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Contribution to the taxonomy of the genus *Amaranthus*: seed observations and characterization of seed oil

Doctoral Coordinator:
Prof. Filippo Santucci De Magistris

Supervisor:
Prof. Paola Fortini

Co-supervisor:
Prof. Duilio Iamonico

PhD Student:
Amara Noor Hussain

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AMARA NOOR HUSSAIN

External rewiers:

Prof.ssa Ivonne Sánchez del Pino
(Centro de Investigación Científica de Yucatán, México)

Prof.ssa Javad Sumera
(Dept. of Botany, Lahore College for Women University, Pakistan)

Committee members:

Prof. Dalila Trupiano
Department of Biosciences and Territory (university of Molise, Italy)

Prof. Nevena Kuzmanovic
Department of Ecology and Geography of Plants (University of Belgrade, Serbia)

Prof. Nida Harun
(University of Okara, Pakistan)

Dedication

To Papa, Mama, and

(My beloved husband) Shoaib Ali Khan

Contribution to the taxonomy of the genus *Amaranthus*: seed observations and characterization of seed oil

Abstract

Amaranths, particularly grain *Amaranthus,* are a substitute for high-nutritional-value crops and are considered the golden crop of the future. The studies of the genus *Amaranthus* that have been recently published indicate that this underused crop has not received the attention it deserves. Taxonomic delimitation in the genus *Amaranthus* has become much more challenging due to a high number of morphotypes, synonyms, germplasm, overlapping morphological traits, and frequent misapplication of names. Hence, the field of *Amaranthus* research is vast, but many taxonomic issues need to be resolved. Most focus was on cultivated *Amaranthus*, whereas no attention was paid to wild species. A comprehensive research strategy is required to project *Amaranthus* as a future golden crop. Thus, the main objectives of the thesis, related to basic and applied research, were the next: (1) to investigate the occurrence and invasion status of *Amaranthus hypochondriacus* in Belarus, Estonia, Italy, the Netherlands, and the North Caucasus to understand its distribution in relation to the bioclimate. (ii) to investigate the taxonomic value of the seed's micromorphological features using the Scanning Electron Microscope (SEM) in order to improve the understanding of the taxonomy of the genus *Amaranthus* at the global scale. (iii) to study oil and squalene content from the seeds of different Italian *Amaranthus* species to contribute to the selection of wild species more effective in producing these compounds. Three kinds of studies were carried out to accomplish the objectives.

In study I, we discussed our observations on *A. hypochondriacus* L. in relation to its distribution and invasion status in Belarus, Estonia, Italy, the Netherlands, and the North Caucasus. For Italy, we modified the status from casual to naturalized based on living individuals who had sustained themselves for 5 to 20 years. Regarding the other countries, we confirmed the species' invasion status (which had been inconsistently described in the literature), indicating it is a casual alien in Belarus, Estonia, and the North Caucasus and naturalized in the Netherlands.

In research II, we investigated the nomenclature and taxonomy of the genus *Amaranthus* and presented a comprehensive study on *Amaranthus* seed micromorphology. The species was identified using a stereomicroscope, the LEICA EZ4W. A scanning electron microscope (JSM5910, 3kv voltage, and secondary electron detector) was used to analyze the micromorphological seed characteristics. The results show that seed micromorphology can be a helpful tool in *Amaranthus* taxonomy. Still, various species cannot be differentiated using these features (for example, the so-called "*deflexus*-type," comprising *A. vulgatissimus*, *A. cacciatoi, A. spinosus*, *A. dubius*, and *A. stadleyanus*). These findings confirm the taxonomic complexity of the genus *Amaranthus* once again.

In experiment three, the chemical characteristics of seed oil, specifically squalene, free fatty acid, tocopherol, and sterol, were extracted from six *Amaranthus* species, collected in situ, and analyzed using the Accelerated Solvent Extraction (ASE) apparatus. This study provides data on the squalene, free fatty acid, tocopherol, and sterol content in the oil of six *Amaranthus* species found in natural habitats for the first time. The smallest seeds (*A. tuberculatus*) have the highest percentages of oil and squalene, while the largest seeds (*A. muricatus*) have the lowest percentages. There is also evidence that specimens from lower altitudes had the highest concentration of fatty acids. According to our findings, the six wild *Amaranthus* species demonstrated features comparable to commercial species, exhibiting medium-high oil and squalene content. This study reveals that the collection site influences the oil and squalene content of the *Amaranthus* species.

Contents

List of publications

This thesis is based on the work contained in the following papers:

I. **On the occurrence and naturalization of *Amaranthus hypochondriacus* (Amaranthaceae) in some European countries, with notes on its climatic features**

Iamonico, D., Fortini, P., & Hussain, A. N. (2022). *Hacquetia*, *21*(1), 211-222. https://doi.org/10.2478/hacq-2021-0028.

II. **Trying to understand the complicated taxonomy in *Amaranthus* (Amaranthaceae). Insights on seeds morphology**

Iamonico, D., Hussain, A. N., Sindhu, A., Kumar, S. A., Shaheen, S., Munir, M., & Fortini, P. (2023). *Plants*, *12*(5), 987. https://doi.org/10.3390/plants12050987**.**

III. **Characterization of seed oil from six in-situ collected wild *Amaranthus* species.**

Hussain, A. N., Geuens, J., Vermoesen, A., Munir, M., Iamonico, D., Marzio, P. D., & Fortini, P. (2023). *Diversity*, *15*(2), 237. https://doi.org/10.3390/d15020237.

