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Full Length Research Paper

Performance of *Vernonia galamensis* as a potential and viable industrial oil plant in Eritrea: Yield and oil content[-1]

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***Vernonia galamensis*, whose seeds can be used to produce high-demand, environmentally friendly oil, can stimulate the economy of a country like Eritrea. The seed from the plant contains oil rich in epoxy fatty acids. A potential market use is as a drying agent for resin paints and can form clear, tough, rubbery plastics or coatings on metal. The general objective was to develop vernonia as a viable industrial plant in Eritrea while the specific objectives were to collect, introduce, and characterize wild vernonia accessions and to evaluate and select the best genotypes with higher seed yield and oil content. A total of 61 wild accessions of vernonia were collected from different parts of Eritrea. Some germplasm materials were also added from Ethiopia in 1995 and collections from the United States Department of Agriculture. The materials were collected in its wild form in valleys, riverbanks, plateaus and hills in many parts of Eritrea. The germplasm was collected, characterized and evaluated. The results of the germplasm collection showed that the mean seed yield (kg/ha), seed size (g/1000 seeds), total oil (%) and vernolic acid (%) were 873, 3.4, 24, and 62, respectively. The variety trial of vernonia tested has shown that ERV-05 (1127 kg ha^{-1}) and 66 BK-OR-1 (1111 kg ha^{-1}) were the best yielding genotypes. The oil content of ERV-05 was better than 66BK-OR-1. The genotypes with smaller seed size had better oil content. There was a positive and significant correlation between oil content, plant height and days to blooming. Therefore, breeders should select genotypes based on these traits for better oil content. The future challenges of the plant are lack of uniform seed maturity, and to develop appropriate technologies for mechanical harvesting, seed cleaning and processing and oil extraction.**

Key words: *Vernonia galamensis*, germplasm collection, varietal evaluation, oil content, seed yield, Eritrea.

INTRODUCTION

Vernonia galamensis is limited in distribution primarily to Eastern Africa, which is grown in a wild form in Eritrea, Ethiopia, Malawi, Tanzania and Kenya. This African species, *V. galamensis* seed contained 42% oil with up to 80% vernolic acid considerably higher than any selection of *Vernonia anthelmintica* originating from India (Perdue et al., 1986; Thompson, 1989, 1990). Attempts to domesticate the Indian species ended when seed shattering before harvest could not be controlled. Seeds from this plant contain oil rich in epoxy fatty acids. Epoxy oils are widely used for plastics and additives. A potential market use is as a drying agent for resin paints. The oil can also form clear, tough, rubbery plastics or coatings on metal. This natural oil is friendly to the environment unlike the synthetic solvents.

Vernonia is a weedy annual, wild in much of tropical Africa resembling a thornless thistle. It has grown in areas with less than 600 mm rainfall and thrives in sandy soils. It is grown for its seeds, which contain 40% oil of which 80% is a “naturally” epoxidized vernolic fatty acid. There is a large industrial market for synthetically epoxidized vegetable oils (such as linseed and soy-bean), but the epoxidation process is expensive. Vernolic acid is epoxided already, and may be able to fill some of those

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market niches. Vernolic acid is much less viscous than the synthetically epoxidized oils. The latter are semi-solids at 10°C and can no longer be poured at 0°C while Vernolic acid can be poured even below freezing point. The low viscosity of vernonia oil should make it a good solvent in paint manufacture, and the highly reactive epoxy group will cause it to become chemically bound in the dried paint rather than evaporating in the atmosphere (Kaplan, 1989). Other potential uses include lubricants, adhesives, plastic formulations, protective coatings, cosmetics, detergents, a raw product for nylons, and much more. Vernonia could also be used as a natural source of plasticizers and stabilizers (binder) for producing polyvinylchloride (PVC plastic), which currently is manufacturing from petroleum. The potential use of vernonia as a petroleum substitute is important since the demand for petroleum each year in the U.S. is approximately 8,500 lbs per person, of which about 500 lbs per person is needed for production of plastics and industrial petrochemicals (Teynor et al., 1992).

Vernonia is an annual plant grown in areas close to the equator and plants with large seed and best seed retention capacity only under short day conditions. Such varieties cannot grow in parts of the world where frost occurs in most parts of the year. A major constraint in the production of vernonia is the indeterminate characteristics with lack of uniform maturity and the problem of shattering. Threshing is also one of the major problems encountered, which is difficult to separate the seed from the cover.

The study done on the varietal evaluation of vernonia is limited. In Zimbabwe and other African and Central American countries near the equator, a yield range between 1345 and 2494 kg ha^{-1} has been reported (Teynor et al., 1992).

The specific objective of this study was to collect, introduce and characterize vernonia accessions as well as evaluate the genotypes further for seed yield, oil quality and quantity.

