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Review Paper on Genetic Diversity of Vernonina (Vernonia Galamensis L.) in Ethiopia

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Abstract: Vernonia galamensis *is a new potential industrial crop with very high content of vernolic acid in the seed oil. The species is known to naturally grow as a weed in fields or in woodlands under a wide range of agroecological conditions of Africa. In order to study the existing variability in Ethiopia, germplasm collection was carried out.* Vernonia *grows wild in various ecosystems. Ten regions were explored from North, South, East, Southeast, Southwest and Central Ethiopia. A diverse range of habitats having different altitudes and ecological conditions was explored. The main objective of this paper was to review the genetic diversity of Vernonia crop through morphological, seed oil content and fatty acid for possible exploitation as an industrial oil crop. Vernonia galamensis is a good source of seed oil rich in epoxy acid, which can be used to manufacture plastic formulations, protective coatings, and other products. Seed from a natural stand in Ethiopia contained 31% epoxy acid.*

Keywords: Epoxy**,** acid**,** *galamensis, diversity, domestication, distribution,* Ethiopia

Introduction

Vernonia (*Vernonia galamensis*) is an annual member of the Asteraceae and is widely distributed in regions of Africa. The *V. galamensis* species complex is now recognized, according to Gilbert (1986), to include six subspecies, namely *galamensis*, *mutomoensis*, *nairobensis*, *afromomntana*, *gibbosa*, and *lushotoensis*. *Subsp. galamensis* is the most widely distributed; it is highly diverse and has four botanical varieties, namely var. *galamensis*, var. *petitiana*, var. *australis and* var. *ethiopica* (Gilbert, 1986). The subspecies *galamensis* and *mutomoensis* in general are found in areas of low rainfall, some as little as 200 mm per year, with no well-defined dry season. Higher elevations and areas of high rainfall are the ideal regions for the subspecies *afromontana* and *lushotoensis* (Perdue *et al*., 1986).

V. galamensis is new potential industrial oilseed crop, which originates from Eastern Africa. This plant was identified for the first time in Eastern Ethiopia by Perdue in 1964 at 7 km south east of Harar town, 9 ° 14' N and 42° 35' E. Due to the high oil and vernolic acid content and its relatively low shattering nature, *var. ethiopica* has been the focus of research and at present its production in some parts of the world reaches semi-commercial scale (Baye *et al*., 2001). *V. galamensis* spp. *galamensis* var. *ethiopica* has a potential to become an industrial oilseed crop.

Vernonia seems to prefer well drained soil, and is fairly drought tolerant once established (Dierig *et al.*, 1996a). There is of variation in terms of vernonia morphology and flower biology. The flower heads (capitula) vary considerably in size. The petals that surround the seeds on the capitula are between 8 and 25 mm long (Thompson *et al.*, 1994a). The colors of the florets are either blue to purple, or white. The capitulum is made up of 50 to 150 florets. This roughly corresponds to the number of seed produced per capitula, depending on pollination events. Hairs attached to the seed (pappus) can be up to 8 mm long. Subspecies are readily hybridised among themselves (Thompson *et al.*, 1994b).

In its natural distribution vernonia in eastern and western Hararghe, which is known to farmers by the local names 'Ferenkundela', 'Dunfare', 'Kefathebogie', and 'Noya', have different connotations in different localities (Tesfaye, 1996). *Vernonia galamensis* produces high quantities of epoxy fatty acids (at least 60 %) in a trivernolin form, useful in the reformulation of oil-based (alkyd-resin) paints to reduce emission of volatile organic compounds (Perdue *et al.*, 1986). About 38% of the Vernonia seed is oil of which about 72 % is vernolic acid. Vernonia has "reactive dilutant" oil properties that produce less pollutants.

Vernonia galamensis (Cass.) Less. produces an unusual fatty acid with an epoxy group attached to 18:1 molecules. *V. galamensis* is limited in distribution and is endemic primarily to East African countries as a weed colonising disturbed areas and bare agricultural lands under a wide range of agroecological conditions (Baye and Becker, 2005a). Vernonia is grown successfully in countries near the Equator because the plants with the largest seed and best seed retention only flower under day-neutral photoperiodic conditions. Therefore, more research is needed to exploit the phenotypic and genotypic variabilities within this species as an alternative crop in countries far from the equator. The phenotypic and genotypic variabilities will reveal about the genetic diversity as a measure of the possible choice of information provided by a gene or genes. The main objective of this paper was to review the genetic diversity of Vernonia crop through morphological, seed oil content and fatty acid for possible exploitation as an industrial oil crop.

