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Leveraging the diversity of *Beta maritima* populations for sugar beet breeding

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[1] Bertram, L., and Frisch, M. (2026) Morphological and genetic diversity of *Beta maritima* populations across Europe and North Africa: a comprehensive review. *Front. Plant Sci.* 16:1731515.

[2] Bertram, L., Gholami, M., Kopisch-Obuch, F., and Frisch, M. (2025a) Exploring the diversity of three Northern Atlantic sea beet populations. *Front. Plant Sci.* 16:1635602.

[3] Bertram, L., Kopisch-Obuch, F., and Frisch, M. (2025b) Crop Wild Relative Populations of *Beta Vulgaris* as Source for Genome-Wide Association Mapping of Complex Traits. *Theor. Appl. Genet.* 138, 157.

Abbreviations

AB-NAM	advanced backcross nested association mapping
AFLP	amplified fragment length polymorphism
DArT	diversity array technology
GPS	global positioning system
GWAS	genome-wide association mapping
LD	linkage disequilibrium
QTL	quantitative trait locus
RFLP	restriction fragment length polymorphism
SNP	single nucleotide polymorphism
SSR	simple sequence repeat

Chapter 1

General Introduction

Domestication and intensive breeding have significantly reduced the genetic diversity of elite sugar beet germplasm, narrowing its genetic base and limiting the adaptive potential of modern cultivars (Jung et al., 1993; Fénart et al., 2008; Panella et al., 2020; Veloso et al., 2021). In contrast, the broader *Beta* gene pool, and particularly the wild relative sea beet [*Beta vulgaris* ssp. *maritima* (L.) Arcang.; short: *Beta maritima*], remains a rich reservoir of genetic variation (Frese et al., 1990; Boudry et al., 2002; Bartsch & Biancardi, 2020). As the closest relative of cultivated beets, sea beet is fully cross-compatible with sugar beet and thrives in diverse, often harsh coastal environments, where it has evolved tolerance to adverse environmental conditions, including salinity and drought, as well as various pathogens – traits that are frequently lost or absent in cultivated material (Monteiro et al., 2013; Panella & Lewellen, 2007; Panella et al., 2020).

In situ conservation preserves *Beta maritima* populations within their native habitats, maintaining their genetic diversity while enabling populations to continuously adapt to changing environmental conditions, ensuring the persistence of valuable traits relevant for crop improvement (Bohra et al., 2022; Zucchini et al., 2024). However, effectively harnessing this diversity requires a comprehensive understanding of the scale and structure of genetic variation within and among sea beet populations.

This thesis aims to bridge the gap between the rich genetic diversity of *Beta maritima* populations and its practical application in sugar beet breeding. By integrating empirical population studies, high-resolution genotyping, and simulation-based breeding design, this work provides new insights into the structure, diversity, and breeding value of three Northern Atlantic sea beet populations.

1.1 *Beta vulgaris* ssp. *maritima*

1.1.1 Range of Distribution

Beta maritima represents the geographically most widespread taxon within the genus *Beta* (Frese & Ford-Lloyd, 2020). Its origin is traced to the Mediterranean region, the primary center of diversity of the species (Romeiras et al., 2016; Frese & Ford-Lloyd, 2020). Accordingly, *Beta maritima* is widely distributed across the Mediterranean and Black Sea coastlines (**Figure 1**), with its presence documented in numerous locations throughout the literature. The species occurs in nearly all coastal countries bordering the Mediterranean, particularly in Spain, Italy, Greece, Turkey, and North African nations such as Morocco, Tunisia, and Egypt, as well as on Atlantic islands including the Canary Islands, Madeira, and Cape Verde (Romeiras et al., 2016; Frese & Ford-Lloyd, 2020; Veloso et al. 2021; Zucchini et al. 2024; Ben Mahmoud et al., 2025).

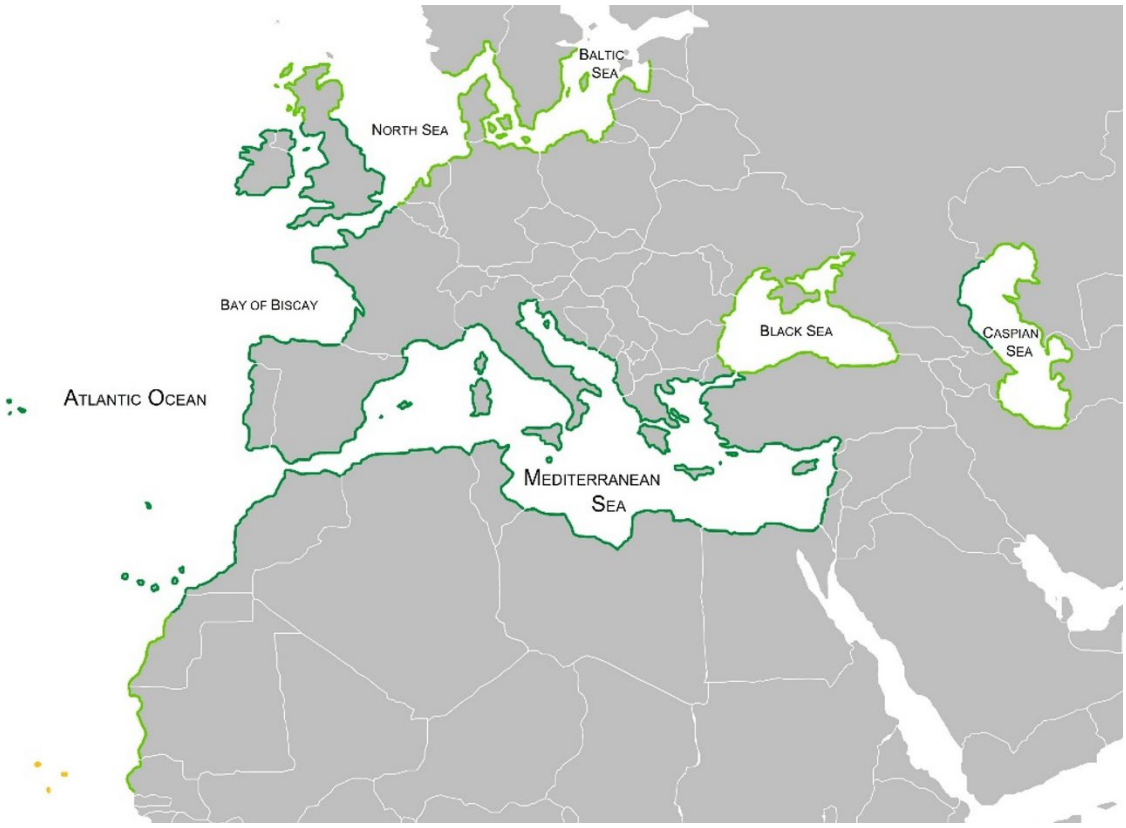

Figure 1 Map showing the distribution of *Beta maritima* along the seashores within its main distribution area in Europe and Northern Africa (dark green: frequent; light green: sparse; yellow: rare). Adapted from Frese & Ford-Lloyd (2020).

Following the last glacial period, *Beta maritima* progressively expanded its range northward along the Atlantic coast, ultimately reaching southern Norway, Sweden, and the western Baltic Sea (Frese & Ford-Lloyd, 2020). In the southern part of this range, the species is relatively common and widespread in Portugal, where it thrives in both natural and ruderal habitats (Monteiro et al., 2013). Moving northward, large and well-established populations are found along the Atlantic and Channel coasts of western France and the British Isles, while populations along the North Sea coasts of Belgium, The Netherlands, and Germany become generally smaller and more scattered (Doney et al., 1990; Letschert, 1993; Boudry et al., 2002; Driessen, 2003; Fievet et al., 2007; Frese & Ford-Lloyd, 2020). Further north in Europe, Denmark harbors relatively large populations of *Beta maritima*, whereas only sparse and scattered occurrences are documented in Sweden (Driessen, 2003; Andersen et al., 2005; Frese & Ford-Lloyd, 2020).

Beta maritima is absent from the eastern coasts of the Americas and the Southern Hemisphere, including African shores south of Morocco's southern (21°N) border (Frese & Ford-Lloyd, 2020). Present-day records confirm its presence along a latitudinal range from approximately 15°N (Cape Verde Islands) to 58°N (southern Norway and southern Sweden), with isolated populations occasionally also reported in the Middle East, India, China, Japan, and California (Bartsch & Ellstrand, 1999; Richardson et al., 2016; Frese & Ford-Lloyd, 2020).

1.1.2 Mode of Distribution

In *Beta maritima*, seed dispersal is primarily mediated by seawater (Letschert & Frese, 1993). The seeds can float for several days without losing viability, facilitating long-distance transport along shorelines (Letschert & Frese, 1993; Fievet et al., 2007). Accordingly, marine currents play an important role in shaping the genetic structure of coastal populations, with oceanographic features such as geographical boundaries and circulation patterns influencing gene flow (Fievet et al., 2007; Leys et al., 2014). A prominent example is the genetic separation between Atlantic and Mediterranean populations, driven by a barrier near the Strait of Gibraltar (Sandell et al., 2022). Similarly, in the Anglo-Norman Gulf, dominant westward currents have led to a clear genetic divide between eastern and western

populations (Fievet et al., 2007). The asymmetric gene flow observed between these latter populations suggests that some populations act as genetic sources while others function as sinks (Fievet et al., 2007).

While marine currents play a significant role in shaping the genetic structure of coastal *Beta maritima* populations, they cannot fully account for genetic patterns observed over larger distances, suggesting additional dispersal mechanisms may be at play (Andrello et al., 2016). Beyond marine dispersal, animals also serve as dispersal agents, especially in inland habitats where seawater-mediated transport is limited (Toll & Hendriksen, 1982; Doney et al., 1990; Bartsch & Biancardi, 2020).

Historical human maritime activities have likely also contributed to the species' broader distribution. In earlier centuries, ships commonly used sand or soil ballast, sourced near harbors, for stabilization. Upon arrival, this ballast was discharged, potentially releasing sea beet seeds into new environments (Bartsch & Ellstrand, 1999). A notable example is the introduction of wild beets to California. Genetic analyses of populations near Santa Barbara revealed close affinities to Spanish accessions from Cartagena, Spain, supporting the hypothesis of transoceanic dispersal by sailing vessels a few centuries ago (Bartsch & Biancardi, 2020). Long-standing maritime trade routes likely also facilitated the spread of *Beta maritima* between the British Isles and the Baltic Sea, the Danish and German coasts, and from the Venetian lagoon to eastern Mediterranean ports (Driessen et al., 2001; Bartsch & Biancardi, 2020).

While seed dispersal plays a crucial role in shaping the distribution of *Beta maritima*, gene flow also occurs through pollen movement. Wind serves as the principal dispersal agent for pollen, capable of carrying it over distances of 5–8 km, and occasionally exceeding 9 km under favorable conditions (Fénart et al., 2008; Biancardi & de Biaggi, 2020). The effectiveness of wind-driven pollination is influenced by environmental factors such as wind speed, direction, and humidity (Biancardi & de Biaggi, 2020). Although less frequent, insect-mediated pollination can reach even greater distances, further contributing to genetic exchange across populations (Biancardi & de Biaggi, 2020).

1.1.3 Reproductive Strategy and Life Cycle

Beta maritima relies on an allogamous reproductive strategy driven by proterandry (Panella & Lewellen, 2007). This temporal separation of male and female maturity within the same flower reduces the likelihood of self-pollination and encourages pollen transfer between different individuals. *Beta maritima* further possesses a complex gametophytic self-incompatibility system (Larsen, 1977; Panella & Lewellen, 2007; Hautekeete et al., 2020). In this system, the compatibility of pollen is determined by the interaction between the genotype of the pollen (male gametophyte) and the genotype of the pistil (female tissue). If both share the same alleles at multiple gametophytic *S*-loci, fertilization is blocked. This prevents self-fertilization and promotes high levels of heterozygosity through outcrossing within the population (Larsen, 1977; Hautekeete et al., 2020).

Beta maritima shows remarkable variation in flowering behavior across its distribution range (van Dijk et al., 1997; Boudry et al., 2002). Although fundamentally perennial, sea beet populations can exhibit annual, biennial, or perennial flowering cycles, depending largely on the presence or absence of a cold period (Hautekeete et al., 2020). This flexibility in life cycle strategy enhances the species' adaptability to diverse and often extreme environments (Letschert & Frese, 1993; Boudry et al., 2002; Ascarini et al., 2021).