IV. **Nuove segnalazioni floristiche italiane 9. Flora vascolare.**

Laface, V. L. A., Musarella, C. M., Spampinato, G., Iamonico, D., Noor Hussain, A., Fortini, P. & Marcucci, R. (2020). *Notiziario della Società Botanica Italiana*, *4*, 1-5. http://hdl.handle.net/11584/294003

V **Notulae to the Italian alien vascular flora: 11.**

Galasso, G., & Sennikov, A. N. (2021). *Italian Botanist*. http://hdl.handle.net/10138/341423

VI. **Notulae to the Italian alien vascular flora: 14.**

Galasso, G., Domina, G., Andreatta, S., Argenti, C., Astuti, G., Bacaro, G., ... & Lastrucci, L. (2022). *Italian Botanist*, *14*, 99-118. http://hdl.handle.net/11368/3035678

1 Introduction

1.1 Family Amaranthaceae:

The Amaranthaceae family, which belongs to the order Caryophyllales, is known as the "Amaranth family." The term *Amaranthus* is derived mainly from the Greek word "Anthos" (Flower), which means never-ending. There are 71 genera and roughly 900 species in the family Amaranthaceae Juss. (Caryophyllales Juss. ex. Berchtold & J. Presl) which are found throughout the world (Pai et al., 2011). They primarily consist of summer annuals and perennial herbs (Blunden et al., 1999).

This family invades various habitats, such as agricultural lands, pastures, rangelands, wastelands, and fencerows. Many species are found in the tropics and subtropics, whereas others are distributed worldwide. Several species are cultivated as grains and ornamentals (Schwartz & College, 2015). These species can survive in unfavorable environmental conditions such as high irradiance, temperature, and limited water supply (Zhigila et al., 2015).

A single plant can generate more than 100,000 seeds even when growing in competition with a crop, and because the seeds are so tiny (averaging 1 mm in diameter), water and wind can quickly disperse them. Other methods of dispersing seeds include machines, animal feed, clothes, birds, and humans (Massinga et al., 2001; Steckel & Sprague, 2004; Tranel & Trucco, 2009). Species of this family have a wide range of characteristics: leaves can be alternate, opposite, simple, or entire, monoecious, and dioecious (Flora of North America 2015). Inflorescences are usually arranged into spikes, often clustered into panicles or glomerules. Fruits can be either dehiscent or indehiscent (Flora of North America 2015).

A lot of research has been done on the systematics of the family Amaranthaceae. Based on morphological and molecular phylogenetic studies (Rodman, 1990; Downie & Palmer, 1994; Cuenoud et al., 2002; Kadereit et al., 2003), the Order Caryophyllales (Takhtajan, 1997; Sánchez-del-Pino et al., 2009) includes the Amaranthaceae (Schinz, 1893) and Chenopodiaceae (Bentham & Hooker, 1883), families and considered to be closely related (Kadereit et al., 2003). Genetic and molecular investigations revealed that traditional classification based on morphological and anatomical characteristics did not always reflect evolutionary relationships. According to recent genetic research, both the families Chenopodiaceae and Amaranthaceae are merged into a single family called Amaranathaceae s.l. (Müller & Borsch 2005; Morales et al., 2021). Many anatomical, morphological, and phytochemical traits, including tiny sessile flowers with five tepals, one whorl of epitepalous stamens, and one basal ovule, distinguish the Amaranthaceae s.l. (Kadereit et al., 2003; Morales et al., 2021).

1.2 Genus *Amaranthus*

1.2.1. Distribution

Amaranthus is the most significant genus in the family Amaranthaceae, subfamily Amaranthoideae, tribe Amarantheae, and subtribe Amaranthinae (Müller & Borsch, 2005). It consists of 70-80 species, of which about half are native to the Americas (Mosyakin & Robertson, 2003; Hernández et al., 2015; Iamonico, 2015). The number of species is still uncertain because of the lack of investigations focused on *Amaranthus* systematics. There is debate over the number of species in this genus (Assad et al., 2017). According to various reports, there are 60 species (Uphof, 1968; Singh et al., 1983; National Research Council, 1984; Budin et al., 1996; Mosyakin & Robertson, 2003), 70 species (Pratt et al., 1999; Costea & DeMason, 2001; Mosyakin & Robertson, 2003), over 75 species (Sauer, 1993), 86 species (USDA, ARS, 1999), and 100 species (Hanf, 1984).