LITERATURE REVIEW

Origin of Vernonia *galamensis* species

The genus Vernonia includes more than 1000 species distributed widely in tropical and subtropical regions of Africa, Asia, and America and has two major centers of origin, South America and tropical Africa [5]. About 200 species ranging from annual herbs and shrubs to perennial trees are found in Africa of which about 50 species of Vernonia have been recorded in Ethiopia (Tadesse, Flora of Ethiopia Vol. 4(1), in prep.).

V. galamensis is known to naturally grow as weeds in fields or in wood lands under a wide range of agro-ecological conditions of Africa (Baye *et al.*,2001; Jeffrey and kew. B.,1988). It is a break crop to prevent pest damage, an alternative crop for farmers and plays a great role in oleo chemical industry. Developing countries can also capitalize on growing it for export or for their own industrial development. It is originally found in Ethiopia, most suitable for dry land areas, essentially noncompetitive with the existing crops and high cash crop as primary source of income for farmers.

The subspecies galamensis is the most widely distributed, highly diverse and has four botanical
varieties, namely verity galamensis, verity petitiana, verity australis and verity ethiopia (Baye T. and Guideta S., 2002 and Grinderg *et al*., 1994). It grows in areas with as little as 20cm of seasonal rainfall (Mohammed *et al., 1995*). A porous, well-drained and sandy soil is best for its growth. Vernonia plant does not dowel on heavy clay. Pest survey of *V. galasmensis* showed that a number of insects and diseases have been recorded (Baye T. and Guideta S., 2002).

There is also a serious problem of Vernonia rust disease on the leaves and pods of the plant at Wendo Genet, in Ethiopia. A superior species of Vernonia in terms of oil and veronica acid content was originally found in Ethiopia (Brent A.,1999).

Taxonomy of Vernonia *galamensis*

Vernonia [Vernonia galamensis (Cass.) Less.; 2n = 18] is a relatively new crop in many parts of
Africa. Vernonia galamensis is an herbaceous annual member of the Asteraceae and is widely distributed in regions of Africa. The *V. galamensis* species complex is now recognized according to Gilbert (1986), to include six subspecies, with one subdivided into four varieties:

1. ssp. *galamensis*
    1. var. *galamensis* M. Gilbert
    2. var. *petitiana* (A. Rich.) M. Gilbert
    3. var. *australis* M. Gilbert
    4. var. *ethiopica* M. Gilbert
2. ssp. *mutomoensis* M. Gilbert
3. ssp. *nairobensis* M. Gilbert
4. ssp. *gibbosa* M. Gilbert
5. ssp. *afromontana* (R.E. Fries) M. Gilbert
6. ssp. *lushotoensis* M. Gilbert

The ssp. *galamensis* and *mutomoensis* in general are found in areas of low rainfall, some as little as 200 mm per year, with no well-defined dry season. The ssp. *nairobensis* and *gibbosa* occur in dry evergreen forests, while ssp. *afromontana* and *lushotoensis* are generally found at higher elevations and areas of highest rainfall (Perdue *et al.* 1986; Perdue 1988). Similar qualities and quantities of vernolic acid are found in the seed oil of all the subspecies of *galamensis.*

Morphology of Vernonia *galamensis*

The morphological characters of the Vernonia plant were described comprehensively by Perdue *et al.,* (1986). The authors described Vernonia as herbaceous, usually annual, varying from small ephemerals 20 cm tall with a single flower head to robust rather diffusely branching somewhat shrubby plants which grow up to 5 m tall with many flower heads. The authors also noted that the stems branch only after the first flower head is formed and the inflorescence consists of a terminal flower head with lateral flower heads from the uppermost axils. The leaves alternate and are membranous, 0.6-5.0 cm wide, up to 25 cm long (Perdue *et al.,* 1986). The classification of the species into six subspecies is based on characters of the phyllaries (Perdue *et al.,* 1986). Vernonia could potentially grow as a seed oil crop in tropical and subtropical environments with frost-free and short-day length for flower initiation and development.