MATERIALS AND METHODS

Germplasm collection and characterization

A total of 61 Vernonia accessions were collected in 1995 from Eritrea and Ethiopia by investigators from Agricultural Research Station of Virginia State University (ARS/VSU) and Eritrea Ministry of Agriculture (MoA). Collections were made at various areas of Yabello in Sidamo, Ethiopia. The plants were available on the road side growing wild along with acacia trees on heavy clay soil. In Eritrea, the plants were found wild in the areas surrounding Keren and Shambuko. The vernonia plants found in Eritrea were also found along the road sides and farms. The collections were done by taking either seed samples of individual flower head or seeds from plants with all matured flowers. The accessions were threshed, cleaned, documented, and cataloged at Paradizo Agricultural Research Station.

During Season 1 (1997), sixty-five accessions were planted in a single row for seed multiplication. Out of the accessions tested 61 were local collections whereas the rest 3 breeding lines were from ARS/USDA, Arizona Experiment Station U.S. on and one breeding line from VER-TECH, Inc.

The vernonia accessions were planted at the rate of 1 g per m^{-1} with each plot consisting of four rows with 0.75 x 3 m. Seeds were planted at a depth of 1.0 to 1.3 cm in furrows. The rows were thinned when the plants reached 15 cm in height. Fertilizer was applied at the rate of 115 kg ha^{-1} Urea.

The data recorded were days to germination, days to full bloom, plant height, lodging, shattering and seed yield. Manual harvesting was done from the center rows before the flower heads open. Seeds were threshed and cleaned to be weighed for seed yield and seed size. The oil quality and quantity was analyzed at Virginia State University.

Varietal evaluations

Genotypes

Nine promising vernonia genotypes were selected from the previous trial (Season 1) on the characterization of accessions and were advanced and tested in the varietal evaluation trial for three years (Season 2 in 1998 and Season 3 in 1999 and Season 4 in 2000) at Halhale Research Station, Eritrea. The genotypes tested were ERVO5, AO437, 29E-OR-1, 66BK-OR-1, VOO1, ETV13, ETV-14, ETV-15 and ETV-17.

Design and plot size

The genotypes were tested in a Randomized Complete Block Design (RCBD) with three replications. Fertilizer (P_2O_5) was added at the rate of 100 kg/ha. Each plot was consisted of four rows with 0.75 x 3 m. Seeds were planted at a seeding rate of one g m^{-1} at 1.0 to 1.27 cm deep in furrows made with hoe or a piece of wood like branch of a tree. The seeds with pappus intact can be drilled in very small furrows less than 1.5 cm deep and lightly covered with soil. The rows were thinned when the plants reached 1.5 cm high to have a space between plants 7.5 to 12.5 cm. Tapping was done when the plants height was 25 cm tall, the upper 2.5 to 5.0 cm of the plants were removed to stimulate branching from the base of the plants. Ammonium nitrate fertilizer at a rate of 150 kg/ha was applied as follows: 1/3 at sowing, 1/3 at four weeks, and 1/3 at eight weeks.

Data collection

The data collected were days to emergence, vigor, days to full bloom, plant height, lodging, shattering and seed yield. At seed maturity each genotypes was harvested manually from the center rows of each four-row plots before the flower heads open. Seeds were threshed, cleaned and characterized for yield and seed size. The seeds samples were taken, ground, and analyzed for oil quality and quantity at VSU.

Methods of oil extraction

Prior to extraction, heating the seeds for 4 min in microwave oven eliminated lipolytic activity of lipase. After drying, the oil extraction and preparation of fatty acids methyl ester was carried. Solvent mixture of hexane/isopropanol (3:2 v/v) was used. One gram of ground seeds was extracted in 10 ml of the solvent. The extraction process was repeated three times. After centrifugation, the combined supernatant was washed twice with 15 ml of a washing solution made of 1% CaCl2 and 10% NaCl in 50% methanol. The hexane layer was separated by centrifugation, removed and dried over anhydrous Na_2SO_4. Total lipids were gravimetrically deter-

Table 1. Seed yield and oil content range and mean values in the vernonia germplasm evaluation in 1997 (Season 1).

Characters	Range	Mean
Seed yield (kg ha $^{-1}$)	64–2848	873
Days to maturity (days)	120–217	171
Reproductive period (days)	033–94	056
Seed size (g/1000 seeds)	2.56– 4.35	3.41
Oil content (%)	14.9–29.5	24.0

mined after solvent evaporation under low streams of N_2 at room temperature.

