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Amaranth Pre-breeding for Northern Hemisphere Climate

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Tiivistelmä – Referent - Abstract

Tämän pro gradu -työn tavoitteena oli tutkia 70 amaranttilajikkeen ydinkokoelman morfologisia, fysiologisia ja fenologisia ominaisuuksia kontrolloiduissa kasvuolosuhteissa, sekä peltoviljelyolosuhteissa. Tavoitteena oli tunnistaa lajien ja lajikkeiden välisiä eroja ja havainnoida lajien sisäistä vaihtelua.

Tulokset osoittivat, että morfologiset, fysiologiset ja fenologiset ominaisuudet vaihtelivat merkittävästi lajien ja lajikkeiden välillä. Merkittäviä eroja kasvihuone- ja kenttäkokeiden välillä voitiin havaita lähes kaikista amaranteilla tehdyistä mittauksista. *A. caudatus* ja *A. blitum* osoittautuivat korkeimmiksi lajeiksi sekä kasvihuoneessa että peltokokeessa. *A. dubius*:lla oli korkeimmat klorofylliarvot ja *A. blitum*:lla merkittävästi korkeimmat antosyaniiniarvot kenttäkokeissa, mikä johtui todennäköisesti stressistä, kuten UV säteilystä tai viileistä lämpötiloista.

Kasvihuone- ja kenttäkokeiden yhdistelmä tarjosi uusia näkemyksiä amarantin viljelymahdollisuuksista pohjoisessa ilmastossa. Löydökset muodostavat vahvan perustan geneettiselle tutkimukselle ja jalostustoiminnalle, jonka tavoitteena on parantaa amaranttien stressinsietokykyä, tuottavuutta ja fenologista sopeutumista. Ottaen huomioon aiempien Suomessa tehtyjen tutkimusten rajallinen määrä, tämä tutkimus lisää merkittävästi tietoisuutta amarantin mahdollisista hyödyistä pohjoisen maatalouden kannalta.

Jatkotutkimusta voitaisiin tehdä selvittämällä, mitkä lajit tai lajikkeet voisivat tuottaa korkeimman vilja- tai vihannessadon Suomessa. Amaranttien kylmänkestävyyttä voitaisiin tutkia kasvukammiossa, jotta saataisiin selville, miten kylmät lämpötilat vaikuttavat amaranttikantojen itämiseen ja kuinka taimet selviytyvät kylmistä yölämpötiloista, jotta saataisiin selville optimaalinen kylvöaika pohjoisen ilmaston kannalta.

Avainsanat — Nyckelord — Keywords

Revonhäntä, Amarantti, esijalostus, pohjoinen ilmasto, ilmastonmuutos

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Tiivistelmä – Referent - Abstract

The aim of this master's thesis was to investigate the morphological, physiological, and phenological characteristics of a core collection of 70 amaranth accessions from six amaranth species (*Amaranthus cruentus*, *A. hypochondriacus*, *A. caudatus*, *A. dubius*, *A. blitum*, and *A. thunbergii)* under controlled climate conditions and field-growing conditions. The goal was to identify differences between species and accessions and to observe variation within species.

The results showed that morphological, physiological, and phenological traits varied significantly among species and cultivars. Significant differences between the greenhouse and the field experiments could be distinguished from almost all measurements made on amaranths. *A. caudatus* and *A. blitum* proved to be the tallest species, both in the greenhouse and in the field experiment. *A. dubius* had the highest chlorophyll values and *A. blitum* significantly the highest anthocyanin values in the field experiment, probably due to stress, such as UV radiation or cold temperature.

The combination of greenhouse and field experiments provided novel insights into the potential of growing amaranth in northern climates. The findings form a strong foundation for genetic research and breeding efforts aimed at improving stress tolerance, productivity, and phenological adaptation in amaranths. Considering the limited number of previous studies in Finland, this research significantly enhances awareness of the potential benefits of amaranth for northern agriculture.

Further research could be carried out by finding out which species or varieties could produce the highest grain or vegetable yield in Finland. The cold resistance of amaranths could be studied in a growth chamber to find out how cold temperatures affect different accessions of amaranths germination and how the seedlings survive cold night temperatures, in order to find out the optimal sowing time for northern climates.

Avainsanat — Nyckelord — Keywords

Amaranth, Amaranthus, pre-breeding, Northern climate, climate change

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ABBREVIATIONS AND CONCEPTS

ANTH	Anthocyanin
CHL	Chlorophyll
FLAV	Flavonoid
GNDVI	Green Normalized Difference Vegetation Index
NBI	Nitrogen Balance Index
NDAI	Normalized Difference Red Edge Index
NDRI	Normalized Difference Red Edge Index
NDVI	Normalized Difference Vegetation Index

1 Introduction

The challenges posed by climate change to plant agriculture have raised concerns about global food security. In Finland, climate change has already brought us unpredictable growing seasons, such as dry hot springs and summers, where traditional crops may not perform well (Finnish Meteorological Institute 2021). The supply of farm inputs such as synthetic chemical fertilizers and irrigation may also become more challenging in the future due to concerns on environmental impact and fluctuations in prices (Seuri 2006).

There is an urgent need for resilient and low input crops to be introduced into cropping systems (Stockholm Environment Institute 2024). One candidate crop is amaranth, which has a good heat tolerance, and they can also manage with a scarce amount of water and also get water from deep in the soil with their developed root system. Amaranths also get along very well in poor nutrient soil and even when fertilized, they need approx. half as much fertilizer as cereals to reach their full yield potential (National Research Council1984, Das 2016).

The emerging plant-based diet and growing population also increase the need for plant-based protein sources. Also, the protein balance of current main crops is not sufficient to provide people with all the essential amino acids (Willett et al. 2019). Amaranth seeds and leaves are packed with all the amino acids. The possibility of also adding a domestic protein source to the livestock has been considered, for which amaranths could be well suited, in various mixtures (Alegbejo 2013, Arendt & Zannini 2013).

Our cropping systems lack potential profitable resilient crops to be used in crop rotation, or at least the farmers do not see enough crop species to be profitable to cultivate. In this case, the crop rotation will be insufficient (Stockholm Environment Institute 2024). Amaranth may act as a new crop that does not have a common disease pressure with other plants cultivated in Finland and also being a low input versatile plant. This makes amaranth a crop with high potential that can be cultivated for its nutritious grain or being used as a leafy vegetable or a feed crop (National Research Council 1984).

Amaranth has been seen as an ornamental crop in Finland for years (Kivirikko 1923). Recently, there has been some attempt for its cultivation in Finland using limited genetic diversity (Yle 2022). To maximize the genetic diversity of amaranth we received the World Vegetable Center (WorldVeg) amaranth core collection consisting of 67 accessions from six species (*Amaranthus blitum* L., *A. caudatus* L., *A. cruentus* L., *A. dubius* Mart. ex Thell., *A. hypochondriacus* L., and *A. thunbergii* Moq.) and representing the global diversity for this crop. In addition to the 67 accessions from the WorldVeg amaranth core collection, three additional *A. cruentus* accessions were included in the study, bringing the total to 70 accessions. This germplasm could be used as a valuable pre-breeding material to be tested in Finland for various applications such as grain, leafy vegetable, ornamental, biomass (bioenergy or peat production), or feed crop.

We strongly believe amaranth has great potential for Finnish agriculture and food and feed in a changing climate. This MSc thesis aims to study the WorldVeg amaranth core collection for various morpho-physiological and phenological traits under controlled and Finnish field conditions.

This research consists of a greenhouse and two field experiments. The goal of the greenhouse experiment is to make observations about accessions growing under optimal conditions to overcome genotype x environment interactions to study morpho-physiological traits as well as multiply seeds for future field experiments.
The field experiments were designed to study the response of amaranth germplasm to short Finnish growing seasons (long days and cool nights). This could allow us to look at phenological responses and study the species interaction under our growing seasons.

In Finland, awareness of amaranth remains limited at both the consumer and cultivation levels, resulting in low consumption despite its well-documented health benefits. Currently, the reliance on small-scale imports from abroad presents challenges in terms of both ecological sustainability and economic feasibility. A key objective is to enhance the visibility of amaranth by disseminating research findings to a wider audience. Establishing domestic cultivation has the potential to increase awareness, promote consumption, and reduce costs, thereby making amaranth more accessible and viable within the Finnish context.

2 Amaranth

Amaranths (*Amaranthus spp.*) belong to the dicotyledonous genus *Amaranthus* L. Recent genetic analyses have identified over 70 distinct species within the genus Amaranthus (Suresh et al., 2014). Amaranths are divided into three subgenera: *Albersia*, *Acnida* and *Amaranthus*. Of these subgenera, *Amaranthus* contains 13 species and all known consumed species (Bayón 2015, Stetter et al. 2017). Most of the species of amaranth are considered weeds commonly known as pigweed globally (Lin et al. 2021).

Amaranths are cultivated as a grain crop and leafy vegetable. Grain amaranths are known as pseudocereal. Pseudocereals are crops yielding similar grains like cereals but belonging to a family other than grasses, such as buckwheat or quinoa. The cultivated grain amaranth species are originally from South, Central and North America (National Research Council 1984). Since ancient times Indians cultivated amaranth species in areas such as Argentina, Bolivia, Peru, and Mexico (National Research Council 1984, Das 2016). Amaranths were cultivated for centuries, but with the arrival of the Spanish, cultivation began to focus on species that are now widely known, such as Corn and beans (National Research Council 1984). Leafy vegetable amaranths have been cultivated mostly in Southeast Asia, from where it already spread to Europe in ancient times (Schafleitner et al. 2022). It has been grown since ancient Greece, the Roman Empire and in medieval gardens. The best-known leaf vegetable amaranth species are *A. tricolor*, *A. blitum* and *A. dubius* (National Research Council 1984, Das 2016).

Amaranths are nowadays cultivated at small scale by small-holder farmers. It is grown especially as a leafy vegetable in Asia and in parts of Africa. In South and Central America and in some areas in Nepal, amaranth is cultivated for grain, mostly in remote areas. The most cultivated species are *A. hypochondriacus, A. cruentus* (from Mexico and Guatemala) for grain and as a vegetable, *A. caudatus* from Peru and other parts of the Andes, mostly for grain and *A. tricolor* which originating from Asia as a leafy vegetable (National Research Council 1984, Das 2016).

Amaranth plants consist of a main stem, which can also form lateral shoots, the number of which depends on the genotype, growing conditions, and cultivation technique. For example, dense planting suppressed the formation of side shoots (Costea 2001, Gimplinger et al. 2007), while cutting off the main stem for repeated harvest induces lateral shoots. Amaranths typically grow 1–2 m tall, with the tallest genotypes reaching more than 3 m (Awe & Osunlola 2013, Das 2016).

Flowers in amaranth are formed as the main inflorescence at the tip of the crown and at the ends of the side shoots (Acosta et al. 2009). The size of the inflorescence varies from the 5 cm small inflorescences of *A. tricolor* to the 1.5 m inflorescences of *A. caudatus* (Das 2016). The color of the inflorescence varies between pink, purple, orange, yellow, brown and green (Lin et al. 2021).

Depending on the species, amaranth leaves are of very different sizes and shapes. Typically, they are, however, 5-15 cm long and 2-10 cm (Das 2016). The shape of the leaves is ovate to rhomboid-ovate or ovate-elliptic, however, they always narrow towards the tip. The leaves are petiolate, which is often almost the same size or shorter than the blade of the leaf (Townsend 1993, Skwarylo-Bednarz et al. 2020). The color of the leaves can also vary from red, yellow to green colors (Adhikari et al. 2021).

The seeds are small, typically 1-1.5 mm in size and lenticular in shape. Their coloring is typically white, cream, yellow, brown or black (Teutonico & Knorr 1985). The color of the seeds is directly related to the purpose of amaranth. Genotypes grown as leafy vegetable and amaranth weeds generally have black seeds, which is the ancestral seed coloring of amaranths. The seeds of grain amaranths have been bred to be pale, especially for species from the Americas (Stetter & Schmid 2020).

The protein content of amaranth seeds varies between 13–21 % (Venskutonis & Kraujalis 2013). For example, in seeds of *A. caudatus* the protein content is 16.5 ± 0.3% (Alvarez-Jubete et al. 2009). On average, amaranth seeds have a higher protein content than any other cereal. For example, wheat (*Triticum aestivum* L.) grain has a protein content of about 11.7%, corn (*Zea mays* L.) 9.2% and rice (*Oryza sativa* L.) 7.0% (Balyan et al. 2013, Das 2016).

In addition to having a higher protein content than traditional grains, the amino acid profile of amaranth is close to optimal for dietary requirements (Arendt & Zannini 2013). Amaranth has high quality protein in, both the seeds and the leaves. The protein value of amaranth alone is 75 (Full score is 100), which is higher than the amino acid balance of any grain protein. The combination of amaranth seed with corn reaches the full protein quality score 100. Amaranth contains over two times more of the essential amino acid lysine, than wheat and 50% more than rice. It is also an excellent source of tryptophan and sulfur-containing amino acids. Of the essential amino acids, threonine and leucine are the most limiting in amaranth (Ferreira et al. 2005, Sunil et al. 2014, Jahan et al. 2022). In addition to protein, amaranth has high concentrations of Fe, Ca and other essential minerals, as well as vitamins for human diets (Ebert et al. 2011, Muriuki 2015).

Amaranths are C4 plants that have a distinguished photosynthetic pathway compared to C3 plants. In the mesophyll cells, CO_2 is initially fixed into a 4-carbon compound (hence the name "C4") by the enzyme PEP carboxylase, which has a higher affinity for CO_2 than the enzyme used in C3 photosynthesis. This forms a 4-carbon compound, usually oxaloacetate, which is then converted into malate or aspartate (Doncaster & Leegood 1990, Urban et al. 2021).

The 4-carbon compounds are transported to special cells called bundle sheath cells, where CO_2 is released and concentrated. The CO_2 is then used in the Calvin Cycle (like in C3 photosynthesis) to produce sugars. This adaptation allows amaranth to grow better in hot and dry conditions than C3 plants, when stomata are typically closed to prevent water loss. The elevated CO_2 concentration in C4 plants reduces the required concentration of Rubisco, the primary protein in leaves, thereby lowering the overall nitrogen requirement of the plant. However, despite their relatively low nitrogen needs, amaranths respond positively to nitrogen fertilization (Urban et al. 2021).

3 Cultivation of amaranth

Often amaranth is cultivated on nutrient-poor lands, while better soils are generally used for other crops. Amaranths are less cultivated in large-scale farming, as it is still a semi-domesticated crop with several agronomic shortcomings, such as seed shattering, which makes harvesting challenging. Further challenges are related to the lack of value chain for this crop. Nowadays in Western countries, amaranth seeds are used mainly in small quantities as a specialty food. In parts of Africa, grain amaranth is used for fortifying maize flour and the demand for grain amaranth is rising. The potential of amaranth is based on the hardiness of the plant, the robustness to abiotic stresses and the good nutritional value, specifically the high protein content (National Research Council 1984, Das 2016).

3.1 Climate adaptation

Grain and vegetable amaranths are grown in tropical highlands in Central and South America, Asia and Africa, and the cultivation of vegetable amaranth is also found in subtropical and tropical lowlands in Asia and parts of Africa, where it grows year-round, but it is less adapted to zones with high precipitation. It is tolerant to heat and can also grow on marginal land. In the Himalayas, grain amaranth is growing at up to 3,500 m altitude (Ebert et al. 2011, Das 2016). *A. blitum* is a vegetable amaranth native to the Mediterranean, which has spread to all continents for cultivation (Ebert et al. 2011, Das 2016).

3.2 Soil requirements

Amaranths are very unpretentious in terms of soil type and are so-called marginal soil plants that get along in all types of soil, but they thrive in well-drained, loamy soils with lots of organic material (Ebert et al. 2011). Amaranth thrives best in soils with a pH of 6.0–7.5, but it can tolerate slightly acidic soils with a pH of 5.5–6.0, which are common in Finland. However, the lower pH may slow growth due to reduced nutrient availability (Live to Plant 2023, Metsähallitus 2024). It also does well in slightly alkaline soil (Nordkalk 2024).

3.3 Temperature and photoperiod requirements

Amaranths grow well in warm temperatures of 15 to 40°C with an optimum temperature above 25 °C. On the other hand, amaranths do not thrive in low temperatures (Ebert et al. 2011). Low temperature will slow down plant growth, weakens growth initiation, prolongs plant development and amaranths stop growing at 8 °C and start to suffer damage below 4 °C (Svirskis 2003, Das 2016). With grain amaranths, it has been found that different types of plants can germinate at temperatures between 16 and 35 °C. In laboratory tests it has been found that 10 °C degrees is the minimum temperature for seed germination, but generally 20 °C degrees is the lowest germination temperature for amaranth seeds used for cultivation (Das 2016).

Amaranths have very variable day length requirements, which are often related to temperature. Vegetable amaranths are day neutral, but the goal in cultivation is to produce as many leaves as rapidly as possible. This occurs best in warm long day conditions, where biomass production is highest (Whitehead et al. 2002, Das 2016).

Grain amaranths have great variation in day length requirement. *A. caudatus* and *A. cruentus* are short day species. *A. cruentus* has a long vegetative growth phase at the equator, but under long day conditions seed production starts very early. *A. caudatus*, on the other hand, usually flowers and produces seeds when day length is less than 8 hours, but as an exception, for example the variety "love-lies-bleeding" produces seeds best during long days. *A. hypochondriacus* shows great variation with regard to optimal growth conditions, but is photoperiod-neutral (Ebert et al. 2011, Das 2016).

3.4 Water requirements for amaranth seed germination and growth

Amaranth seeds need moist soil to germinate and establish a root system, as amaranths are sensitive when they are planted (Zubillaga et al. 2021). After this, grain amaranths begin to grow with very little irrigation or rain in hot temperatures. They typically grow in lowlands with an annual rainfall of 200 mm (Das 2016), but to achieve a satisfactory yield, a minimum precipitation of 500 mm is required (Mukuwapasi et al., 2024). With vegetable amaranths the soil must always remain moist to ensure optimal conditions even after germination and root formation. The areas where vegetable amaranths typically grow get an annual rainfall of 3000 mm (Das 2016), but 1270–1524 mm is enough for a good harvest (Qiu & Liu 2021).

4 Amaranth - a versatile plant

Amaranth has been used in a variety of ways worldwide. It is widely used as a vegetable, an ornamental, and a grain crop known as a pseudocereal. In addition, it has potential for lesser-known uses such as animal feed, a source of vegetable oil, a dye, and as a fiber similar to hemp and flax (National Research Council 1984, Das 2016).

4.1 Leafy vegetable

Amaranth leaves are an excellent source of vitamins including provitamin A, C, and K. Amaranth leaves also have calcium, iron, and zinc. As a leafy vegetable, amaranth is either harvested by uprooting young plants, by multiple harvesting by cutting the top of the shoots, or by harvesting leaves. Dual-use varieties are available, where during the vegetative phase leaves are harvested, and at maturity, the grain is obtained (Ebert et al. 2011, Muriuki 2015). The leaves are often lightly bruised, steamed, boiled or fried in oil reducing nitrate, oxalate and bitter taste. After cooking, they are mixed with other ingredients. The leaves can also be eaten fresh, but due to the nitrate and oxalate concentrations, fresh leaves are often less popular in fresh salads (Ebert et al. 2011). Instead, the seedlings are eaten fresh like spinach, because they have a milder taste, have less nitrate and oxalate and a high vitamin and nutrient content. Oxalate in amaranth acts as anti-nutrient inhibiting the uptake of cations including Ca, Fe, Zn, and Mg. Food preparation strongly influences nutrient bioavailability. For amaranth, incorporating vitamin C and boiling of the vegetable significantly improves the mineral bioavailability (Nyonje et al. 2021). Amaranth vegetables are popular crops, especially in parts of Asia and Africa. Commonly cultivated species include *A. tricolor*, *A. dubius*, *A. viridis*, *A. hybridus*, *A. cruentus*, and *A. blitum* (National Research Council1984, Das 2016).

4.2 Ornamental plant

Amaranths are popular as ornamental plants, due to their large colorful blooms and leaves of various colors and sizes (Das 2016). Amaranths' popularity as ornamental plants has a long history. While they were primarily grown as food crops by the Incas, they were also important as ornamental plants in their ceremonies. After that they

reached Europe and quickly became ornamental plants sometime in the 16th or 17th century. In Finland, they have been used as ornamental plants at least since the 19th century (Kivirikko 1923, National Research Council 1984).

A. caudatus is known for its long, drooping inflorescences, which can be up to 1.5 m long. Their inflorescences also come in many beautiful colors, such as pink, purple or yellow. *A. tricolor* is popular because of its wide range of leaf colors from reddish, yellow to green. *A. hypochondriacus* and *A. cruentus* are known for their large, multi-colored, upright inflorescences and multi-colored leaves (Das 2016, Thapa & Blair 2018).

4.3 Grain amaranth (pseudocereal)

A. caudatus, *A. hypochondriacus* and *A. cruentus* have been cultivated as pseudocereals for centuries, particularly in Central and South America by native Americans. Today, in addition to the Americas, they are cultivated in parts of Africa (Kenya) and some regions in Asia (National Research Council 1984, Arendt & Zannini 2013). Amaranth grain is known for its high protein content with high concentrations of lysine and tryptophane. As a gluten-free grain, it's especially valuable as a cereal replacement to people with gluten sensitivities or celiac disease. It is also a good source of nutrients for a vegan diet (Arendt & Zannini 2013). Flour made from amaranth seeds can be used to make bread, porridge or gluten-free products. Breads and porridges often also use wheat or corn flour as a mixture. In India and its neighboring countries, as well as in Latin America, amaranth seeds are used like popcorn, by popping them open by heating (Das 2016).

4.4 Amaranth as a feed crop

Amaranth can be used as animal feed. High protein content, minerals such as iron and calcium and good quality carotenoids can bring a healthy diet to livestock. The biggest advantage would be the utilization of the biomass of grain amaranths, since the cattle could eat the parts that are not suitable for humans, such as the leaves and the soft stem (Alegbejo 2013, Patel et al. 2024).