1.2.2. Origin

Amaranthus is believed to be originated in Central America, with evidence of its cultivation as far back as 6700 BC (Putnam et al., 1989; Sauer, 1993; Kigel, 1994; Mposi, 1999). *Amaranthus cruentus* and *Amaranthus hypochondriacus* are native to Guatemala and Mexico, respectively, and have been domesticated in Central America. *Amaranthus caudatus* was domesticated by the people of the Andes area. *Amaranthus* was used for the first time during the Aztec civilization in central Mexico between 1400 and 1500 AD. Their primary source of food, *Amaranthus*, which they referred to as "huautli," was also used in religious rituals. Its seeds were mashed by Aztec women, combined with honey, other delights, and sometimes with human blood (National Research Council, 1984), then shaped into different forms (including animals, natural characteristics, and Gods) for eating during religious ceremonies and other events (Brenner et al., 2000). The Spanish endeavored to suppress Aztec culture and religion after they arrived in Mexico at the beginning of the 1500s AD; as a result, *Amaranthus* as a crop nearly vanished in America after their conquest (Sauer, 1950). However, around 50 years after the Spanish arrived, according to Spanish missionaries, *Amaranthus* was still used in small amounts as food and in traditional cultural rituals (Early, 1992). According to Sauer (1967), *Amaranthus* was introduced into Spain in the fifteenth century, from where it spread throughout Europe. By 1700 AD, it was known as a minor grain plant in central Europe and Russia, and by the early nineteenth century, it reached Africa and Asia. Both *A. hypochondriacus* and *A. caudatus* are cultivated in this region (Sauer, 1950). Until the mid-1990s, South Asia was the only location in the globe where *Amaranthus* production was expanding (Brenner et al., 2000). In the United States in the 1970s, research on this plant began, and new evidence proved that grain amaranth is rich in good-quality protein (Senft, 1980; Lehmann, 1996). It has spread worldwide, particularly in Europe, Asia, and Africa (Putnam et al., 1989; Mposi, 1999). Thus, *Amaranthus* is a historical as well as modern plant.

1.2.3. Phylogeny

Carl Linnaeus, a Swedish botanist, published the first formal description of the genus *Amaranthus* in his book "Species Plantarum" in 1753. *A. blitum, A. caudatus, A. hybridus, A. hypochondriacus,* and *A. retroflexus* are the only five *Amaranthus* species that Linnaeus discussed in this book. Since then, many other amaranth species have been discovered, and the taxonomy of the genus has undergone several revisions throughout its history and even now. The controversy over the origin of the grain *Amaranthus* focuses on two conflicting hypotheses: the independent domestication concept and the single progenitor concept (Sauer, 1967). No research has shown evidence for the independent domestication hypothesis (Kietlinski et al., 2014), whereas other research has shown that *A. hybridus* is the ancestor of grain *Amaranthus*, which supports the single progenitor hypothesis (Mallory et al., 2008; Maughan et al., 2011; Kietlinski et al., 2014).

According to (Stetter et al., 2017), some grain *Amaranthus* are incompletely domesticated species either because they were not firmly chosen, or they had high levels of gene flow from their sympatric wild relatives that counteracted the fixation of key domestication traits found in the domesticated *A. caudatus*. Another recent work on the phylogeny of *Amaranthus* is by Waselkov et al.(2018). According to this research, the genus originated in the Americas with a single long-distance dispersal event to the Old World. The nuclear and chloroplast trees recover three to four major clades, closely matching the three subgenera recognized based on morphology. Due to this genus' high phenotypic and genotypic variability, which is reflected in the complex taxonomy, there are currently nomenclatural disorders and misapplication of several names (Costea et al., 2001; Arroyo et al., 2006; Minuto et al., 2006; Amini et al., 2011; Sadeghian et al., 2014; Iamonico, 2015; Iamonico, 2016a; Iamonico, 2016b; Iamonico, 2016c; Iamonico, 2020a; Iamonico, 2020b; Iamonico, 2020c; Iamonico & Palmer, 2020; Lemus et al., 2021).

1.2.4. Taxonomy

Linnaeus described the genus *Amaranthus* for the first time (1753). Many species of the genus were once considered to be separate genera, notably the dioecious and monoecious species having dehiscent or indehiscent fruits (Linnaeus, 1753; Kunth, 1838). Sauer(1967) identified two subgenera *Acnida* (which comprised dioecious species) and *Amaranthus* (which comprised monoecious species). According to the most recent taxonomic studies, the genus has been separated into three subgenera: *Acnida*, *Amaranthus*, and *Albersia* (Mosyakin & Robertson, 1996; Costea et al., 2001). Subgenus *Acnida* typically includes all dioecious *Amaranthus* species. However, subgenus *Amaranthus* and subgenus *Albersia* includes the monoecious species based on a combination of inflorescence position, number of tepals, and fruit dehiscence (Mosyakin & Robertson, 1996). Some authors have hypothesized that this infrageneric classification does not match well with evolutionary history (Eliasson, 1988; Mosyakin & Robertson, 2003). Müller and Borsch (2005) and Sage et al. (2007) classified *Amaranthus* as belonging to the order Caryophyllales, family Amaranthaceae, subfamily Amaranthoideae, tribe Amarantheae, sub-tribe Amaranthinae, and according to Sauer (1967) into the section *Amaranthus*, respectively.

Amaranthus is often difficult to characterize because of the few differentiating features among its species, small and difficult-to-see diagnostic features, vast geographical range, and a significant number of hybrid forms. These attributes complicate the taxonomy of the genus, and it has been regarded as a problematic genus by systematists (Costea & DeMason, 2001; Pinto & Velasquez, 2010; Bayón, 2015; Iamonico, 2015a; Iamonico, 2016a, 2016b, 2016c; Waselkov et al., 2018; Iamonico, 2020a, 2020b, 2020c; Iamonico & Palmer, 2020).