The fatty acid profile was determined as methyl ester (FAME) using diazomethane. A supelcowax 10 Capillary Column (30 m x 0.25 mm i.d. and 0.25 um film thickness) and a Hewlett- Packard Model 5890A Gas Chromatography (GC) equipped with a flame ionization detector (FID) and mass spectrometer detector (MSD) model HP5971A were employed. The GC was connected to an HP3396A integrator for FID and a HP59970 Chemistry Station for MSD. Helium was used as a carrier gas at a flow rate of 52.5 ml/min. with the split ratio of 100:1. The oven temperature was isothermal at 200℃, injector and detector temperature set at 250 and 280℃, respectively. Identification of fatty acid methyl ester was based on comparison of retention time and mass spectrum of unknown peaks to FA methyl ester standards. Quantification was made by the aid of heptadecanoic acid (17:0) as an internal standard. Fatty acid concentration was reported as a relative weight percentage of total fatty acids. A normalization technique was used to calculate absolute response factors for all identified fatty acids.

RESULTS AND DISCUSSION

Germplasm collection and characterization

Accessions ERV-06-82, ERV-05-89 and ERV-05-86 gave the highest yield (Table 1). The mean oil content of the accessions with higher seed yield ranged from 24.3 - 24.6%. The high yielding accessions were not one of the best in oil content. The reverse is true that the accessions with lower seed yield relatively produced higher oil content.

The mean oil content of the accessions was 25% and ranged from 20.6 to 29.7% (Table 2). The majority of the accessions had high oil content with the exception of ERV-01-9-13 had the lowest and ETV-15-13 had the highest.

The mean seed yield of the accessions was 873 kg ha^{-1} and ranged from 64 to 2880 kg ha^{-1} (Table 2). The variation in seed yield was also reflected in seed size and the mean seed size was 3.41 g/1000 seeds. The total mean oil was 24% and ranged from 14.9 to 29.5% where the majority (46%) of the accessions falls within the overall mean. The vernolic acid mean of the accessions was 62% and ranged from 38 to 77 and 49% of the accessions had vernolic acid content which exceeded the mean. The accession ETV-13 produced the lowest vernolic acid and the breeding lines 66BK-OR-1 and V001 had higher than the overall mean. The accession 66BK-OR-1 is early maturity type that could be used in areas with limited rainfall. Preliminary evaluation of these new collections has indicated the presence of wide genetic variability that could enhance the future breeding program.

Varietal evaluation

The seed yield (kg ha^{-1}) of the varietal evaluation conducted at Halhale Research Station is shown in Table 3. When averaged over the two years the best yield was obtained from ERVO5 (1127 kg ha^{-1}), followed by 66BK-OR-O1 (1111 kg ha^{-1}) and ETV 14 (1099 kg ha^{-1}). It can be noted that the seed yield was better in Season 4 (2000) due to improved rainfall situation compared to the previous year. The seed yield of the best performing varieties was higher in Season 4 (2000) than in Season 3 (1999). Based on the performance of the genotypes, the MoA distributed the seeds of promising variety 66BK-OR-1 to three farmers for seed multiplication.

The agronomic characters of the varieties are shown in Table 4. It can be seen that 66BK-OR-1 took less number of days to blooming and was shorter than the rest of the vernonia genotypes. The number of days to harvesting was also lower. This is a suitable variety for areas with short growing period. It can be realized also that this variety was one of the good performing ones in seed yield. However, the oil content was lower than the rest with 17.8%.

Genotypes with better oil content, ETV 14, seem to be relatively better in seed yield (1099 kg ha^{-1}). The genotypes with the highest oil content mentioned above have taken longer period of time to be harvested despite the poor yield. Such genotypes are not expected to give adequate seed yield under short rain fed conditions.

There was a positive and significant correlation between seed size (r = 0.533*) and seed yield (Table 5). The result suggested that an increase in seed size would bring about a substantial increase in seed yield. Therefore, the seed size has contributed positively to the increase in seed yield. However, the rest of the agronomic traits have affected the seed yield negatively. Besides that there was a negative and significant correla-

Table 2. Mean seed yield and oil content of the best Vernonia accessions planted at Halhale Research Station in Season 2 (1998).

Genotype	Seed yield (kg ha^{-1})	Days to maturity[†]	Seed size (g/1000 seeds)	Reproductive period (days)[¥]	Oil content (%)
ERV-06-82	2848	126	3.97	46	24.3
ERV-05-89	2448	107	3.81	34	24.4
ERV-05-86	2330	124	3.20	47	24.6
ERV-05-88	2325	123	3.79	48	23.9
29E-OR-2	2264	199	3.35	94	27.0
ETV-15-66	1979	199	4.02	58	24.8
ERV-05-85	1952	124	3.40	44	24.2
ERV-05-87	1819	123	4.10	46	25.3
ERV-05-83	1792	124	3.60	47	27.7
ETV-15-3	1776	199	3.83	56	29.7
ERV-01-9-13	1717	124	3.57	40	20.6
ETV-15-67	1456	199	3.54	58	26.2
ERV-17-1	1376	---	3.42	---	24.6
ETV-15-75	1365	214	3.79	76	26.0
ERV-05-84	1335	124	3.34	44	25.0
66-BK-OR-1	800	185	3.39	83	26.1

[†]Number of days from planting to seed maturity;
[¥]Number of days between flowering and seed maturity.

