant, and 1000 seed weight and the number of seed per plant (Table 2). This implies that unfavourable phenotypic correlations were not found in noug and thus the plant architectural constraints could not be a factor to hider breeding noug for larger and more seed. Correlations between 1000 seed weight and the number of seed per head and the number of seed per plant were not significant, and showed different trends in the two sites. This might due to the slight environmental difference between the two locations where Ginchi is found at lower altitude (by 115 meter), receive less rainfall (250 mm mean rainfall) and has heavier soil than Holetta.

3.2 Principal component analysis

According to the principal component, noug populations such as N07027 and N07028 collected from Benishangul Gumuz, N07001, N08009 and N08002 from Amhara, N08048 and N07009 from Oromia regions were found to be the most extreme outliers that indicates their unique nature or differ in genetic make-up from other populations (Fig. 3). These noug populations were found from previously uncollected districts of the above regions.

3.3 Non-metric multidimensional scaling (NMDS) analysis

Among the 19 environmental variables that mostly correlated with the observed spread phenotypic diversity is precipitation. As we observed from NMDS analysis, three precipitation variables: - i) Precipitation of Wettest Quarter, ii) Precipitation of Wettest Month and iii) Precipitation of Coldest Quarters are highly correlated with each other that is why they seem to be align in parallel as shown in the figure 4. This indicates that the level of precipitation during the growing season of noug is likely the most important environmental factor that drives noug phenotypic diversity. The analysis of NMDS is also revealed that precipitation in the wettest quarter seems to be the environmental factor most strongly correlated with phenotypic diversity. This indicates that there is likely local adaptation to different precipitation regimes.

3.4 Distribution and area coverage of noug

Based on ecological niche modeling using locations of geo-referenced noug populations and GIS data, noug is dominantly distributed in the northern and western part of the Ethiopia where the crop grown with altitude ranges from 750-3397m a.s.l (Fig 5 and Table 1). Oromia region followed by Amara occupy large area for noug production whereby the former region cover an average area of 154,833 ha while the latter 100434 ha during the last ten years (Fig 6.) According to geospatial map of the continent Africa, noug is assumed to be grown in other Africa countries such as Kenya and Eritrea (Fig 7). Such modeling attempts are important in the context of promoting and facilitating the conservation and use of neglected and underutilized crops like noug.

3.5 Towards a breeding program for noug

A wide range of data for important traits of noug was generated and noug populations relevant to the breeding efforts were identified based on their useful agronomic traits (Table 3). A total of 100 new accessions were obtained and such collections significantly enhance the *ex situ* collection in Ethiopia. It should be noted that there are some additional areas in Ethiopia that could not be addressed due to their remoteness and that might harbor further interesting materials.

4. Conclusions

In general, this study has clearly shown that there is a considerable amount of noug diversity present in farmers' fields that can be used for breeding program. Such diversity was mainly within population which implies the need of on-farm conservation efforts in all major noug growing regions. It could be suggested that future breeding efforts of noug should focus on strong selection for more heads and larger seeds to boost its yield in addition to other relevant traits such as synchronous flowering and reduced shattering. Germplasm evaluation would result in identification of valuable noug germplasms with useful agronomic traits. It will be important for noug breeders to be aware of the high levels of phenotypic plasticity of the crop, which requires testing of the cultivars in a wide variety of different environments.

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Authors Bibliography

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Table 1. Noug accessions collected from various areas of major regions of Ethiopia in 2007 and 2008