Vernonia mode of pollination

The capitula of *V. galamensis* contain hermaphroditic florets that are protandrous. The pistil emerges through an anther sheath as pollen is shed. As the stigma emerges and fully opens, pollen has reached its surface. This would appear to be an important opportunity for auto-fertility. Controlled crosses of ten capitulum on each of five plants were made on a number of different subspecies accessions. Only one accession (V013) uniformly set viable seed on all five self-pollinated plants. Five of ten accessions tested appear to be completely auto-sterile, and produce essentially no seed following self-pollinations (V003, V004, V021, V022, V018). Four accessions appear to segregating for this trait and contain both auto-fertile and auto-sterile plants. Variability of this characteristic allows selection and breeding for the desired type of mating system. Even though there appears to be a high amount of variation among plants within a given accession, there was little variation in response among flowers within a single plant. In most cases, the amount of selfed seed was a low percentage of the total possible seed produced in a given capitulum. An average number of total possible seeds per head is about 80, with a range between 60 and 155. In contrast to greenhouse grown plants, most open-pollinated plants in the field have percentages of 95 to 100% viable seed. Under favorable environmental conditions, high activity of bees and other pollinating insects are readily observed in field plantings.

We have concluded that low seed set following controlled self-pollinations of certain accessions, and among certain plants within other accessions is due to self-incompatibility. This is most likely a genetic trait that may have a selective advantage. Even though high pollinating insect activity is observed in the field, we believe that auto fertility would be most desirable for maximization of seed set in field plantings.

Description and Domestication of Vernonia

Description of Vernonia *galamensis*

There are limited germplasm containing naturally occurring epoxy oils, with good potential for commercialization (Gunstone, 1993). In recent years many new oilseed species have been investigated as potential sources of vegetable oils for oleochemical uses (Knapp, 1990). Many of them contain a high proportion of industrially desirable fatty acids, such as linoleic (*Mandia sativa* olina), linolenic (*Lepidium sativum* L. and *Camelina sativa* (L.) Crts.), calendic (*Calendula officinalis* L.), epoxy-(*Euphorbia lagascae* Sprengel and *Vernonia galamensis* (Cass.) Less.), hydroxyl-(*Lesquerella fendleri* (Gray) Wats.) and petroselinic (*Coriandrum sativum* L.) acids (Angelini *et al.,* 1997).

Vernonia galamensis (Cass.) Less. produces an unusual fatty acid with an epoxy group attached to 18:1 molecules. *V. galamensis* is limited in distribution and is endemic primarily to East African countries as a weed colonising disturbed areas and bare agricultural lands under a wide range of agroecological conditions (Baye and Becker, 2005a). Recent studies on herbarium materials in Addis Ababa, Ethiopia, and Kew, London, indicated the existence of about 40–50 species in Ethiopia (Naliaka, 1990).

Domestication of Vernonia *galamensis*

Vernonia galamensis (Cass.) Less., Asteraceae is an annual native to Africa. Taxa include six subspecies. One subspecies, *galamensis*, is divided into four botanical varieties (Gilbert 1986). Literature sometimes still refers to this species as *V. pauciflora* (Willd.) Less. Gilbert (1986) revised the taxonomic treatment and included three taxa that had not previously been described. Eastern Africa appears to be the center of diversity.

Development of an epoxy fatty acid source from plants began in the late 1950s to mid-1960s when *Vernonia anthelmintica* (L.) Willd., native to India, was seen as a possible candidate. Vernolic acid in this species was discovered and isolated by Gunstone (1954). Excessive seed shattering prevented further development of this species. Collections of *V. galamensis* and subsequent evaluation (Carlson et al. 1981; Thompson *et al.* 1994b, c) indicated that this species is substantially better because of the quantity and quality of the seed-oil and better seed retention. The plant is native to equatorial Africa. To develop this crop for temperate zones, the short day-length flowering requirement needed to be altered (Dierig and Thompson 1993).

Distribution of Vernonia *galamensis*

Most of *Vernonia* species occur in South America but more than 300 species from Africa have been described with most occurring in Ethiopia and Madagascar. Apart from these two countries, vernonia is also grown in Cape Verde, Eritrea, Mozambique, northern Tanzania and Senegal. The greatest diversity of vernonia is found in east Africa while a single variety occurs in West Africa. The genus vernonia comprises of more than a thousand species which vary from annual herbs and shrubs to perennial trees (Baye *et al.,* 2001). There are six major subspecies namely *afromontana, galamensis*, *gibbosa*, *nairobensis*, *lushotoensis* and *mutomonesis*.