Limiting factors for amaranth as a feed crop are its nitrate content, which in large quantities is harmful to livestock. Other harmful substances are phenolics, saponins,

tannins, phytic acid and oxalates. The easiest solution to this is to use amaranth as part of a versatile diet, and not as the main food source (Alegbejo 2013). However, one potential method to reduce these antinutritional factors is lactic acid fermentation, which can decrease the levels of certain harmful substances, such as saponins, tannins, and oxalates (Naseem et al. 2023).

5 Amaranth in Finland

The first mention of amaranth in Finnish literature dates back to 1683, when Elias Til-Landz presumably mentioned *A. caudatus* for the first time. This is referred to in the book "Suomalaiset kasvinnimet" published in 1936, which mentions " Amaranthus caudatus — Ketunhäntä Lönnr. 1866. — Punainen revonhäntä Hiit. K. — Punainen yrtti Jusl. 1745. — Punajnen yrti Turku (Till. 1683).” Elias Til-Landz is considered the father of botany in Finland (Ruoff 2016).

By 1936, *A. albus, A. angustifolius, A. caudatus, A. chinensis, A. hybridus, A. lividus, A. paniculatus* and *A. retroflexus* were already known in Finland (Suhonen 1936) of which *A. chinensis* probably refers to *A. hybridus* (Srivastava 2017). At the end of the 19th century, approx. In 1890, amaranth had already been introduced to Finland as an ornamental plant. (Kivirikko 1923). Over sixty years ago, Alli Väre observed amaranth as a weed in a cornfield in Finland. She characterized the plants, identified the species as *A. hybridus* and *A. retroflexus*, and concluded that the amaranth seeds had traveled to Finland with the corn seeds (Societas pro Fauna et Flora Fennica 1962).

In 1970–1980, when amaranth became more common in the world, it also came to Finland with a slight delay, for example as amaranth-based dye E123 (National Research Council 1984, Helsingin Sanomat 1995). In the 1990s, awareness of amaranth's nutrient richness began to increase in Finland, after which its use in Finland has gradually increased (Helsingin sanomat 1999). At the beginning of the 2000s, the popularity of the ornamental amaranth rose, when numerous new accessions for gardens were introduced (Helsingin Sanomat 2002).

Raiskio’s (2020) bachelor’s Thesis found that grain amaranth can be cultivated in Finland in 2018. Amaranth has a great potential to be grown (Yle 2022) and can be

used as porridge, casseroles, soups, and in salads, which increased the interest in amaranths in Finland (Kasi 2018).

According to the Finnish Meteorological Institute (2021) climate change will bring irregular precipitation patterns and hot summers to Finland. The precipitation will come in the form of heavy plum rains, followed by long dry periods. It can be difficult to grow traditional grains, so pseudocereals, especially amaranth, which is known to grow even in harsh conditions, have the potential to enter the Finnish cropping system. Amaranth is able to utilize water when it is available by growing at a fast pace and tolerates hot and dry periods with the help of its well-developed roots. The general optimum growing condition for amaranths is above 25 °C, which is already classified as warm in Finland. Grain amaranths can, however, be at their best in photosynthesis efficiency and biomass growth at even 40 °C depending on species (Ebert et al. 2011, Das 2016).

The European Commission (2022) highlights that fertilizers are becoming more difficult to obtain, prices are rising, and due to the polluting properties of chemical fertilizers, efforts have been made to reduce their use in the EU and Finland. The amaranth crop can be obtained with less fertilization than corn or wheat. Wheat and maize need 100-150 kg of nitrogen per hectare, while amaranth needs 50 kg of nitrogen per hectare. Fertilizer administration for amaranth therefore is less than half as for wheat and corn. However, when comparing nitrogen use in relation to yields, the difference between amaranth and cereal becomes less pronounced. (Pospišil et al. 2006, Das 2016).

The possible large-scale cultivation of amaranth still needs to be researched. While breeding for uniformity, reduced seed shattering and mechanical harvesting capacity has already been carried out since the 1980s in the United States of America. A significant issue still is the plant's requirement to dry down for mechanical harvesting. However, amaranth, as a semi-domesticated crop without well-established value chain remains a small-scale cultivation crop but has the to be cultivated on a large scale in Finland, provided the right species and genotypes are identified and breeding yields well adapted varieties that are accepted by farmers, markets and consumers (National Research Council 1984, Gimplinger et al. 2007).

6 Research Objectives

The aim of this study was to screen a core collection of amaranth for morpho-physiological and phenological traits under controlled and field conditions and assess the potential of amaranth for cultivation under Finland's climate conditions. The goal was to find amaranth species and\or accessions with potential to thrive in Finland's relatively short growing seasons.

Hypothesis

1. Amaranth species and genotypes differ in leaf morphological and physiological properties (under controlled climate conditions)
2. Amaranth species and genotypes respond differently to Finland’s growing conditions such as long days and cool nights (phenological response, field studies)

7 Materials and Methods

The germplasm survey study involved 70 accessions belonging to *A. cruentus* (24), *A. hypochondriacus* (20), *A. caudatus* (12), *A. dubius* (11), *A. blitum* (2), and *A. thunbergii* (1). All those accessions were provided by the World Vegetable Center genbank, Shanhua, Taiwan. These germplasms were grown both under greenhouse and field conditions.

Table 1. List of accessions, origin and taxonomy of Amaranthus species used in this study. More information about accessions can be found at https://genebank.worldveg.org/

Accession ID	Accession number	Accession DOI	Biological satus	Provenance of material	Taxon
VI033451	1	10.18730/12C210	Traditional cultivar/Landrace	Peru	*A. caudatus L.*
VI033452	2	10.18730/12C221	Traditional cultivar/Landrace	Peru	*A. caudatus L.*
VI033453-B	3	10.18730/12C2CB	Traditional cultivar/Landrace	Peru	*A. caudatus L.*
VI033454	4	10.18730/12C243	Traditional cultivar/Landrace	Peru	*A. caudatus L.*
VI033455-A	5	10.18730/12C254	Traditional cultivar/Landrace	Peru	*A. caudatus L.*
VI033455-B	6	10.18730/12C2DC	Traditional cultivar/Landrace	Peru	*A. caudatus L.*
VI033457-A	7	10.18730/12C276	Traditional cultivar/Landrace	Peru	*A. caudatus L.*
VI033457-B	8	10.18730/12C2ED	Traditional cultivar/Landrace	Peru	*A. caudatus L.*
VI033458	9	10.18730/12C287	Traditional cultivar/Landrace	Peru	*A. caudatus L.*
VI033460	10	10.18730/12C2GF	Traditional cultivar/Landrace	United States of America	*A. hypochondriacus L.*
VI033462-A	11	10.18730/12C2A9	Traditional cultivar/Landrace	Ecuador	*A. caudatus L.*
VI033462-B	12	10.18730/12C2FE	Traditional cultivar/Landrace	Ecuador	*A. caudatus L.*
VI033463	13	10.18730/12C2BA	Traditional cultivar/Landrace	Peru	*A. caudatus L.*
VI036228	14	10.18730/12F1ZU	Traditional cultivar/Landrace	Hungary	*A. hypochondriacus L.*
VI036229	15	10.18730/12F200	Traditional cultivar/Landrace	Hungary	*A. hypochondriacus L.*
VI036230	16	10.18730/12F211	Traditional cultivar/Landrace	Austria	*A. hypochondriacus L.*
VI036231	17	10.18730/12F222	Traditional cultivar/Landrace	Austria	*A. cruentus L..*
VI042947	18	10.18730/12MM86	Traditional cultivar/Landrace	Indonesia	*A. cruentus L.*
VI042948	19	10.18730/12MM97	Traditional cultivar/Landrace	Indonesia	*A. cruentus L.*

VI042949	20	10.18730/12MMA8	Traditional cultivar/Landrace	Indonesia	*A. cruentus L.*
VI044367	21	10.18730/12PQXS	Traditional cultivar/Landrace	Tanzania	*A. cruentus L.*
VI044369	22	10.18730/12PQYT	Traditional cultivar/Landrace	Ghana	*A. cruentus L.*
VI044397	23	10.18730/12P6T~	Traditional cultivar/Landrace	India	*A. cruentus L.*
VI044399	24	10.18730/12P6W=	Traditional cultivar/Landrace	India	*A. hypochondriacus L.*
VI044437-A	25	10.18730/12PQNH	Traditional cultivar/Landrace	Malaysia	*A. cruentus L.*
VI044439	26	10.18730/12QD63	Traditional cultivar/Landrace	Nigeria	*A. dubius Mart. ex Thell.*
VI044456	27	10.18730/12PQQK	Traditional cultivar/Landrace	Zimbabwe	*A. cruentus L.*
VI044468-B	28	10.18730/12P79B	Traditional cultivar/Landrace	Mexico	*A. hypochondriacus L.*
VI047539	29	10.18730/12S64C	Traditional cultivar/Landrace	Vietnam	*A. hypochondriacus L.*
VI047552	30	10.18730/12S6HS	Traditional cultivar/Landrace	Vietnam	*A. cruentus L.*
VI047576	31	10.18730/12S77A	Traditional cultivar/Landrace	Vietnam	*A. dubius Mart. ex Thell.*
VI048583	32	10.18730/12SP24	Traditional cultivar/Landrace	United States of America	*A. hypochondriacus L.*
VI048676	33	10.18730/12V0BS	Wild	Thailand	*A. dubius Mart. ex Thell.*
VI050128	34	10.18730/12VJWT	Traditional cultivar/Landrace	Kenya	*A. hypochondriacus L.*
VI050158	35	10.18730/12X3BQ	Traditional cultivar/Landrace	Taiwan ROC	*A. dubius Mart. ex Thell.*
VI050449	36	10.18730/12VJRP	Traditional cultivar/Landrace	Na	*A. hypochondriacus L.*
VI050451	37	10.18730/12X3DS	Traditional cultivar/Landrace	Tanzania	*A. dubius Mart. ex Thell.*
VI050457	38	10.18730/12WYTT	Traditional cultivar/Landrace	NA	*A. cruentus L.*
VI050458	39	10.18730/12X3HX	Traditional cultivar/Landrace	NA	*A. dubius Mart. ex Thell.*
VI050460	40	10.18730/12X3KZ	Traditional cultivar/Landrace	Tanzania	*A. dubius Mart. ex Thell.*
VI050463	41	10.18730/12X3P$	Traditional cultivar/Landrace	NA	*A. dubius Mart. ex Thell.*
VI050608-B	42	10.18730/12VJNK	Traditional cultivar/Landrace	Vietnam	*A. blitum L.*
VI050611-B	43	10.18730/12VJPM	Traditional cultivar/Landrace	Vietnam	*A. hypochondriacus L.*

VI050998	44	10.18730/12XEE8	Traditional cultivar/Landrace	Cameroon	*A. thunbergii Moq.*
VI051005	45	10.18730/12WYVV	Traditional cultivar/Landrace	Cameroon	*A. cruentus L.*
VI054577	46	10.18730/13025Q	Traditional cultivar/Landrace	Philippines	*A. cruentus L.*
VI054579	47	10.18730/13027S	Traditional cultivar/Landrace	Philippines	*A. cruentus L.*
VI054580	48	10.18730/13028T	Traditional cultivar/Landrace	Philippines	*A. cruentus L.*
VI058957	49	10.18730/133CMT	Traditional cultivar/Landrace	NA	*A. dubius Mart. ex Thell.*
VI058963	50	10.18730/133CHQ	Traditional cultivar/Landrace	NA	*A. dubius Mart. ex Thell.*
VI059037-A	51	10.18730/132MX7	Traditional cultivar/Landrace	NA	*A. hypochondriacus L.*
VI059412	52	10.18730/132N3D	Traditional cultivar/Landrace	Tanzania	*A. hypochondriacus L.*
VI059414	53	10.18730/1331ZJ	Traditional cultivar/Landrace	Tanzania	*A. cruentus L.*
VI061487	54	10.18730/135JWH	Traditional cultivar/Landrace	NA	*A. cruentus L.*
VI061497	55	10.18730/135RYT	Traditional cultivar/Landrace	Malawi	*A. dubius Mart. ex Thell.*
VI061501	56	10.18730/134MB2	Traditional cultivar/Landrace	NA	*A. hypochondriacus L.*
VI061504	57	10.18730/135K1P	Traditional cultivar/Landrace	Cameroon	*A. cruentus L.*
VI061505	58	10.18730/135K2Q	Traditional cultivar/Landrace	NA	*A. cruentus L.*
VI061511-A	59	10.18730/134M8U	Traditional cultivar/Landrace	Malawi	*A. blitum L.*
VI061517	60	10.18730/135JZM	Traditional cultivar/Landrace	Tanzania	*A. cruentus L.*
VI062425	61	10.18730/13680V	Traditional cultivar/Landrace	Madagascar	*A. hypochondriacus L.*
VI062427	62	10.18730/13681W	Traditional cultivar/Landrace	Tanzania	*A. hypochondriacus L.*
VI064070	63	10.18730/1387DN	Traditional cultivar/Landrace	Tanzania	*A. hypochondriacus L.*
VI064071	64	10.18730/1387EP	Traditional cultivar/Landrace	Tanzania	*A. hypochondriacus L.*
VI064076	65	10.18730/138RPK	Traditional cultivar/Landrace	NA	*A. cruentus L.*
VI064080	66	10.18730/13878G	Traditional cultivar/Landrace	Madagascar	*A. hypochondriacus L.*
VI064527	67	10.18730/138RQM	Traditional cultivar/Landrace	Tanzania	*A. cruentus L.*

Three accessions (“Azhurnyy”, “Opopeo”, and “Orange”) of *A. cruentus* were used for the field studies only.

7.1 Greenhouse (Experiment1)

7.1.1 Growing seedlings

The study was started 08.04.2024 by planting the seeds in the seed tray to ensure uniform germination. Seed trays were 3 cm x 3 cm x 3 cm. wide, deep and long. The seed trays were filled with peat from Kekkilä professional growing medium with VHM 620 and pH 6.0 (Kekkilä Oy, Vantaa, Finland). Approximately three seeds were sown per cell in five cells per accession, because the germination percentage was unknown (Figure 1).

Figure 1. Seedlings growing in seed trays (Photo: Ilja Koli, 17.04.2024).

The seed trays were kept in the greenhouse at temperatures of 22 °C day/18 °C night ± 1 °C and a photosynthetic photon flux density (PPFD) of approximately 300 µmol m^{-2} s^{-1}. The photoperiod was 14 h light and 10 h dark. The plants were watered every day

until the seedlings started to grow. On 19.04.2024, the seedlings were transplanted to 10 L pots.

7.1.2 Preparation of pods

10 L white-colored pots were used. We used Kekkilä professional growing medium with VHM 620 at a pH 6.0 peat was used to fill the pots, as for all treatments. To ensure the homogeneity of the peat and equal growth conditions, substrate from five 50 L bags of Kekkilä was mixed to fill the pots. The peat was compacted in the pot by light pressure with the pot's circumferential, flat-bottomed lid.

The experimental design was a randomized complete block design (RCBD) with three replicates. Each replication consisted of a single plant from each accession, with the most vigorous seedling selected. The transplanting date was 19.04.2024.

7.1.3 Photoperiod and temperature

The photoperiod was 14 h light and 10 h dark. From 19.04.2024 to 15.05.2024, the greenhouse temperature was maintained at 22.0 ± 1°C during the day and 18 ± 1°C at night. Starting on 16.05.2024, the warm season commenced and continued throughout the summer. 16.05.2024 to 30.08.2024, average daytime temperatures were about 27.4 ± 2.5°C during the day, while the night temperature was about 18.7 ± 0.9°C.

7.1.4 Irrigation and fertilizer

A drip irrigation system was used to apply a consistent water quantity. The amount of watering was 2 dl per pot twice a day at 08:00 and 12:00. On 24.06.2024, watering was increased by 2 dl per day so that the plants would get enough water, resulting in an irrigation amount of 3 dl twice a day.

Fertilization was performed using Kekkilä Professional Suprex NPK fertilizer (Kekkilä Oy, Vantaa, Finland), which was applied separately from the regular watering by

mixing it with 1 dl of water per pot. Fertilization occurred on the following dates: 05.06.2024, 10.06.2024, 24.06.2024, 28.06.2024, and 15.07.2024. Each pot received 3 g of fertilizer every time, all together 15 g. The fertilizer contains 10% nitrogen.

7.1.5 Measurements in greenhouse (experiment 1)

The measurement included morphological, physiological and phenological characterization to assess differences between species and accessions. All measurements of leaves were done at the two fully opened leaflets at the top of the plants. Tools and measurements are listed in Table 2.

Measurements started on 19.05.2024 by quantifying chlorophyll, anthocyanin, flavonoid, and nitrogen in the leaves. The measurements were performed three times in five-days intervals on 19.05.2024, 24.05.2024, and 29.05.2024.

Stomatal conductivity stomata was measured using a porometer and by determining the leaf temperature. Stomatal conductivity was measured twice (04–06.06.2024 and 10–13.06.2024), and each time two reads per fully developed leaf were used. Leaf temperature was measured also from fully developed leaves (17.06.2024).

The flowering of the plants was monitored at least twice a week to visually record the heading and the beginning of flowering in days after transplanting. Observations were carried out throughout the growing season.

Pictures were taken from leaves with a cell phone camera on 20.06.2024. The pictures were taken from a height of 30 cm above the surface, so that the distance to all leaves was the same. A 13.7 cm pencil was placed next to the leaves to help illustrate the size of the leaves.

Leaf morphological measurements including area, length, and width were taken on 27.06.2024. Leaf length and width were measured with a ruler from three leaves per accession, and their area was measured using a Leaf Area Meter.

The height of the plants was measured using a measuring tape on 08.08.2024. The color of the stem, seeds, and flowers was observed before the collection of the seeds

and the disposal of the plants, from which the differences among accessions were noted.

Table 2. Equipment used in the greenhouse experiment.

Measurements	**Instrument used**	**Note**
CHL, FLAV, ANTH & NBI	The DUALEX® SCIENTIFIC	From two fully expanded top leaflets
Stomatal conductance	Leaf Porometer METER Group; SC-1 Leaf Porometer, USA	From two fully expanded top leaflets
Leaf temperature	Infrared thermometer; IRT, FLUKE® thermometer gun 574, Everett, WA, USA	From two fully expanded top leaflets
Plant height	Measuring tape	Of all accessions, in every replica
Leaf area	Leaf area meter; LI-3000A, with transparent belt conveyor, S/N: PAM 1703, Germany	From two fully expanded top leaflets
Leaf length	Ruler	From two fully expanded top leaflets
Leaf width	Ruler	From two fully expanded top leaflets
Flowering time	Visual inspection	From the tips of the stem and side shoots.

7.2 Field experiments (Experiment 2 & 3)

Two field experiments were conducted at the Viikki Experimental Farm, University of Helsinki, in Southern Finland (60°13'31.5"N, 25°01'13.3"E) during the summer of 2024. Field Experiment 2 began on 30.05.2024, and field experiment 3 began on 24.05.2024. Field experiment 2 consisted of 67 amaranth accessions received from the WorldVeg along with cvs. Azhurnyy, Opopeo, and Orange. For this experiment, due to limited seed availability, the seedlings were germinated in the greenhouse and transplanted in the field. In the field experiment 3, only the three amaranth accessions Azhurnyy, Opopeo, and Orange were planted by direct seeding in the field.

The soil at the experimental site is classified as fine sandy soil and is rich in organic material. The soil is acidic with pH=5.7. Other notable soil characteristics include the following mineral concentrations: lead (Pb) 0.9 mg/kg, calcium (Ca) 1800 mg/kg,

phosphorus (P) 17 mg/kg, potassium (K) 360 mg/kg, magnesium (Mg) 180 mg/kg, sulfur (S) 18 mg/kg, copper (Cu) 33 mg/kg, manganese (Mn) 9.7 mg/kg, and zinc (Zn) 11 mg/kg.

Because the pH of the soil was low (5.7), liming was performed to provide better growing conditions. The liming was carried out late on 10.06.2024, i.e. after transplanting. For this reason, approximately 8000 kg/ha was used by giving each row the same amount of lime mixed with water.

7.3 Field (Experiment 2)

7.3.1 Growing seedlings

The study was started by planting the seeds in 10 holes per plant in seed trays on 08.05.2024 (Figure S1). The Kekkilä professional growing medium with VHM 620 and pH 6.0 peat was used to fill the trays, as for the seedlings for the greenhouse experiment. The seed trays were kept in the greenhouse under the same growing conditions explained in Section 7.1.1. On 30.05.2024, the seedlings were transplanted to the field.

7.3.2 Transplanting the seedlings

An area of 100 square meters was reserved from the field for the experiment. Rows with a length of 1.5 m separated by 0.5m between rows and 75 cm per column were arranged as shown in Figure 2. Seedlings were planted approximately 5 cm deep at 15 cm intervals, ten plants per row.