S.No	Acc. designation	Altitude (m)	Region of collection	S.No	Acc. designation	Altitude (m)	Region of collection
1	N08027	1251	Amhara	53	N08040	3397	Amhara
2	N08031	1216	Amhara	54	RC-2	1804	Oromiya
3	N08051	1333	Oromiya	55	N08003	2778	Amhara
4	N08028	1659	Amhara	56	N07-005	1493	Oromiya
5	N08032	1690	Tigray	57	N07-006	1487	SNNP
6	N08033	2149	Tigray	58	N07-012	1494	Oromiya
7	N08034	1896	Tigray	59	N07-013	1494	Oromiya
8	N08038	1716	Amhara	60	N07-014	1787	Oromiya
9	N08055	1410	Oromiya	61	N07-015	1525	Oromiya
10	N07-046	2544	Amhara	62	N07-016	1525	Oromiya
11	N08001	2481	Amhara	63	N07-033	1227	Oromiya
12	N08002	2612	Amhara	64	N07-036	1343	Oromiya
13	N08039	2171	Amhara	65	N07-037	1557	Oromiya
14	N08042	2917	Amhara	66	N07-038	1557	Oromiya
15	N08043	2826	Amhara	67	N08009	1500	Amhara
16	N08044	2531	Oromiya	68	N08010	1276	Amhara
17	N07-025	1335	Oromiya	69	N08011	1212	Amhara
18	N07-026	1414	BSG	70	N07-003	1669	Oromiya
19	N07-027	1445	BSG	71	N07-003	1669	Oromiya
20	N07-028	1293	BSG	72	N07-004	1764	Oromiya
21	N07-029	1411	BSG	73	N07-009	1635	Oromiya
22	N07-029	1411	BSG	74	N07-010	1635	Oromiya
23	N07-030	1334	BSG	75	N07-017	1763	Oromiya
24	N08012	1208	Amhara	76	N07-018	1829	Oromiya
25	N08013	1484	Amhara	77	N07-019	1829	Oromiya
26	N08016	872	Amhara	78	N07-020	1832	Oromiya
27	N08017	872	Amhara	79	N07-021	1832	Oromiya
28	N08018	820	Amhara	80	N07-022	1672	Oromiya
29	N08019	770	Amhara	81	N07-023	1521	Oromiya
30	N07-001	2022	Oromiya	82	N07-023	1521	Oromiya
31	N07-001	2022	Oromiya	83	N07-023	1521	Oromiya
32	N07-002	2070	Oromiya	84	N07-024	1510	Oromiya
33	N07-011	1562	Oromiya	85	N07-039	2203	Oromiya
34	N07-031	1482	BSG	86	N07-040	1846	Oromiya
35	N07-032	1462	BSG	87	N07-042	1909	Amhara
36	N08014	1784	Amhara	88	N07-043	1952	Amhara
37	N08020	1929	Amhara	89	N07-044	2056	Amhara
38	N08021	1878	Amhara	90	N07-045	1868	Amhara
39	N08022	1833	Amhara	91	N08005	2143	Amhara
40	N08023	1833	Amhara	92	N08006	2086	Amhara
41	N08024	1762	Amhara	93	N08007	2086	Amhara
42	N08026	2116	Amhara	94	N08008	2078	Amhara
43	N08029	1842	Amhara	95	RC-12	1569	Oromiya
44	N08030	2462	Amhara	96	RC-19	1868	Amhara

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Vol.9, No.5, 2019

S.No	Acc. designation	Altitude (m)	Region of collection	S.No	Acc. designation	Altitude (m)	Region of collection
45	N08035	2597	Amhara	97	RC-3	1804	Oromiya
46	N08036	1591	Amhara	98	RC-4	1832	Oromiya
47	N08037	2099	Amhara	99	RC-9	1636	Oromiya
48	N08041	2375	Amhara	100	N08004	3062	Amhara
49	N08046	2057	Oromiya	101	Fogera	-	-
50	N08047	1887	Oromiya	102	Esete	-	-
51	N08048	2004	Oromiya	103	Kuyu	-	-
52	N08049	2006	Oromiya	104	Shambu	-	-