A key genetic factor underlying this variation is the major gene *B*, which controls vernalization requirements; the need for cold exposure to trigger flowering (Boudry et al., 1994). The *B* allele, which promotes bolting and early flowering, is more prevalent in southern populations, where rapid reproduction provides a selective advantage. Consequently, Mediterranean populations often flower in their first year. In contrast, northern populations predominantly carry the *bb* genotype, which requires vernalization and long-day conditions to initiate flowering (van Dijk et al., 1997; Boudry et al., 2002). Environmental pressures such as frequent rainfall and low winter temperatures favor this biennial behavior, allowing plants to delay reproduction until their second year of growth (Hautekeete et al., 2020). In northern regions, such as in the British Isles, populations may even exhibit perennial behavior, characterized by the persistence of seed stalks across multiple seasons (Doney et al., 1990; van Dijk et al., 1997). The species hence shows significant regional differences in its need for vernalization, with a clear north–south cline along the Atlantic coast of Europe (van Dijk et al., 1997). While the influence of gene *B* is substantial, other

genetic and environmental factors also contribute to the species' diversity regarding bolting behavior and flowering time. Ultimately, the realized lifespan and reproductive strategy of *Beta maritima* are shaped by both genetic composition and environmental influences, including climate, habitat stability, and the length of the growing season (Letschert & Frese, 1993; Boudry et al., 2002; Ascarini et al., 2021).

1.1.4 Habitat

Beta maritima thrives in coastal environments and demonstrates a remarkable ability to colonize a wide range of habitats, from loamy beaches to salt marshes (Doney et al., 1990; Stevanato et al., 2001). It shows a preference for heavy alluvial soils and clays at the upper part of beaches, where wastage is deposited by the high tide (Letschert & Frese, 1993). In northern parts of their distribution area, sea beets are typically confined to a narrow coastal strip, usually within 10 to 20 meters of the high-water mark (Doney et al., 1990; Letschert, 1993; Bartsch & Schmidt, 1997). These habitats, often characterized by high salinity, limit competition from other plant species, allowing sea beet to grow in isolation or alongside other wild flora such as *Brassica* species (Doney et al., 1990; Bartsch & Schmidt, 1997; Stevanato et al., 2001). *Beta maritima* thrives particularly in exposed coastal habitats such as pebble or shingle beaches, banks and rocky cliffs (Bertram et al., 2025a), whereas less favored habitats include dense grasslands or sandy shores (Doney et al., 1990; Letschert, 1993; Bartsch & Schmidt, 1997). Occasionally, *Beta maritima* has been reported in atypical habitats, including unpaved parking areas and vacant lots adjacent to beaches (Doney et al., 1990; Bartsch & Schmidt, 1997).

In contrast, populations in the Mediterranean and Middle Eastern regions display broader ecological diversity. While coastal habitats remain predominant, inland populations are frequently observed (Letschert, 1993; Boudry et al., 2002), for example in Sicily (Toll & Hendriksen, 1982) and southeastern Spain (Frese et al., 1990). These inland occurrences are found at ruderal sites, on mountain slopes up to 800 meters, in dry riverbeds, on roadsides, and plantation borders, with environments ranging from open, disturbed grounds to densely vegetated areas (Toll & Hendriksen, 1982; Letschert, 1993; Monteiro et al., 2013).

The multigermity of *Beta maritima* is considered a key factor in its ability to colonize new and remote habitats. Because multiple seeds are housed within a single glomerule, newly established populations are often founded by genetically related but distinct individuals (Fievet et al., 2007). This inherent genetic diversity helps the species overcome its high degree of self-sterility, enabling reproduction and increasing the likelihood of successful establishment even in isolated environments (Bartsch & Biancardi, 2020). This may account for the presence of sea beet in otherwise inaccessible locations, such as Mount Etna (Letschert & Frese, 1993).

1.2 *Beta maritima In Situ* Populations

1.2.1 The Importance of *In Situ* Conservation

Given the narrow genetic base of modern sugar beet cultivars (Fénart et al., 2008; Panella et al., 2020; Veloso et al., 2021), wild relatives such as *Beta maritima* are indispensable sources of adaptive traits for future breeding efforts. The remarkable adaptability of this taxon to diverse environmental conditions, along with its tolerance to various biotic and abiotic stresses, makes it a vital reservoir for sugar beet improvement (Panella et al., 2020). While material conserved *ex situ* in germplasm banks and botanical gardens represents an important source of genetic variation for breeding purposes (Hoban et al., 2018), this often fails to capture the full ecological complexity and evolutionary dynamics present in natural populations, potentially resulting in a gradual loss of genetic diversity over time (Bohra et al., 2022; Zucchini et al., 2024).

In contrast, *in situ* conservation preserves *Beta maritima* populations within their native habitats, maintaining their genetic diversity while enabling ongoing evolutionary processes driven by selection pressures (Zucchini et al., 2024). This dynamic context enables populations to continuously adapt to changing environmental conditions, ensuring the persistence of valuable traits relevant for crop improvement (Bohra et al., 2022).

However, despite its adaptability, *Beta maritima* populations face significant threats from habitat destruction, urban expansion, civil unrest, and natural disasters such as wildfires, floods, droughts, and volcanic activity (Bohra et al., 2022). Studies have observed a decline in both sea beet populations and the natural habitats essential for their survival over time,

due to both environmental changes and human activities (Doney et al., 1990; Stevanato et al., 2001; Monteiro et al., 2013; Zucchini et al., 2024). Coastal areas are increasingly being exploited for tourism, leading to habitat destruction and fragmentation (Toll & Hendriksen, 1982; Stevanato et al., 2001). The construction of cement sea walls, ports or other barriers to preserve the coastline and the recreational use of beaches and estuaries have altered the natural landscape, making it difficult for sea beet to thrive (Doney et al., 1990). Grazing by livestock, especially sheep or goats, poses another major threat to the species (Toll & Hendriksen, 1982; Doney et al., 1990). These activities degrade the habitats and restrict the natural distribution range of *Beta maritima* and increasing the vulnerability to environmental changes and genetic erosion (Monteiro et al., 2013). Ultimately, preserving the genetic diversity of *Beta maritima* through robust *in situ* conservation is essential for sustaining the evolutionary potential and breeding value of this crop wild relative, especially in the face of climate change and emerging agricultural challenges.

1.2.2 Collecting Populations: Practical and Legal Considerations

Collecting *in situ* populations of sea beet requires careful planning to ensure that the genetic diversity present in natural habitats is effectively captured and that collections are both scientifically robust and legally compliant, while ensuring that local populations are preserved and not harmed through sampling.

The potential for future research and breeding applications of conserved wild populations is fundamentally shaped by the sampling strategy employed during collection. Bulk sampling, in which seeds from multiple individuals within a population are collected and pooled, can provide a useful estimate of certain parameters such as allelic diversity but limits the ability to investigate finer-scale population characteristics (Hoban et al., 2018). In contrast, more intensive strategies, such as sampling multiple populations across a species' range and keeping seeds from individual maternal plants separate, enable a much broader array of downstream analyses, such as studies of genetic structure or geographic patterns. Well-planned, systematic sampling that captures both spatial and environmental diversity, and that preserves information on maternal genotypes, maximizes the potential for future research, breeding, and conservation, ensuring that collections remain relevant as new analytical methods and breeding needs emerge (Hoban et al., 2018).

When designing a sampling strategy, it is essential to sample individuals across the full spatial extent of each population to avoid collecting closely related plants and to maximize genetic diversity (Hoban et al., 2018). For outcrossing species such as sea beet, this means selecting well-spaced individuals that collectively represent the entire area occupied by the population. A representative sample size, adjusted to the total population size, is essential at each site to effectively capture both frequent and rare alleles. Additionally, recording detailed metadata such as GPS (global positioning system) coordinate for each sample is crucial to support more detailed analyses (Hoban et al., 2018).

When sampling wild beet populations *in situ*, researchers must also comply with international and regional legal frameworks, particularly the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization. In the European Union, this is implemented through Regulation 511/2014, which outlines compliance measures for users of genetic resources. Before collecting any material, researchers must determine whether the country of origin has ratified the Nagoya Protocol and established national access rules (Regulation 511/2014). If so, prior informed consent and mutually agreed terms must be obtained from the competent national authority (Regulation 511/2014). In our study, all sampling activities were conducted in full compliance with the Nagoya Protocol and relevant national legislation, and all procedures adhered to the principles of ethical and lawful access to genetic resources.

1.2.3 Studied Populations: Rationale and Relevance

For this study, seed of the three Northern Atlantic sea beet populations from Denmark, France, and Ireland was collected. The selection for these populations was guided by both scientific and practical considerations. These populations had already been partially characterized in previous studies, making them suitable candidates for further investigation. For instance, the population from Kalundborg in Denmark has been characterized in previous studies (Driessen, 2003; Andersen et al., 2005) and has further been used to identify the rhizomania resistance gene *Rz2* (Capistrano-Gossmann et al., 2017). The population from Brighton in France has previously been included in scientific studies (Fénart et al., 2008) and was also used in the validation of the *Rz2* resistance gene (Capistrano-Gossmann et al., 2017).

Further, these populations represented some of the remaining accessible sea beet populations in Northern Europe that had not yet been significantly affected by habitat degradation or urban development. In many coastal regions, wild beet populations have become increasingly difficult to locate due to environmental degradation or local extinction, making reliable *in situ* sampling a significant challenge (Zucchini et al., 2024). Given the limited time and resources available within the scope of a Ph.D. project, we prioritized sites with confirmed population presence to ensure efficient and successful collection. The populations were further selected for their predominantly biennial life forms, a characteristic attributed to their northern locations, which enhances their potential suitability for breeding applications (Van Dijk et al., 1997).

Sampling strategies were designed to capture the full extent of genetic diversity. Each population comprised more than 1,000 individuals, and plants were found in a wide range of habitats, from the edges of sandy beaches and gravel areas to rocky cliffs (Bertram et al., 2025a). Collections were carried out wherever the coastline was accessible, targeting all three populations along distinct coastal stretches; approximately 2 km in France, 11 km in Ireland, and 16 km in Denmark. Seeds from individual maternal plants were collected separately, with GPS coordinates recorded for each sample to support future spatial analyses. Further details regarding the collected populations are provided in chapter 3 (Bertram et al., 2025a).

1.3 The Theoretical Framework of Genome-Wide Association Studies

Genome-wide association studies (GWAS) are a statistical approach used to identify associations between genetic variants and phenotypic traits across a population. GWAS work by surveying the genomes of a large number of individuals, searching for genetic variants that occur more frequently in those with a specific phenotype compared to those without it (Korte & Farlow, 2013; Santure & Garant, 2018). The presence of the genetic variants, such as single nucleotide polymorphisms (SNPs), is correlated with trait variation (Santure & Garant, 2018). Importantly, GWAS identifies statistical associations, not causation. Such associations can be detected when the causative variants are in linkage disequilibrium with genotyped markers (Korte & Farlow, 2013). For instance, while populations with low linkage disequilibrium provide higher mapping resolution because the regions associated with traits are smaller, they also require a much higher marker density to achieve sufficient coverage of the genome (Garnier-Géré & Chikhi, 2013). Once associated variants are identified, they can be used to search for nearby variants that contribute directly to the trait.

Unlike traditional QTL mapping, which relies on controlled crosses and segregating populations such as F2-populations or recombinant inbred line families, GWAS can exploit naturally occurring genetic variation within a diverse set of individuals (Korte & Farlow, 2013). The statistical power to detect associations in GWAS is shaped by several factors, such as the experimental sample size, the effect sizes and frequencies of causal variants within the population, the number of loci influencing the trait, or the degree of linkage disequilibrium between genotyped markers and unknown causal variants (Visscher et al., 2017). To achieve robust and meaningful results, GWAS requires a sufficiently large and genetically diverse sample as well as adequate marker density to ensure comprehensive genome coverage and high mapping resolution (Korte & Farlow, 2013; Santure & Garant, 2018). High-quality phenotypic data and substantial phenotypic variation are also essential for accurately capturing trait differences (Korte & Farlow., 2013). Furthermore, careful consideration of population structure and relatedness is necessary, as these factors can confound associations and increase the risk of false positives (Santure & Garant, 2018). When these conditions are met, GWAS can serve as a powerful tool for uncovering genetic variants.

