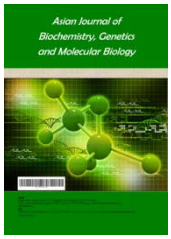

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Garlic (*Allium sativum L.*): Overview on its Biology and Genetic Markers Available for the Analysis of Its Diversity in West Africa

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Authors' contributions

This work was carried out in collaboration among all authors. Author TKAS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RA and YB managed the analyses of the study. Authors AKT and ISS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Garlic belongs to the *Allium* genus, which includes more than 750 species divided into more than 60 taxonomic groups. It is cultivated in many countries throughout the world for the bulb and used as a spice and functional food. The plant vegetatively propagates. This review will focus on origins, biology, analysis of genetic diversity, pharmacological properties of garlic. It appears from this synthesis that the *Allium sativum* species is derived from *Allium longicuspis* and is native to Central Asia. Studies on the analysis of genetic diversity through morphological markers revealed a wide variation in the color, shape and number of cloves and the ability to flower. Biochemical markers

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such as Esterase (EST), Phosphoglucomutase (PGI), Malate Deshydrogenase (MDH), and Diaphorase (DIA as well as molecular markers such as *Random Amplified Polymorphic DNA* (RAPD), *restriction fragment length polymorphism* (RFLP), *Amplified Fragment Length Polymorphism* (AFLP), and *Simple Sequence Repeats* (SSRS) *Inter-Simple Sequence Repeat* (ISSR) were successfully used. RFLPs or RAPD are the most used for assessing genetic variability within asexually reproducing garlic species. Work using SSRs markers is limited in garlic relative to other crops.

Keywords: Garlic (Allium sativum); genetic makers; genetic diversity; West Africa.

1. INTRODUCTION

Garlic (*Allium sativum L.*) belongs to the genus Allium. In this genus more than 750 species are identified and divided in 60 taxonomic groups [1]. It is a bulbous plant whose domestication is very old. Its primary center is in Central Asia while the Mediterranean and Caucasian regions are recognized as the secondary center of garlic [2]. The species are cultivated in many countries of the world mainly for its bulb. In 2018, the world production of garlic was 26,639,081 tons/year. The world's biggest producers of garlic are China with 21,263,237 tons, India with 1,400,000 tons, and Bangladesh (USA) with 381,851 tons. In Africa, the main garlic producing countries are Egypt with 280. 216 tons, Ethiopia with 138. 664 tons and Algeria with 103. 627 tons. Niger's production is estimated at 3,761 tons for an area of 317 hectares with a yield of 11.86 kg/ha [3]. Cultivated garlic varieties show wide morphological and agronomic variations in characteristics such as bulb color and size, plant height, time of flowering, the number and size of cloves, dormancy of the cloves, and adaptation to agro-climatic conditions [4]. Cultivars that are grown in the tropics are not subject to high dormancy [5]. However, relatively low temperatures 6 to 7°C are necessary for optimal plant development [6]. Garlic is consumed by many populations to treat a variety of disorders including fever, cough, ulcers, bronchitis and other respiratory problems, rheumatism, tuberculosis, typhoid, arteriosclerosis, diabetes, hyperlipidemia, and the prevention of atherosclerosis [7]. Studies on pharmacological properties have revealed that garlic has significant antibacterial, antifungal, antiviral, and heart disease activity [8,9]. Garlic is known to be hepatoprotective, antihelmintic, anti-inflammatory, and antioxidant [10,11]. Several studies exposing the therapeutic as well as pharmacological virtues can be found on garlic [12-14], but there are very few that include work on the analysis of the genetic diversity of the species. The purpose of this work is to state most judicious informations on the species to better valorize the crop in West Africa. It reports on the origin and biology of the species, the markers used for genetic characterization, and the pharmacological properties of garlic.

2. BIOLOGY

2.1 Origin and Domestication

Garlic has its primary center of origin in Central Asia (Kazakhstan), and the Mediterranean and Caucasian zones are considered as the secondary centers [2]. Allium longicuspis is designated as its ancestor because of its many similarities with the species [15]. Evidence for its use as a medicinal plant dates to more than 1550 B.C and its distribution to other parts of the world was made possible by nomadic traders and great conquests [2,16] Today, it is cultivated in many countries of the world in Asia, Europe, America and Africa where it is consumed as a spice or therapeutic food.