Note: BSG = Benishangul Gumuz, SNNP= South Nation and Nationalities People

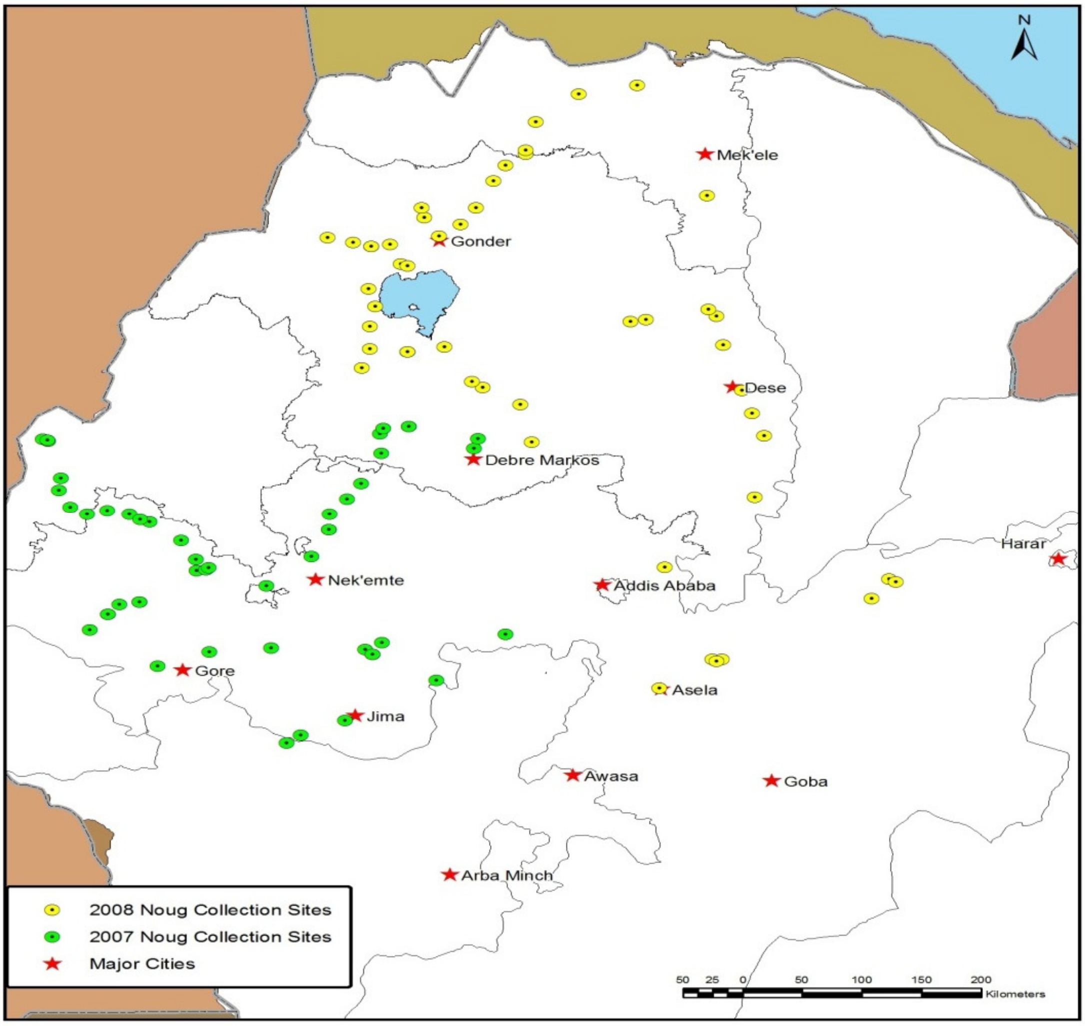

Fig.1: Noug collections made in year 2007 are shown in green, those made in 2008 are shown in yellow.