1.4 Objectives

The aim of this Ph.D. project was to investigate the genetic diversity of *Beta maritima* populations from the Northern Atlantic coast, using high-resolution genotypic data and simulation-based approaches. This work supports the integration of sea beet diversity into sugar beet improvement by identifying optimal strategies to develop mapping populations. Specifically, the objectives were:

1) To assess the current state of research on the characterization and diversity of *Beta maritima* populations.
2) To characterize the genetic diversity and population structure of three Northern Atlantic *Beta maritima* populations using high-density SNP markers.
3) To evaluate how the population characteristics of the three Northern Atlantic *Beta maritima* populations influence the power and accuracy of GWAS in detecting QTL.
4) To simulate and compare crossing strategies between wild and elite beet genotypes for the development of mapping populations optimized for QTL discovery in GWAS, particularly for traits with minor effects such as yield and drought tolerance.

Chapter 2

Morphological and Genetic Diversity of *Beta maritima* Populations Across Europe and North Africa: A Comprehensive Review[1]

[1] Bertram, L., and Frisch, M. (2026) Morphological and Genetic Diversity of Beta maritima Populations Across Europe and North Africa: A Comprehensive Review. *Front. Plant Sci.* 16:1731515.

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Morphological and genetic diversity of *Beta maritima* populations across Europe and North Africa: a comprehensive review

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Beta vulgaris ssp. *maritima* (sea beet), the wild ancestor of cultivated beet, represents a key reservoir of adaptive genetic diversity for sugar beet breeding. This review synthesizes research on morphological and genetic variation of *Beta maritima* populations across Europe and North Africa to (1) summarize regional diversity patterns, (2) assess the correspondence between phenotypic traits and genetic structure, and (3) identify knowledge gaps. Morphological studies show wide variation in sea beet. Growth habits range from prostrate to erect. Coastal plants often have thicker leaves and prostrate forms while inland types are adapted for water efficiency. Traits like pigmentation, inflorescence, and root shape also differ, reflecting adaptation to local environments. Bolting and flowering occur early in Mediterranean populations but are delayed in northern regions. Genetic analyses further identify a distinct Atlantic/Mediterranean divide. Mediterranean populations exhibit greater genetic diversity, while Baltic populations show low diversity and high homogeneity, presumably due to recent establishment and founder effects. Comparative findings suggest phenotypic variation often exceeds genetic differentiation and is strongly influenced by environmental factors. This review identifies research gaps among sea beet populations in Mediterranean regions particularly along the southern and eastern coasts of Spain, Italy, Greece, Turkey, and the eastern Mediterranean. As the first comprehensive review focused solely on *Beta maritima in-situ* populations, this work delivers a full account of the regions, traits, and genetic patterns studied to date. It establishes a foundation for future research and is an indispensable resource for advancing breeding, conservation, and scientific understanding of this important wild relative.

KEYWORDS

***Beta vulgaris* ssp. *maritima*, crop wild relatives, genetic diversity, genetic resources, sea beet**

Introduction

Beta maritima (*Beta vulgaris* ssp. m*aritima* (L.) Arcang.; sea beet), the wild ancestor of cultivated beet, is the most widespread taxon within the genus *Beta* (Frese and Ford-Lloyd, 2020; Figure 1). Its origin is traced to the Mediterranean region (Romeiras et al., 2016). Its distribution covers nearly all Mediterranean coastal countries, several Atlantic islands, and much of the Atlantic coast of Europe (Frese and Ford-Lloyd, 2020; Veloso et al., 2021; Ben Mahmoud et al., 2025). Following the last glacial period, Be*ta maritima* expanded northward, establishing populations along the Atlantic and North Sea coasts (Doney et al., 1990; Boudry et al., 2002; Fievet et al., 2007; Monteiro et al., 2013). Some populations are large and well-established, while others are small and scattered, reflecting both historical dispersal and recent colonization events (Letschert, 1993; Driessen, 2003; Andersen et al., 2005; Frese and Ford-Lloyd, 2020).

The broad distribution of *Beta maritima* reflects its evolutionary success and its ecological adaptability. It thrives in highly variable and often harsh coastal habitats, including salt marshes, beaches, and inland ruderal sites (Doney et al., 1990; Stevanato et al., 2001). This environmental heterogeneity, combined with strong local selection pressures, drives high morphological variation within and among populations (Toll and Hendriksen, 1982; Letschert and Frese, 1993; Abdelhameed et al., 2024; Ben Mahmoud et al., 2025). Traits such as growth habit, leaf morphology, and bolting behavior are shaped by adaptation to salinity, drought, and temperature extremes (Letschert and Frese, 1993; El Manhaly et al., 1996; Ben Mahmoud et al., 2025). The species' ability to colonize diverse habitats and maintain dynamic, *in-situ* populations preserves adaptive alleles that may be lost in *ex-situ* collections (Bohra et al., 2021).

Understanding the diversity of wild crop relatives like *Beta maritima* is essential for conservation and breeding efforts. Sea beet populations provide a reservoir of adaptive genetic variation, contributing valuable traits to sugar beet improvement (Frese et al., 1990; Panella et al., 2020). Over the past decades, numerous studies have documented the morphological variation among sea beet populations. In parallel to morphological investigations, advances in molecular genetics have enabled deeper insights into the population structure and genetic diversity of *Beta maritima*.

This review provides an overview of current research on the morphological and genetic diversity of *Beta maritima* populations across Europe and North Africa. It aims to (1) summarize regional patterns of morphological and genetic variation, (2) evaluate the relationship between observed phenotypic traits and underlying genetic structure, and (3) identify knowledge gaps and underexplored populations to support the utilization of *Beta maritima* as a genetic reservoir for sustainable sugar beet improvement.

Morphological variation

Morphological diversity in *Beta maritima* has been extensively studied, with numerous investigations exploring how geographic and environmental factors shape variation within and among populations (Table 1). Across its native range, sea beet populations exhibit pronounced variability in growth habit, leaf morphology, inflorescence structure, pigmentation, and root traits

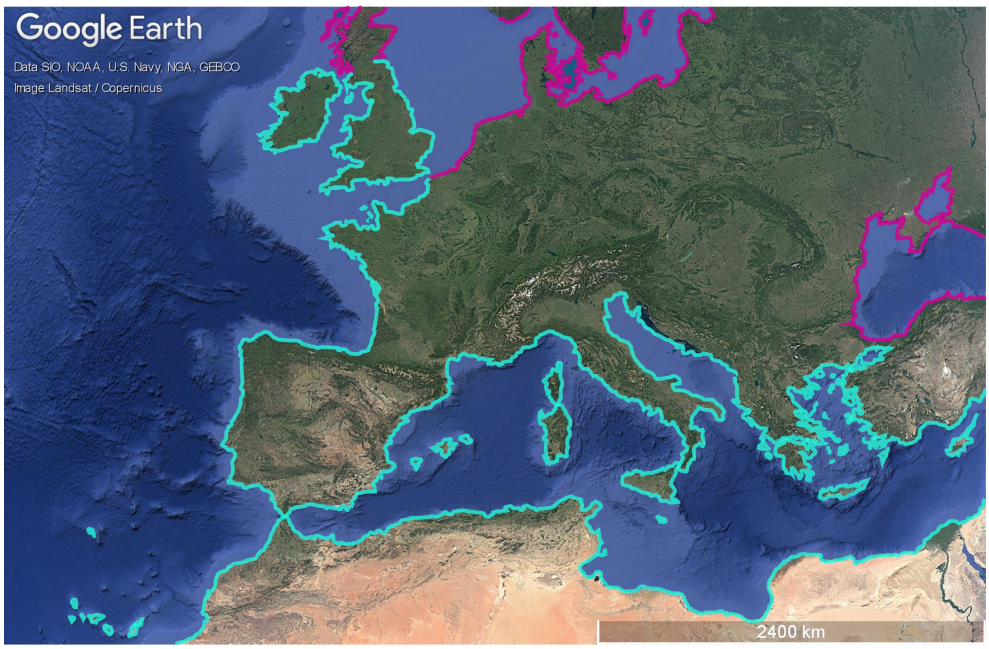

FIGURE 1
Map showing the general distribution of *Beta maritima* along the seashores within its main distribution area in Europe and Northern Africa (blue: frequent; purple: sparse). The map was generated using GoogleEarthPro. Adapted from Frese and Ford-Lloyd (2020).

TABLE 1 Summary of morphological diversity in *Beta maritima* populations examined across different studies.

Traits examined	Patterns of variation and key findings
Toll and Hendriksen, 1982 (Italy)	
	■ High variability in growth habit, leaf size, inflorescence, seedball size, and pigmentation ■ Variation in root tapering and side root number ■ Greater variation between populations than within ■ Morphology strongly linked to environment: ■ Dry sites: small plants with thick leaves ■ Open habitats: procumbent or prostrate growth
Doney et al., 1990 (England, Ireland, North Ireland, Wales)	
	■ British Isles sea beets: few leaf hairs, heavy waxy cuticle, very green appearance ■ Life cycle variation: annual and perennial forms observed; some populations highly uniform, others highly variable (older, established) ■ Greater morphological differences with increasing distance and geographic barriers
Frese et al., 1990 (Portugal, Spain)	
	■ Growth habit varied: erect types frequent on Iberian Peninsula ■ Plant size highly variable; old populations produced large seed quantities ■ Despite geographic barriers, little morphological differentiation - spatial separation does not seem to prevent gene flow ■ Bay of Aerosa: exceptionally high morphological variability observed; may result from admixture of South and North Atlantic gene pools
Letschert and Frese, 1993 (Italy)	
15 morphological characters: pigmentation, leaf pubescence, bract shape, multigermicity, growth habit, flowering stage (2 periods), lamina (length, width, thickness), petiole (length, width), stem diameter, biomass (fresh weight), plant height	■ High variation in lamina length, width, and thickness, petiole length and width, stem diameter, biomass, plant height, flowering (both periods) ■ Tendency for decreasing leaf length and width from north to south ■ No single trait reflected clear regional pattern ■ Significant differences between adjacent populations; coastal populations showed large fluctuations ■ Inland vs. maritime groups differed for some characters
El Manhaly et al., 1996 (Egypt)	
18 morphological characters: growth habit, leaf (erectness, hairiness, thickness), leaf blade (length, width, pigmentation), petiole (length, width), petiole color, hypocotyl pigmentation, external root color, main color flesh, root shape, flower stern pigmentation, male sterility, multigermicity, bolting tendency *9 evaluators for resistance:* curly top, Rhizoctonia, leaf spot, cyst nematode, root aphid, Rhizomania, virus yellows, root maggot, Aphanomyces	■ Variation in growth habit, bolting behavior, leaf shape, roots ■ Two main groups identified: ■ Delta: prostrate, small thick leaves, no leaf hair ■ Luxor/Fayum: segregating for leaf size, growth habit, root swelling, bolting, and red types ■ Inland types had longer, narrower petioles and less succulent leaves than coastal types ■ Bolting occurred very early, complicating disease resistance scoring ■ Moderate curly top resistance found in Fayum/Luxor accessions; root aphid resistance noted in some populations
Bartsch and Schmidt, 1997 (Italy)	
8 morphological characters: bigermity, hypocotyl color, leaf (color, pubescence), pollen sterility, seed (cluster weight, emergence), annuality	■ Differentiation into three types based on morphological characters ■ Evidence of hybridization between wild beets and cultivars found (i.e. supported by bigermous individuals, low proportion of annual individuals [26.5%] among offspring)
Van Dijk et al., 1997 (Belgium, England, France, Guernsey, Jersey)	
vernalization requirements, sensitivity for vernalizing factors, flowering date	■ Strong variation in flowering time is linked to latitude and vernalization requirement (mainly determined by a single gene *B/b* and quantitative trait loci) ■ Southern populations: high frequency of *B* allele (no vernalization needed); northern populations: strong vernalization requirement ■ High heritability (0.33) for flowering time → potential for evolutionary change.