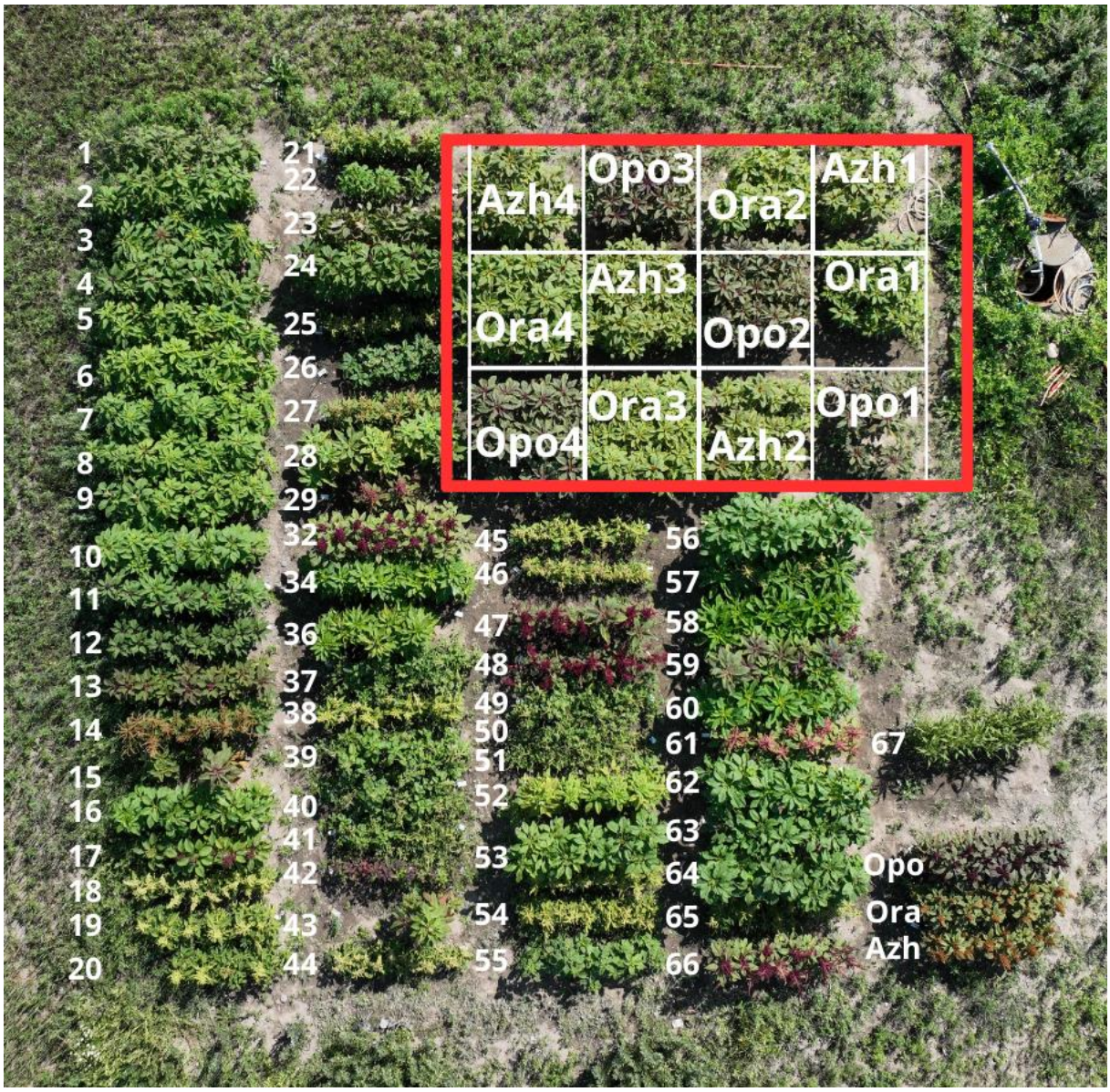

Figure 2. Overhead view of field experiments 2 and 3. Number 1-67 refers to the accessions listed in Table 1. The three cultivars are labeled with abbreviations (Opo=Opopeo, Ora=Orange, and Azh=Azhurnyy). The red area is the field experiment 2 (Photo: drone, taken by Iiro Miettinen, 09.08.2024).

7.3.3 The photoperiod and temperature

The photoperiod was over 15.5 h light and less than 8.5 h dark from May to beginning of August, with a maximum on 21 June 19.5 h light and the minimum of 4.5 h dark. The photoperiod was less than 12 h of light after 21 of September. Temperature and precipitation are listed in Table 3.

Table 3. Weather statistics during the trial period (Experiment 2).

month	Precipitation (mm)	Average temperature (°C)	Minimum temperature	Maximum temperature
May	2	17.0	4.9	26.8
June	50	17.2	6.0	27.2
July	74	18.9	10.0	26.0
August	148	17.9	8.0	26.6
September	259	14.7	-0.2	25.7

7.3.4 Irrigation and fertilizer

Amaranth seedlings were watered every day for a week, keeping the soil moist to ensure seedling establishment. After this, no more irrigation was provided, and the plants were grown under rain-fed conditions.
Fertilization was performed using Kekkilä Professional Suprex NPK fertilizer (Kekkilä Oy, Vantaa, Finland), which was applied to the plants by mixing it with water on the following dates: 12.06.2024, 25.06.2024, and 03.08.2024. The plants received 30 kg/Ha of nitrogen every time. The fertilizer contained 10% nitrogen.

7.3.5 Measurements in field (experiment 2)

The study used the same measurements as in the greenhouse experiment. In addition, field measurements were also done with a DJI Mavic M3 multispectrum drone to get more data (09.08.2024). All measurements from leaves were done on the top fully opened leaflets of five plants. Tools and measurements used are listed in Table 4.

Measurements were made in the field in the same way as in the greenhouse including chlorophyll, anthocyanin, flavonoid and nitrogen activity were measured four times, the first time with MPM-100 and then three times with Dualex. The Dualex was the main measuring device, so that the results could be compared to the greenhouse plants. Due to the fact that Dualex was not available at the optimal time, the first measurement was made with the MPM-100 for the plants at the same relative to time of sowing the

seeds in the greenhouse plants. This ensured that greenhouse and field trial data can be compared in the future. Measuring dates were 24.07.2024, 30.07.2024, 02.08.2024, and 03.08.2024.

Table 4. Equipment used in field experiments.

Measurements	**Instrument used**	**Note**
CHL, FLAV, ANTH & NBI	The DUALEX® SCIENTIFIC	From two fully expanded top leaflets
CHL, FLAV, ANTH & NFI	MPM-100 Multi-Pigment-Meter	From two fully expanded top leaflets
NDVI	DJI Mavic M3 multispectrum drone	4 multispectral sensors (G, R, RE, and NIR)
GNDVI	DJI Mavic M3 multispectrum drone	4 multispectral sensors (G, R, RE, and NIR)
NDRE	DJI Mavic M3 multispectrum drone	4 multispectral sensors (G, R, RE, and NIR)
NDAI	DJI Mavic M3 multispectrum drone	4 multispectral sensors (G, R, RE, and NIR)
Height	DJI Mavic M3 multispectrum drone	20mp RGB camera

7.4 Field (experiment 3)

On 24.05.2024 a direct seed sowing trial was conducted with three amaranth accessions *A*zhurny, Opopeo and Orange. The experimental design was a RCBD with four replicated plots. Each plot was 1m^2 and contained three 1 m long rows of plants. Planting was done in 5 cm depth, and fine sand was added to protect the seeds. Seeds were sown abundantly to compensate for the unknown survival rate. Each plot was separated by 30 cm to ensure clear distinction (Figure 2).

7.4.1 Irrigation and fertilizer

Amaranth plots were irrigated for two weeks manually to ensure germination and provide seedlings with a strong start. No supplementary irrigation was provided later on.

Fertilization was performed using Kekkilä Professional Suprex NPK fertilizer (Kekkilä Oy, Vantaa, Finland), which was applied to the plants by mixing it with water on the same dates as field experiment 2: 12.06.2024, 25.06.2024, and 03.08.2024. The

fertilizer contained 10% nitrogen, and the plants received 30 kg/Ha of nitrogen every time.

7.4.2 Measurements in field (experiment 3)

The study used the same measurements as in the field experiment 2 and tools and measurements used are listed in Table 4.
In field experiment 3, chlorophyll, anthocyanin, flavonoid, and nitrogen measurements were done on 24.07.2024 with MPM-100, and on 02.08.2024 and 03.08.2024 with Dualex.

7.5 Statistical analysis

The analyses were performed using R software (Version 4.3.3). The analyses performed were one-way analysis of variance (ANOVA) and Tukey-test for experiments 1 and 3. In the ANOVA test, the explanatory factors were species and cultivar. The analysis examined the main effects of these factors and their possible interaction effects on the dependent variables. Tukey test was performed to further compare differences between species to refine significant differences. The t-test was used to compare the field experiment and the greenhouse experiment by species. Experiment 2 (field data) was analysed using an augmented block design.

8 Results

8.1 Greenhouse (Experiment 1)

8.1.1 Physiological properties of leaves

Under greenhouse conditions, significant differences were observed for species and accessions for the ANTH, CHL, FLAV, and NBI values. Stomatal conductance measurements revealed only little differences between genotypes. According to the ANOVA results (Tables S1 & S2), leaf width and leaf temperature were the only leaf morphology measurements that did not show significant differences between species and accessions.

Anthocyanin content

A. blitum had the highest anthocyanin content and the widest variation among accessions. Interestingly, *A. blitum* was the only species that showed significant variation between accessions in anthocyanin content compared to all other species. In contrast, *A. dubius* and *A. thunbergii* had distinctly lower concentrations (Figure 3), but no significant differences were found between these species in the Tukey test. The results are probably explained by the differences of the *A. blitum* accession between green and purple leaves, while the dubius only had green leaves. Overall, the average anthocyanin content across all species was similar. When excluding *A. blitum*, intra-species variation in anthocyanin content was relatively small.

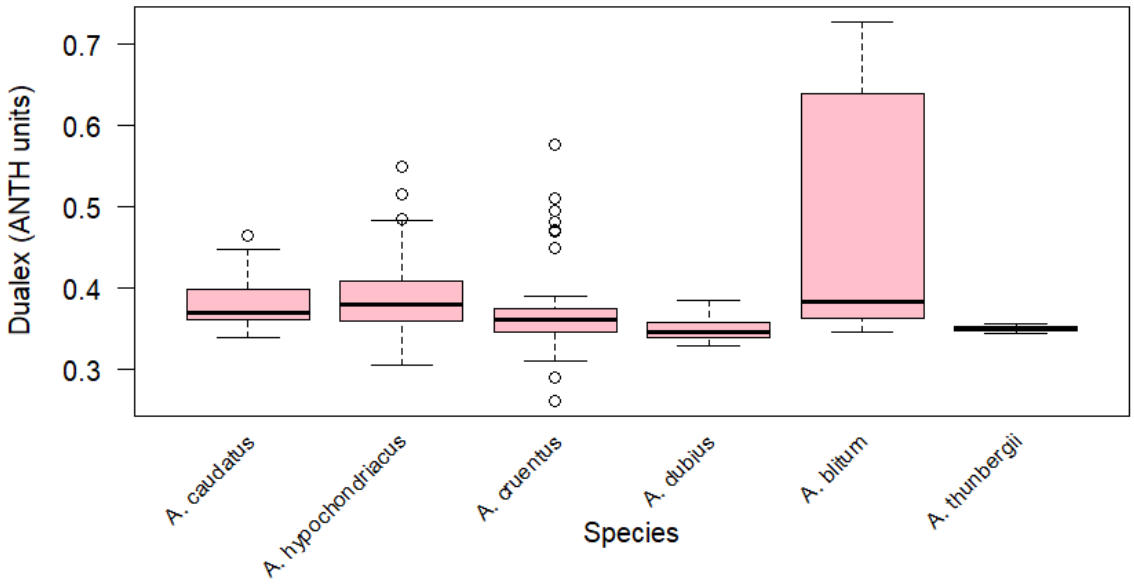

Figure 3. Average of anthocyanin measurements taken at the end of May 2024. The graphs show the differences between species and the range of variation within each species across different accessions. Data from greenhouse (Experiment 1).

Chlorophyll content

The highest mean chlorophyll content was found in *A. dubius*, while the lowest is in *A. blitum* and *A. hypochondriacus*, and differences were observed between all species, as shown in Figure 4. The most significant differences are between *A. dubius* and the other species P value 0.03. A significant difference was also observed between *A. hypochondriacus* and A. cruentus, alongside *A. dubius*.

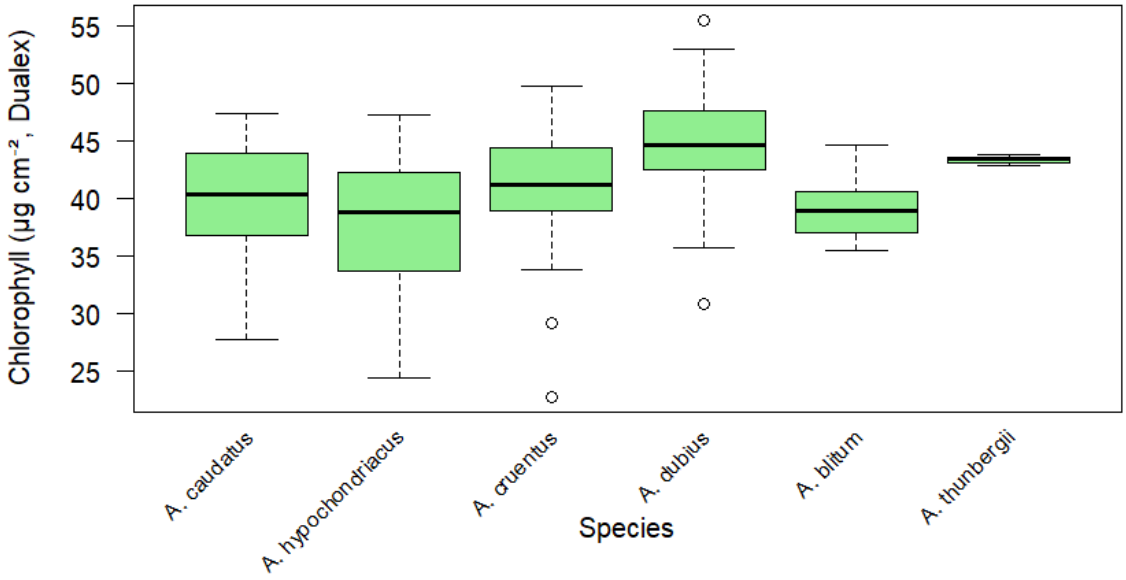

Figure 4. Average of chlorophyll measurements. The graph shows the differences between species and the range of variation within each species across different accessions. Data from greenhouse (Experiment 1).

Flavonoid content

A. thunbergii had the highest flavonoid content. *A. hypochondriacus* and *A. caudatus* showed very similar flavonoid contents. Despite that, only *A.* hypochondriacus was significantly different from *A. cruentus* among these two species (Figure 5).

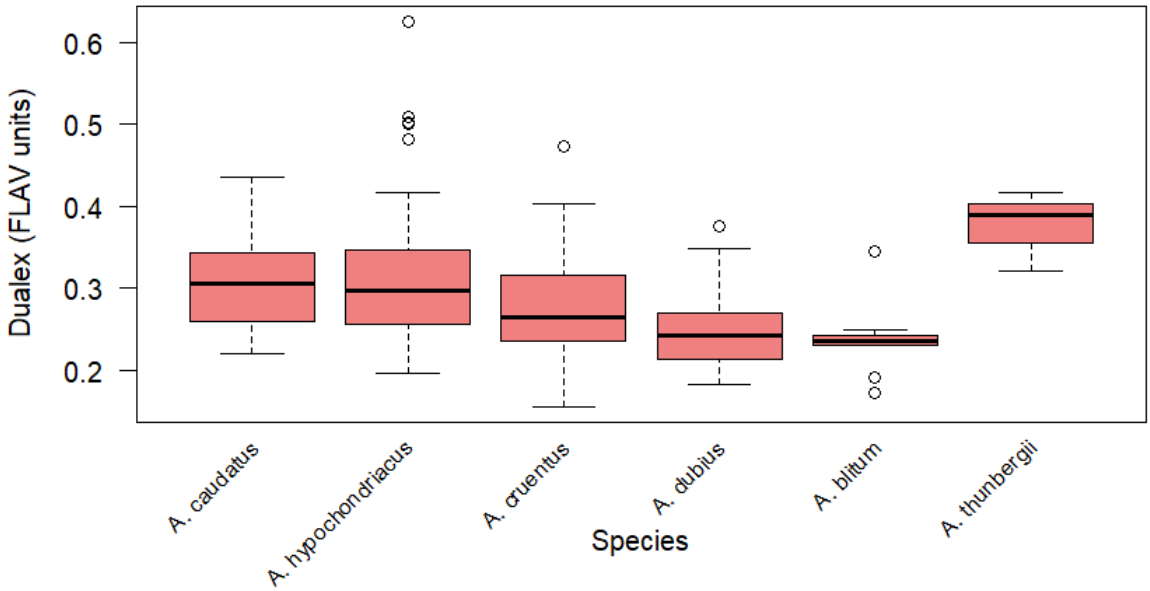

Figure 5. Average of flavonoid (FLAV) measurements. The graph shows the differences between species and the range of variation within each species across different accessions. Data from greenhouse (Experiment 1).

NBI nitrogen value

Nitrogen content in leaves showed a similar trend like chlorophyll content. *A. dubius* has the highest nitrogen content in leaves and *A. thunbergii* with *A. caudatus* and *A. hypochodriacus* the lowest nitrogen content (Figure 6) and the differences are significant. Also *A. cruentus* and *A. blitum* showed significant differences with other species.

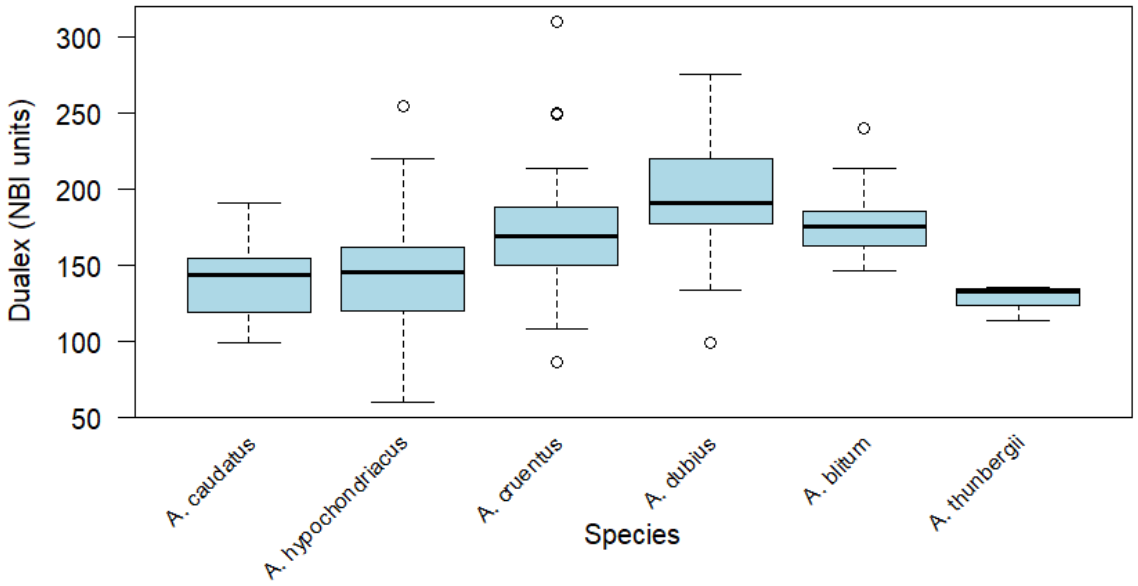

Figure 6. Average of Nitrogen Balance Index (NBI) measurements. The graph shows the differences between species and the range of variation within each species across different accessions. Data from greenhouse (Experiment 1).

stomatal conductance (g_s)

There is a clear difference in stomatal conductance between *A. caudatus* and the other species (Figure 7). *A. caudatus* had higher values and was the only species with large variation among accessions. All the other studied species had similar g_s without significant differences.

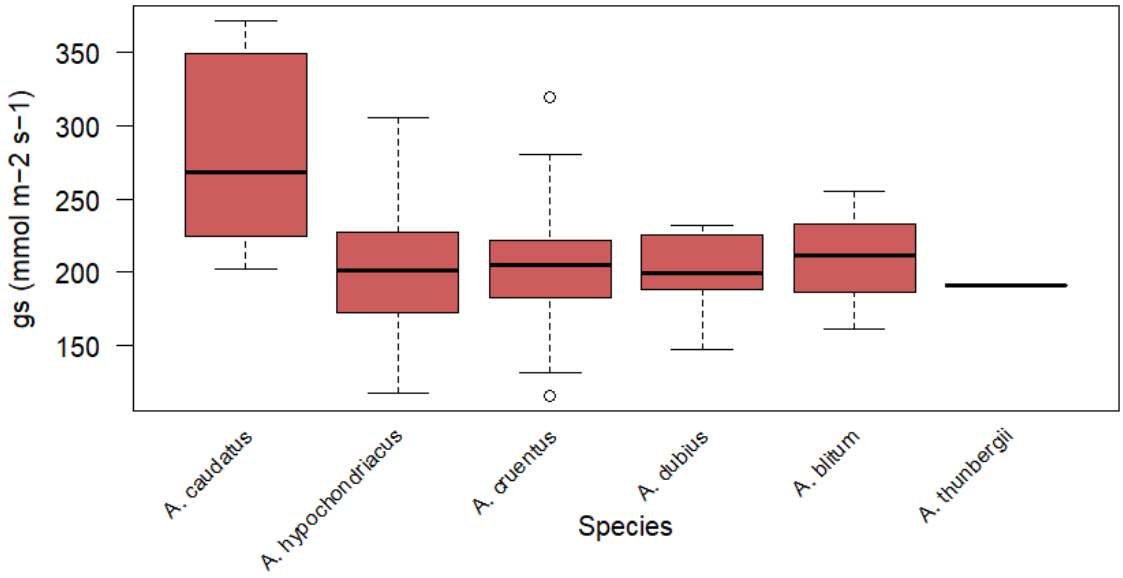

Figure 7. Average stomatal conductance (g_s). The graph shows the differences in gs between species and the range of variation within each species across different accessions. Data from greenhouse (Experiment 1).

8.1.2 Leaf size

A.hypochondriacus (168 cm^2) had larger leaves than *A. dubius* (126 cm^2). Their Tukey test revealed that the difference was 45 cm^2, lower 14 cm^2 and upper 77 cm^2 (Figure 8). Leaf size also correlates with Flavonoid (Figure 5).