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Vol.9, No.5, 2019

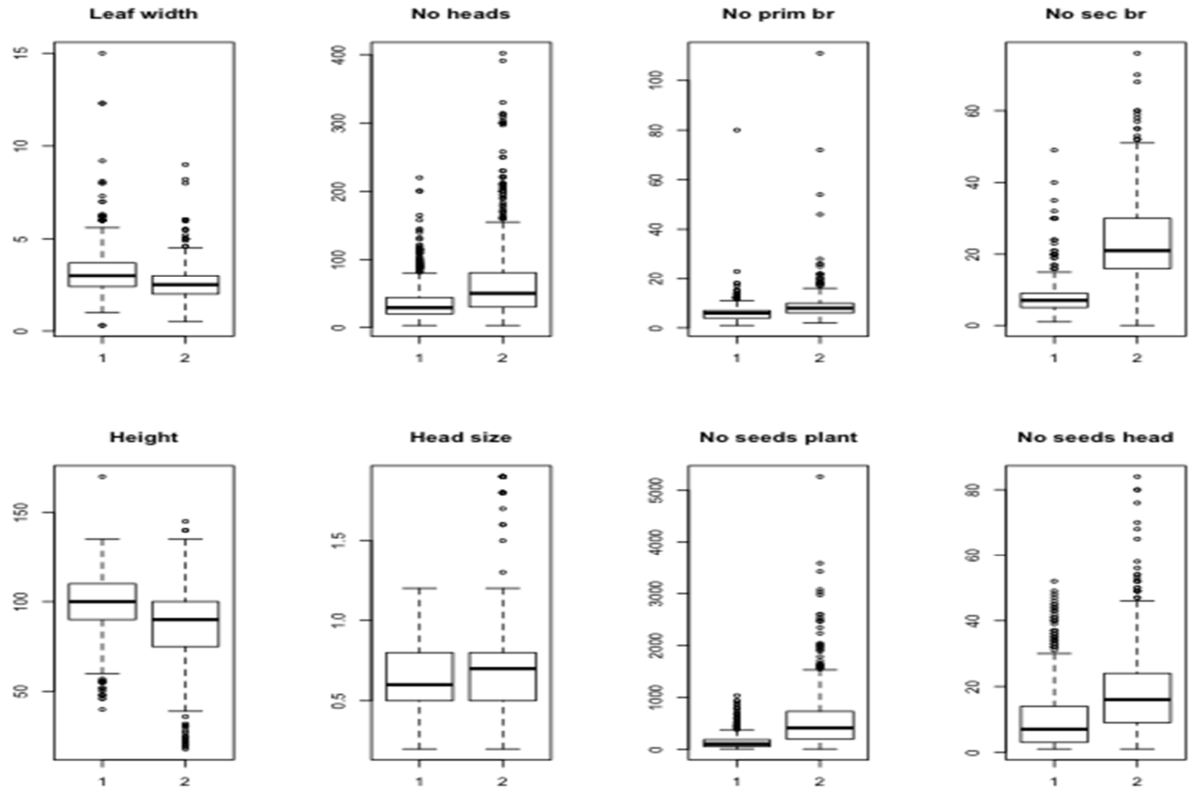

Fig.2: Boxplots showing differences between the two sites, Holetta (1) and Ginchi (2) for several phenotypic traits that were assessed.

Table 2. Phenotypic trait correlations of selected traits at Holetta and Ginchi

Traits	NH	NPB	NSB	PH	HS	NSPP	NSPH
NH	**1.00/1.00**	**-**	**-**	**-**	**-**	**-**	**-**
NPB	**0.6515***/0.4085*****	**1.00/1.00**	**-**	**-**	**-**	**-**	**-**
NSB	**0.355***/0.6466*****	**0.332***/0.5758*****	**1.00/1.00**	**-**	**-**	**-**	**-**
PH	**0.1705***/0.484*****	**0.143***/0.357*****	**0.1251***/0.3923*****	**1.00/1.00**	**-**	**-**	**-**
HS	**-0.0008NS/0.005NS**	**0.0235NS/0.05218NS**	**0.0409NS/0.0861****	**0.0174NS/0.1325*****	**1.00/1.00**	**-**	**-**
NSPP	**0.4413***/ 0.2406*****	**0.368***/0.2656*****	**0.2118***/0.2098*****	**0.0505NS/0.0485NS**	**-0.0158NS/0.0143NS**	**1.00/1.00**	**-**
NSPH	**0.0083NS/-0.1111****	**0.0359NS/-0.0481NS**	**0.049NS/-0.0743***	**0.024NS/-0.1831*****	**0.0758**/-0.0235NS**	**0.3652***/0.1373*****	**1.00/1.00**
TSW	**0.0666NS/-0.1677NS**	**0.1743NS/-0.0463NS**	**0.145NS/0.2769****	**0.2947*/0.1731NS**	**0.0666NS/0.4582*****	**-0.0884NS/0.06441NS**	**0.0714NS/-0.785NS**

Note : Values stated above as Holetta followed by Ginchi separated by slash '/', *, ** and *** denote the strength significance as significant, highly significant and very highly significant, respectively at P<0.05. NH: Number of heads, NPB: Number of primary branch, NSB: Number of secondary branch, PH: plant height, HS: head size, NSPP: Number of seed per plant, NSPH: Number of seed per head, TSW: Thousands Seed Weight.