(Continued)

TABLE 1 Continued

Traits examined	Patterns of variation and key findings
Stevanato et al., 2001 (Italy)	
	■ Significant morphological variability in leaf shape and size; deep green leaves, occasional red streaks ■ Variability in the reproductive structure (seed stalks) ■ Populations showed resistance to Cercospora and Rhizomania
Boudry et al., 2002 (Belgium, France, The Channel Islands)	
Vernalization requirements, flowering response to cold periods at young seedling stage	■ Vernalization requirement increased with latitude; northern plants require more vernalization ■ Young seedlings more difficult to vernalize than plants which already developed vegetative rosettes ■ Penetrance of the annual habit in *Bb* genotypes was affected by both environmental and genetic factors
Ascarini et al., 2021 (Portugal)	
11 morphological characters: number of basel stems, plant height, inflorescence height, distance of the first branch from the basis, number of branches, leaf (length, width), petiole (length, width), stem diameter, average number of glomerulus per branch	■ Morphological characterization showed a high quantitative variation among populations ■ Plant height and inflorescence height parameters had the highest influence in the separation of populations ■ Populations in less exposed sites tend to have bigger and more developed aerial parts; plants closer to the sea or under dry/saline conditions are more prostrate with smaller leaves
Abdelhameed et al., 2024 (Egypt)	

35 morphological characters: plant length, stalk (length, diameter), number of branches/stalk, branch length, lower & upper leaf lamina (length, width), lower & upper leaf petiole (length, width), inflorescence bract lamina (length, width), inflorescence bract petiole (length, width), lower & upper glomerule bract lamina (length, width), lower & upper glomerule bract petiole (length, width), lower & upper glomerule (length, width), lower & upper glomerule bract length/glomerule length, inflorescence/branch, number of glomerule/inflorescence, number of flowers/glomerule	■ Notable morphological diversity observed within and among populations
	■ Significant differences in 17 of 35 traits; branch length and inflorescence traits showed highest variability
	■ Two groups identified:
	  ■ *var. glabra:* glabrous, erect, large basal leaves
	  ■ *var. pilosa:* hairy, prostrate, smaller basal leaves
	■ Soil parameters significantly influenced population morphological variability; especially strong correlation to soil organic carbon

Ben Mahmoud et al., 2025 (Tunisia)	
23 morphological characters: growth habit, stem (color, pigmentation, hairiness), leaf (color, pigmentation, curliness, hairiness, shape, blade length, blade width), petiole (color, length, width), cuticle thickness, bract (shape, thickness), inflorescence (color, height), multigermicity, flowering pattern between plants, glomerule diameter, 1000 seed weight	■ Substantial morphological variability within and between populations ■ Traits like glomerule diameter, seed weight, and inflorescence height showed high between-population variability ■ Island populations: prostrate habit, red inflorescences ■ Mainland populations: erect habit, hairy curly leaves ■ Strong phenotypic plasticity; Adaptation to harsh conditions (salinity, heat, drought) evident, i.e. reducing leaf size allows plants to reduce water loss by evapotranspiration

The table lists the main traits analyzed, key findings, and principal patterns of variation observed within each study. Countries in brackets indicate the origin of the analyzed populations.

(Toll and Hendriksen, 1982; Letschert and Frese, 1993; Stevanato et al., 2001; Abdelhameed et al., 2024; Ben Mahmoud et al., 2025).

Morphological differentiation is strongly influenced by environmental factors. Traits such as plant size, leaf thickness, and growth form are strongly linked to habitat conditions. Plants in dry or exposed environments tend to be smaller with thicker leaves, while those in open, resource-rich habitats often develop more expansive, procumbent forms (Toll and Hendriksen, 1982; Abdelhameed et al., 2024; Ben Mahmoud et al., 2025). Exposure to stressors like high salinity, drought, or heat further drives adaptive changes, including reduced leaf size and increased cuticle thickness (Ben Mahmoud et al., 2025). Soil properties, especially organic carbon content, also significantly influence morphological variability (Abdelhameed et al., 2024).

A consistent pattern emerges when comparing inland and coastal populations. Inland types typically have longer, narrower petioles and less succulent leaves, whereas coastal populations display shorter, thicker leaves and a more prostrate growth habit (Letschert and Frese, 1993; El Manhaly et al., 1996). Coastal populations are also more likely to bolt and flower early, while inland types often show delayed generative development. These patterns reflect adaptation to contrasting environmental pressures. Coastal habitats favor compact, robust morphology, while inland environments select for traits that enhance water use efficiency and competitive ability (Letschert and Frese, 1993; El Manhaly et al., 1996).

The extent to which morphological variation correlates with geography is variable. Some studies report substantial diversity

within short coastal stretches (Abdelhameed et al., 2024), while others find only minor divergence across broader regions (Letschert and Frese, 1993). In the British Isles, morphological variation increases with geographic distance and physical barriers, highlighting the influence of dispersal mechanisms and environmental heterogeneity (Doney et al., 1990).

A clear distinction is observed between Atlantic and Mediterranean populations. Mediterranean populations generally exhibit greater morphological and genetic diversity, with a wider range of growth forms and adaptive traits, while Atlantic populations, especially those in the Baltic and North Sea regions, are more uniform and often display traits associated with recent colonization and founder effects (Frese et al., 1990; Richards et al., 2014; Andersen et al., 2005). This regional contrast is also reflected in the frequency of key adaptive alleles, such as gene *B*, which controls vernalization requirement and flowering time. The *B* allele, which eliminates the need for vernalization and promotes early bolting, is frequent in Mediterranean populations but largely absent in northern populations, contributing to the observed differences in life history strategies (Van Dijk et al., 1997; Boudry et al., 2002).

Much of this diversity is attributable to phenotypic plasticity – *Beta maritima's* ability to adapt its morphology in response to environmental variation (Ribeiro et al., 2016; Ascarini et al., 2021). Nevertheless, genetic control is also evident, as shown by the identification of major genes (e.g. gene *B*) and quantitative trait loci affecting flowering and growth (Van Dijk et al., 1997; Boudry et al., 2002). The interplay between plasticity and genetic differentiation complicates the interpretation of regional patterns and underscores the need for integrative approaches.

Beyond basic morphology, few studies also described variation in agronomically relevant traits such as disease resistance and stress tolerance. For example, resistance to important pathogens such as Cercospora leaf spot and Rhizomania has been documented in certain wild populations (Stevanato et al., 2001). Moderate resistance to curly top virus and root aphid has also been identified in populations from Egypt (El Manhaly et al., 1996). However, most findings are based on phenotypic screening rather than genetic confirmation. Nevertheless, these findings underscore the value of *Beta maritima* as a genetic resource for breeding programs, offering a reservoir of adaptive traits that can be harnessed to improve stress tolerance and disease resistance in cultivated beets.

Genetic diversity

Following extensive research on morphological variation in wild sea beet, recent studies have increasingly focused on genetic diversity and population structure using molecular techniques (see Table 2 for an overview of diversity measures across studies). These genetic analyses have uncovered both broad-scale patterns and distinct regional differences within *Beta maritima* populations. Importantly, genetic differentiation across Europe and North Africa reflects a complex, multi-layered process. Rather than being determined solely by geographic distance, population structure in *Beta maritima* populations is shaped by a combination of historical events, dispersal mechanisms, and environmental heterogeneity.

A key pattern of variation, the Atlantic–Mediterranean divide, originates from glacial history. Mediterranean regions acted as refugia during the last glacial period, preserving high allelic richness and heterozygosity. Northern populations on the other hand were recolonized more recently, resulting in reduced diversity and greater genetic uniformity within these populations (Driessen et al., 2001; Driessen, 2003; Andersen et al., 2005). Comparative studies confirm that Mediterranean populations hold more alleles and show stronger substructure, whereas Northern Atlantic and Baltic groups exhibit high gene flow and low differentiation (Desplanque et al., 1999; Leys et al., 2014; Richards et al., 2014; Veloso et al., 2021; Bertram et al., 2025a).

Specifically Baltic and North Sea populations exhibit genetic homogeneity and low polymorphism, reflecting founder effects and bottlenecks associated with recent colonization and seed dispersal via ocean currents (Driessen et al., 2001; Driessen, 2003). Danish and Swedish populations show high gene flow and no internal structure, consistent with wind pollination and long-distance dispersal (Andersen et al., 2005; Bertram et al., 2025a). These northern groups remain distinct from Mediterranean and Atlantic populations, reflecting their restricted genetic base and recent origin.

Different dispersal mechanisms also influence population structure. Coastal populations showed strong geographic clustering shaped by marine currents (Leys et al., 2014). Inland ruderal populations exhibit more complex genetic structures due to admixture and human activities, such as soil and plant movement and habitat modification (Letschert, 1993; Leys et al., 2014). These actions introduce and mix genetically distinct individuals, increasing gene flow and hybridization, and disrupting clear geographic genetic patterns. Nuclear genes, spread by both pollen and seeds, enable broader gene flow and genetic mixing across regions. In contrast, mitochondrial genes, dispersed only through seeds, are more restricted in their movement, resulting in stronger spatial genetic structuring (Fénart et al., 2008). For example, along the French Atlantic and Channel coasts, an asymmetric gene flow shaped by marine currents and differences in nuclear versus cytoplasmic dispersal was observed (Fievet et al., 2007). This demonstrates how the mode of gene transmission shapes genetic patterns in wild plant populations.

Within these broader patterns, distinct regional variations introduce additional complexity. Admixture signals, such as clustering between French Atlantic and Corsican individuals, highlight ongoing connectivity despite distance (Richards et al., 2014). Similar dynamics occur in Iberian and Macaronesian systems, where marine currents and isolation create admixture gradients. Northern groups are more differentiated, while southern and insular populations form mixing zones (Veloso et al., 2021).

Environmental heterogeneity also plays a role in genetic differentiation of populations. For instance, the presence of mixed ploidy levels in Portuguese *Beta* taxa (Castro et al., 2013) and the

TABLE 2 Overview of diversity measures reported across studies. .

Study	Country												Number and type of genetic marker	N_A	A_R	N_P	H'	H_O	H_E	F_{IT}	F_{ST}	F_{IS}
	SE	DK	DE	IE	NL	GG	JE	FR	PT	ES	IT	MA										
Desplanque et al., 1999								X					5 RFLP & 1 microsatellite	3 – 26				0.508 – 0.796	0.609 – 0.900		0.020 – 0.204	0.085* – 0.318**
Driessen et al., 2001		X	X										75 RAPD-PCR fragment loci			8.1 – 27.0	0.046 – 0.144					
Driessen, 2003	X	X	X										78 RAPD-PCR fragment loci & 1 AFLP			6.4 – 34.6	0.038 – 0.182					
Andersen et al., 2005	X	X		X	X			X			X		8 SSR	1 – 8				0.040 – 1.000	0.040 – 0.810		(0.310***)	-0.800* – 0.530^{NS}
Fievet et al., 2007						X	X	X					7 micro- & 4 minisatellites	3.85 – 7.14	3.350 – 5.580				0.410 – 0.600	0.086*** – 0.207*** (0.151***)	0.015^{NS} – 0.119*** (0.089***)	-0.012^{NS} – 0.100*** (0.068***)
Fénart et al., 2008								X					5 micro-, 4 minisatellites & 1 PCR-RFLP	1 – 6	1.000 – 5.410				0.000 – 0.880		0.147***	-0.441^{NS} – 0.439***
Leys et al., 2014								X	X	X		X	8 micro- & 4 minisatellite	3 – 56	3.650 – 12.043				0.418 – 0.831	0.132* – 0.223*	0.126* – 0.177*	0.007^{NS} – 0.087*
Richards et al., 2014								X	X				13 SSR	3 – 31	2.020 – 6.320			0.290 – 0.690	0.400 – 0.690		(0.140)	
Ribeiro et al., 2016									X				6 SSR	3 – 15	7.670 – 10.170			0.480 – 0.906	0.300 – 0.900		0.019 – 0.121 (0.052)	
Ascarini et al., 2021									X				8 SSR	22 – 38			0.856 – 1.381	0.400 – 0.650	0.575 – 0.787			-0.423 – 0.622 (0.186)
Veloso et al., 2021								X	X	X			6 SSR	7 – 25	2.333 – 10.167			0.044 – 0.783	0.074 – 0.789		0.000 – 0.277	-0.041 – 0.287
Bertram et al., 2025a		X		X				X					16,201 SNPs					0.099 – 0.167	0.430 – 0.780			

Countries from which populations were examined, SE, Sweden; DK, Denmark; DE, Germany; IE, Ireland; NL, The Netherlands; GG, Guernsey; JE, Jersey; FR, France; PT, Portugal; ES, Spain; IT, Italy; MA, Morocco. Diversity measures include N_A, number of alleles per locus; A_R, allelic richness; N_P, polymorphic fragments (%); H′, Shannon Index; H_O, observed heterozygosity; H_E, expected heterozygosity; and F-statistics (F_{IT}; F_{ST}; F_{IS}). Ranges of minimum and maximum values are shown; mean values are provided in brackets where available. Significance levels; if specified in the original studies: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; NS, non-significant.