Table 3. Mean seed yield of vernonia variety trial tested at Halhale Research Station, Eritrea, in Season 3 (1999) and Season 4 (2000).

Genotype	Seed yield (kg ha^{-1})		
	Season 3 (1999)	Season 4 (2000)	Mean Season 3and 4
ERVO5	824	1430	1127
AO437	809	973	891
29-E-OR-1	706	956	831
66BK-OR-1	685	1537	1111
VOO1	715	835	775
ETV-13	516	1096	806
ETV-14	592	1305	949
ETV-15	337	1174	756
ETV-17	549	1082	816
Mean	637	1154	896
LSD (0.05)	207	379	293
CV %	34.5	33.5	34.0

tion between seed size (r = –0.574*) and oil content.

Genotypes such as ETV 13 (25.0%), ETV 15 (25.7%) and ETV 14 (25.7%) with the highest oil content did not show good performance in terms of seed yield. The accessions with bigger seed size tended to have lower oil content. For example 66BK-OR-1 had larger seed size (3.45 g/1000 seeds) but lower oil content (17.8%). The genotypes with smaller seed size had higher oil content. Therefore, seed size has not contributed to better oil content. The traits that seem to contribute to higher oil content are days to half bloom, days to full bloom and plant height. This study showed that breeders should concentrate on selecting traits such as plant height, days to full bloom and half bloom in screening genotypes for better oil content (Table 5).

Conclusions and Recommendations

In conclusion, three promising varieties of vernonia namely 66BK-0-1, ERV 05, and ETV 14 were identified. The seed multiplication of the accessions is underway at

Table 4. Mean agronomic traits in the vernonia variety trial tested at Halhale Research Station averaged over the years.

Genotype	DE (days)	DHBL (days)	DFBL (days)	RP (days)	PH (cm)	DH (days)	SS (g/1000 seeds)	OIL (%)
ERVO5	26	111	133	54	131	157	3.20	26.3
AO437	24	108	120	39	132	159	3.00	25.5
29-E-OR-1	25	111	135	58	140	166	3.35	25.5
66BK-OR-1	26	91	101	73	94	172	3.45	17.8
VOO1	29	126	147	45	122	174	3.00	26.3
ETV-13	24	126	148	54	152	190	3.30	25.0
ETV-14	20	126	148	43	155	181	3.40	25.7
ETV-15	25	117	127	46	128	184	3.15	25.7
ETV-17	24	125	149	41	143	176	3.05	26.1

DE, Days to emergence; DHBL, Days to half bloom; DFBL, Days to full bloom; RP, Reproductive period; PH, Plant height; DH, Days to harvest; SS, seed size.

Table 5. Simple correlation coefficient between agronomic traits, seed yield and oil content pooled over two years.

Character	Seed yield	Oil content
Seed size	0.533*	–0.574*
Days to harvest	–0.381	–0.025
Reproductive period	0.412	–0.807*
Days to emergence	–0.269	–0.175
Days to half bloom	–0.483	0.778*
Days to full bloom	–0.372	0.765*
Plant height	–0.237	0.739*
Oil content	–0.441	-----

*Significant at 5% probability level.

Halhale Research Station and it is expected that they will be disseminated to farmers. Seed production has started with the effort of domesticating the plant from its wild state. However, there are challenges– lack of uniform seed maturity, and to develop appropriate technologies for mechanical harvesting, seed cleaning and processing and oil extraction.

The following recommendations can be given: (i) Germplasm collection on vernonia to be continued on places where the collection has not been done such as in areas like Guluj and surrounding areas. It is expected that there is enormous genetic variability of vernonia in some of these places which has not yet been explored. (ii) Seed of germplasm collection available in the gene bank should be maintained so that the accessions could be rejuvenated. (iii) Seed production on promising vernonia accessions should continue and this will also serve as a demonstration to farmers and policy makers. (iv) Further research activities need to be ensured on the insecticidal use of vernonia apart from the oil content of the seed for industrial use. The research on mutation breeding can reduce the problem of shattering in vernonia and reduce the problem of non-uniform maturity of the plant. (v) Technology transfer on farmers' fields should continue on varieties and agronomic packages in outreach programs.

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