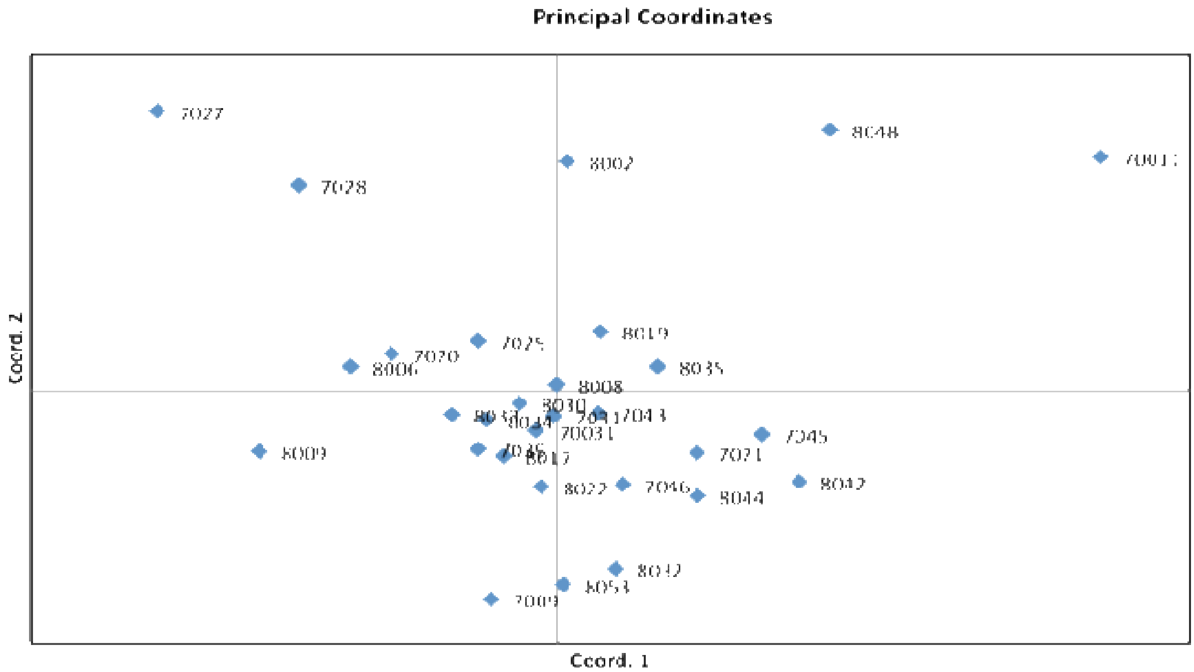

Fig.3 Principal coordinates analysis based on a Fst distance matrix, which has been calculated from the generated genotypic data.