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high genetic diversity found in salt marsh populations (Ribeiro et al., 2016) point to a strong adaptive potential linked to diverse habitats. Such habitat heterogeneity creates a mosaic of selective pressures, fostering local adaptation and maintaining genetic variation. Additionally, the pronounced variability observed in Madeira and Porto Santo demonstrates that environmental selection can sometimes override the effects of geographic distance, leading to distinct population characteristics even within relatively small regions (Ascarini et al., 2021).

Overall, geographic distance alone is an unreliable proxy for genetic differentiation. Population structure results from glacial history, dispersal mechanisms, and ecological selection.

Discussion

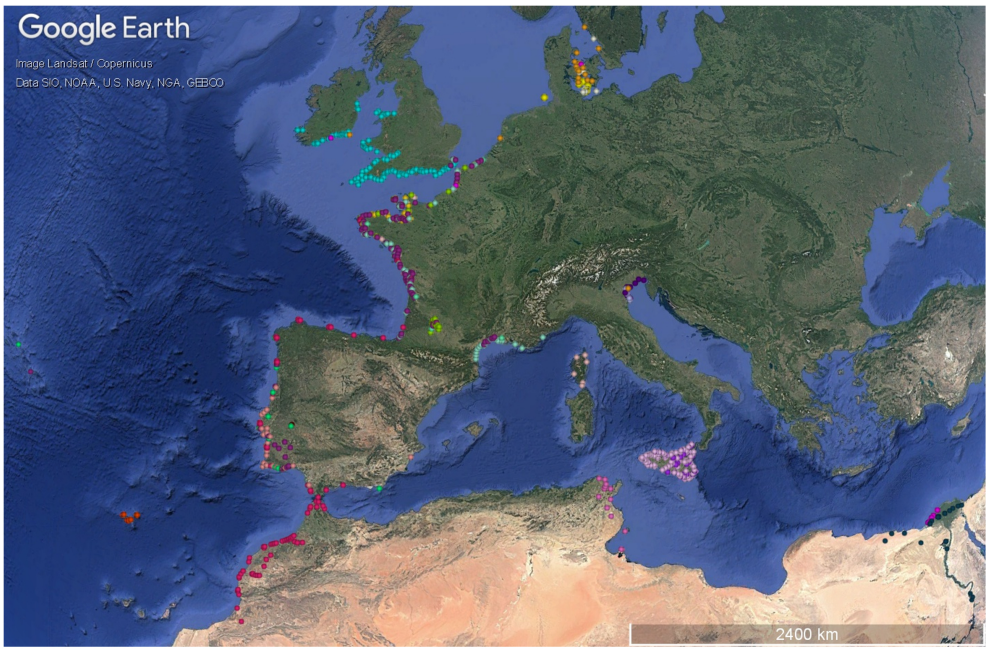
Research on sea beet populations has progressed from early morphological studies to increasingly sophisticated genetic analyses. Initial work focused on phenotypic traits to infer diversity and adaptation, revealing substantial variation shaped by geography, environment, and local selection pressures. With the advancement of molecular techniques, research has shifted toward examining genetic diversity and population structure, offering deeper insights into evolutionary processes and genetic diversity (Desplanque et al., 1999; Andersen et al., 2005; Leys et al., 2014; Bertram et al., 2025a).

A key observation is that phenotypic variation in sea beet populations often exceeds genetic differentiation and is strongly influenced by environmental conditions (Ribeiro et al., 2016; Ascarini et al., 2021; Abdelhameed et al., 2024). The species' outcrossing mating system, along with high pollen and seed dispersal, promotes gene flow and hence genetic mixing among populations. At the same time, the high phenotypic plasticity of *Beta maritima* enables individuals to respond flexibly to strong environmental gradients across their range. This plasticity complicates the interpretation of morphological data, especially when environmental variation is pronounced. As a result, morphological traits may not reliably reflect underlying genetic relationships. While some studies have identified major genes, such as the *B* gene for vernalization requirement, comprehensive integration of phenotypic and genetic data remains limited. Notably, there are successful examples of wild alleles being introgressed into cultivated beet, such as the *Rz2* gene for Rhizomania resistance (Capistrano-Gossmann et al., 2017). However, most research has focused on either morphological or genetic variation in isolation, so the extent to which genetic architecture explains morphological patterns is only partially examined. Agronomic traits, especially disease and pest resistances, have been evaluated only minimally across sea beet populations. This gap, likely also due to the difficulty of evaluating such traits in wild material directly (Bertram et al., 2025b), highlights the need for further studies to systematically assess and characterize this diversity.

Comparative studies have consistently identified two distinct genetic groups among sea beet populations – one from the Atlantic and one from the Mediterranean region, with the latter generally exhibit greater genetic and morphological diversity (Desplanque et al., 1999; Richards et al., 2014). This divide has been further confirmed by sequence analyses of 239 sea beet *ex-situ* accessions from germplasm banks (Sandell et al., 2022; Felkel et al., 2023). This division reflects the influence of both historical events and evolutionary processes on population structure. During the last glacial period, Mediterranean regions acted as refugia and preserved high ancestral diversity. In contrast, post-glacial recolonization toward the north caused genetic bottlenecks and reduced allelic richness in northern populations (Driessen et al., 2001; Driessen, 2003; Andersen et al., 2005).

Nevertheless, this does not mean that northern populations lack valuable alleles for breeding. For example, Capistrano-Gossmann et al. (2017) identified the *Rz2* resistance gene to Rhizomania, which is of major importance for breeding, in a Danish sea beet population. Other studies have also reported unique alleles and polymorphisms in northern Atlantic populations (Andersen et al., 2005; Bertram et al., 2025a). While it remains unclear which of these alleles hold practical value for breeding, the implications are significant. This highlights the importance of *in-situ* conservation, specifically of genetically unique micro-populations, to preserve unique genetic variants. It also underscores the need for broad sampling and comprehensive testing to fully uncover useful genetic diversity across all regions. Genomic tools, including high-density SNP arrays and whole-genome sequencing, can aid with the identification of candidate alleles for introgression and the systematic assessment of genetic resources (Andrello et al., 2017; Felkel et al., 2023).

Mediterranean populations, with their high diversity and high amount of unique alleles, surely represent valuable sources for crop improvement. Especially their ability to thrive even under challenging conditions, such as drought or high salinity, makes them especially valuable for breeding programs aiming to develop more resilient cultivars. However, despite evidence of rich diversity, Mediterranean sea beet populations remain notably underrepresented, especially in genetic studies (Figure 2). Notable gaps exist along the southern and eastern coasts of Spain, the Italian coastline and Sardinia, all of Greece, the western coast of Turkey, and other eastern Mediterranean regions. Although genebank accessions sampled from these areas confirm the historical presence of *Beta maritima* (Andrello et al., 2016), many of these populations have not been genetically characterized. Zucchini et al. (2024) also noted discrepancies between the presence of *in-situ* populations and the original collection sites of *ex-situ* accessions. One possible reason for this is the ongoing decline of natural habitats, which threatens the survival of sea beet populations (Doney et al., 1990; Stevanato et al., 2001). However, this does not fully explain the lack of data, as recent studies still report widespread occurrences, for example along the Italian coast (Zucchini et al., 2024). This highlights a significant research gap. Although Mediterranean populations are known to exist and contribute substantial diversity, they remain largely uncharacterized. Future research should prioritize these regions to better capture the full spectrum of sea beet diversity and its breeding potential.



Study	P	N	M	G	Country													
					SE	DK	DE	BE	GB	IE	NL	GG	JE	FR	PT	ES	IT	MA	TN	EG
Toll & Hendriksen, 1982	98		X														X			
Doney et al., 1990	143		X						X	X										
Frese et al., 1990	29		X												X	X				
Letschert & Frese, 1993	35		X														X			
El Manhaly et al., 1996	26		X																	X
Bartsch & Schmidt, 1997	21		X														X			
Van Dijk et al., 1997	93	1–30	X					X	X			X	X	X						
Desplanque et al., 1999	54	~2–5		X										X						
Driessen et al., 2001	11	~10		X		X	X													
Stevanato et al., 2001	13		X														X			
Boudry et al., 2002	7	8–9	X					X				X		X						
Driessen, 2003	23	3–10		X	X	X	X													
Andersen et al., 2005	18	~25		X	X	X				X	X			X			X			
Fievet et al., 2007	33	18–62		X										X						
Fénart et al., 2008	23	27–52		X										X						
Castro et al., 2013	21	≤26													X					
Leys et al., 2014	88	ø19.5		X										X	X	X		X		
Richards et al., 2014	28	12		X										X						
Ribeiro et al., 2016	3	30–34		X											X					
Ascarini et al., 2021	11	5	X	X											X					
Veloso et al., 2021	14	9–45		X											X	X				
Abdelhameed et al., 2024	6		X																	X
Ben Mahmoud et al., 2025	10		X																X	
Bertram et al., 2025a	3	388–509		X		X				X				X						

FIGURE 2
Geographic distribution of *Beta maritima* populations sampled within the studies covered by this literature review. Locations are based either on GPS coordinates provided in the original publications or inferred from maps therein. Populations are color-coded by study. Only *Beta maritima* populations are shown. P = total number of sea beet populations (locations) sampled, N = number of individuals per sampled population. Type of study: M = morphological: G = genetical characterization. Countries from which populations were evaluated with each study are indicated: SE, Sweden; DK, Denmark; DE, Germany; BE, Belgium; IE, Ireland; NL, The Netherlands; GG, Guernsey; JE, Jersey; FR, France; PT, Portugal; ES, Spain; IT, Italy; MA, Morocco; TN, Tunesia; EG, Egypt. The map was generated using GoogleEarthPro.

Despite recent progress, the value of many genetic studies on sea beet populations is limited by small sample sizes and a narrow set of genetic markers, restricting insights into the full genetic architecture of these populations (Figure 2). While these studies provide useful estimates of heterozygosity and allelic richness in specific regions, they offer only a partial view of overall diversity. The work by Bertram et al. (2025a) represents a major advancement, applying high-density SNP genotyping across large and diverse populations. This approach enables a more comprehensive analysis of genetic variation, population substructure, and mapping potential, setting a new benchmark for future research. However, while genome-wide SNP data represent a significant improvement over single-marker approaches, SNP panels are often developed based on cultivated material and may suffer from ascertainment bias (Andrello et al., 2017), potentially underrepresenting rare or novel alleles in wild populations. To fully capture the genetic diversity in *Beta maritima* populations and detect also structural variants such as insertions and deletions

(Felkel et al., 2023), whole-genome sequencing would be preferable. Sequencing technologies are becoming increasingly accessible and are likely to become the method of choice for future diversity studies.

Ultimately, the value of sea beet for crop improvement depends not only on the presence of genetic diversity but also on the ability to identify and utilize alleles conferring desirable traits. A key challenge remains: How to evaluate breeding potential without extensive, resource-intensive testing? First studies have begun to tackle this question. Capistrano-Gossmann et al. (2017) demonstrated an approach to identify resistance genes directly within sea beet populations without time-consuming material development. Building on this, Bertram et al. (2025b) used simulation studies to design suitable development schemes for evaluating even complex traits like yield.

Looking forward, addressing current knowledge gaps through integrated genomic, phenotypic, and environmental research is essential to fully harness the potential of *Beta maritima* for sugar beet improvement. Combining morphological, genomic, climatic, and soil data will provide a more comprehensive understanding. Genome-wide scans and landscape genomics can reveal adaptive variants and clarify the environmental drivers of genetic differentiation. Additionally, systematic phenotyping for stress and disease traits, together with the integration of *ex-situ* and *in-situ* datasets, will help resolve inconsistencies and maximize the utility of wild genetic resources for both breeding and conservation.