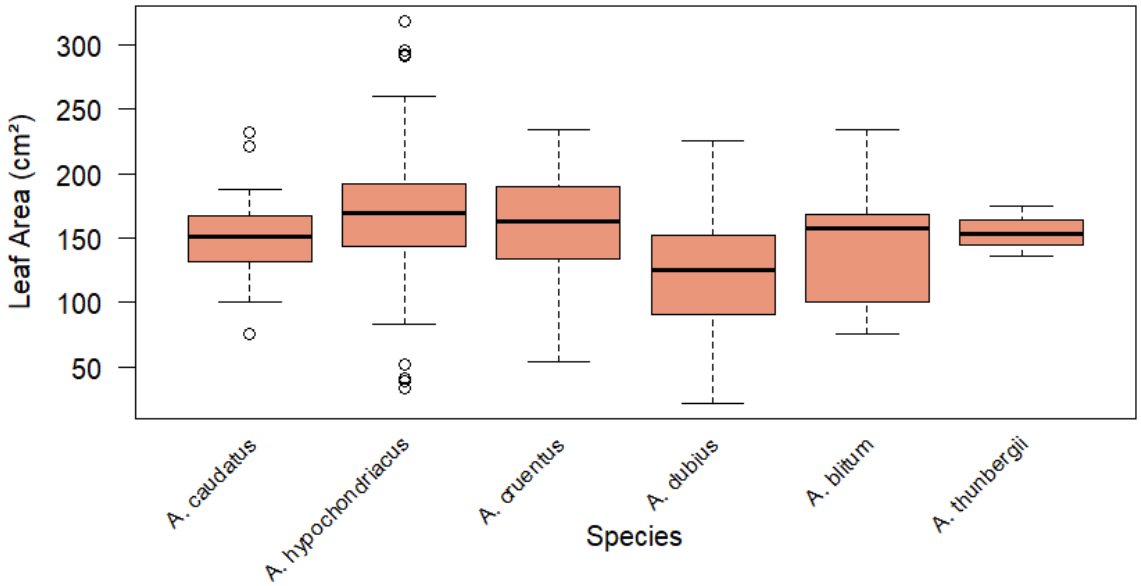

Figure 8. Mean of leaf area measurements taken 49 days after sowing. The graphs show the differences between species and the range of variation within each species across different accessions. Data from greenhouse (Experiment 1).

Leaf length is strongly correlated with leaf area (Figures 8 & 9), but *A. dubius* leaves are clearly shorter than those of other species, although there is a large variation of leaf lengths (Figure 9). Statistically significant differences in between *A. dubius* and species like *A. cruentus*, *A. hypochodriacus* and *A. caudatus* were found. Also *A. thunbergii* had significantly longer leaves than *A. dubius*, but because there was only a single *A. thunbergia* accession part of the experiment, the Tukey test P value remained insignificant (P-value = 0.073).

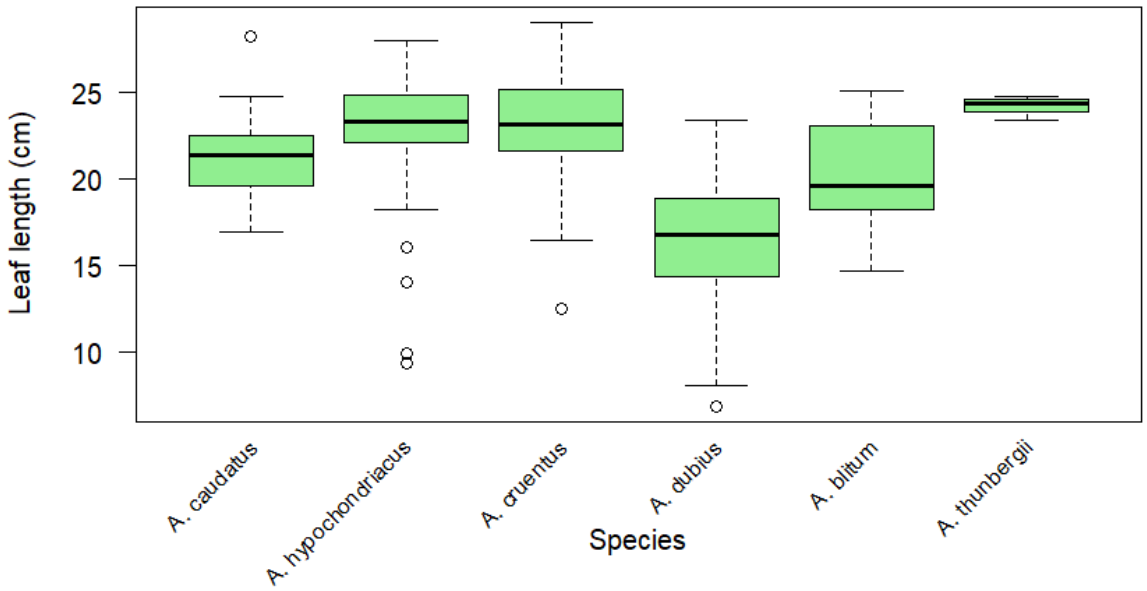

Figure 9. Mean of leaf length measurements taken 49 days after sowing. The graphs show the differences in leaf length between species and the range of variation within each species across different accessions. Data from greenhouse (Experiment 1).

There are no statistically significant differences in leaf width. *A. thunbergii* on average had the narrowest leaves, but its small sample size (only one accession was used) is

not sufficient to get significant results. Also the differences between the other species remained insignificant due to the large intraspecies variation (Figure 10).

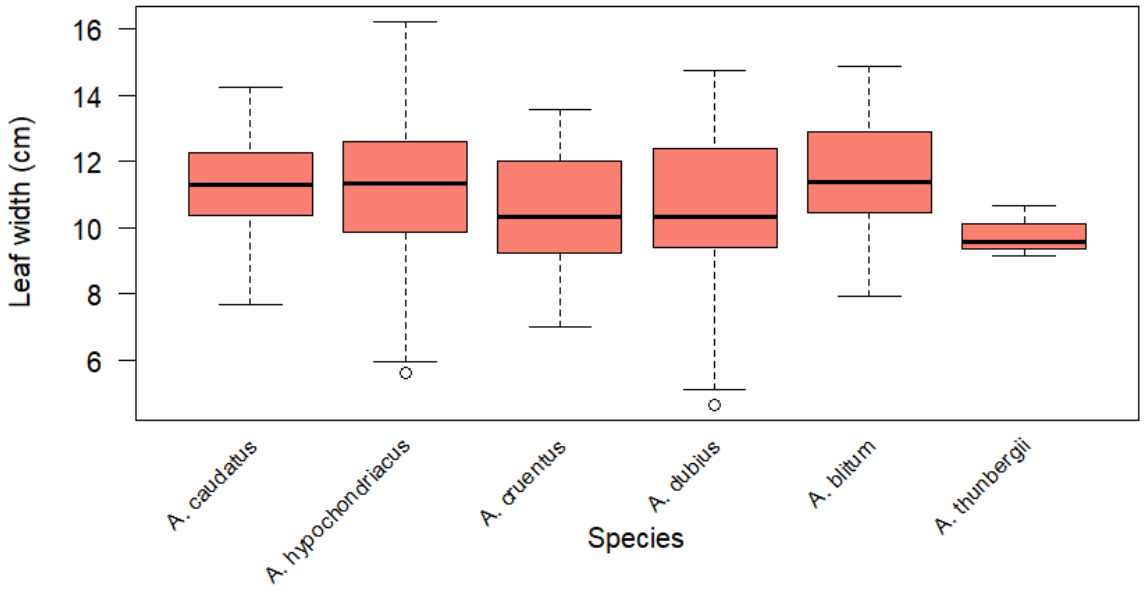

Figure 10. Leaf width (LW) measurements taken on 27.05.2024, 49 days after seed sowing on 08.04.2024, based on three leaves per species. The graphs show the differences in leaf width between species and the range of variation within each species across different accessions. Data from greenhouse (Experiment 1).

8.1.3 Plant height

Plant height varied greatly between species and accessions, but clear height differences were noticeable between some species.

A. caudatus and *A. blitum* were clearly the tallest species, *with A. blitum* having a mean plant height of 219 cm and *A. caudatus* 207 cm. These two species did not differ significantly from each other. However, *A. blitum* had a larger range of plant height, while *A. caudatus* accessions were all high, as shown in figure 11. The longest accession was *A. blitum* accession VI061511-A, with a length of 310 cm. The shortest *A. blitum* was accession 42, with a length of 137 cm. So, the length variation is up to 173 cm within *A. blitum*.

The shortest species turned out to be *A. dubius* (123 cm) and *A. thunbergii* (117 cm). However, there was only one accession of *A. thunbergii* in the study, so the differences between accessions within the species are unknown. The shortest accession belonged to *A. dubius,* accession VI058963, with an average length of 77 cm. However, the size difference of the single *A. thun ii* accession with *A. caudatus* and *A. blitum* was so large that it was significant. With the other species this difference remained insignificant. The difference between *A. cruentus* and *A. hypochodriacus* also remained insignificant.

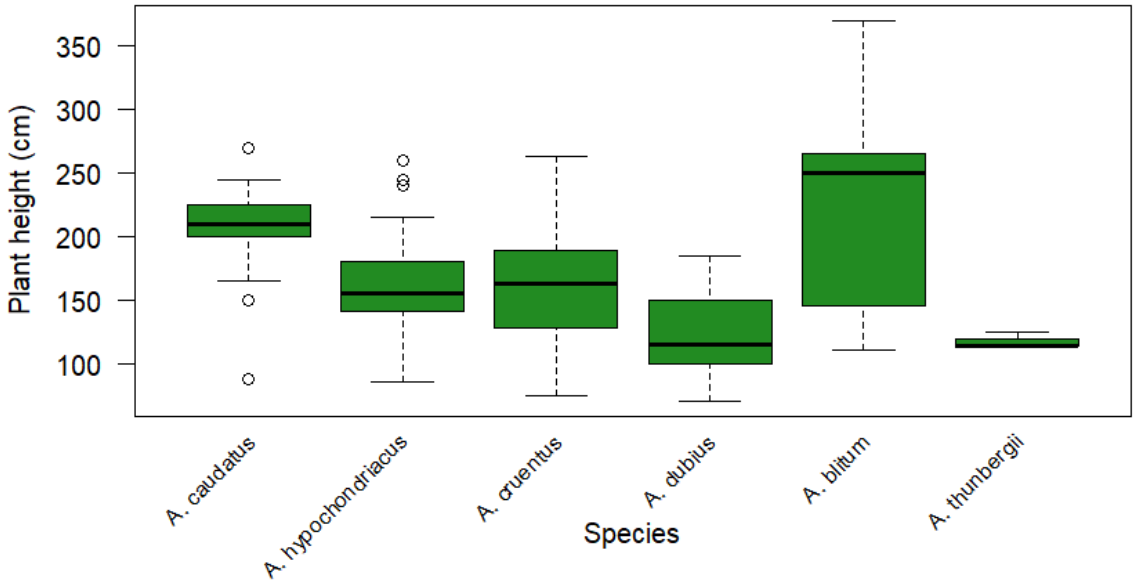

Figure 11. Plant height measurements taken 123 days after sowing. The graphs show the differences in plant height between species and the range of variation within each species across different accessions. Data from greenhouse (Experiment 1).

8.1.4 Number of tillers

Tiller numbers are notably higher in *A. dubius* and *A. thunbergii* compared to other species. In Figure 12 (Tiller Numbers), an inverse trend is observed compared to Figure 11 (Plant Height). Most accessions of grain amaranths (*A. caudatus*, *A. cruentus*, and *A. hypochondriacus*) and *A. blitum* exhibit low tiller numbers. Notably, *A. blitum* had only three accessions: one with 12–14 tillers and two with no tillers at all.

A. dubius showed a significant difference in tiller numbers compared to all low-tiller species. Among grain amaranths, the highest and lowest tiller counts in *A. caudatus* and *A. cruentus* were significantly different.

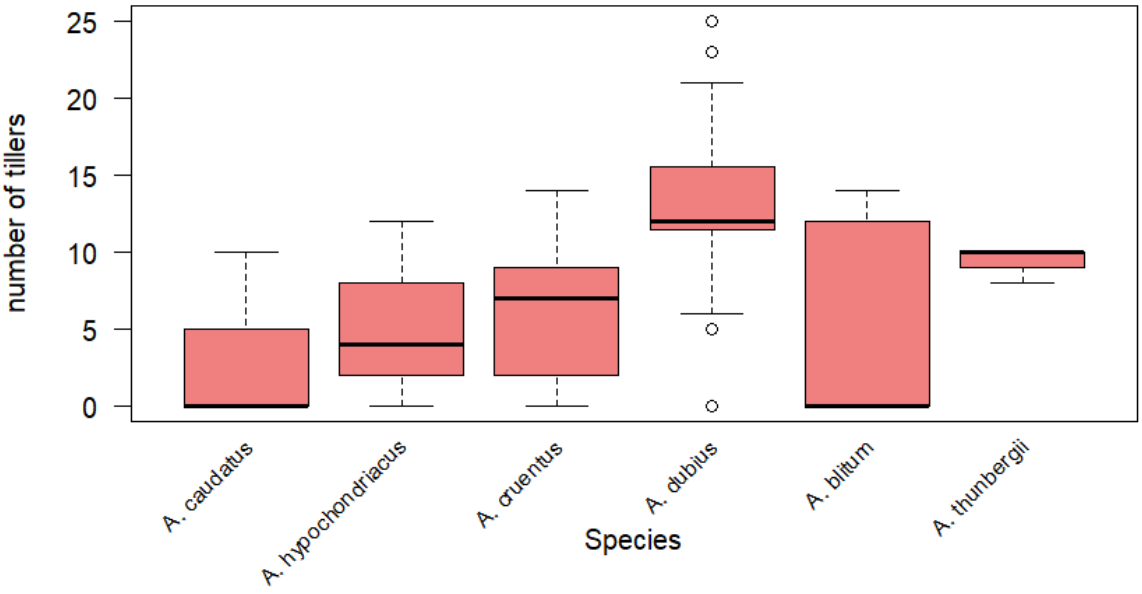

Figure 12. Tillers measurements taken 112 days after sowing. The graphs show the differences in tiller count between species and the range of variation within each species across different accessions. Data from greenhouse (Experiment 1).

8.1.5 Heading and flowering times

Comparing the heading and flowering times of amaranths, it is noticeable that when comparing grain (*A. caudatus*, *A. cruentus*, and *A. hypochondriacus*) and vegetable (*A. blitum*, *A. dubius*, and *A. thunbergii*) amaranth species they proceed consistently in the same order from heading to flowering (Figures 13 & 14). Early and late species can be observed in terms of flowering. The flowering time of grain amaranths is later than that of vegetable amaranths (Figure 14).

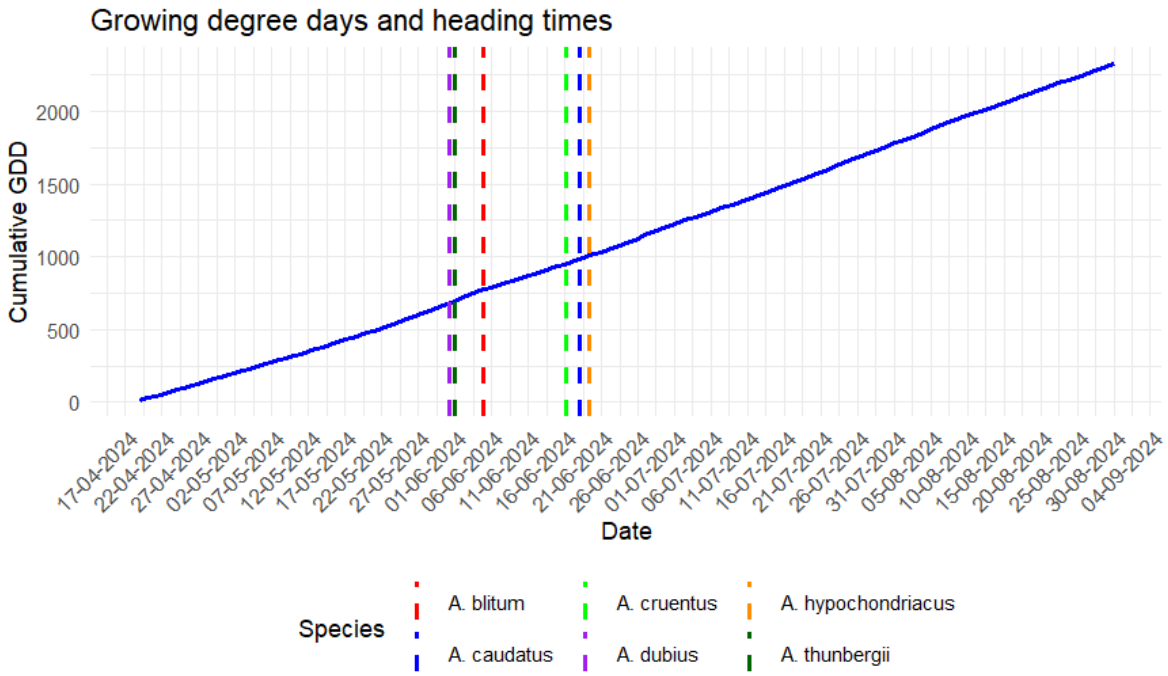

Figure 13. Growing Degree Days (GDD) progression with species-specific heading time averages indicated by colored dashed lines. The graph shows the cumulative heat sum (GDD) over time and the corresponding average heading dates for different species, highlighting the variation in heading time across species under the observed conditions. Data from greenhouse (Experiment 1).

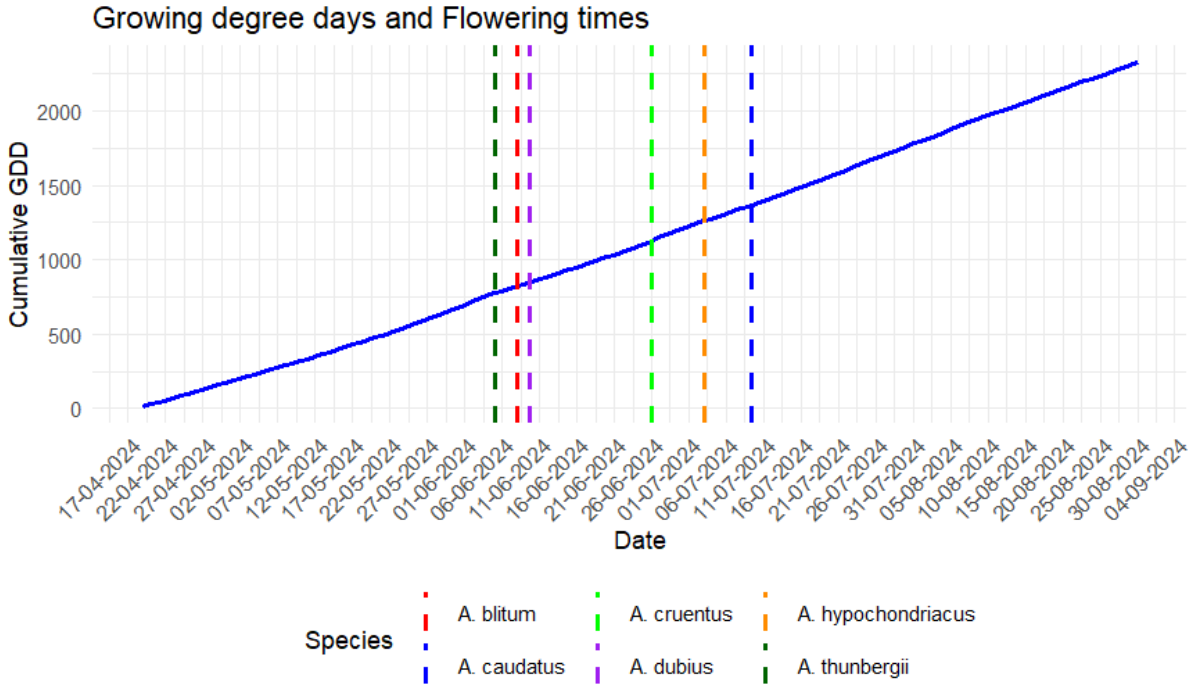

Figure 14. Growing Degree Days (GDD) progression with species-specific flowering time averages indicated by colored dashed lines. The graph shows the cumulative heat sum (GDD) over time and the corresponding average flowering dates for different species, highlighting the variation in flowering time across species under the observed conditions. Data from greenhouse (Experiment 1).

Figure 15 shows that *A. blitum* and *A. caudatus* did not produce seeds well under greenhouse conditions in long day conditions. Most of the other species accessions' produced seeds. In *A. dubius* and *A. thunbergii* species all accessions produced seeds.

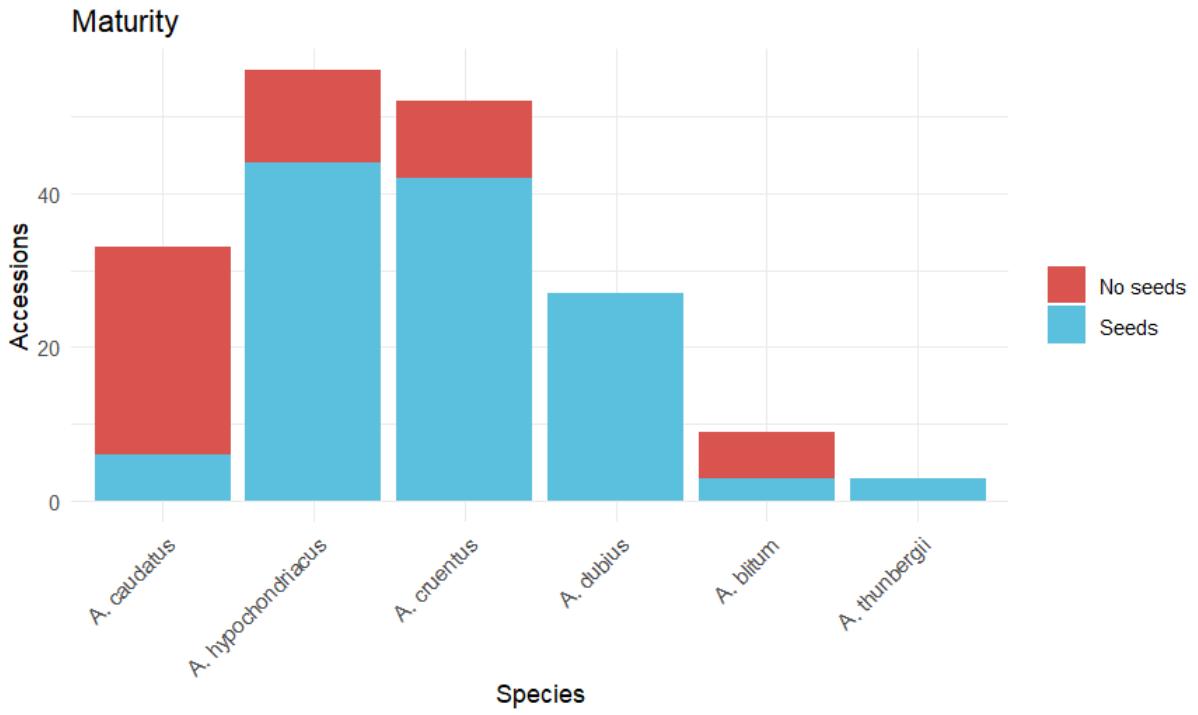

Figure 15. Maturity distribution of accessions as a stacked bar chart. "No seeds" refers to those accessions that did not produce any seeds (red). "Seeds" refers to those accessions that went to seed production stage (blue). The graph illustrates how many accessions were available for each species and the balance between mature and non-mature plants. Data from greenhouse (Experiment 1).