Among these, *galamensis* shows the highest genetic diversity (Gilbert, 1986). It contains four botanical varieties namely *australis*, *ethiopica*, *galamensis* and *petitiana* (Gilbert, 1986).

Economic importance of Vernonia *galamensis*

The development of industrial crop for semiarid zones is important in both developing and developed countries. Many plants suitable for arid and semiarid zones are regarded as having high potential as industrial crops [2]. *V. galamensis* is an oil seed crop and as a candidate crop for arid and semiarid zones in both developing and developed countries (Grinderg et al 1994).

Chemically, Vernonia oil is similar to epoxidized soybean and linseed oils. Soybean oil contains only 50% of linoleic acid as its principal fatty acid and linseed oil contains just 57% of linoleic acid as its principal fatty acid, whereas Vernonia oil is rich in a single fatty acid known as Vernolic acid. This makes it an especially attractive raw material for industry. In addition, Vernonia oil has several unique properties: it is a transparent homogenous liquid at room temperature with a low viscosity of about 110 cps, and it is even pourable at room temperatures below 0^0C. In contrast, epoxidized soybean oil and epoxidized linseed oils are highly viscous, with a viscosity of about 300-1500 cps; they are semi-solid at 10^0C and are non-pourable below 0^0C (Grinderg *et al* 1994).

Nowadays there is a large industrial market for synthetically epoxidized vegetable oils such as linseed or soybean oils, but the epoxidation process is expensive and has a lot of environmental problems. Vernonia oil, on the other hand, is already epoxidized in nature by enzymatic action, and may be able to fill some of those market niches or could be used as a substitute for currently used epoxy oils (Mills D. and Grinberg S., 1997)

The presence of epoxy group, the low viscosity and polymerizing characteristics of this oil makes it especially valuable as a solvent in industrial coatings and paints (Kaplan K.C, 1989). Some of the products that are being developed from Vernonia oil are also degradable lubricants and lubricant additives, epoxy resins, plastic formulations of polyvinyl chloride, adhesives, insecticides and insect repellants and reactive monomers in polymer synthesis (Kaplan K.C, 1989) and, Grinberg et al 1996). Other applications of the products that developed from Vernonia oil are for the construction of polyurethane foams, for the synthesis of interpenetrating polymer networks, as PH stabilizers, waxes, glues, emulsifiers and rust suppression, and in organic formulation of carriers for slow-release pesticides and herbicides. Vernonia oil has also been used as a source of hydroxyl alkoxy fatty esters and for the synthesis of epoxy secondary amides (Grinderg *et al* 1994). Current Vernonia product on the market includes Vernola super Gloss, a car-care product used on tires, Vinyls, flash boards, leather, and rubber bumpers (Cunningham, 1997).

The unique and special structure of epoxy acid within the triglyceride enables a wide variety of reaction characteristics of the ester group, the double bond, and the epoxy group to occur as shown below (Grinderg *et al* 1994).

Genetic diversity at a molecular (DNA) level

Molecular markers are becoming essential tools in plant breeding (Staub *et al.*, 1996; Mohan *et al.*, 1997; Gupta *et al.*, 1999). They have several advantages over the traditional phenotypic markers that are difficult or time-consuming to select by plant breeders. These DNA markers are not influenced by environmental conditions and are detectable at all plant growth stages.

During the last few years, complementary molecular methods have been developed for strain typing including: (I) PCR amplification of antigen-encoding genes (gp63 and cpb) followed by analysis of restriction fragment length polymorphism (PCR-RFLP, (Harrington and Wingfield, 1995), (II) PCR-RFLP of kDNA minicircles (Morales *et al*., 2001), (III) random amplification of polymorphic DNA (RAPD) (Chague *et al.*, 1996) and (IV) multilocus microsatellite typing (MLMT) (Bulle *et al*., 2002; Ochsenreither *et al.*, 2006).