In summary, sea beet populations exhibit remarkable morphological and genetic diversity shaped by geography, environment, and dispersal dynamics. The transition from morphological to genetic characterization has greatly enhanced our understanding of wild sea beet diversity. Nevertheless, significant gaps remain, particularly in the underexplored Mediterranean populations and in translating genetic variation into breeding value to unlock the full value of *Beta maritima* populations for resilient and sustainable crop development.

Author contributions

LB: Conceptualization, Data curation, Visualization, Writing – original draft, Writing – review & editing. MF: Writing – review & editing.

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Conflict of interest

Author LB was employed by KWS SAAT SE & Co. KGaA.

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Chapter 3

Exploring the Diversity of Three Northern Atlantic Sea Beet Populations[1]

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Exploring the diversity of three Northern Atlantic sea beet populations

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Sea beet [*Beta vulgaris* ssp. *maritima* (L.) Arcang.] populations exhibit high genetic diversity and possess valuable traits that could enhance resilience, disease resistance, and tolerance to abiotic stress. In contrast, the genetic variation in the sugar beet breeding gene pool is limited. Our objectives were to examine sea beet population diversity using high-density marker data and substantial sample sizes within a population simultaneously. Their genetic diversity and population structure were investigated to evaluate their potential for direct mapping of traits with association mapping. Within this study, a total of 1,363 genotypes across three Northern Atlantic sea beet populations from Denmark, France, and Ireland were analyzed using 16,201 SNP markers. The findings reveal genetic variation among the populations, with the Irish population exhibiting the highest genetic diversity and pronounced population structure. The Danish population showed low genetic diversity and minimal population structure, while the French population displayed intermediate levels of both, genetic diversity and population structure. Based on its high genetic diversity, the Irish population appears to have the most potential for use to directly map traits by association mapping, provided that the challenges posed by the severe population structure can be adequately addressed within analysis.

KEYWORDS

***Beta vulgaris* ssp. *maritima*, beta maritima, sea beet, crop wild relatives (CWR), genetic diversity, population structure, association mapping**

1 Introduction

Sea beets or wild beets [*Beta vulgaris* ssp. *maritima* (L.) Arcang.] are wild relatives of cultivated sugar beet. Sea beets have a large area of distribution along the Mediterranean and North Atlantic coasts, from the British Isles to the Canary Islands (Doney et al., 1990). A difference in vernalization requirements and hence flowering time related to latitude can be observed across their area of distribution (Van Dijk et al., 1997). While populations from the Mediterranean Sea and the Atlantic coast up to Brittany are observed to have little to no vernalization requirement, Northern Atlantic populations exhibit a low frequency of

FIGURE 1
(A) Map showing the geographic locations of the three collected sea beet populations in northern Europe: **(B)** Denmark, **(C)** Ireland, and **(D)** France. Each marker represents the geographical location of one sampled sea beet. The maps were created using Google Earth Pro.

flowering within the first year (Van Dijk et al., 1997). This facilitates the use of Northern Atlantic populations in breeding.

The breeding gene pool of sugar beet is narrow, and it is considered to lack sufficient genetic variation to cope with stress (Fénart et al., 2008; Panella et al., 2020; Veloso et al., 2021). Sea beet populations possess a high level of genetic diversity with useful traits that can be harnessed to improve crop resilience, disease resistance or tolerance against abiotic stress (Ribeiro et al., 2016; Panella et al., 2020; Ascarini et al., 2021; Veloso et al., 2021; Sandell et al., 2022). At the same time, the low linkage disequilibrium observed in sea beet populations due to many generations of outcrossing generally allows for the direct use of these populations in association mapping (Capistrano-Gossmann et al., 2017). Hence, sea beet populations hold great potential for crop improvement in breeding programs.

There is an increasing number of studies on the genetic diversity of sea beet populations. Some of these studies focus on assessing the diversity based on morphological characteristics of sea beet populations sampled along the coasts of England and Ireland (Doney et al., 1990), Northern Italy (Bartsch and Schmidt, 1997), in Egypt (Abdelhameed et al., 2024) or Tunesia (Ben Mahmoud et al., 2025).

Other studies focus on the genetic diversity of sea beet populations. Sea beet populations along the coast of Dorset (Poole Harbour and adjacent coast) were analyzed for seven polymorphic isozyme loci and six polymorphic RFLP loci (Raybould et al., 1996, 1998). A total of 300 individuals across 54 populations from France was analyzed using five single-copy RFLP and one microsatellite marker (Desplanque et al., 1999). Twelve Danish, two Swedisch, one French, one Italian, one Dutch and one Irish population of sea beets were screened with eight microsatellite markers to evaluate genetic variation and gene flow of around 25 individuals per population (Andersen et al., 2005). Sea beet populations alongside the coast of the English Channel in Guernsey, Jersey and Northern France were analyzed with seven microsatellite loci, with sample sizes ranging from 18 to 62 individuals per population (Fievet et al., 2007). Eleven sea beet populations, each consisting of 27 to 49 individuals were sampled along the Channel French coastline and genotyped with five nuclear microsatellite loci (Fénart et al., 2008). Three Portuguese sea beet populations were evaluated based on six microsatellite markers (Ribeiro et al., 2016). Fourteen plants from each of eleven Madeiran sea beet populations were characterized using morphological descriptors and eight polymorphic Simple Sequence Repeats markers (Ascarini et al., 2021). Wild beet populations from fourteen locations in western Iberia, the Azores and Madeira islands were analyzed based on 9 to 35 plants per populations using six SSR loci amplifying a total of 100 alleles (Veloso et al., 2021). While these studies provide a first insight into the diversity of sea beet populations, they rely on either a limited number of individuals per population, a small set of markers, or both. This limits the possibility of generalizing the results.

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FIGURE 2
Pictures of the *Beta vulgaris* ssp. *maritima* plants found in the sampled regions: **(A–G)** Ireland, **(H–L)** France, and **(M–P)** Denmark. Not all plants shown were sampled and are included in analysis (due to seed availability). The pictures demonstrate the diversity of phenotypes within the sampled regions.

Few newer studies analyze sea beet accessions from gene banks. 1,054 *Beta vulgaris* ssp. *maritima* accessions were analyzed with 4,436 DArT markers (Andrello et al., 2016). Around 240 sea beet accessions were analyzed with short-read sequencing data to study genomic relationships within the genus *Beta* (Sandell et al., 2022; Felkel et al., 2023). While these analyses are based on multiple markers or even sequencing data, they are based on one or few plants per accession and, hence, cannot capture the full diversity of the corresponding sea beet populations or give insight into their structure.

While most of these studies have compared populations from different geographic origins, a comprehensive diversity analysis of sea beet populations using large sample sizes per population and high-density markers has yet to be conducted.

The aim of this study is to deepen the understanding of the diversity and genetics of sea beet populations to enable their use as a

TABLE 1 Characteristics of the genetic data of the analyzed populations after quality control.

Population	Genotypes	Monomorphic markers	Polymorphic markers	Monomorphic only in this population	Polymorphic only in this population
Denmark	388	1,073	15,128	524	163
France	509	1,476	14,725	942	178
Ireland	466	668	15,533	264	308

All populations were analyzed using the same set of 16,201 markers. All markers remaining after quality control were heterozygous in at least one individual of one population, resulting in some markers being monomorphic across one or two populations.

source of genetic variation in sugar beet breeding. While genetic diversity is an indicator of the potential of a sea beet population to contribute new, valuable variation, the structure of a population affects how this variation can be used in breeding. For this purpose, three sea beet populations form the Northern Atlantic coast were chosen due to their predominantly biannual lifeform and analyzed with 16,201 SNP markers. Our objectives were to (1) analyze the genetic diversity of these populations, (2) assess their population structure and detect possible subpopulations, and (3) evaluate the implications for their use in sugar beet breeding, particularly concerning their suitability for direct trait mapping through association mapping.

2 Materials and methods

2.1 Plant material

This study is based on three wild beet populations from the coast of the Northern Atlantic Sea. Seeds were sampled from the populations in their natural habitat along coastal regions in Denmark, France and Ireland. All three populations were collected from a rather small stretch along the coastline: France ~2km, Denmark ~16km, Ireland ~11km. All populations consisted of >1000 individuals. Details on the locations of the populations are given in Figure 1. Populations from these regions have already been described to some extent in other studies (Andersen et al., 2005; Fénart et al., 2008; Capistrano-Gossmann et al., 2017). Plants were found in very different types of habitat, ranging from edges of sandy beaches, gravel areas or even rocky cliffs. Pictures were taking during sampling. Some examples are shown in Figure 2, demonstrating the phenotypic diversity of the collected material.

2.2 Genetic data

One random offspring per sampled wild beet was grown and plant DNA was extracted using silica-membrane technology following the NucleoSpin® 96 Plant II protocol from Macherey-Nagel. The samples were fingerprinted using a proprietary Illumina SNP array of KWS SAAT SE & CO KGaA. For Denmark N = 388 were genotyped, for France N = 509, and for Ireland N = 466. This resulted in a total of 1,363 genotypes across the three populations. Markers with more than two alleles and more than 1% missing values as well as all markers that were monomorphic across all three populations were discarded. All markers remaining after quality control were heterozygous in at least one individual of one population, resulting in some markers being monomorphic across one or two populations (Table 1). This resulted in a set of 16,201 SNP markers, that were used consistently across all datasets, enabling a comparison of results across the three populations. The markers span the entire sugar beet genome across nine chromosomes for a total length of 625 cM. The average genetic distance between markers was 0.0386 cM with a maximum of 1.8834 cM.

2.3 Data analysis

All analysis were conducted using R version 4.0.5 (R Core Team, 2021).

2.3.1 Linkage disequilibrium

Linkage disequilibrium (LD) analysis was performed for each chromosome by computing r^2 values for all pairwise marker comparisons. The correlation coefficient (r^2) was calculated using the st.calc.ld function of SelectionTools (v 23.1; https://population-genetics.uni-giessen.de/~software/) which contains the simulation routines of software Plabsoft (Maurer et al., 2008).

2.3.2 Genetic diversity estimates

The observed heterozygosity H_{O_i} was calculated for each individual by dividing the number of heterozygous markers by the sum of all heterozygous and homozygous markers $\left(H_{O_i} = \frac{N_{Het_i}}{N_{Het_i} + N_{Hom_i}}\right)$. Markers with missing information for the respective individual were not considered.

The average expected heterozygosity over all loci is an estimate of the extent of genetic variability in the population. It was calculated over all 16,201 marker loci using SelectionTools. For each locus, the function st.plot.gene.diversity calculates the expected heterozygosity by subtracting the expected frequencies of homozygotes at the locus from 1 ($H_{E_i} = 1 - p_i^2 - q_i^2$), with p_i and q_i being the frequency of the two alleles at locus i. The operation was repeated for all loci. The expected heterozygosity ranges from 0 to 1 and is maximized when there are many alleles at equal frequencies. For plotting, haploblocks were built using the function st.def.hblocks based on ten adjacent markers each.

The minor allele frequency was calculated as the frequency of the less common SNP allele within each population.