Several studies on the genetic variability and phylogenetic relationships of *Amaranthus* species have been done from a molecular perspective. These studies used several molecular markers, including RAPDs (Random amplified polymorphic DNA) and isozymes (Chan & Sun, 1997), low-COT DNA sequences (Sun et al., 1999); ITS DNA sequences (Internal transcribed spacers), AFLPs (Amplified fragment length polymorphism) and SSRs (Simple sequence repeats) (Xu & Sun, 2001), SNPs (Single nucleotide polymorphism (Maughan et al., 2011) Genotyping by sequencing (GBS), Whole genome sequencing (WGS), microsatellites (Mallory et al., 2008; Kietlinski et al., 2014; Stetter et al., 2017; Wu & Blair, 2017) and DNA sequencing of particular genes or areas, such as chloroplast and nuclear markers. These studies proved that *A. hybridus* was the progenitor of the domesticated grain amaranths (*A. caudatus, A. cruentus,* and *A. hypochondriacus*). All species from Australia and South Africa, and several others in the genus, have never undergone genetic or phylogenetic analysis. According to molecular investigations, this genus has a high level of genetic diversity within and among species, suggesting that this genus has had a complicated evolutionary history.

1.2.5. Why studying wild Amaranths is essential:

The detailed examination of wild *Amaranthus* can provide key information on the conservation of biodiversity, genetic diversity, nutrition, and environmental adaptation.

Conservation of biodiversity: Wild *Amaranthus* species are a vital component of our planet's biodiversity. We can learn more about them by researching their diversity, distribution, and ecological role. With this knowledge, we can develop conservation strategies and prevent their extinction (Keller et al., 2005).

Genetic variability: Wild *Amaranthus* species have a higher genetic diversity than cultivated species. New crop varieties with enhanced features, such as disease resistance, drought tolerance, and nutritional quality, can be developed using this genetic diversity (Keller et al., 2005).

Nutritional value: Wild species of *Amaranthus* are frequently more nutrient-dense than their cultivated counterparts. They are an excellent source of fiber, vitamins, minerals, and protein. We can create new food products that are healthier and more sustainable if we study their nutritional composition.

1.2.6. Habitat preferences

Several *Amaranthus* species have the capacity to spread outside of their natural habitat and form self-replicating populations, which is harmful to both agricultural systems and uncultivated vegetation (Costea et al., 2001; Iamonico, 2015; Das, 2016). *Amaranthus* are primarily self-pollinated but also outcross to varying degrees (Yeshitila et al., 2023). The C4 photosynthetic pathway helps *Amaranthus* species thrive at high temperatures and light levels and resist drought and low fertility (Tantawy et al., 2023). They can seal the stomata and therefore manage the osmotic pressure, preserving water in the cells and allowing photosynthesis even in drought (Kelly & Martin, 1983). Amaranth is also particularly sensitive to moistening (Zheleznov et al., 1997). They vigorously compete with warm-season plants for light, moisture, and nutrients. Their negative impacts include decreased crop quality and yield, toxicity, the development of harmful secondary chemicals, and the spread of infections (Iamonico, 2010). Amaranth is an essential plant because of all these qualities, especially in areas with limited water supplies (Sindhu et al., 2021).

They are locally well suited to marginal land and need less agricultural input than traditional crops (Das, 2012). *Amaranthus* has great genetic variability, with variation in plant shape (erect to prostrate), plant height, number of inflorescences (one to multiple), seed color, protein content, seed yield, pest and disease resistance, and adaptability to soil type, pH, climate, rainfall, and day length (Kulakow, 1990). Although *Amaranthus* may grow in a variety of soil types and moisture levels, it has been observed to grow well in loamy, sandy-loam, or silty-loam soils with high water holding capacity (Bharat & Whitehead, 1993; Ghorbani et al., 1999; Palada & Chang, 2003) with a pH range of 4.5 to 8.0. (National Research Council,1984; Stallknecht & Schulz-Schaeffer, 1993; Palada & Chang, 2003).

Field studies have indicated that it grows well in soils with a wide range of nutrient levels (National Research Council, 1984; Myers, 1998) and reacts well to good soil fertility and organic matter (Schippers, 2000).

Annual plants like *Amaranthus*, which grow naturally in open or disturbed places and receive full sunshine, thrive in agricultural fields (Pratt et al., 1999). The abundance of *Amaranthus* species in agricultural areas is related to high nitrate levels and low phosphate and potassium levels (Dieleman et al., 2000; Assad et al., 2017). The weedy species of Amaranths, such as *A. hybridus, A. powellii, A. retroflexus,* and *A. viridis* are the ones that are most well-known (Holm et al., 1977; Holm et al., 1997; Tranel & Trucco, 2009). The few weedy amaranth species introduced to the Americas from the Old World are *A. blitum* (subsp. *blitum*) and *A. graecizans.* One species imported to North America is *A. blitum,* which needs special care because it commonly causes problems for irrigated crops in Europe, Africa, and Asia (Holm et al., 1977; Hügin, 1986; Costea, 1998a, b; Teitz et al., 1990) reported that in the United States, *A. lividus* = *A. blitum* has lately become Ohio's most troublesome weed in vegetable cultivation, harming crops including lettuce, spinach, celery, and carrot. In addition, *A. blitum* is a vital leaf vegetable crop throughout the Pacific islands, Asia, and Africa.