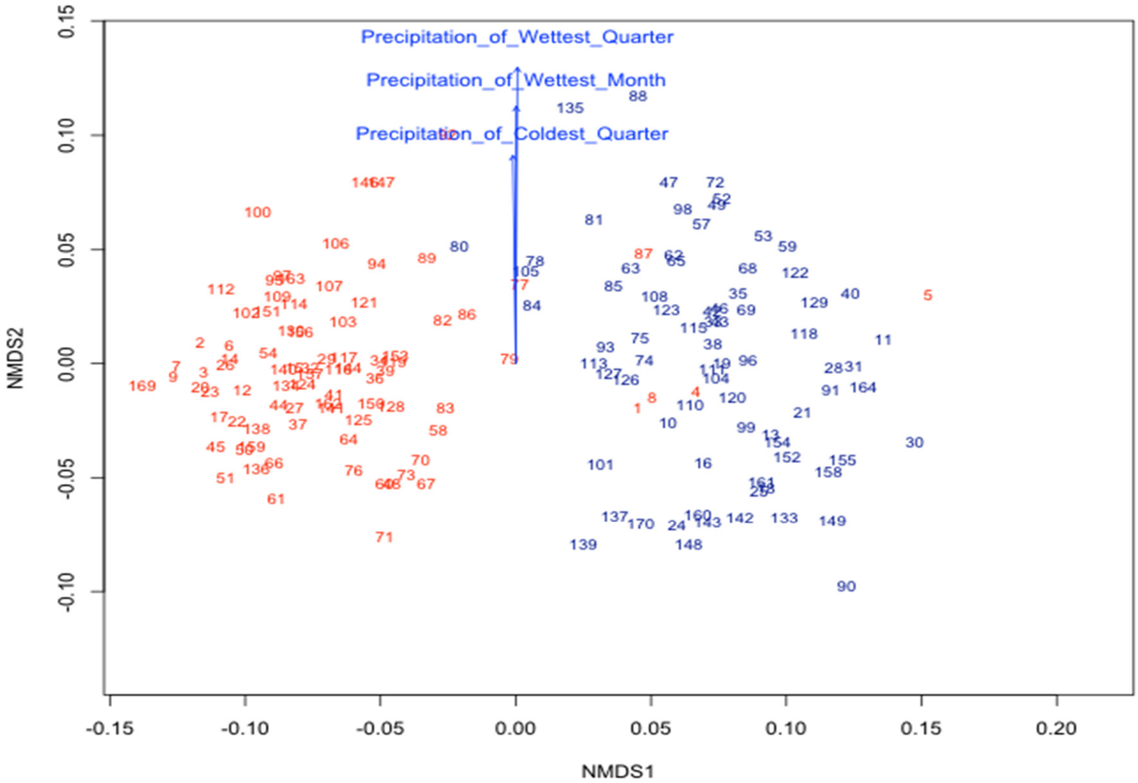

Fig. 4. NMDS analysis of phenotypic data of both sites (red = site 1 and blue = site 2) showing correlations with environmental variables.

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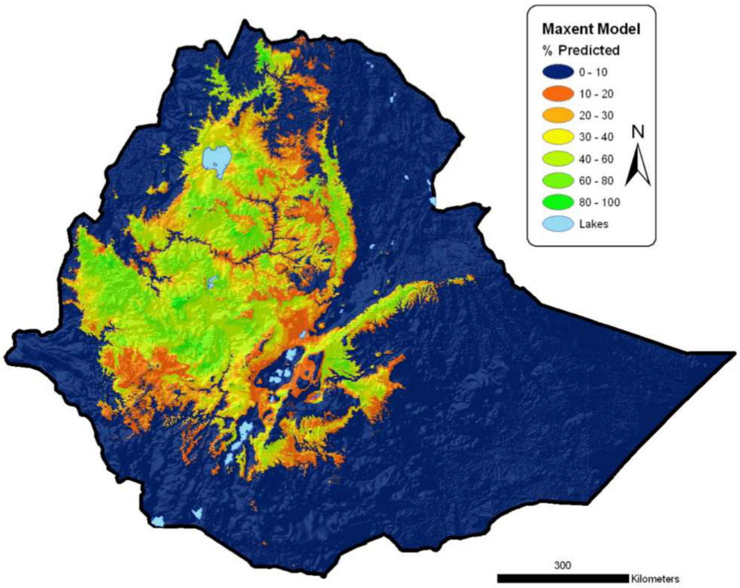

Fig.5: Map of Ethiopia showing the predicted agro-ecological niche for noug.