2.2 Classification of *Allium sativum*

Table 1. Classification of *Allium sativum*

Kingdom	Plantae
Subkingdom	Tracheobionte
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Equisetopsida
Subclass	Magnoliidae
Superorder	Lilianae
Order	Asparagales
Family	Amaryllidaceae
Genus	Allium

2.3 Vernacular Names

Table 2. Vernacular names

Benin: Gungbé – Ayo, Fonbgé – Tchiayo
Burkina Faso: Mooré – Gando, Layi, Dioula – Laii, Fulfuldé – Toumé
Ghana: Twi – Gyene Kankan, Ga Adangbe – Aya, Hausa – Tafarmuwa
Mali : Bambara – Tumé, Tamachek – Teskart
Nigéria : Hausa – Tafárnúúwáá, Igbo – Oy Ayón, Ayún, Yoruba – Àlubósa, Ayúu
Sénégal: Wolof – Laji, Manding Bambara – Layi
Togo: Ewe – Ayo, Kabyè – Ayo, Moba- Gabdjak
Niger: Hausa- Tafárnúúwáá, Zarma-Tafárnúúwáá, Fulfuldé; Toumé, Tamachek – Teskart

Table adapted from [17].

2.4 Morphological Description

Allium sativum L. is an annual herbaceous bulbous plant. It's a plant that can be erect or prostrate at heights ranging from 20 to 70 cm [4,18,19].

2.4.1 The stem

The stem or pseudo-stem is very short forming a tray at the base from which adventitious roots start. It consists of a succession of leaves that fit together through their leaf sheaths [20].

2.4.2 The root

The root system of garlic is of the adventitious type, rather thick and little branched, with an epidermis, a multicellular cortex, and an endoderm that surrounds the central stele [20]. The root development of the plant is sensitive to soil moisture and temperature [21]. The poor development of its root system is one of the factors limiting its capacity to absorb nutrients [22].

2.4.3 The leaves

The leaves are linear and alternate with a tubular sheath and their number varies from 9 to 12 in the species [23]. They can measure up to 40 cm long and 2 cm wide [19]. The limbs are broad and streamlined and the bases of all the leaves are located at the bulb [20].

2.4.4 The bulb

The bulb (or head) is characterized by a great diversity of shape and color [24-26]. It can be white, brown, light brown, violet, light violet or dark violet while the observed shapes can be rounded, elliptical or circular, transverse wide elliptical and transverse narrow elliptical [4,27]. Each bulb is formed of several cloves or pods which represents the reproductive organ of the garlic. Each clove consists of a protective tunic, often colored, a single fleshy leaf sheath, and a small bud [28]. The number of cloves per bulb varies considerably depending on the variety. Some varieties have 4 to 6 cloves per bulb, while in others the number can reach 10 or even 14 cloves per bulb [26,27].

2.4.5 The inflorescences

The inflorescences are umbels composed of perfect flowers with 6 petals, 6 anthers and 3 locules composed of 2 ovules each [29-30]. They can be large or small containing a more or less important number of sterile flowers and bulbils. However, the ability to produce inflorescences is not observed in all varieties. This ability is most common in central Asian and Spanish varieties [2].

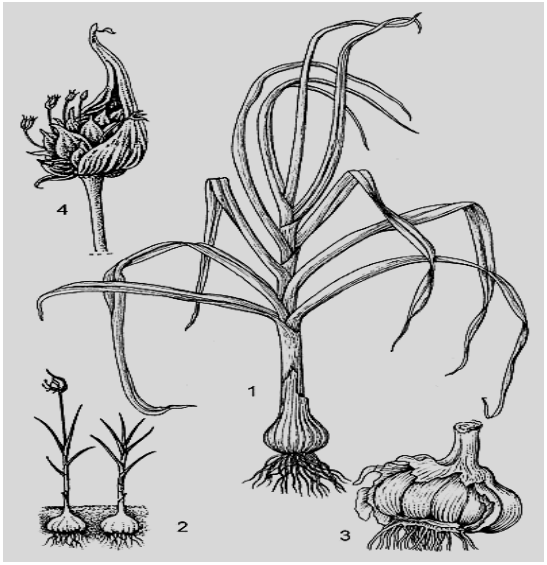

Fig. 1. Ail (*Allium sativum*)
1, 2, plant habit; 3, bulb; 4, inflorescence [31].

2.5 Growth and Development

Cultivated garlic reproduces mainly vegetatively [32]. The cloves of bulbs are used to propagate the plant. The growth and development of the plant is strongly influenced by the environmental conditions, especially temperature and photoperiod [6,33]. The dormancy of cloves is eliminated by exposure to temperatures of 6 to 7°C. However, cultivars growing in the tropics are not subject to this requirement [5]. Germination is epigerminated and emergence is observed one or two weeks after sowing. The optimum temperature for vegetative growth is around 18 and 20°C [6]. Thus, differentiation of apical meristem from the vegetative to the reproductive state occurs after the formation of 6 to 7 leaves [33]. High temperatures close to 25°c or 30°c and a photoperiod of 14h to 16h are necessary for good bulb development [34]. Bulb formation takes place in two phases marked by different thermal needs. A first phase of differentiation of the axillary buds which will become cloves with temperatures around 7°c. The second phase corresponds to the bulging of the cloves which requires temperatures between 20 and 25°C. Maturity is reached when the first leaves start to dry out. The complete cycle varies from 4 months in the tropics to 9 months in a northern Mediterranean climate.