8.1.6 Principal Component Analysis

Based on PCA analysis, no specific clustering for species was observed. There was an association between days to flowering and stomatal conductance. It can also be seen that *A. caudatus* showed less variation for some physiological traits. In other species, the diversity is really wide (Figure 16).

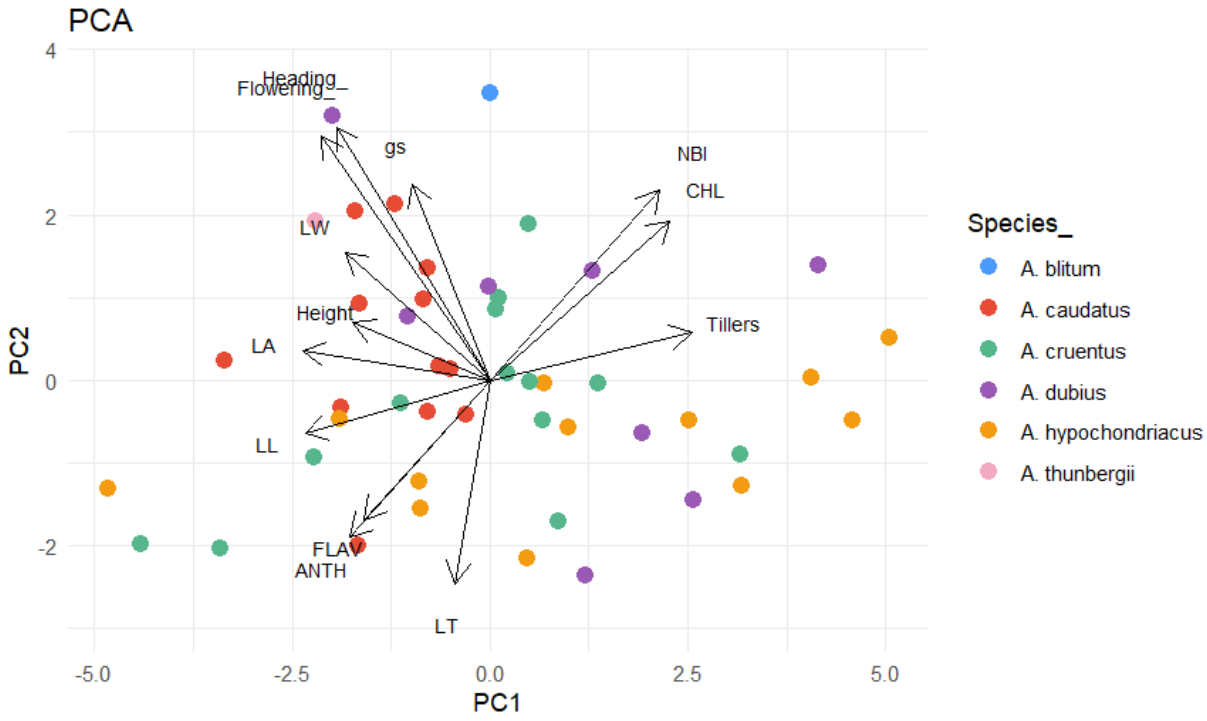

Figure 16. Principal component analysis (PCA) plot showing species differentiation based on various variables. Each species is represented by a different color, and the plot highlights the correlations between these variables, illustrating the similarities and differences between species. Data from greenhouse (Experiment 1).

8.1.7 Morphological observations

Amaranth accessions showed diversity in stem and inflorescence color, leaf shape, and seed color. There was often a species-specific uniformity in stem color, which was often consistent with the inflorescence. Green stems typically had a yellow

inflorescence, and purple / pink ones had variably purple or pink inflorescences. This can be seen in Table 5.

Table 5. shows the variation in stem, inflorescence, and seed colors across *Amaranthus* species, summarizing the most common pigmentation observed within each species. Data from greenhouse (Experiment 1).

Species	**Stem color**	**Inflorescence color**	**Seed color**
A. blitum	Purple/Pink	Purple/Pink	Black
A. caudatus	Green/Purple/Green; Purple	Yellow/Purple	Yellow/Cream/Pale
A. cruentus	Green	Yellow	Black
A. dubius	Green/Green; Brown	Yellow	Black
A. hypochondriacus	Green/Purple	Yellow/Purple	Pale/Orange/Black
A. thunbergii	Green	Yellow	Black

The leaf shape was unique for almost every accession. Typically, however, either ovate, deltoid-ovate, deltoid, rhomboidal-ovate or elliptic (Figure S2). *A. dubius* turned out to be a deltoid-leaved species (Figure 17). There was particularly much variation between species between *A. cruentus*, *A. hypochodriacus*, *A. dubius* and *A. blitum* and intraspecific variation within *A. hypochodriacus* (Figure 18). The picture also shows differences in colour and shade between the leaves.

Figure 17. Collage of leaves from four accessions of *A. dubius*, highlighting their deltoid shape.

Figure 18. illustrates the significant variation in amaranth leaf morphology, even within individual species. The accessions included in the figure are as follows: VI054579 and VI064527 (*A. cruentus*), VI048583, VI050449, VI064080, and VI050128 (*A. hypochondriacus*), VI050460 (*A. dubius*), and VI061511-A (*A. blitum*).

The colors of the seeds were very diverse (Figure 19) (Table 5). From grain amaranth seeds *A. caudatus* had pale (cream, yellow orange) colored seeds and from *A. hypochondriacus* seeds half was black. While *A. cruentus* was black seeded with one pale accession. The vegetable amaranths (*A. blitum*, *A. dubius*, and *A. thunbergii*)

were almost all black or sometimes black mixed with brown seed accessions. Only one accession of *A. dubius* was pale seeded. Based on visual inspection, the pale seeds were bigger than the black seeds.

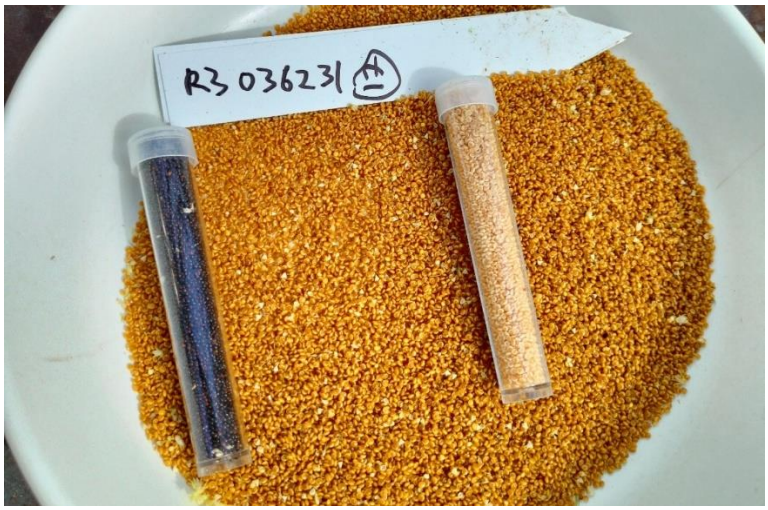

Figure 19. The picture shows three different amaranth seeds and their range of colors.

8.2 Field Experiment (Experiment 2)

8.2.1 The physiological properties of the leaves

The NBI/NFI, CHL, FLAV, and ANTH levels measured with MPM-100 (Figure 20) were very similar to those measured with Dualex. Only the ANTH levels between *A. blitum* and the other species differed greatly.

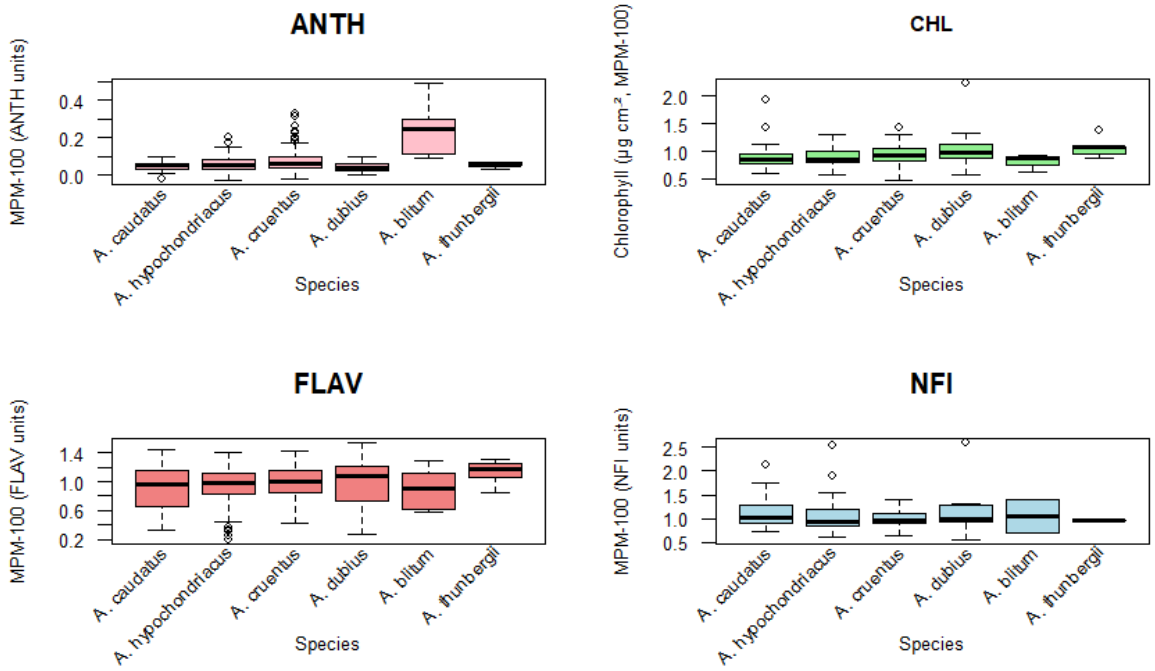

Figure 20. Average of anthocyanin (ANTH), chlorophyll (CHL), flavonoid (FLAV), Nitrogen-Flavonol Index (NFI) measurements. The graphs show little differences between species and the range of variation within each species across different accessions. Data from field (Experiment 2).

Chlorophyll content was similar in the outdoor experiment as in the greenhouse, but there is a greater variation between species (Figure 21). There were clear differences in *A. dubius* and *A. cruentus* compared to lower values. AugmentedRCBD (Table S5) also shows no significant differences between accessions.

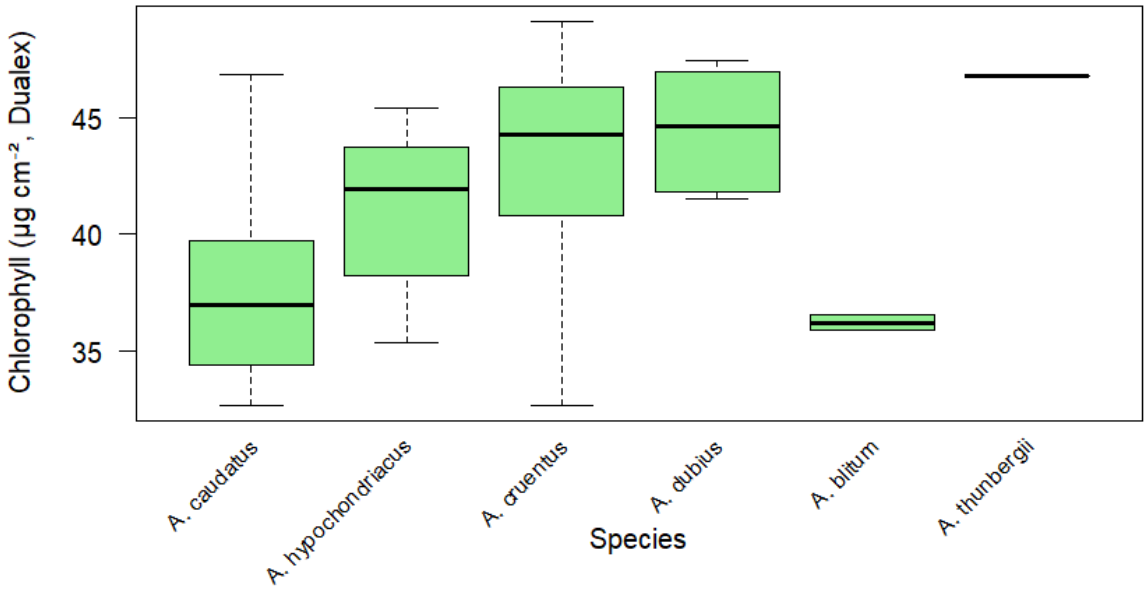

Figure 21. Average of chlorophyll measurements taken in the field between 27.05.2024 and 30.05.2024. The graph shows the differences between species and the variation range within each species across different accessions. Data from Field (Experiment 2).

The anthocyanin values of the plants grown in the field the biggest difference was with *A. blitum* that had the highest average values in the field (Figure 22). AugmentedRCBD (Table S5) also shows significant differences between accessions.

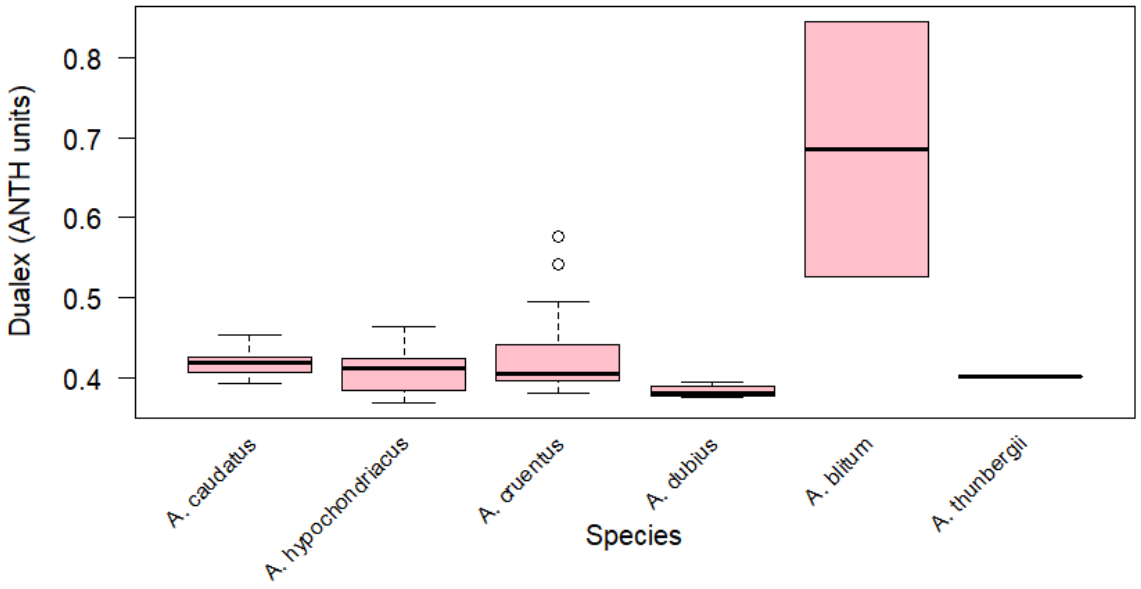

Figure 22. Average of anthocyanin measurements taken in the field between

30.07.2024 and 03.08.2024. The graph shows the differences between species and the variation range within each species across different Accessions. Data from field experiments (Experiment 2).

Flavonoid values are higher outdoors compared to indoors greenhouse results (Figures 5 & 23), approximately four times higher than in the greenhouse experiment. The species-specific differences are similar indoors and outdoors, with *A. blitum* had the lowest values and *A. thunbergii* had the highest values. AugmentedRCBD (Table S5) also shows significant differences between accessions.

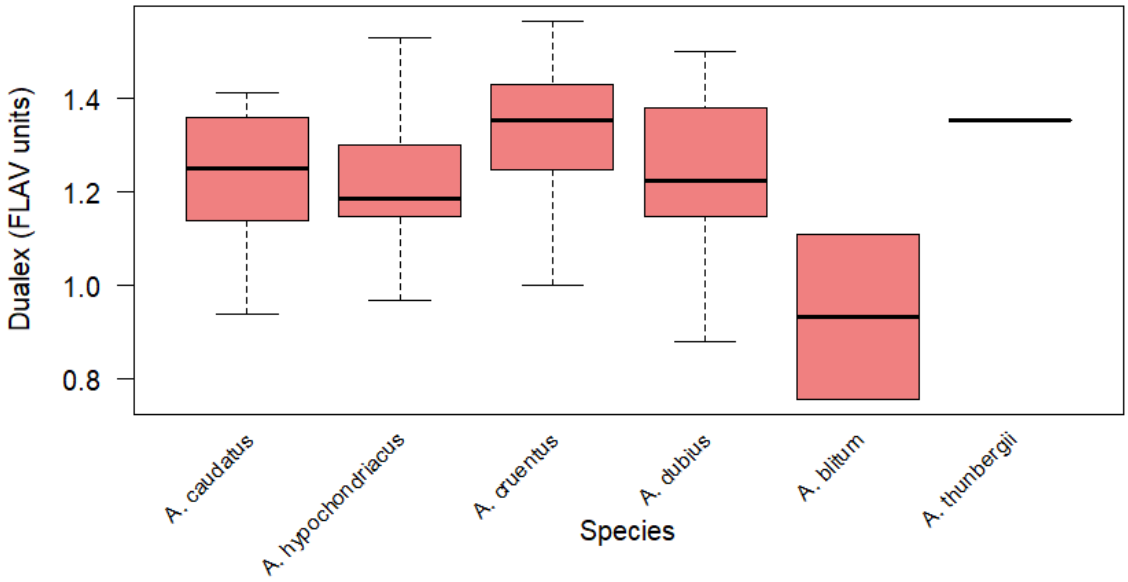

Figure 23. Average of flavonoid measurements taken in the field between 27.05.2024 and 30.05.2024. The graph shows the differences between species and the variation range within each species across different Accessions. Data from Field (Experiment 2).

The largest difference in nitrogen concentrations by species is in *A. blitum*, whose readings in the outdoor experiment turned out to be the largest (Figure 24). However, the most significant difference is in the nitrogen concentration amounts between the outdoor and indoor experiments. Indoor values were more than four times higher in the

greenhouse. AugmentedRCBD (Table S5) also shows no significant differences between accessions.

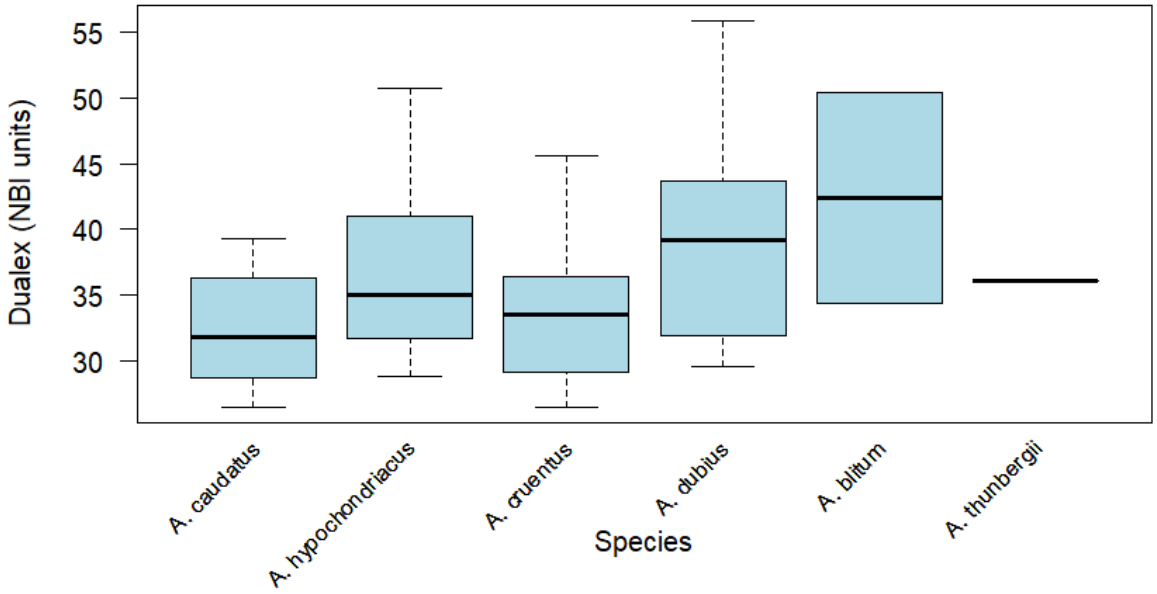

Figure 24. Average of Nitrogen Balance Index (NBI) measurements taken in the field between 27.05.2024 and 30.05.2024. The graph shows the differences between species and the variation range within each species across different accessions. Data from Field (Experiment 2).

8.2.2 Plant height

The plant height in the field conditions compared to the greenhouse was about half. For *A. blitum* the difference is up to 2.7 times. In field conditions, *A. caudatus* grew the highest on average (Figure 25). Apart from *A. caudatus*, the order of the height in the field and in the greenhouse was the same. There was somewhat more variation in height than in the greenhouse.