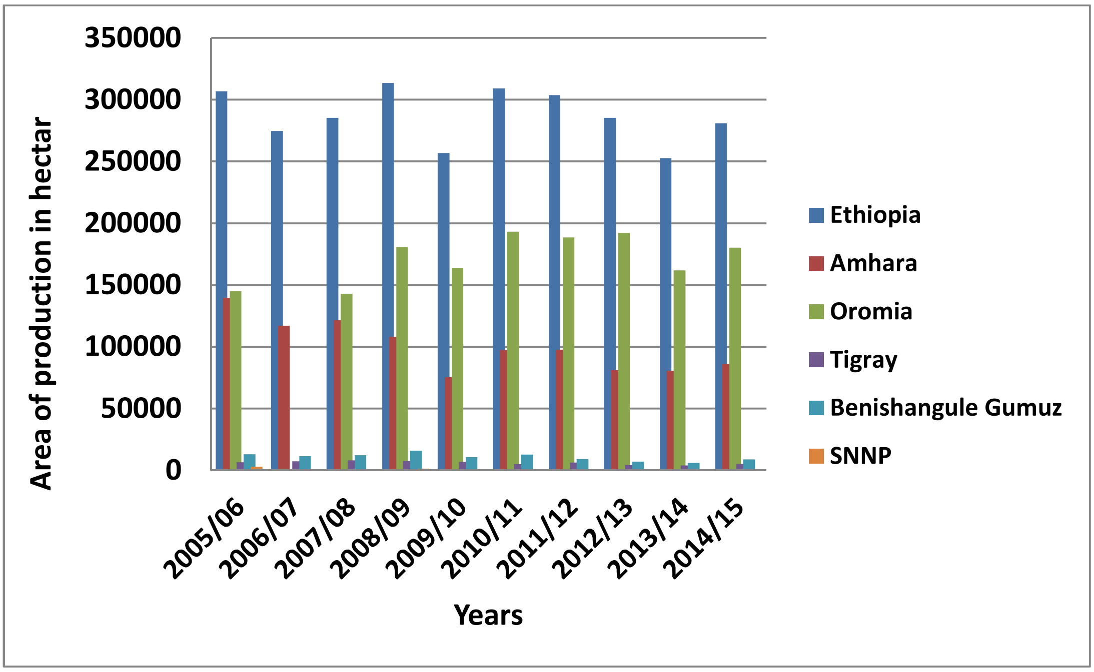

Fig 6. Area of production of noug in Ethiopia for the last 10 years

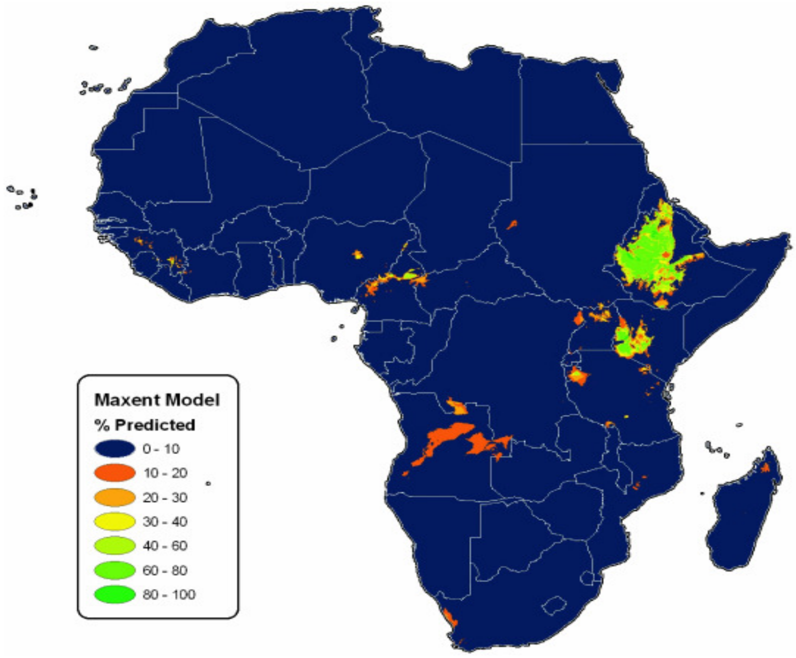

Fig.7. Map of Africa, indicating which areas would be suitable for noug production.

Table 3. Noug genotypes identified as potential interest to breeding efforts

Useful traits	Collections with useful trait
Maximum number of heads (observed range: 8 to 144 heads; mean value: 50)	N07-024 (144); N08-019 (136); N07-029-1 (135); N07-032 (126) and N07-026 (125)
Height – shortest (observed range: 24cm to 117cm; mean value: 93cm)	N08-037 (24cm); N08-034 (27cm); N08-043 (54cm); N08-034 (57cm); N08-037 (58cm)
Number of seeds per plant (observed range: 34 to 1465; mean value: 337)	N07-015 (1465); N07-020 (1008); N07-004 (1385); N07-002 (1134) and N08-001 (994)
Number of seeds per head (observed range: 0 to 52; mean value:14)	N08-001 (52); N07-012 (32); N08-042 N08-008 (34); N07-005 (32);
1000 seed weight (observed range: 0.7 to 4.9g;mean value: 3.1g)	N08-028 (4.9); N08-48 (4.9); N08-017 (4.8); N08-026 (4.8) and N08-030 (4.8)

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