2.6 Phylogenetic Resources

Many classifications have been proposed, thus testifying the complexity of the taxonomy of the species. The genus *Allium* belongs to the class *Liliopsidae*, the subclass *Liliideae*, the superorder *Liliianae*, the order *Amaryllidales*, the family *Alliaceae*, the subfamily *Allioideae*, and the tribe *Allieae* [1]. The genus *Allium* is represented by more than 750 species and more than 60 taxonomic groups [1]. The forms of food interest within the genus are practically all found in the two subgenera*: Rhizirideum*, and *Allium* divided into 4 sections: the *Rhizirideum, Schoenoprasum*, and *Cepa* sections for the *Rhizirideum* subgenus and the *Allium* section for the *Allium* subgenus [5].

The *Allium* section includes all forms with flat, semi-circular or hollow leaves. This section includes *A. Vineale, A. Guttatum, A. Mareoticum, A. Sphaerocephalum, A. Ascalonicum, A. Sativum, A. Ampeloprasum, A. Dregeanum, A. Baeticum* [35].

[12] classified the species *Allium sativum* into four groups: the Mediterranean sativum group, the middle and eastern European *Ophioscorodon* group, the central European *Longicuspis* group, Asia and the subtropical group of south Asia. The *longicuspis* group includes *A*. *Longicuspis* and the East Asian subgroup *Pekinense*. The *Longicuspis* group is the most primitive group and could be the group from which the other groups derive.

3. ANALYSIS OF GENETIC DIVERSITY

3.1 Morphological Markers

Morphological markers are the basis for the description of plant material. They are the most used to assess species diversity. Several studies on the genetic diversity of garlic have revealed a high diversity in the color, shape and number of bulb cloves as well as the ability to form a floral scape [4,18,19,33]. The bulbs of garlic varieties differ considerably in purple, pink, dark purple color [4,28] while the bulb shape can be regular or irregular [36]. The number of cloves contained in the bulbs is also a criterion which makes it possible to discriminate the various varieties and varies considerably from one variety to another [19,27]. Studies carried out by Jabbes et al. [37], on 31 accessions of local Egyptian garlic showed significant differences in the color of the cloves, the number of leaves per plant, the length of the pseudo-stem, dry weight of bulb, cloves number per bulb and the yield. Similar results were obtained by [18,38] further confirming the discriminating character of the numbers of cloves. However, [39], obtained different results from these authors pointing out that Iranian varieties are significantly different for the weight of bulbs and cloves without detecting a significant difference for the number of cloves per bulb**.**

3.2 Biochemical Markers

Isoenzymes were the first molecular markers to be studied and the discovery of their important polymorphism, more than 20 years ago, is still the subject of passionate debate [40]. These markers have also been widely used to assess diversity in garlic [41-43]. [44] used 20 isoenzymes to characterize 65 garlic varieties from 25 countries and results showed significant polymorphism for only 4 enzymes: Esterase (EST), Phosphoglucomutase (PGI), Malate Deshydrogenase (MDH), and Diaphorase (DIA).

These authors obtained 4 types of EST enzymes, 5 types of PGI enzymes, 3 types of MDH enzymes, and 2 types of DIA enzymes [42] classified a collection of fifty-two ecotypes into 6 groups using these same markers. However, [45] obtained a two-group classification of the ecotypes from Brazil with the markers ADH, EST, PRX and PGI. The analysis of two ecotypes of summer and winter garlic by [43] with the Esterase (EST) marker allowed the ecotypes to be separated into two groups. This variability corresponded to that observed for morphological characteristics and length of the vegetative cycle. This classifies the EST marker as useful for the identification and differentiation of different garlic ecotypes. Also [46] reports that Leucine Amin opeptidase (LAP) and Phosphoglucoiso merase (PGI) are polymorphic within 140 varie ties of garlic consisting of varieties of Japanese origin and diverse.

In general, there is a weak correlation between morphological traits and isoenzymes [41]. However, the polymorphism of the enzymes Esterase (EST), Alcohol Dehydrogenase (ADH), Phosphoglucoisomerase (PGI) has been used to evaluate the genetic diversity of onion [47], rice [48] and millet [49].