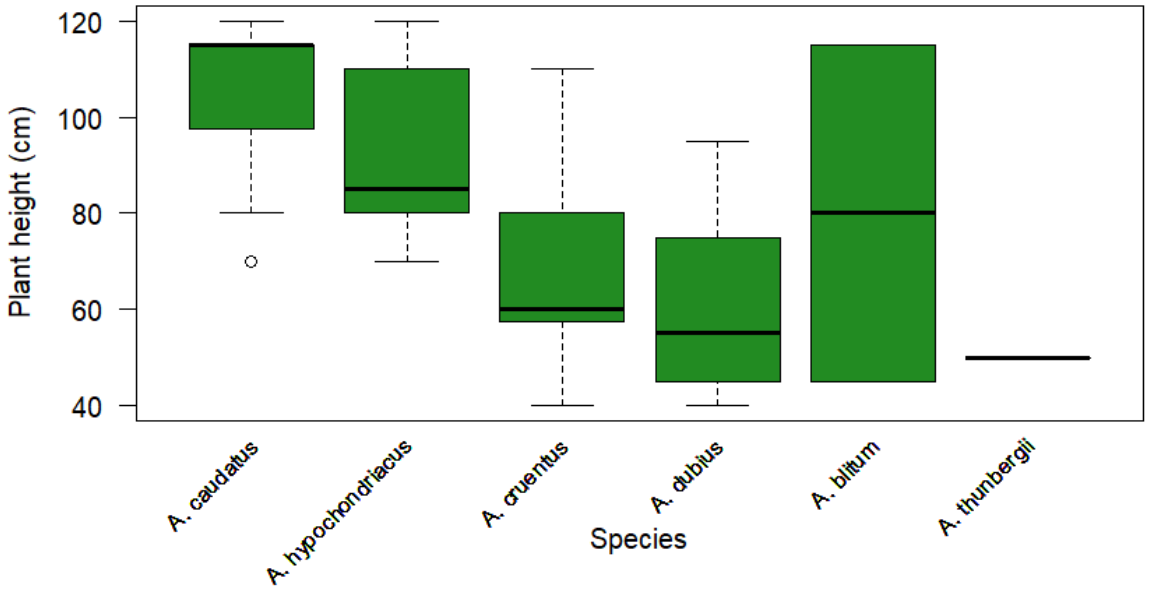

Figure 25. Plant height measurements taken on 09.08.2024 by drone, 93 days after seed sowing on 08.05.2024. The graphs show the differences in plant height between species and the range of variation within each species across different accessions. Data from Field (Experiment 2).

8.2.3 Heading and flowering times

Comparing the heading and flowering times of amaranths on the field, it is noticeable that they proceed consistently in the same order from heading to flowering (Figures 26 & 27). Early, middle and late flowering species can be observed. The time to flowering of *A. caudatus* and *A. hypochodriacus* is longer than that of the other amaranths (Figure 27). *A. blitum* and *A. cruentus* are between early and late flowering species. *A. dubius* and *A. thunbergii* show similar flowering behaviour in both environments and are very early flowering species.

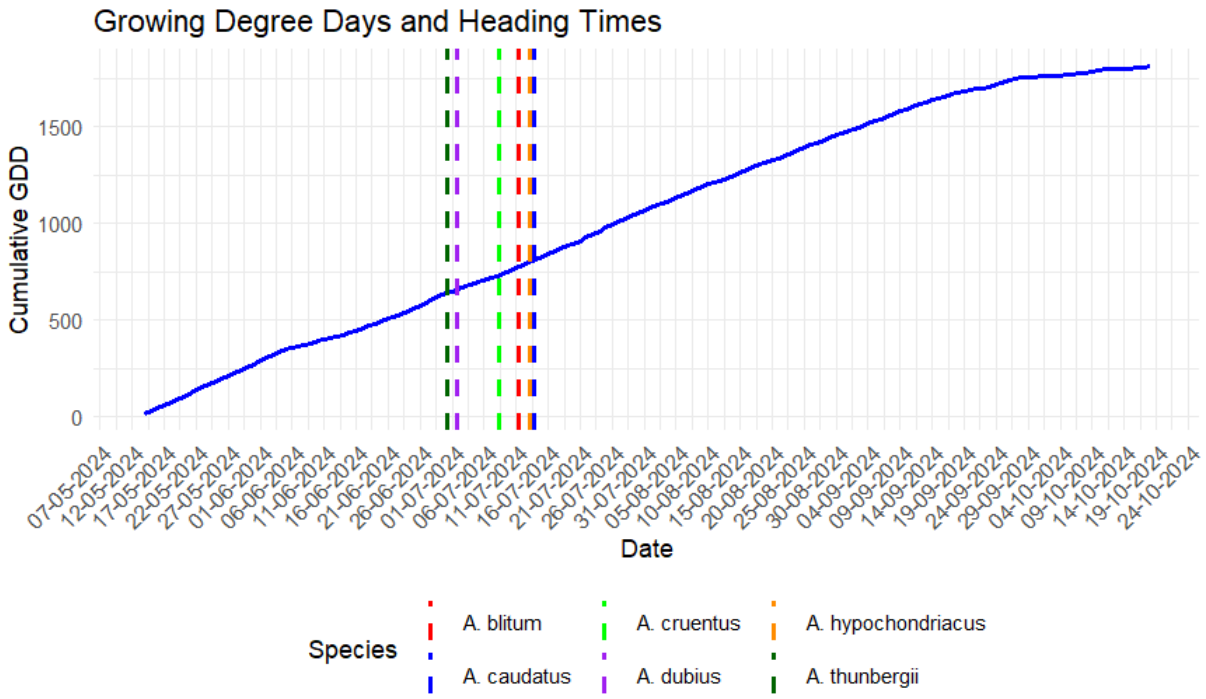

Figure 26. Growing Degree Days (GDD) progression with species-specific heading time averages indicated by colored dashed lines. The graph shows the cumulative heat sum (GDD) over time and the corresponding average heading dates for different species, highlighting the variation in heading time across species under the observed conditions. Data from field (Experiment 2).

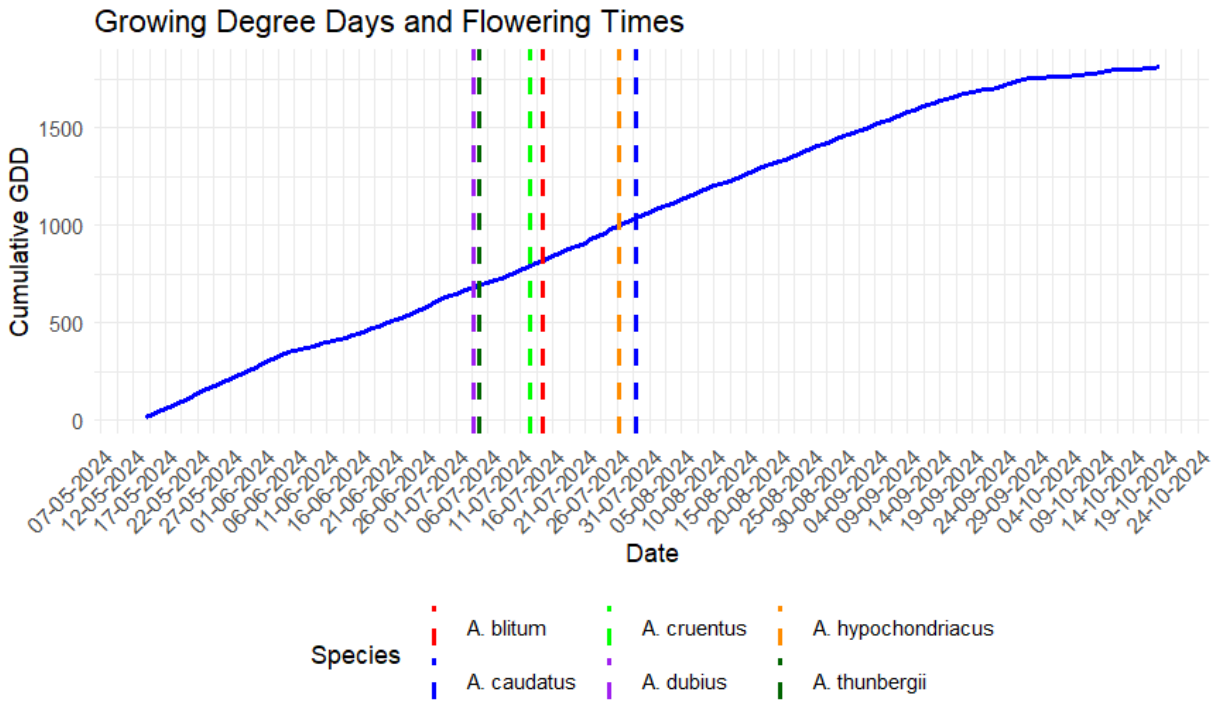

Figure 27. Growing Degree Days (GDD) progression with species-specific flowering time averages indicated by colored dashed lines. The graph shows the cumulative heat sum (GDD) over time and the corresponding average flowering dates for different species, highlighting the variation in flowering time across species under the observed conditions. Data from field (Experiment 2).

The maturity results of the field experiment closely mirrored those of the greenhouse experiment, with the species maintaining the same trends observed indoors. Species that had less than 50% seed production in the greenhouse also produced less than 50% in the field, while those exceeding 50% seed production indoors followed the same pattern outdoors, albeit at reduced levels. The most notable difference was in *A. hypochondriacus*, whose seed production dropped from 79% in the greenhouse to 55% in the field (Figure 28).

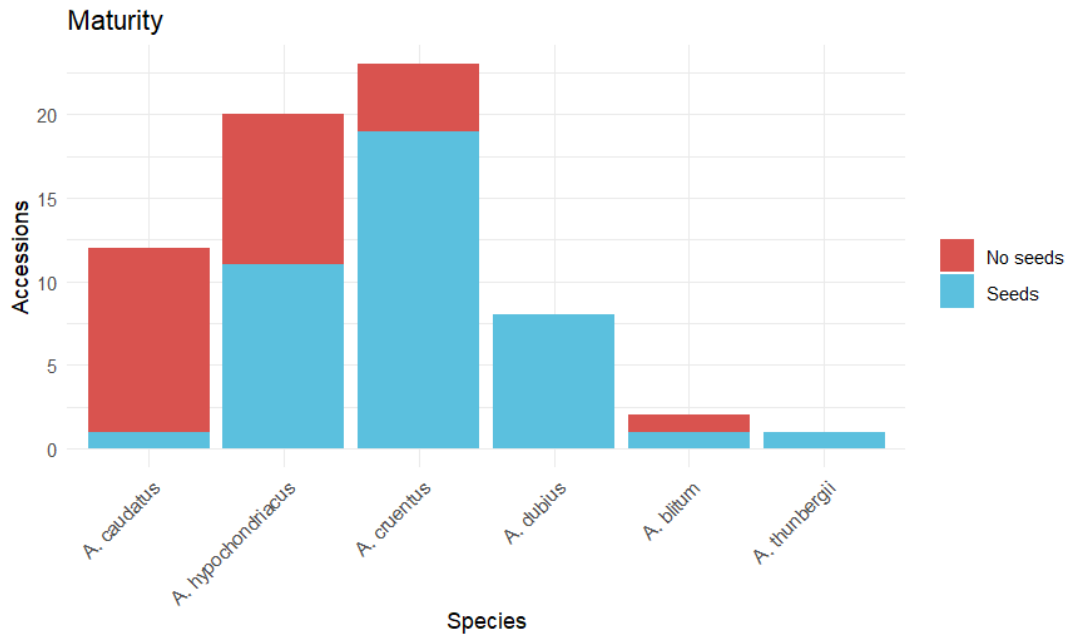

Figure 28. Maturity distribution of accessions as a stacked bar chart. “No seeds” refers to those accessions that did not produce any seeds (red). “Seeds” refers to those accessions that went to seed production stage (blue). The graph illustrates how many accessions were available for each species and the balance between mature and non-mature plants. Data from field (Experiment 2).

Based on the PCA results from the field experiment, plants that grow tall start flowering later than species that are short (Figure 29). This correlation is much more pronounced in the field experiment than in the greenhouse. Chlorophyll content was also negatively correlated with height in the field. Outdoors, the intraspecific uniformity of *A. caudatus* is even more pronounced than in the greenhouse. NBI and anthocyanin values are significantly more correlated in the field experiment.

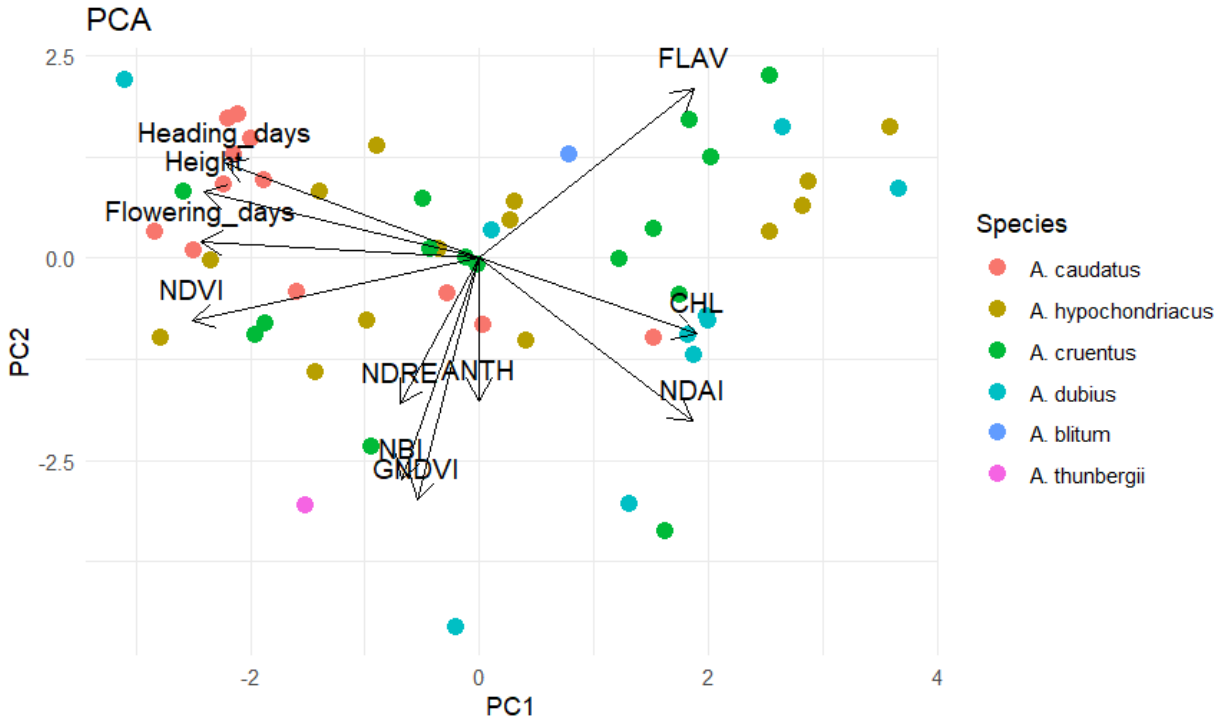

Figure 29. Principal Component Analysis (PCA) plot showing species differentiation based on various variables. Each species is represented by a different color, and the plot highlights the correlations between these variables, illustrating the similarities and differences between species. Data from Field (Experiment 2).

8.3 Field experiment 3

In experiment 3, no significant differences were observed in terms of height (Figure 30) and flowering time between Azhurnyy on 05.08.2024, Opopeo on 07.08.2024, and Orange on 04.08.2024 (Figure 31). The same applies on mature ratio. The three varieties performed well in direct sowing and grew moderately tall compared to the same accessions in experiment 2. All individuals matured and produced seeds.

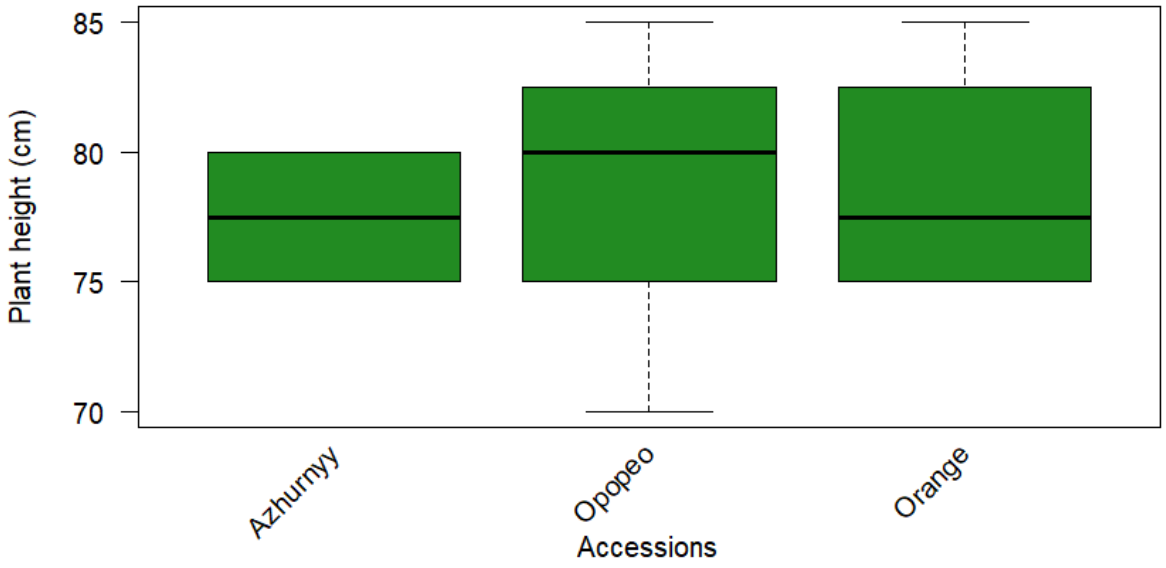

Figure 30. Plant height measurements taken on 09.08.2024 by drone, 77 days after seed sowing on 24.05.2024. The graphs show the differences in plant height between species and the range of variation within each species across different accessions. Data from Field (Experiment 3).

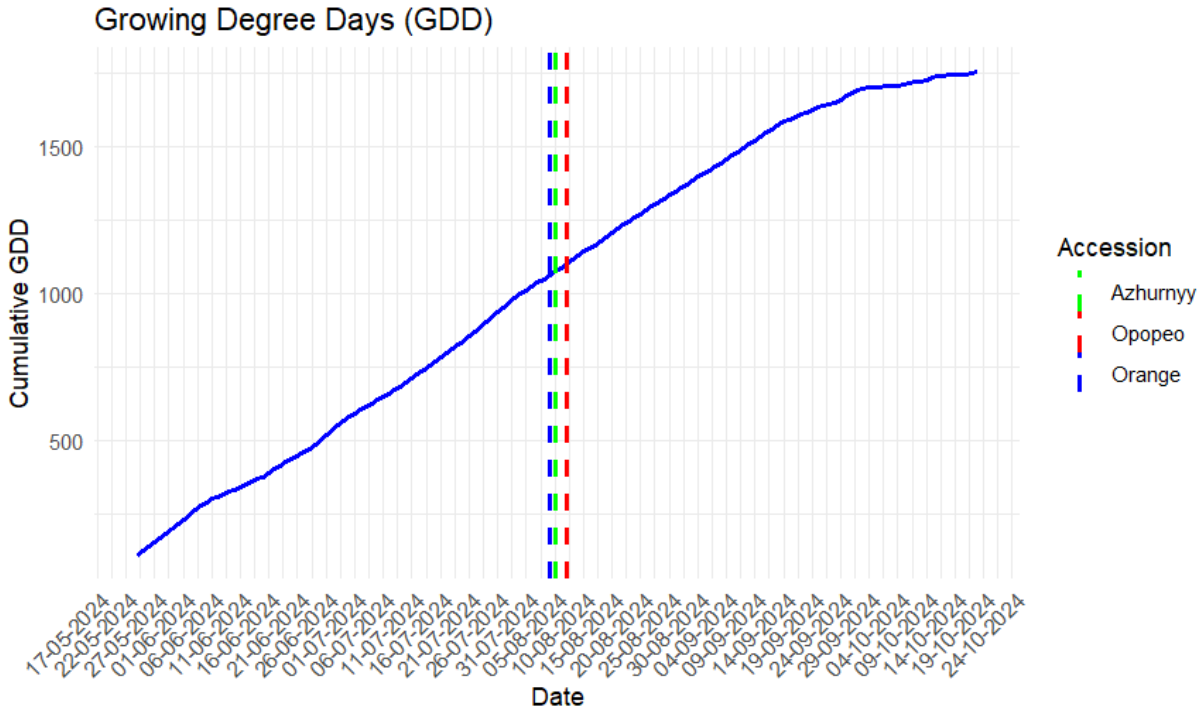

Figure 31. Growing Degree Days (GDD) progression with species-specific flowering time averages indicated by colored dashed lines. The graph shows the cumulative heat sum (GDD) over time and the corresponding average flowering dates for different

species, highlighting the variation in flowering time across species under the observed conditions. Data from field (Experiment 3).

9 Discussion

Three experiments were set up to study morphological, physiological, and phenological properties of an amaranth collection consisting of 70 accessions of six species. Two growing distinct conditions, one under controlled-climate greenhouse and one under boreal field conditions, were employed. Based on the research, there are large morphological, physiological, and phenological differences between the species. The results also revealed variation for each species (except for *A. thunbergia*, which was represented by only one entry). For all traits except leaf temperature, there was significant variation between accessions.

The tallest plants in the greenhouse were by far *A. blitum* and *A. caudatus,* and other grain amaranths were moderately tall. In the field experiment, the highest species was *A. caudatus*. *A. blitum* and *A. hypochondriacus* were high compared to other species. This is supported by Gins et al. (2024) observations, where *A. caudatus* was by far the tallest species and *A. blitum* the second tallest. There was also plenty of variation within species in their experiment as well.

Grain amaranths had the largest leaf area in the greenhouse and *A. hypchocondriacus* had the largest leaves. Gins et al. (2024) showed that some grain amaranth accessions had larger than average leaves, but *A. hypchocondriacus* had a smaller than average leaf area.

Chlorophyll values vary widely between species and accessions. Gins et al. (2024) study has found similar results. In their study, *A. cruentus* and *A. hypochondriacus* obtained high values, which matches the obtained results. However, contradictory results are observed in Osei-Kwarteng et al. (2022) in a study where chlorophyll values were the highest in *A. cruentus* compared to *A. dubius* and *A. hypochondriacus*. In our results, *A. dubius* had the highest values in both greenhouse and in field experiment 2. This could be attributed to variations in the accessions sampled, as there can be significant differences in chlorophyll content even within the same species.

Stomatal conductance is an indicator of water evaporization and carbon dioxide uptake. This physiological parameter reflects a plant's ability to regulate gas exchange and water loss under different environmental conditions. This thesis measured the stomatal conductance under controlled environment. In a study by Osei-Kwarteng et al. (2022) *A. cruentus* clearly differed from *A. dubius* and *A. hypochondriacus*, in terms of stomatal conductance. In the present species all species except *A. caudatus* showed very similar g_s values. These differences might be due to differences in the watering regime, the time of day the measurements were taken, and germplasm used in each study.