1.2.7. Phenology

Phenology is the study of periodic biological phenomena that occur at several levels, such as organs, tissues, and cells (Alm et al., 1991). Analysis of phenological phases permits precise estimation of crop-weed competition (Ghersa & Holt, 1995). Hence, phenological surveys are vital to weed research (Brainard et al., 2005) and may aid in the construction of a realistic and applicable model for weed management (Elmore, 1996; Swanton et al.,1999). Several authors have reported on the phenology of numerous *Amaranthus* species. For most *Amaranthus* species, the growth cycle starts with seed germination in the spring, is followed by a phase of vegetative growth in the summer, and then blooming and seed production in the late summer. Amaranth growth depends on the species as well as environmental factors, including temperature, light, and moisture (Forcella et al., 1997; Assad et al., 2017).

1.2.8. Morphology

The following characteristics define the genus *Amaranthus*: Herbaceous habit with prostrate to erect stem, annual or (rarely) short-lived perennial life cycle. The leaves are alternating, oval to linear, with a notched or concave apex and smooth edges. Flowers are imperfect, with complex dichasia packed into inflorescences. *Amaranthus* plants are either monoecious (*A. albus, A. blitum, A. caudatus, A. graecizans, A. hybridus, A. powellii, A.*

retroflexus, and *A. viridis*) or dioecious (*A. palmeri, A. rudis,* and *A. tuberculatus*). The terminal and/or axillary inflorescence has three to five tepals and stamens. Monoecious species are typically wind-pollinated and self-pollinated. Fruit is utricle or pyxidium (Assad et al., 2017).

1.2.9. Biological, Chemical and economical importance of *Amaranthus*

The rational and efficient exploration of sustainable plant resources is essential to maintaining global food security in the future. Humans have used more than 10,000 edible species over the years, yet just 150 plant species are commercially accessible globally. Only 12 species — rice, wheat, maize, and potato provide more than 60% of the world's protein and calorie needs (FAO, 2005). As a result, many researchers from throughout the world have concentrated on these essential but underutilized crops in recent decades (Ruth et al., 2021; Aderibigbe et al., 2022). *Amaranthus* are a group of plants with various uses, including ornamental materials, medicinal, and food. Due to their wide range of nutrients, these grains are chosen over conventional cereals to produce nutritious, sustainable food items, including plant-based dairy, vegan meat, and gluten-free products (Balakrishnan et al., 2022; Yeşil & Levent, 2022).

Amaranthus is a particularly significant crop for developing countries since it contains necessary and non-essential amino acids, vitamins, polyunsaturated fatty acids, vitamins, carotenoids, bile acids, alcohols, steroids, and squalene (Kislichenko, 2004; Johns & Eyzaguirre, 2007). Grain *Amaranthus* is a gluten-free pseudocereal, non-grass but cereal-like grain (true cereals are classified as grasses). It is suitable for celiac disease patients because it contains no gluten (Nasirpour et al., 2020).

The following are some of the primary benefits of *Amaranthus: Amaranthus* are grain and leafy vegetables. Various nations, including Asia, Africa, and South America, boil the leaves of various amaranth species, including *A. dubius*, *A. hybridus,* and *A. tricolor*. Protein-rich seeds of a few species (*A. caudatus* L., *A. cruentus* L., and *A. hypochondriacus* L.) are used as pseudocereals; these species are nutritious and are known as grain Amaranths. *A. caudatus*, *A. cruentus,* and *A. hypochondriacus* are endemic to Guatemala, Mexico, and the Andes area, respectively. The ability of grain Amaranths to supplement traditional economic crops is well documented (Das, 2012).

The conventional primary sources are Shark and whale liver oils containing 40 to 86% squalene. Scientists and commercial organizations have made numerous attempts to find vegetable sources of squalene, which are simpler to handle than animal sources, due to population issues for these marine species. *Amaranthus* seed oil is getting greater attention due to its high amount of squalene and rate of unsaturated fatty acids (Das, 2016; Szabóová et al., 2020; Tarhan, 2021).

Amaranthus oil is unique among organic vegetable oils because it has the most balanced amino acid content as well as the most active form of Vitamin E and Squalene (Venskutonis & Kraujalis, 2013; Soriano et al., 2019; Rivero et al., 2023). Amaranth oil has a considerable therapeutic effect on the body, saturating it with oxygen, which enhances the performance of all human organs. Amaranth oil is now used to treat the cardiovascular and digestive system, cancer prevention and therapeutic interventions, accelerated recovery after radiotherapy, the kidneys and liver, skin diseases (eczema, psoriasis, lichen, ulcer), tuberculosis, and many infectious viral and fungal diseases. Amaranth oil contains squalene, vitamin E, phytosterols, and magnesium, which prevent the development of inflammatory processes in the body, reduce the risk of blood clots, and lowers blood levels of low-density lipoproteins, which promote the development of atherosclerotic plaques in blood vessels (Konyk et al., 2002; Zaremba, 2009).