3.3 Molecular Markers

Molecular markers have been applied to assess genetic diversity in many crops because they are unlimited in number, unaffected by the environment and can be organized into genetic maps [50]. Studies of garlic genetic diversity with molecular markers are very patchy in Africa. At the current state of our knowledge, no studies on species diversity with molecular markers have been carried out in west Africa. Genetic markers such as *Random Amplified Polymorphic DNA* (RAPD), *restriction fragment length polymorphism* (RFLP), *Amplified Fragment Length Polymorphism* (AFLP), *Simple Sequence Repeats* (SSR_S) *Inter-Simple Sequence Repeat* (ISSR) have been used to identify genetic markers associated with pollen fertility [51-52], determine the stability of vitro plants [53], group accessions according to their photoperiodic needs [54]. They have also been used to reveal variation in the genome [50,55,56,57], and variability within cultivated garlic varieties [58]. All these markers have been recognized as important tools in marker assisted selection and analysis of genetic diversity between and within species.

3.3.1 AFLP makers

AFLP markers have been used to accurately characterize variation in many species of the *Alliaceae* family [59]. They have been used by [60] to successfully differentiate varieties of *Allium sativum*. The AFLP markers allowed [61] to demonstrate genetic differentiation between the species *Allium sativum* var. *sativum*, *Allium sativum* var. *Ophioscorodon* and *Allium ampeloprasum*. The results of [62] showed, in addition to high similarity indices between varieties, a genetic structuring of the collection in two groups that corresponded to the classification of farmers and dealers.

3.3.2 RAPD makers

The RAPD markers allowed to observe a genetic differentiation between garlic varieties of different origin. [63] used RAPD markers on eighteen garlic ecotypes from Egypt and China. They were able to differentiate Egyptian and Chinese ecotypes according to their origin. Similar results were obtained by [15] who classified the different genotypes into five groups according to their geographical origins. However, [64] did not detect any difference in genetic structuring according to the geographical origins of the Iranian ecotypes. Afterward, [65] emphasized the importance of environmental selection in Iranian ecotypes with RAPD markers. Their study revealed that geographical and environmental factors together created more genetic differentiation than isolation by distance alone. Genetic variation and relationship among 25 garlic of different sources have been assessed by [66]. They found high proportions of polymorphic loci (79.16%) and classified the collection in three groups. Their result showed a differentiation according the origins of the collection. [67] investigated the genetic variation between economically important species of the genus *Allium.* The species *Allium Cepa, A. sativum, A. porum, A. tuberosum, A. stracheyi* have been used in the study with their varieties using the RAPD and ISSR techniques. This study showed considerable polymorphism and discriminating capacity. It was also detected a very close relationship between *Allium sativum* and A. porrum.

3.3.3 SSR markers

Today SSR markers have become the markers of choice because of their high polymorphism, codominance and high reproducibility. They are

used to identify variability between varieties, but also within varieties. [68] highlighted an important polymorphism in their study of the genetic differentiation of one hundred-and twenty varieties from the republic of Korea, China, Japan, Kazakhstan and Spain. These authors used seven SSRs markers and generated with them a total of thirty-seven alleles allowing to divide all the varieties into four main groups. All loci tested deviated significantly from Hardy Weinberg's equilibrium suggesting that there was selection pressure in the collection tested. [69] characterized and compared SSR_S in the garlic genome and related alliums. The work showed that garlic had the highest overall density, the lowest frequency of tri-nucleotides, and the highest frequency of di- and tetranucleotides. A total of thirty-six alleles were obtained with 2 to 5 alleles per locus. The classification of the varieties resulted in five groups according to flowering ability, botanical traits and ecophysiological characteristics. However, work using these types of markers is limited in garlic compared to other crops.

3.3.4 ISSR markers

Regarding ISSR markers, [58] analyzed more than one hundred and thirty-one Indian varieties separated the entire collection into two groups. The authors pointed out less genetic variability within the analyzed varieties. However, [70] used these two markers SSR and ISSR in a garlic collection to study the relationship of parentage between thirty-one varieties from Egypt and China, Brazil, Italy, Mexico and the United States of America. The authors used sixteen and three primers for SSR and ISSR respectively. The results obtained reveal a percentage of 100% polymorphism for all ISSR primers and a variation of 33.3% to 100% for SSR primers. Also, a high similarity coefficient between the different analyzed varieties was found.

4. PHARMACOLOGICAL PROPERTIES

The garlic bulb can be eaten fresh, dehydrated, or as steam distilled oil. It is used to treat many disorders, including bacterial, fungal and viral infections, parasitic and intestinal infections, rheumatism, digestive disorders, and heart disease [8,9,11,71,72,73]. Garlic is used as an excellent blood cleansing agent and as an anti-aging agent [74]. Another advantage of garlic is its ability to relax smooth muscles, which reduces hypoxia [75].

5. CONCLUSION

Garlic is a vegetable very appreciated for its many therapeutic virtues. The species have adapted over the years to different ecological conditions, but remains very sensitive to variations in temperature and photoperiod. Studies have revealed a great diversity of shapes and color of the bulb, size, date of maturity. The most used markers for genetic analysis are morphological markers and molecular markers such as RFLP or RAPD.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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