Anthocyanin amounts vary significantly between species and accessions. Havugimana et al. (2022) observed the same phenomenon with amaranth, but in their study the species with high anthocyanin content differed from those in the present study. They also mention in their article that anthocyanins are among the flavonoids, which probably explains why as the amount of anthocyanins increases, the amount of other flavonoids decreases if they are measured separately. Flavonoids and anthocyanins are plant compounds that may accumulate under various stress situations (Dixon & Paiva 1995), which explains the increase in their amount in the field experiment compared to the greenhouse experiment.

The small sample of *A. blitum* cultivars did not prevent the appearance of a clear difference in anthocyanin concentrations compared to other species. Under greenhouse conditions, anthocyanin values were higher especially due to the purple pigmentation of *A. blitum* cultivars. However, under field conditions, *A. blitum* anthocyanin concentrations were more than 50% higher than other species. The biggest difference is especially explained by the strong increase in anthocyanin content of accession VI050608-B In this case, high concentrations can be caused by environmental factors such as high UV radiation, drought or cool nights, which can cause stress to the plant and promote anthocyanin production as a protective reaction (Bendokas et al. 2020).

The flavonoid concentration in the plants was clearly higher outside compared to the greenhouse. It is likely that the same stress factors as in the increase of anthocyanin in *A. blitum* are the explanatory factors for the increase of flavonoids. This is because anthocyanin is a flavonoid and an increase in its amount is likely to decrease the others

(Bendokas et al. 2020). This is supported by the fact that the flavonoid concentrations of *A. blitum* were not high but correlated negatively with flavonoids.

From the t-test results of comparing the greenhouse experiment 1 and the field experiment 2, it was found that except for the small accession number of *A. blitum* and *A. thunbergii* and not significant anthocyanin differences of *A. hypochondriacus*. The differences were very significant in NBI, flavonoid, anthocyanin, and height values. The differences in CHL values of *A. dubius* inside and outside were not significant. There were not significant differences in seed production inside the same species in the field or in the greenhouse.

10 Conclusions

The goal of this master's thesis was to study the morphological, physiological, and phenological characteristics of a core collection of 70 amaranth accessions under long-day controlled climate conditions and under Finland field conditions with long days and cool nights. The goal was to find differences between species and accessions and observe variation within the species. The results revealed that morphological, physiological, and phenological characteristics differ significantly among species and cultivars. The results showed that in the greenhouse, *A. caudatus* and *A. blitum* differ significantly from the other species in terms of their plant height. The largest leaf area was found in grain amaranths (*A. caudatus* and *A. cruentus*, and *A. hypochondriacus*), but the differences were not statistically significant. CHL, FLAV, ANTH, NBI, and g_s values measured from leaves differed significantly between several species. In the outdoor experiment, only ANTH values differed greatly between species, but FLAV, ANTH, NBI, and height differed significantly compared to the greenhouse experiment. No significant differences were observed in seed maturity between these experiments.

Such an extensive study of six species and 70 varieties has not been carried out before in Finland, examining morphological, physiological and phenological differences. The greenhouse experiment and the parallel outdoor experiment provided completely new information about growing amaranth in the northern climate. This study provides a good basis for genetic research and breeding of stress tolerance, productivity and phenological adaptations. Considering the minor number of previous studies in

Finland, this study greatly increases awareness of the potential benefits of amaranth for northern farming.

Although the research brings a lot of new information about amaranth to the fore. It is still limited to only one greenhouse experiment and one summer's observations from the field experiments. To get more accurate results, it would be necessary to study different years and locations. Among the amaranth species, *A. blitum* and *A. thunbergii* contained a limited number of accessions, which leaves uncertainty for the results obtained from them. The research was limited to examining physiological and phenological observations, but genetic and biogenetic research could still be carried out. Further research could be carried out by finding out which species or varieties could produce the largest grain or vegetable yield in Finland. Research could be carried out in different parts of the northern climate in different years. The cold resistance of amaranths could be studied in a growth chamber, finding out how cold temperatures affect different accessions of amaranths germination and how the seedlings survive cold night temperatures, in order to find out the optimal sowing time for northern climates.

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12 APPENDIX 1: Greenhouse (Experiment 1)

ANOVA RESULTS OF MEASUREMENTS

Table S1. Results of ANOVA from Experiment 1 comparing species of Amaranth under controlled greenhouse conditions.

Variable	df	Sum Sq	Mean Sq	F value	P value
NBI	5	76630.05	15326.01	12.15014	4.58E-10
CHL	5	828.1337	165.62674	6.44805	1.64E-05
FLAV	5	0.152874	0.0305749	6.672402	1.07E-05
ANTH	5	0.112238	0.0224476	7.270416	3.42E-06
gs	5	279572.2	55914.434	9.732135	3.38E-08
LL	5	896.7054	179.34107	14.69588	4.77E-12
LW	5	36.85173	7.3703466	1.766504	0.122011
LA	5	42591.71	8518.3424	3.858716	0.002416
Height	5	140450.5	28090.1	16.28268	3.66E-13
Maturity	5	13.68597	2.7371946	19.39556	2.62E-15
Tillers	5	1697.52	339.50393	18.05966	2.07E-14
Heading	5	5.65E+13	1.131E+13	15.45786	3E-12
Flowering	5	1.44E+14	2.882E+13	33.37154	9.5E-23
LT	5	4.62718	0.9254361	1.705014	0.135924

df, degrees of freedom

Table S2. Results of ANOVA from Experiment 1 comparing accessions of Amaranth under controlled greenhouse conditions.

Variable	df	Sum Sq	Mean Sq	F value	P value
NBI	64	183113.1	2861.1414	2.949878	3.02E-07
CHL	64	3008.222	47.003468	2.392696	2.86E-05
FLAV	64	0.533413	0.0083346	2.327691	4.87E-05
ANTH	64	0.522462	0.0081635	8.048737	2.05E-21
gs	64	693508.7	10836.074	2.134748	0.000222
LL	64	1869.516	29.211182	2.903381	2.7E-07
LW	64	460.4489	7.1945143	2.695975	1.56E-06
LA	64	227605.7	3556.3387	2.03986	0.000414

Height	64	332765.1	5199.4543	5.543548	9.9E-16
Maturity	64	37.43389	0.5849046	100.0187	2.3E-77
Tillers	64	3410.55	53.289844	3.933461	8.53E-11
Heading	59	1.26E+14	2.128E+12	5.019372	1.54E-12
Flowering	59	2.36E+14	4.005E+12	10.44949	3.47E-23
LT	64	37.74121	0.5897064	1.106313	0.316764

TUKEY TEST RESULTS

Table S3. Tukey test results for NBI the measurements in Greenhouse experiment (Experiment 1).

Comparison	T value	P value	Conf int lower	Conf int upper	Mean diff	Mean Group 1	Mean Group 2
A.cauda-A.hypo	-0.15	0.87764	-14.18	12.14	1.02	139.85	140.87
A.cauda-A.cruen	-4.31	4.46E-05	-42.94	-15.83	29.38	139.85	169.23
A.cauda-A.dubius	-5.95	5.81E-07	-71.57	-35.27	53.42	139.85	193.27
A.cauda-A.blitum	3.78	0.002961	17.10	64.59	-40.84	139.85	180.69
A.cauda-A.thun	1.55	0.197662	-10.15	35.22	-12.53	139.85	127.32
A.hypo-A.cruen	3.90	0.000171	13.93	42.79	-28.36	140.87	169.23
A.hypo-A.dubius	5.62	1.09E-06	33.62	71.18	-52.40	140.87	193.27
A.hypo-A.blitum	3.58	0.003532	15.72	63.92	-39.82	140.87	180.69
A.hypo-A.thun	1.60	0.174070	-8.64	35.75	-13.55	140.87	127.32
A.cruen-A.dubius	-2.54	0.014462	-43.08	-4.99	24.04	169.23	193.27
A.cruen-A.blitum	1.02	0.326065	-12.80	35.72	-11.46	169.23	180.69
A.cruen-A.thun	4.86	0.004545	19.80	64.03	-41.91	169.23	127.32
A.dubius-A.blitum	-0.99	0.332892	-39.08	13.93	12.58	193.27	180.69
A.dubius-A.thun	6.33	0.000106	42.59	89.32	-65.95	193.27	127.32
A.blitum-A.thun	4.44	0.001567	26.22	80.53	-53.37	180.69	127.32

Table S4. T-test p-value results comparing greenhouse experiment 1 and field experiment 2 results comparing species to species.

Species	CHL	FLAV	ANTH	Height	NBI	Maturity
A. blitum	0.294	0.24	0.295	0.322	0.041	1.000
A. caudatus	0.264	1.55E-08	0.001	1.14E-12	3.81E-15	0.558
A. cruentus	0.001	9.03E-16	0.008	9.25E-10	1.12E-14	0.957

A. dubius	0.858	1.72E-06	0.001	3.78581E-05	4.39E-10	N/A
A. hypochondriacus	0.021	6.67926E-15	0.182	1.05E-09	4.77E-14	0.096
A. thunbergii	N/A	N/A	N/A	N/A	N/A	N/A

Species	**Heading**	**Flowering**
A. blitum	N/A	N/A
A. caudatus	0.00156	0.00143
A. cruentus	0.337	0.334
A. dubius	0.234	0.250
A. hypochondriacus	0.0435	0.240
A. thunbergii	N/A	N/A

Figure S1. Seedlings of experiment 2 planted in seed trays (Photo: Ilja Koli, 10.05.2024).

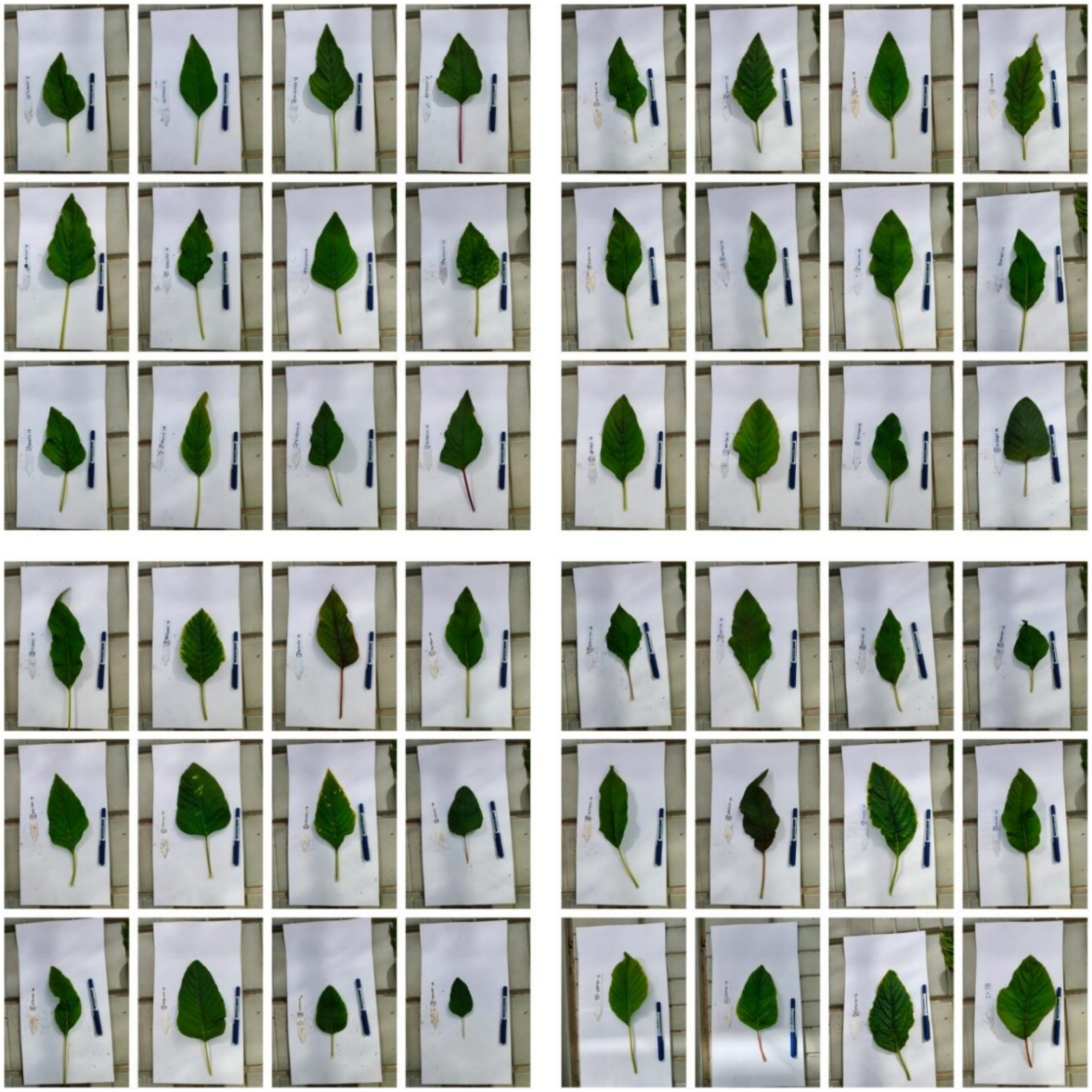

Figure S2. Different shapes and sizes of leaves Experiment 1 (Photo: Ilja Koli, 20.06.2024).

13 APPENDIX 2: Field (Experiment 2)

Table S5. ANOVA, Block Adjusted

Source	df	Mean.Sq									
		Height		CHL		FLAV		ANTH		NBI	
Treatment (ignoring Blocks)	65	589.40	**	45.34	*	0.05	**	0.00	**	85.25	**
Treatment: Check	2	61.67	ns	18.76	ns	0.01	ns	0.00	ns	22.13	ns
Treatment: Test	62	615.93	**	27.23	ns	0.05	**	0.00	**	80.38	**
Treatment: Test vs. Check	1	0.11	ns	1221.30	**	0.17	**	0.06	**	513.39	**
Block (eliminating Treatments)	4	172.50	ns	10.45	ns	0.02	ns	0.00	ns	10.55	ns
Residuals	8	51.25		10.16		0.01		0.00		11.19	

ns P > 0.05; * P <= 0.05; ** P <= 0.01

Table S6. ANOVA, Treatment Adjusted

Source	df	Mean.Sq									
		Height		CHL		FLAV		ANTH		NBI	
Block (ignoring Treatments)	4	3496.05	**	71.14	*	0.01	ns	0.00	*	46.18	*
Treatment (eliminating Blocks)	65	384.87	**	41.61	*	0.05	**	0.00	**	83.06	**
Treatment: Check	2	61.67	ns	18.76	ns	0.01	ns	0.00	ns	22.13	ns
Treatment: Test and Test vs. Check	63	395.13	**	42.33	*	0.05	**	0.00	**	84.99	**
Residuals	8	51.25		10.16		0.01		0.00		11.19	

ns P > 0.05; * P <= 0.05; ** P <= 0.01

Table S6. ANOVA, Block Adjusted

Source	df	Mean.Sq			
		NDVI	GNDVI	NDRE	NDAI
Treatment (ignoring Blocks)	65	0.00 ns	0.00 ns	0.00 *	0.02 **
Treatment: Check	2	0.00 ns	0.00 ns	0.00 ns	0.04 **
Treatment: Test	62	0.00 ns	0.00 ns	0.00 *	0.02 **
Treatment: Test vs. Check	1	0.00 ns	0.00 ns	0.01 **	0.05 **
Block (eliminating Treatments)	4	0.00 ns	0.00 ns	0.00 **	0.02 **
Residuals	8	0.00	0.00	0.00	0.00

ns P > 0.05; * P <= 0.05; ** P <= 0.01

Table S7. ANOVA, Treatment Adjusted

Source	df	Mean.Sq			
		NDVI	GNDVI	NDRE	NDAI
Block (ignoring Treatments)	4	0.00 *	0.00 ns	0.01 **	0.04 **
Treatment (eliminating Blocks)	65	0.00 ns	0.00 ns	0.00 *	0.02 **
Treatment: Check	2	0.00 ns	0.00 ns	0.00 ns	0.04 **
Treatment: Test and Test vs. Check	63	0.00 ns	0.00 ns	0.00 *	0.02 **
Residuals	8	0.00	0.00	0.00	0.00

ns P > 0.05; * P <= 0.05; ** P <= 0.01

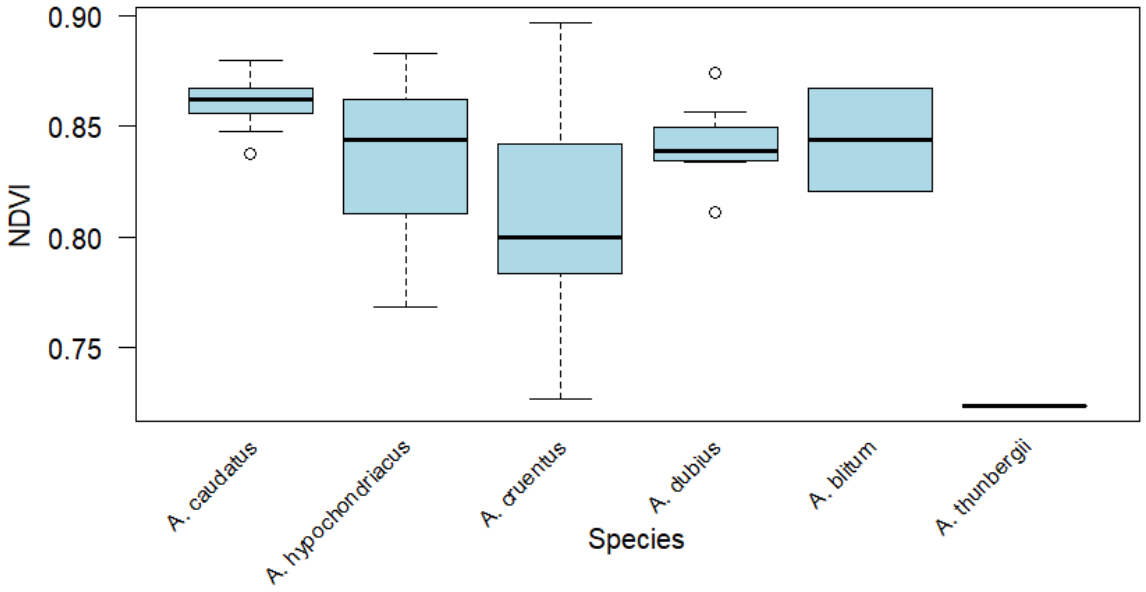

Figure S3. Normalized Difference Vegetation Index measurements taken on 09.08.2024 by drone, 93 days after seed sowing on 08.05.2024. The graphs show the differences in NDVI between species and the range of variation within each species across different accessions. Data from Field (Experiment 2).

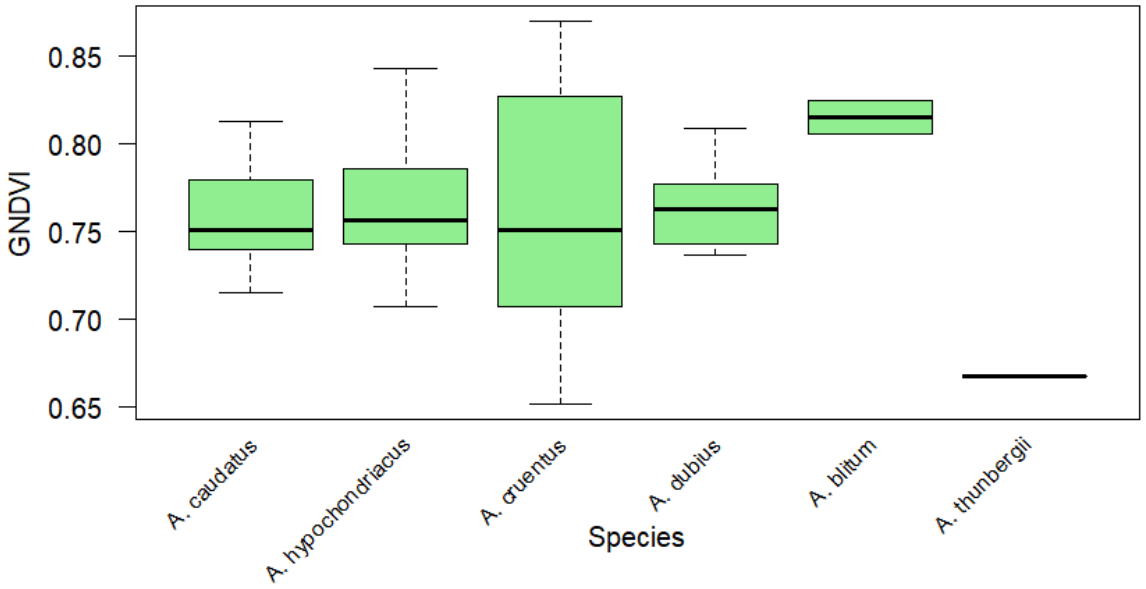

Figure S4. Green Normalized Difference Vegetation Index measurements taken on 09.08.2024 by drone, 93 days after seed sowing on 08.05.2024. The graphs show the differences in plant GNDVI between species and the range of variation within each species across different accessions. Data from Field (Experiment 2).