1.2.10. Why the taxonomy of *Amaranthus* is important

Many people in underdeveloped nations rely on medicinal plants to meet their basic and primary healthcare requirements. Hence phytomedicines have gained popularity for historical and traditional reasons (Aburjai et al., 2007). The quality of these medications is based on the appropriate authentication of the plant material used, and pharmacognostic assessments are one of the fundamental processes used to standardize the herbal material. Macroscopic characterization of plant material is used to provide the initial authentication. There is a risk of adulteration during collection because of the resemblance of various species within the genus. As a result, plant material standardization, including qualitative and quantitative assessments, is required (Ishtiaq et al., 2016).

1.2.11. Seed Micromorphology

Every mature seed has an exterior layer called the seed coat, sometimes known as the “testa.” It interacts with both the external environment and the internal structures of the seed. Seed coats show complex and incredibly variable morphology and structure, providing important taxonomic characteristics (Barthlott, 1981, 1990). Seeds of *Amaranthus* species are nutrient-dense and used as human and animal food (Tucker, 1986). Knowledge of the taxonomy, evolution, and ecology of angiosperm species is improved by the studies on the morphology and anatomy of seeds (Cortez & Carmello-Guerreiro, 2008). As most species cannot be distinguished vegetatively and are thus worthless for taxonomic identification, it is challenging to investigate these groups. It is well-acknowledged that seed surface ultrastructure is a reliable method for resolving taxonomic issues (Heywood, 1971; Buth & Ara, 1983; Brochmann, 1992; Koul et al., 2000). It has always been recognized that the morphology of seeds is essential for classification (Hooker & Thomson, 1861).

1.2.12. Effect of Climate on *Amaranthus*

The climate has a considerable impact on the growth and development of Amaranths. Amaranths are tolerant of environmental conditions and can be cultivated in various habitats. Nevertheless, amaranth growth and output may be regulated by a variety of environmental conditions, such as temperature, water availability, and light intensity. The potential effects of climate change on global agriculture production, such as poor crop yields, desertification, deforestation, erosion, water quality degradation, and water resource depletion, have been widely projected to impede future crop productivity levels, complicating the challenge of maintaining food security in agriculturally vulnerable areas (Taub et al., 2000; Araus et al., 2008; Delgado et al., 2011). The combination of global population expansion and the issues mentioned above, as well as rising energy prices and accompanying increases in agricultural input costs, will make maintaining global food security even more difficult.

Some of the proposed climate change mitigation and adaptation techniques include the creation of more varied cropping systems, including drought and heat-stress-resistant crops, to better adjust to climatic unpredictability and new pest and disease pressures (Morton, 2007; Delgado et al., 2011). Amaranth is a good option for incorporating proper climate change mitigation techniques. As a result, amaranth should be researched further as a possible crop for extending the use of the region's marginal agricultural lands and improving the agricultural systems of dry and semiarid areas in eastern Africa. In contrast, using both the seed and the leaf production for food security purposes (Fomsgaard et al., 2011). The globalization of agriculture has resulted in a situation in which the majority of cultivation and research efforts are concentrated on a few major crops around the world. In contrast, minor crops, which offer unique benefits for global food security and climate change mitigation, have been ignored and underutilized globally (FAO, 2009; Jacobsen et al., 2013). World crop production currently depends on only nine crops to produce more than 75% of the world's plant-derived energy, with wheat, rice, and maize accounting for more than half of total production (FAO, 2009; Schmidt et al., 2010).

This reliance on a small number of species is a severe problem for agriculture (Frison, 2006; Raschke & Cheema, 2008). For example, in 2007, a lack of food supplies to satisfy increased demand led to agricultural product price fluctuations and chaos in global food markets, driving people farther into the current situation of food insecurity (Clapp & Helleiner, 2012). Several high-profile multinational programs are ongoing in Africa to boost the agricultural output of food, livestock feed, and biofuel (Devereux, 2009; Vermeulen & Cotula, 2010; Clapp & Helleiner, 2012; Fanzo, 2012). As a result of climate change, there is an urgent need to investigate alternative crops because they have the potential to play an important role in satisfying the future food demands of a growing global population. Amaranth (*Amaranthus* spp.) and quinoa (*Chenopodium quinoa* Willd.) are two underused plants having such potential (Jacobsen et al., 2003a,b). Because of its great genetic diversity and variety of phenotypic plasticity, amaranth is well suited to marginal terrain and has the potential for further adaptation and development (Ecker et al., 2010; Emire & Arega, 2012; Rastogi & Shukla, 2013).

When these features combine with Amaranth's innate resilience to high temperatures, drought, poor soil conditions, and a lack of severe disease concerns, it becomes a particularly appealing crop for unfavorable growing circumstances (Jacobsen et al., 2003a,b). *Amaranthus* is a crop with characteristics that can support food security, mitigate the effect of climate change, and provide the immediate dietary needs of rural communities in agriculturally vulnerable areas (Martínez et al., 2020). External markets may provide a consistent income for subsistence farmers because of Amaranth's comparatively high selling prices and growing popularity in the Western world. While the usage of *Amaranthus* is not new to East Africa, it may be introduced as an alternate, supplemental crop to the region's current farming practices (Ecker et al., 2010; Emire & Arega, 2012). All of the above traits make *Amaranthus* an attractive crop to promote as a method of assisting small-holder farmers in East Africa. The current study focuses on the origin, beneficial characteristics, existing and potential usage of *Amaranthus* in East Africa, as well as future possibilities for cultivation and its involvement in climate change mitigation and agricultural development initiatives in the region (Alemayehu et al., 2015).