Testing genetic markers for Hardy-Weinberg equilibrium was performed using a Chi-square test for goodness-of-fit as the

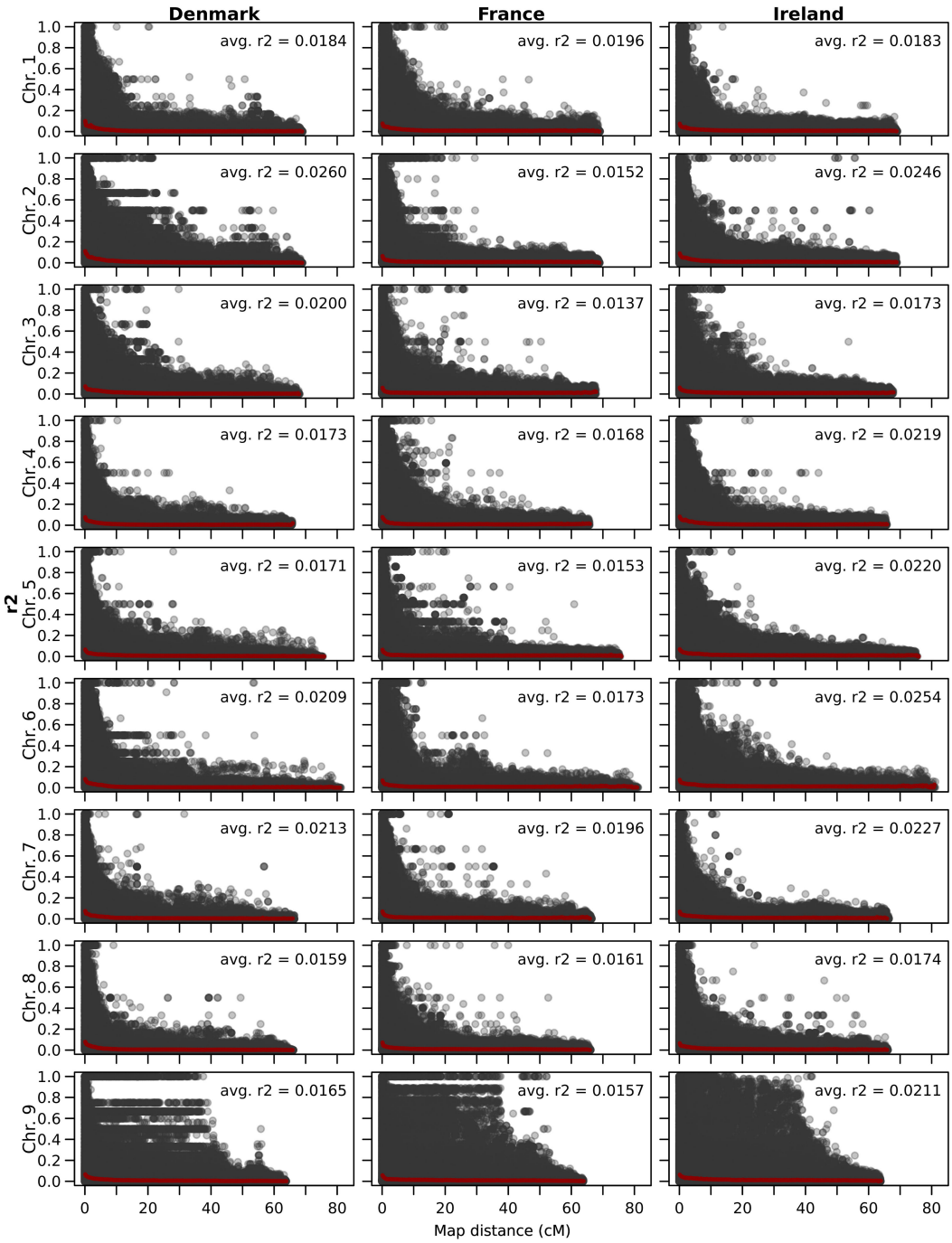

FIGURE 3
Scatter plot showing the linkage disequilibrium decay per chromosome of all three sea beet populations. The genetic distance (cM) on the x-axis is plotted against estimates of the linkage disequilibrium correlation coefficient (r^2) for pairs of markers on the y-axis. In red, a trend line is shown and the average correlation coefficient is given for every chromosome.

classical test for HWE (Crow and Kimura, 1970; Graffelman, 2015), with the R package HardyWeinberg (v. 1.7.8; Graffelman, 2015) at a significance level of 0.05. Ternary plots for three-way genotypic compositions (AA, AB, BB) of all marker loci were generated for each population and also for subpopulations of France and Ireland. The assignments to subpopulations were based on the calculated admixture coefficients for the inferred number of optimal clusters per population. For the population from Ireland, Hardy-Weinberg equilibrium was also calculated for subpopulations assigned based on geographic origin.

2.3.3 Genetic distances and population structure

Genetic distances were calculated as modified Roger's distance for each possible pair of individuals using SelectionTools. A

hierarchical cluster analysis was performed on these genetic distances using the hclust() function of the R package stats. The heatmap with the attached dendrogram was then produced using the R package ComplexHeatmaps (v 2.15.4; Gu et al., 2016; Gu, 2022).

Principal coordinate analysis was performed to investigate the relationship among the populations based on the previously estimated genetic distances using the cmdscale function in R (R Core Team, 2021).

As a complementary approach, the assignment of individuals from the three sea beet populations to genetic clusters was inferred using R package LEA (v 3.2.0; Frichot and François, 2015). The snmf function estimates population genetic structure from the genotype matrix using sparse Non-Negative Matrix Factorization algorithms and provides a least-squares estimate of ancestry proportions rather than maximum likelihood estimates (Frichot et al., 2014; Frichot and François, 2015). Admixture coefficients for each individual were estimated for K = 3 to 10 based on allelic data of 16,201 SNPs for all three populations. For every value of K, 100 repetitions were carried out. The estimated individual admixture coefficients for the run with the lowest cross entropy value was plotted using the barplot function of R package ggplot2 (v 3.4.4; Wickham, 2016).

To infer the number of major components within the data for up to K = 10 clusters, kmeans as clustering method was used (R package factoextra v 1.0.7; Kassambara and Mundt, 2020). The plot represents the variance within the clusters, which decreases as K increases. The Elbow method was used to select the number of clusters by minimizing the within-cluster sum of squares. With this graphical approach, the within-cluster sum of squares is plotted against the different K-values. The optimal K is identified at the point where the graph bends, forming an elbow.

To infer the number of subpopulations in Ireland and France, admixture coefficients were estimated based on the results of the analysis across all three populations for K = 2 to 5 in Ireland and for K = 2 to 4 in France. The optimal number of clusters within each of the populations was inferred also using kmeans clustering and the Elbow method.

3 Results

3.1 Linkage disequilibrium

The linkage disequilibrium, calculated as the average pairwise correlation coefficient (r^2), is consistently low across all three analyzed populations and throughout all chromosomes (Figure 3). The lowest value was observed in France (0.0178), followed by Ireland (0.0190), with Denmark exhibiting the highest value (0.0194). The average linkage disequilibrium varies across chromosomes, and the chromosome with the highest or lowest values differs between populations. Overall, the lowest LD was observed on chromosome 3 in France (0.0137), while the highest was recorded on chromosome 2 in the Danish population (0.0260).

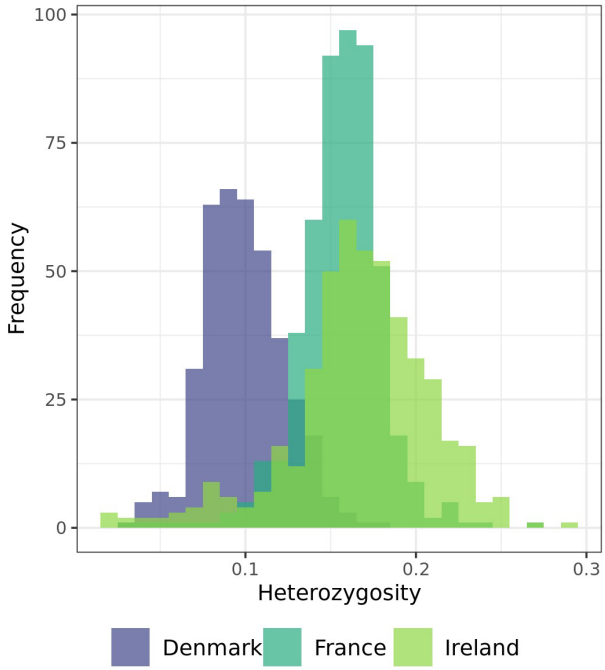

FIGURE 4
Distribution of individuals of each population by their heterozygosity (y-axis shows the heterozygosity of the individuals in %).

3.2 Heterozygosity

Observed heterozygosity is low across all individuals analyzed within this study (Figure 4). None of the individuals within all three populations showed a heterozygosity of more than 30%. On average 9.89% of the analyzed markers in the population from Denmark, 15.58% on average in the population from France, and 16.68% on average in the population from Ireland were heterozygous. While the standard deviation of minor allele frequencies is relatively low in the Danish (2.35%) and French (2.50%) populations, the Irish population exhibits greater variability, with a standard deviation of 4.12%.

Expected heterozygosity (Figure 5) is higher than the observed heterozygosity. Sea beets from France and Ireland exhibit greater expected heterozygosity compared to those from Denmark. While in Denmark the average expected heterozygosity ranges from 0.43 on (chr. 2) to 0.60 (chr. 5), in France from 0.69 (chr. 6) to 0.78 (chr. 8), and in Ireland from 0.68 (chr. 4) to 0.78 (chr. 5 and chr. 9). Hence, the variation between chromosomes is higher within the population from Denmark. Different regions and different chromosomes seem to be under selection within the different populations. As can be seen also in Table 1, each population has a certain number of exclusive polymorphisms.

3.3 Minor allele frequencies

Minor allele frequencies observed within the three analyzed populations are low (Figure 6). On average they lie between 6.99% in Denmark, 11.91% in France, and 12.84% in Ireland. While the

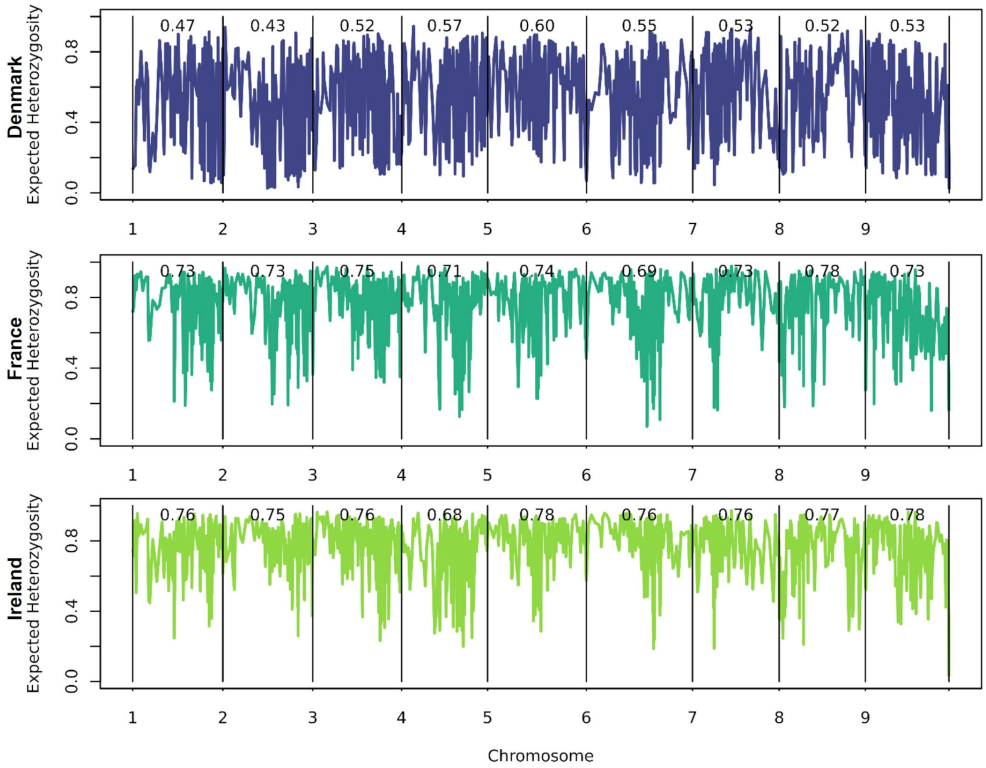

FIGURE 5
Average expected heterozygosity over all loci as an estimate of the extent of genetic variability in the population calculated over all 16,201 marker loci. Haploblocks were built based on 10 adjacent markers each.

distributions of minor allele frequencies in the Irish and French populations are similar, the Danish population exhibits a distinct pattern. The interquartile ranges for France and Ireland are comparable, whereas Denmark has a noticeably smaller interquartile range. Additionally, the variance (132.21) and standard deviation (11.49%) within the Danish population are lower than those observed in the French (187.67; 13.70%) and Irish (170.43; 13.05%) populations.