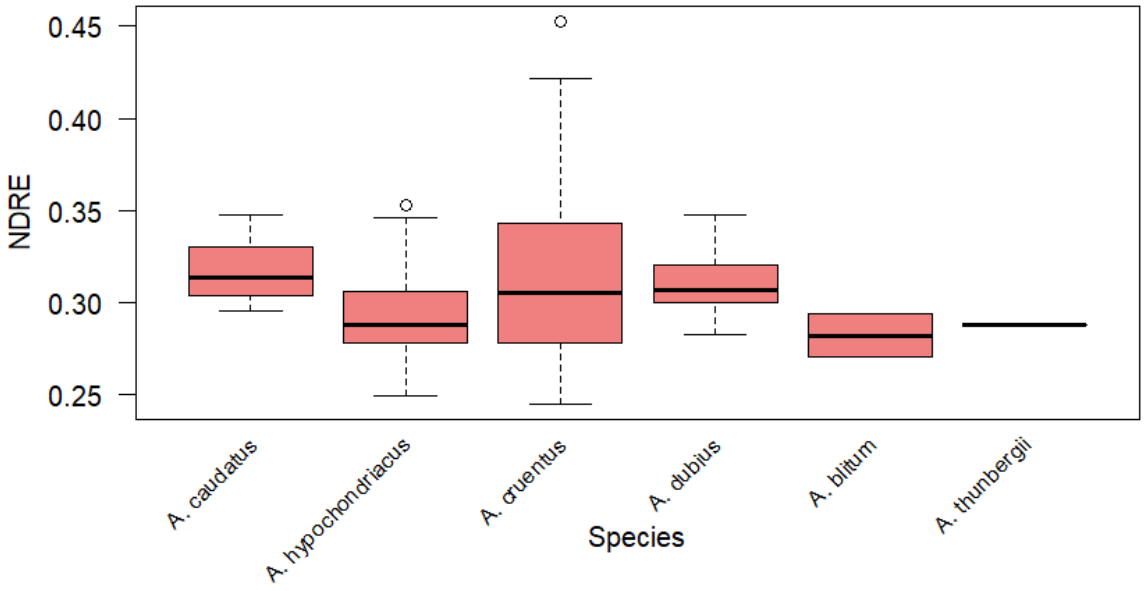

Figure S5. Normalized Difference Red Edge Index measurements taken on 09.08.2024 by drone, 93 days after seed sowing on 08.05.2024. The graphs show the differences in plant NDRE between species and the range of variation within each species across different accessions. Data from Field (Experiment 2).

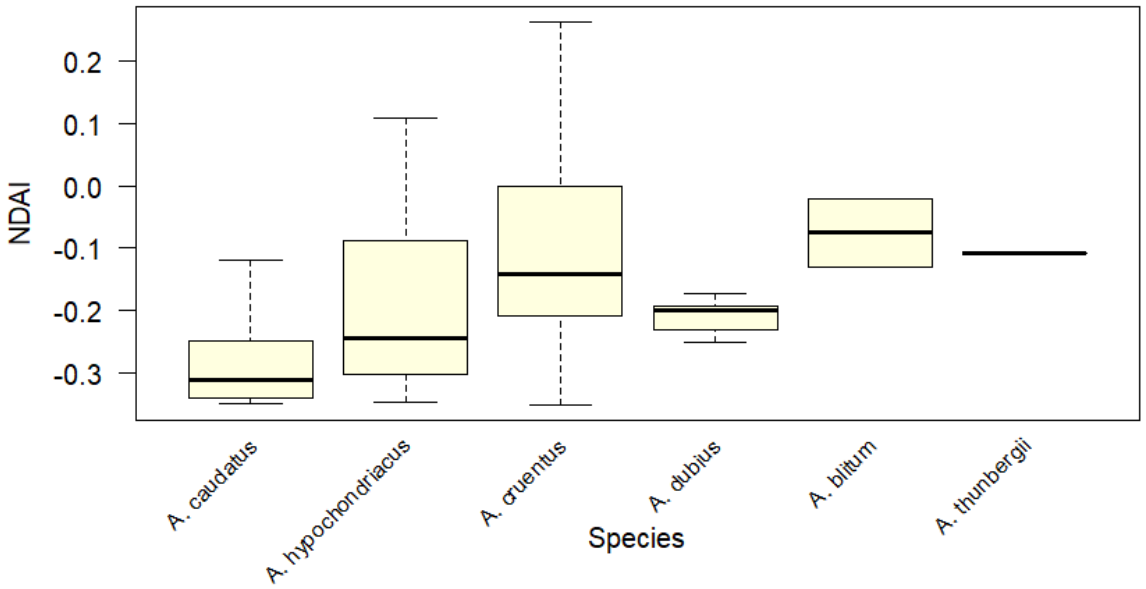

Figure S6. Normalized Difference Red Edge Index measurements taken on 09.08.2024 by drone, 93 days after seed sowing on 08.05.2024. The graphs show the differences in plant NDAI between species and the range of variation within each species across different accessions. Data from Field (Experiment 2).

14 APPENDIX 3: Field (Experiment 3)

Table S5. Results of ANOVA for the measurements in the field (Experiment 3).

Variable	df	Sum Sq	Mean Sq	F value	P value
CHL Dualex	2	31.528667	15.7643334	1.434869	0.246617455
FLAV Dualex	2	0.0282207	0.01411035	0.404227	0.669390789
ANTH Dualex	2	0.0123269	0.00616345	4.488708	0.015476311
NBI Dualex	2	63.372711	31.6863554	0.723376	0.48951105
CHL MPM-100	2	0.01621	0.008105	0.534535	0.588850013
FLAV MPM-100	2	0.13117	0.065585	1.446622	0.243873845
ANTH MPM-100	2	0.02569	0.012845	9.426613	0.000290518
NFI MPM-100	2	0.33996	0.16998	0.944904	0.394720333
Flowering	2	1.393E+11	6.9673E+10	0.913043	0.435481282

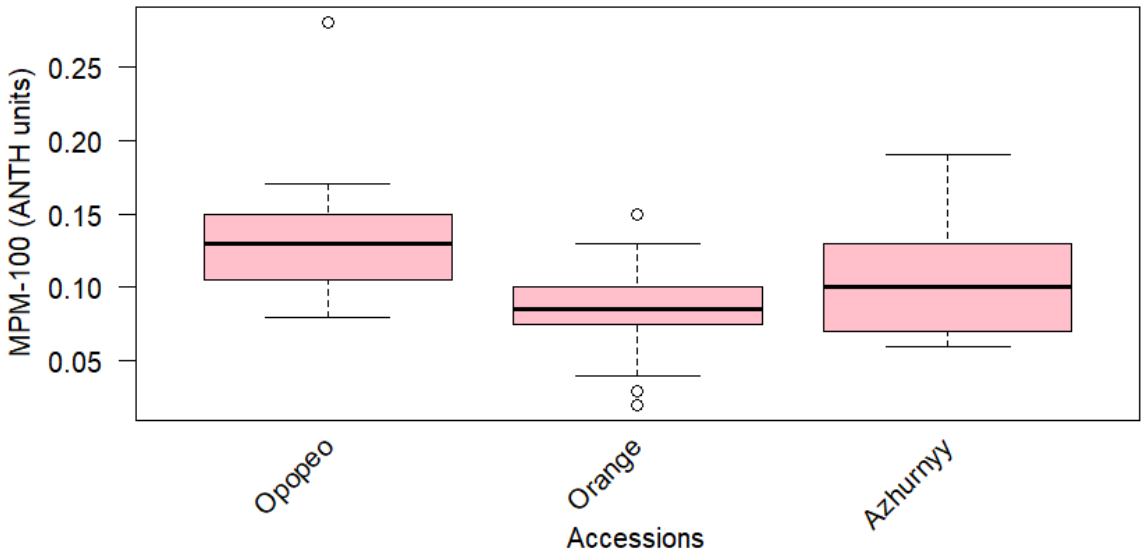

Figure S7. Anthocyanin measurements taken in the field 24.07.2024. The graph shows the differences between species and the variation range within each species across different accessions. Data from field experiments (Experiment 3).

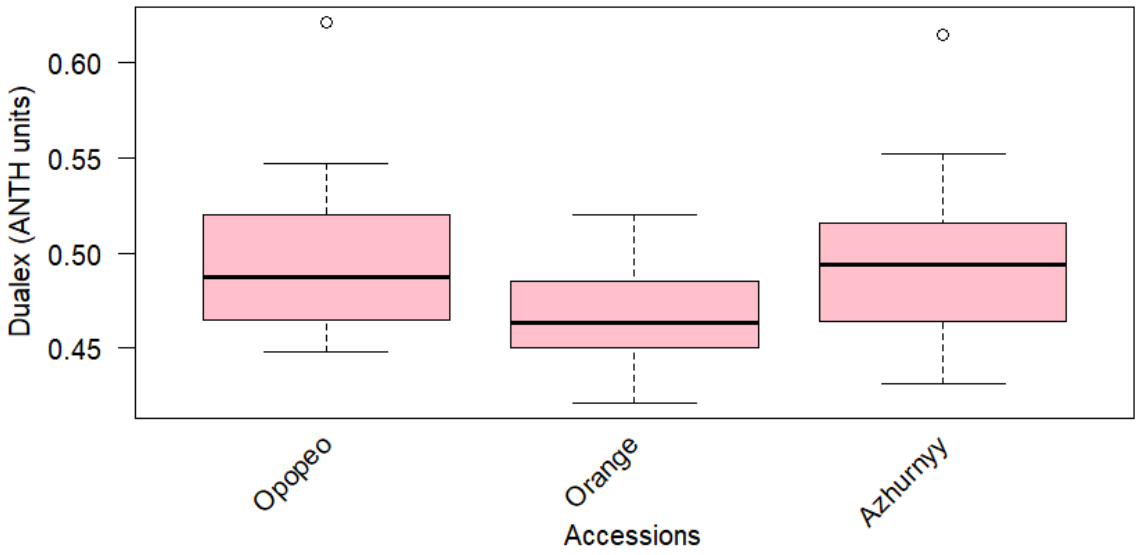

Figure S8. Average of anthocyanin measurements taken in the field between 30.07.2024 and 03.08.2024. The graph shows the differences between species and the variation range within each species across different accessions. Data from field experiments (Experiment 3).

Chlorophyll, Flavanoids, NBI/NFI

The three accessions seem to be very similar to each other. This supports the visual observations that the only difference between the accessions was the purple color of Opopeo.

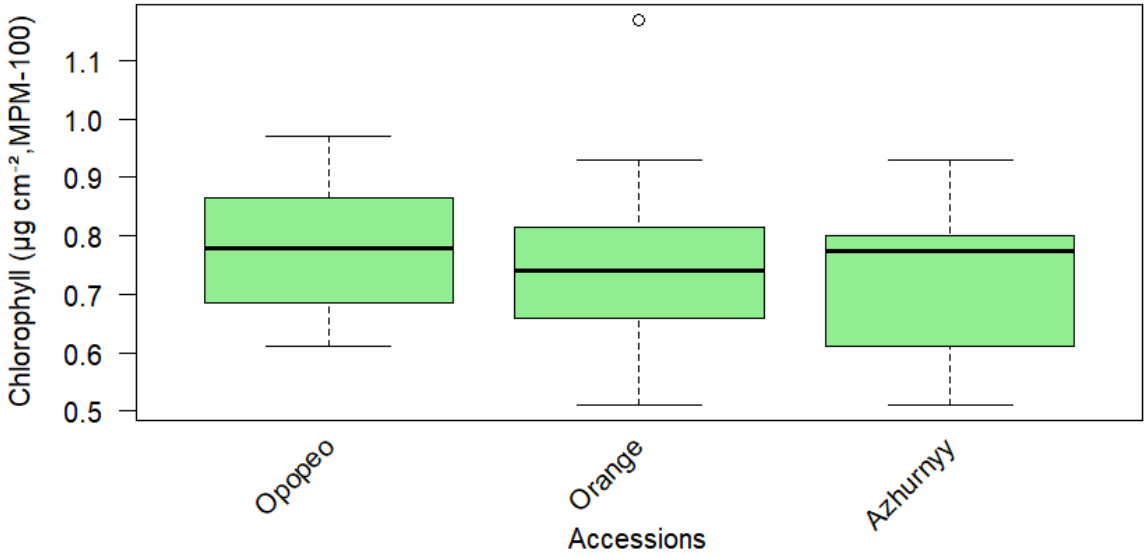

Figure S9. Chlorophyll measurements taken in the field 24.07.2024. The graph shows the differences between species and the variation range within each species across different accessions. Data from field experiments (Experiment 3).

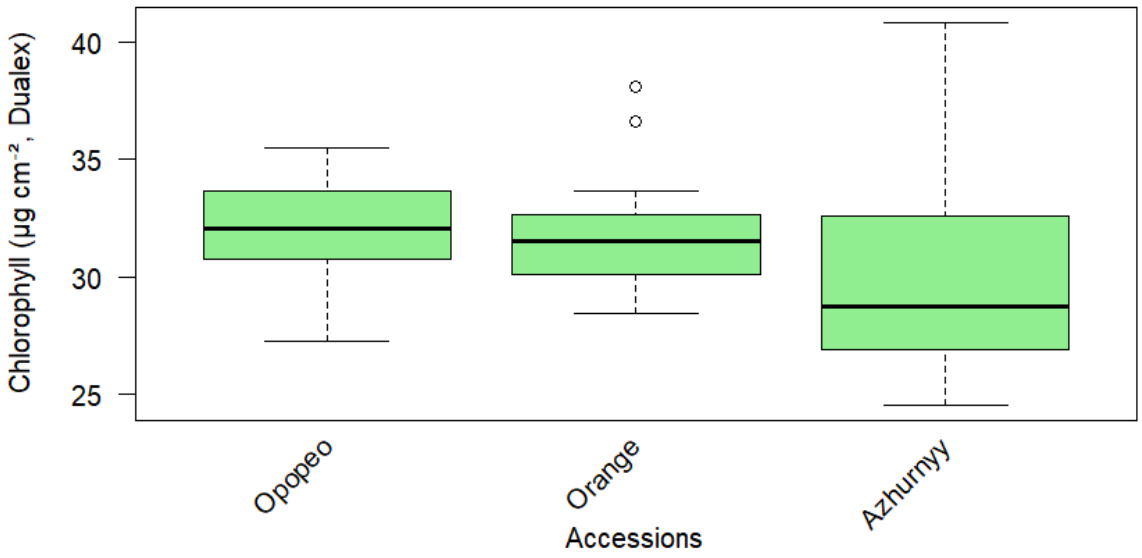

Figure S10. Average of chlorophyll measurements taken in the field between 30.07.2024 and 03.08.2024. The graph shows the differences between species and the variation range within each species across different accessions. Data from field experiments (Experiment 3).

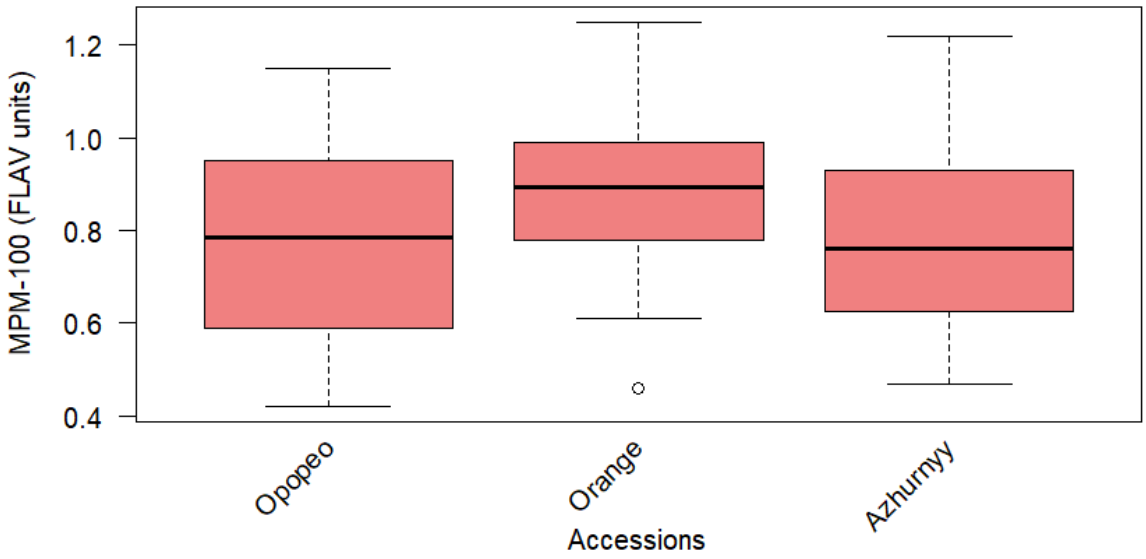

Figure S11. Flavonoid measurements taken in the field 24.07.2024. The graph shows the differences between species and the variation range within each species across different accessions. Data from field experiments (Experiment 3).

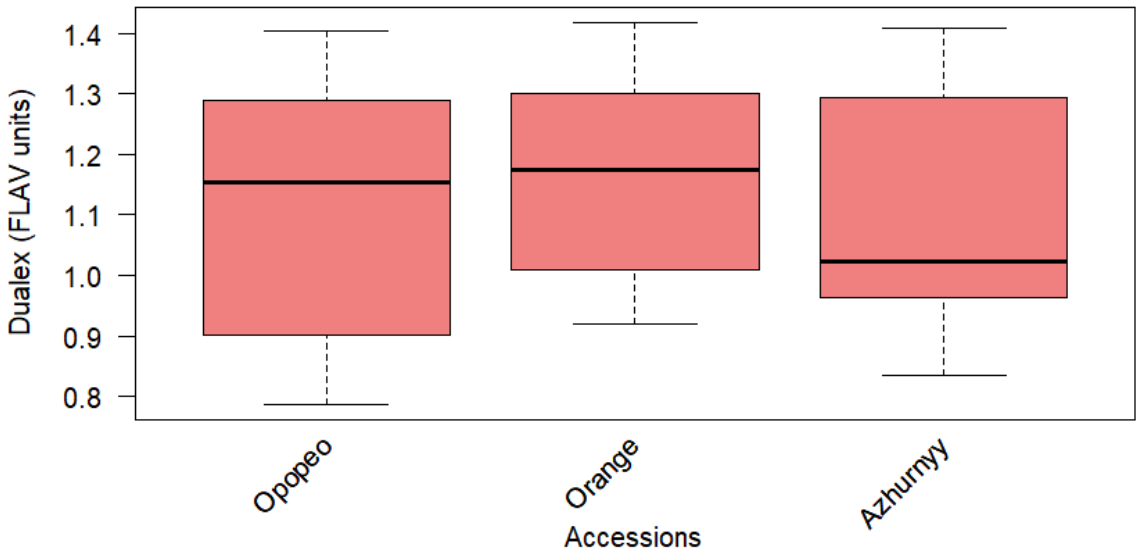

Figure S12. Average of Flavonoid measurements taken in the field between 30.07.2024 and 03.08.2024. The graph shows the differences between species and the variation range within each species across different accessions. Data from field experiments (Experiment 3).

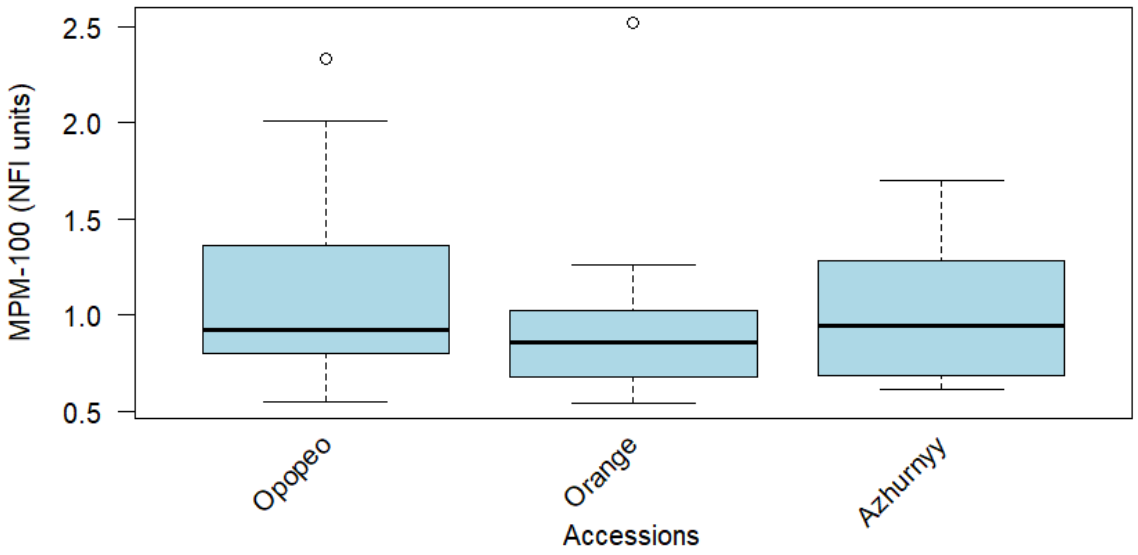

Figure S13. Nitrogen-Flavonol Index (NFI) measurements taken in the field 24.07.2024. The graph shows the differences between species and the variation range within each species across different accessions. Data from field experiments (Experiment 3).

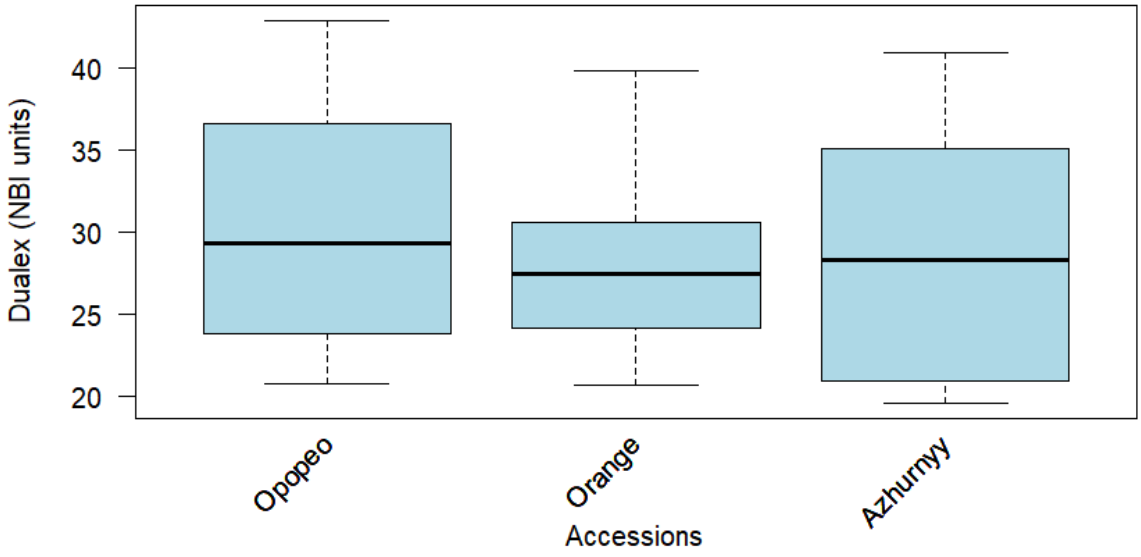

Figure S14. Nitrogen balance index measurements taken in the field between 30.07.2024 and 03.08.2024. The graph shows the differences between species and the variation range within each species across different accessions. Data from field experiments (Experiment 3).