Restriction fragment length polymorphism (RFLP) markers have routinely been used for agronomic crops linkage analysis and genome mapping (Tanksley *et al.*, 1989). However, construction of RFLP maps has been very difficult due to the low level of polymorphism in a self-pollinated crop such as wheat (Chao *et al.*, 1989). DNA markers such as RFLPs have been applied to interspecific studies of the genus *Helianthus* (Choumane and Heismann, 1988; Riesberg *et al.*, 1998; Gentzbittel *et al.*, 1992).

AFLP markers are highly polymorphic and reproducible and thus represent a powerful technique for DNA analysis that has revolutionized fingerprinting and diversity studies (Vos *et al*., 1995). AFLP analysis detects genetic variation throughout the genome by using a pair of specific restriction enzymes and their corresponding adapters combined with 2 selective rounds of PCR. AFLP technique has been used to identify markers linked to disease resistance genes (Thomas *et al*., 1995; Harlt *et al.*, 1999) and assess genetic diversity in several important agronomic crops including wheat (Breyne *et al.*, 1997; Gupta *et al.*, 1999).

SSR markers or microsatellites are tandem repeats interspersed throughout the genome and can be amplified using primers that flank these regions (Grist *et al*., 1993). The primers for SSRs can be synthesized based on a repeat sequence of (CA)n (Godwin *et al*., 1997). SSR has been successfully used to construct detailed genetic maps of several organisms and to study genetic variation within populations of the same species, such as grapes, honeybees and tropical trees (Brown *et al*., 1996).

RAPD reactions are PCR reactions, but they amplify segments of DNA which are essentially unknown to the scientist (random). Randomly amplified polymorphic DNA (RAPD) analysis is widely used for studying taxonomy of various genera (Devas and Gale, 1992), species (Faroog *et al.*, 1995; Igbal *et al*., 1995), for differentiation of intraspecies (Mackil, 1995; Sweeny and Danneberger, 1995) and to study the genetic diversity of various cultivars and lines. RAPD (Williams *et al*., 1990) is based on the polymerase chain reaction (PCR) and is widely adapted in genetic diversity analysis. Recently, RAPDs have also been extensively used for assessment of genetic variation in inbred lines of sunflower (Isaacs *et al.,* 2003). These markers are of particular interest and DNA profiles based on arbitrary primed PCR are both time- and cost-effective (Williams *et al*., 1990).

Principal component analysis and a dendrogram constructed from the Shannon-Weaver diversity index (H') indicated the close relationship of some of the Vernonia populations both at molecular (RAPD) and morphological marker level (Baye, 2004). Though clustering matches, there was a low correlation between the RAPD based molecular diversity and phenotypic traits diversity. RAPD analyses (Welsh and McClelland, 1990) are capable of detecting differences among strains of a single species. The simplicity and fast sample processing of RAPD technique makes it useful for assessing population genetic parameters such as within-population and between-population genetic diversity. An additional advantage is that knowledge of the DNA sequences is not necessary to apply this technique (Weising *et al*., 1995).

Summary and Conclusion

Ethiopia is rich in gcnetic diversity of both wild and domesticated plants as a result of its diverse agro-climatic conditions that often occur within relatively short distance and its various ethnic groups with diverse culture. Many wild plants of Ethiopia arc considered as weed and ignored by agricultural researchers. At best they remain untouched but more often they are burnt or cleared to give way for domesticated plants and/or to prevent their spread. Unlike cultivated plants, wild plants must also compete with other vegetation in their natural habitat, and are subjected to fluctuating environmental factors. This causes genetic erosion of the lesser known but potentially valuable wild spccics like Vernonia galamensis.

Vernonia galamensis, Asteraccae, is a new potential industrial oilseed crop. It has unique properties, which makes it economically and ecologically interesting. The seeds contain about 40% oil, of which 80% is trivemolin that consist of vcmolic acid (12, 13-epoxy-cis-9- octadecenoic acid), a naturally epoxidized fatty acid which may replace the chcmically modified soybean/linseed oils or petrochemicals for a wide range of use in the olcochcmical industry. Vernonia is a weedy plant naturally growing in semi-arid and arid (marginal and disturbed) areas which makes it ideal for developing countries in the tropics as it reduces possible competition for land with other food crops, creatcs diversification of products, serves as a break crop to prevent pest damage, as an insurance against crop failure and as a new cash crop. So far, most research on Vernonia galamensis has focused on the oil chemistry and potential uses specially in the olcochcmical industry while information about its performance for agronomic and seed quality traits are rather limited.

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