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3. Objectives and outputs

The taxonomy of *Amaranthus* is complicated, and there has not been extensive molecular and morphological research published. The most recent phylogenetic work based on cp and nuclear DNA was published by Waselkov & al. (2018), who included a sampling of 58 taxa. Recent taxonomic revisions are rare [the last published papers were by Iamonico 2015 for Italy and Bayón 2015 for South America [only subgen. Albersia (Kunth) Gren. & Godr. Overall, research on this genus still needs to be completed from a floristic and taxonomic point of view. *Amaranthus* requires comprehensive investigation since different amaranth species may influence the environment and the economy (Camarda et al., 2005). They fight fiercely with crops, which negatively affects crop quality, yield, synthesis of secondary chemicals, and disease transmission. Biological and ecological studies are essential since the genus *Amaranthus* contains worldwide invasive species and studying the characteristics that favor the alien species in the naturalization invasion process is the primary goal of biological invasion research (Iamonico, 2010). The current work aims to classify this problematic genus using micromorphology and molecular-based approaches.

The majority of sexual characteristics, particularly those related to the bracts and tepals (structure, length, ratios; see, for example, Mosyakin & Robertson, 2003; Bayón, 2015; Iamonico, 2015), are now used to identify Amaranths. These characteristics enable us to distinguish between different phenotypes and taxa. The characteristics that have already been poorly examined are related to the seeds. Every plant has a unique seed character. However, some authors believe that seeds may be used to describe new species and distinguish some taxa (Sindhu & al., 2019, 2020). The seeds of the *Amaranthus* genus (Family: Amaranthaceae) are small and difficult to distinguish with the naked eye. Although some research has been done specifically on seed micromorphology, very little has been done on seed morphology. Keeping all of these considerations in mind, we have made an effort to observe potential microfeatures using a scanning electron microscope (SEM). Scanning electron microscopy was used to examine the micro-morphological traits of seed surfaces in 25 species of *Amaranthus*.

Many Amaranths were chosen by the National Academy of Sciences of the United States of America as important prospective crops from a list of 36 of the most desired crops in the world (National Academy of Sciences 1985). Due to these qualities, amaranth would be a desirable alternative crop in the Mediterranean Basin as well. *Amaranthus* is currently regarded as a third-millennium food crop that contains significant amounts of vitamins, minerals, high-quality proteins, and bioactive substances with antioxidant properties, such as phytosterols, saponins, flavonoids, squalene, and phenolic acids (Paśko et al., 2009; Pavlík, 2012). The highest concentration of squalene, the primary component of skin surface polyunsaturated lipids, is another noteworthy benefit of amaranth. Squalene lowers blood cholesterol levels, protects against cardiovascular disease, and has antitumor and anticancer effects against ovarian, breast, lung, and colon cancer (Rao et al., 1998).

In detail, the specific objectives of the experiments on which the doctoral thesis was based were:

- To investigate the occurrence and invasion status of *Amaranthus hypochondriacus* in Belarus, Estonia, Italy, the Netherlands, and the North Caucasus to understand its distribution in relation to the bioclimate (Paper I).

- To investigate the taxonomic value of the seeds micromorphological features using the Scanning Electron Microscope (SEM) in order to improve the understanding of the taxonomy of the genus *Amaranthus* at a global scale (paper II).

- To study oil and squalene content from the seeds of different Italian *Amaranthus* species in order to contribute to the selection of wild species more effective in the production of these compounds (paper III).

3.1 *Amaranthus* herbarium (IS)

During the field investigations carried out in Italy in 2021. Nine *Amaranthus* species [*Amaranthus blitum* L., *Amaranthus cruentus* L., *Amaranthus deflexus* L., *Amaranthus hybridus* L., *Amaranthus hypochondriacs* L., *Amaranthus muricatus* (Gillies ex Moq.) Hieron., *Amaranthus retroflexus* L. *Amaranthus tuberculatus* (Moq.) J.D. Sauer., *Amaranthus. viridis* L.] were collected only in wild fields in Italy (Table 1). For the common species *A. hybridus* and A. *hypochondriacus,* two and three collections were made in North Central (NC, Italy), where these species spread in the agricultural landscape. 40-50 individuals per site were collected in the fruiting stage. The voucher specimens were deposited in the Herbarium of the University of Molise IS (Table 1).

Table 1. List of the specimens collected from wild Italian habitats.

***Amaranthus* species**	**Voucher specimen**	**Italian region (Code)**	**Coordinates**	**Altitude m (a.s.l.)**	**Date of collection**	**Substrate**	**Habitat**
A. blitum	12010	Molise (MOL)	41°35'31"N 14°13'42"E	385	16/07/2021	Soil rich in nitrogen	Roadside
A. cruentus	12002	Veneto (VEN)	45°17'49.2"N 11°53'31.2"E	5	10/21/2021	Clastic, soil with fertilizers	Cereals and vines crops
A. deflexus	12012	Isernia (MOL)	41°35'56.4"N 14°14'09.6"E	462	10/07/2021	Soil rich in nitrogen	Roadside
A. hybridus	12005	Friuli Venezia Giulia (FVG)	46°03'14.4"N 13°04'18.8"E	144	08/08/2021	Clastic, soil with fertilizers	Crop in full sun
A. hybridus	12006	Piedmont (PIE)	45°05'29.0"N 7°22'53.3"E	348	09/12/2021	Sandy silty soil with coarse pebble component deriving from river flooding	Abandoned garden in the alluvial plain
A. hybridus	12007	Veneto (VEN)	46°06'39.2"N 12°08'20.4"E	380	9/17/2021	Calcareous matrix	Corn crop
A. hypochondriacus	12008	Veneto (VEN)	46°07'60.0"N 12°15'32.5"E	440	9/22/2021	Calcareous matrix	Corn crop
A. hypochondriacus	12009	Lazio (LAZ)	41°49'44.4"N 13°08'24.0"E	625	10/15/2021	Soil rich in nitrogen	Roadside
A. muricatus	12004	Molise (MOL)	42°00'14.4"N 14°59'45.6"E	14	7/17/2021	Calcareous matrix	Stony wall of the Svevo castle
A. retroflexus	12011	(Trieste) FVG	46°03'18.0"N 13°04'22.8"E	146	08/08/2021	Soil rich in nitrates and phosphates	Cultivated field

A. tuberculatus	12001	Marche (MAR)	43°48'39.0"N 13°02'21.0"E	5	9/23/2021	Terrigenous matrix	Gravelly riverbed, in full sun
A. viridis	12003	Campania (CAM)	40°51'07.2"N 14°16'22.8"E	12	7/21/2021	Soil rich in nitrogen	Roadside

3.2 *Amaranthus* seed bank

Nine *Amaranthus* species [*A. blitum* L., *A. cruentus* L., *A. deflexus* L., *A. hybridus* L., *A. hypochondriacs* L., *A. muricatus* (Gillies ex Moq.) Hieron., *A. retroflexus* L. *A. tuberculatus* (Moq.) J.D. Sauer., *A. viridis* L.] were collected from wild fields during the field investigations conducted in Italy in 2021 (Table 2). For the common species *A. hybridus* and A. *hypochondriacus*, two and three collections were made in North Central (NC, Italy), where these species spread in the agricultural landscape. 40-50 individuals per site were collected in the fruiting stage. The seeds were deposited at the seed bank of the University of Molise for the preservation and conservation of the genetic diversity of *Amaranthus*. These seeds can be used for further studies in the future.

Table 2: List of specimens collected from wild Italian habitats.

***Amaranthus* species**	**Italian region (Code)**	**Coordinates**	**Altitude m (a.s.l.)**	**Date of collection**
A. blitum	Molise (MOL)	41°35'31"N 14°13'42"E	385	16/07/2021
A. cruentus	Veneto (VEN)	45°17'49.2"N 11°53'31.2"E	5	10/21/2021
A. deflexus	Isernia (MOL)	41°35'56.4"N 14°14'09.6"E	462	10/07/2021
A. hybridus	Friuli Venezia Giulia (FVG)	46°03'14.4"N 13°04'18.8"E	144	08/08/2021
A. hybridus	Piedmont (PIE)	45°05'29.0"N 7°22'53.3"E	348	09/12/2021
A. hybridus	Veneto (VEN)	46°06'39.2"N 12°08'20.4"E	380	9/17/2021
A. hypochondriacus	Veneto (VEN)	46°07'60.0"N 12°15'32.5"E	440	9/22/2021
A. hypochondriacus	Lazio (LAZ)	41°49'44.4"N 13°08'24.0"E	625	10/15/2021

A. muricatus	Molise (MOL)	42°00'14.4"N 14°59'45.6"E	14	7/17/2021
A. retroflexus	(Trieste) FVG	46°03'18.0"N 13°04'22.8"E	146	08/08/2021
A. tuberculatus	Marche (MAR)	43°48'39.0"N 13°02'21.0"E	5	9/23/2021
A. viridis	Campania (CAM)	40°51'07.2"N 14°16'22.8"E	12	7/21/2021

3.3 Articles published

Below are the three papers reporting the results obtained during the doctorate program:

I. **On the occurrence and naturalization of *Amaranthus hypochondriacus* (Amaranthaceae) in some European countries, with notes on its climatic features**

Iamonico, D., Fortini, P., & Hussain, A. N. (2022). *Hacquetia*, *21*(1), 211-222. https://doi.org/10.2478/hacq-2021-0028.

II. **Trying to understand the complicated taxonomy in *Amaranthus* (Amaranthaceae). Insights on seeds morphology**

Iamonico, D., Hussain, A. N., Sindhu, A., Kumar, S. A., Shaheen, S., Munir, M., & Fortini, P. (2023). *Plants*, *12*(5), 987. https://doi.org/10.3390/plants12050987**.**

III. **Characterization of seed oil from six in-situ collected wild *Amaranthus* species.**

Hussain, A. N., Geuens, J., Vermoesen, A., Munir, M., Iamonico, D., Marzio, P. D., & Fortini, P. (2023). *Diversity*, *15*(2), 237. https://doi.org/10.3390/d15020237.

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