3.4 Hardy-Weinberg equilibrium

The percentage of markers in Hardy-Weinberg equilibrium varies across populations (Figure 7). The highest percentage of markers in Hardy-Weinberg equilibrium was observed within the population from Denmark (81.53%). The population from France has fewer markers in Hardy-Weinberg equilibrium (55.81%), while the population from Ireland has the lowest percentage of markers in Hardy-Weinberg equilibrium (44.64%). In both populations a heterozygote deficiency (excess of homozygotes) can be observed.

3.5 Principal coordinate analysis

Principal coordinate analysis was conducted to investigate relationships among the populations and their individuals. The first three coordinates collectively accounted for 26.59% of the total variation. The first two axes explained 11.20% and 6.09% of the total variation, respectively, and identified three distinct genetic groups (Figure 8). The analysis revealed that genetic diversity between populations is considerably greater than within them. The three populations were clearly differentiated, as observed primarily in PC1 and PC2. In contrast, PC3 indicated some genetic admixture between Denmark and Ireland, as well as France and Ireland, while populations from France and Denmark remained distinctly separate (Figure 8). Furthermore, whereas individuals from Ireland exhibited a more dispersed clustering pattern along PC1 and PC2 compared to other populations, PC3 revealed the presence of subpopulations within the Irish population.

3.6 Genetic distances

The heatmap and dendrogram of genetic distances (Figure 9) indicate a genetic separation of the Danish population from the Irish and French populations. The latter two exhibit closer genetic relatedness. Additionally, individuals within the Danish population are more genetically homogeneous compared to those in the other two populations. In contrast, individuals from the French, and to an even greater extent those from the Irish population, show a higher genetic divergence within their population. This pattern is evident in both the dendrogram and the genetic distance measurements.

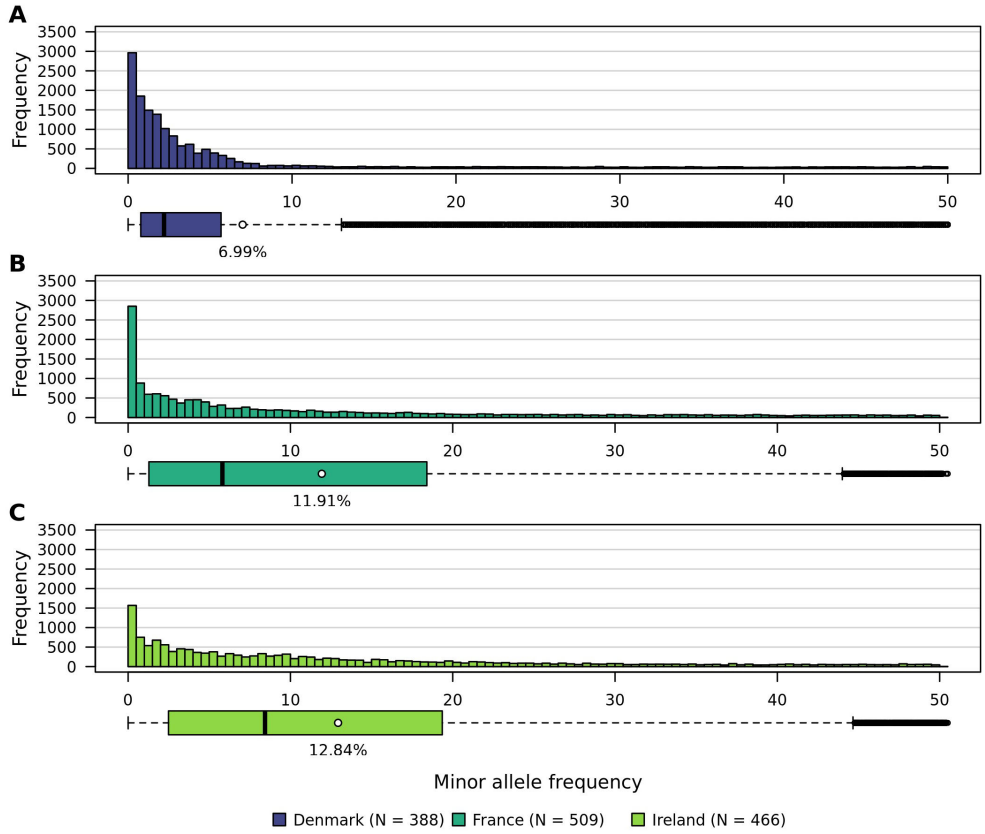

FIGURE 6
Histograms showing the distribution of minor allele frequencies within all three sea beet populations. **(A)** Denmark, **(B)** France, and **(C)** Ireland.

The average genetic distance, estimated as modified Roger's distance, was 0.231 within the Danish population, 0.304 within the French population, and 0.324 within the Irish population. Notably, the average modified Roger's distance across all three populations was 0.320, slightly lower than the value observed within the Irish population.

3.7 Admixture coefficients

As a complementary approach, admixture coefficients for each individual were calculated for K = 2 to 10 (Figure 10). Admixture analysis also revealed clear genetic differentiation among the three populations. Accordingly, k-means clustering identified K = 3 as the

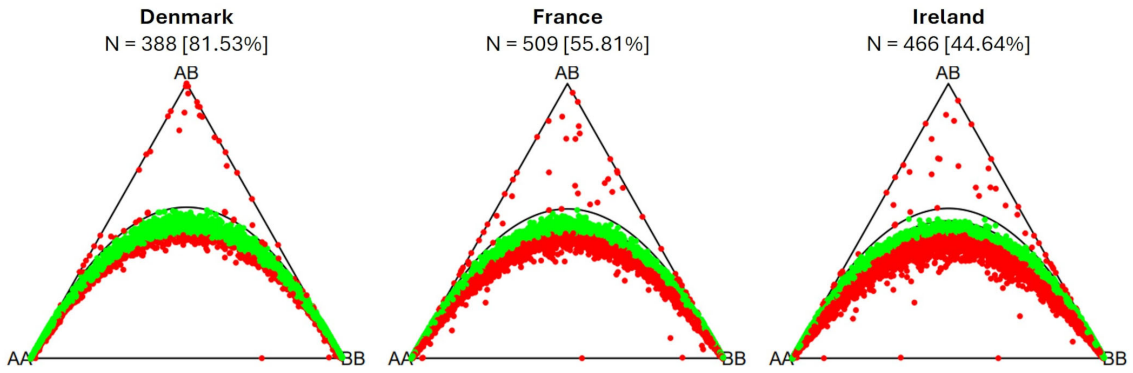

FIGURE 7
Ternary plots for three-way genotypic compositions (AA, AB, BB) of all 16,201 SNP marker loci. The parabolas within the plot represent the acceptance region corresponding to the Chi-square test for Hardy-Weinberg equilibrium. The (non-)significance of the test can be inferred from the position of the markers in the ternary plot. Significant markers are indicated by red points, non-significant markers by green points. Significance level is 0.05. The number of individuals within each subpopulation (N) is depicted above each plot. Values in brackets determine the percentage of markers in Hardy-Weinberg equilibrium.

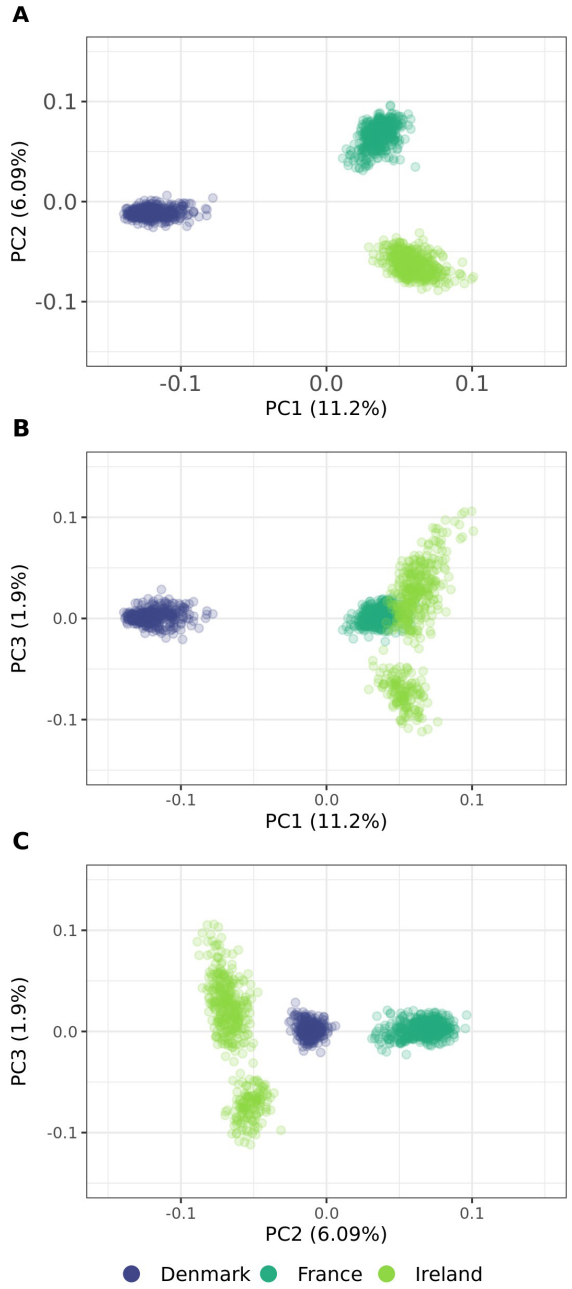

FIGURE 8
(A–C) Principal coordinate analysis plots of 1,363 individuals from three sea beet populations. Genetic distances are calculated as modified Roger's Distance based on 16,201 SNP marker loci. PC1, PC2 and PC3 are the first, second, and third principal coordinate, respectively, and the values in parentheses refer to the proportion of variance explained. Colors are assigned according to population in all graphs.

most likely number of clusters within the dataset. Furthermore, the populations from France and Ireland exhibited greater genetic similarity to each other than to the Danish population as can be seen for K = 2. Despite being sampled across the largest geographical range, individuals from Denmark demonstrated a high degree of genetic homogeneity, with no detectable subpopulations even for K = 10. In contrast, substructure was observed in the French population for K ≥ 6. K-means clustering estimated the optimal number of clusters for this population to be K = 3 (results not shown). The Irish population, however, exhibited pronounced genetic substructure, which is consistent with its deviation from Hardy-Weinberg equilibrium. The most probable number of subpopulations within the Irish dataset was estimated to be K = 4.

4 Discussion

4.1 Linkage disequilibrium

The low linkage disequilibrium observed in sea beet populations allows for the direct use of these populations in association mapping, rather than constructing biparental populations for quantitative trait locus analysis. Many generations of outcrossing result in a low linkage disequilibrium within these populations. The calculated average pairwise correlation coefficient (r^2) regarding all chromosomes is lowest in France, followed by Denmark and highest in Ireland. Overall, the linkage disequilibrium within all three analyzed populations is very low across all chromosomes (Figure 3). This is expected for sea beet populations of outcrossing species which have undergone many generations of random mating and has been described in sea beet populations also in other studies (Fievet et al., 2007; Capistrano-Gossmann et al., 2017). Lower linkage disequilibrium allows for higher resolution in detecting regions associated with traits (Garnier-Géré and Chikhi, 2013), as demonstrated by the successful mapping of the Rhizomania resistance gene Rz2 within the sea beet population sampled near Kalundborg in Denmark (Capistrano-Gossmann et al., 2017). Therefore, while developing biparental populations for QTL analysis can be time-consuming and costly, sea beet populations with low linkage disequilibrium are highly suitable for association mapping due to their inherited high resolution.

4.2 Heterozygosity

Observed heterozygosity is low across the three analyzed sea beet populations, with none of the individuals showing a heterozygosity of more than 30% (Figure 4). This is contrary to most of the literature, where *Beta vulgaris* ssp. *maritima* (L.) Arcang. is described as a naturally outcrossing species with a high degree of self-incompatibility and hence usually high levels of heterozygosity within the populations (Hautekeete et al., 2020; Veloso et al., 2021; Felkel et al., 2023). While the observed heterozygosity is low, the average expected heterozygosity is high (Figure 5). Expected heterozygosity estimates the probability that two alleles randomly chosen from a population will be different, indicating genetic diversity. Expected heterozygosity is highest within the populations from France and Ireland and lowest the population from Denmark (Figure 5). Lower observed heterozygosity compared to expected heterozygosity, as found in this study, suggests inbreeding or population structure. Certain

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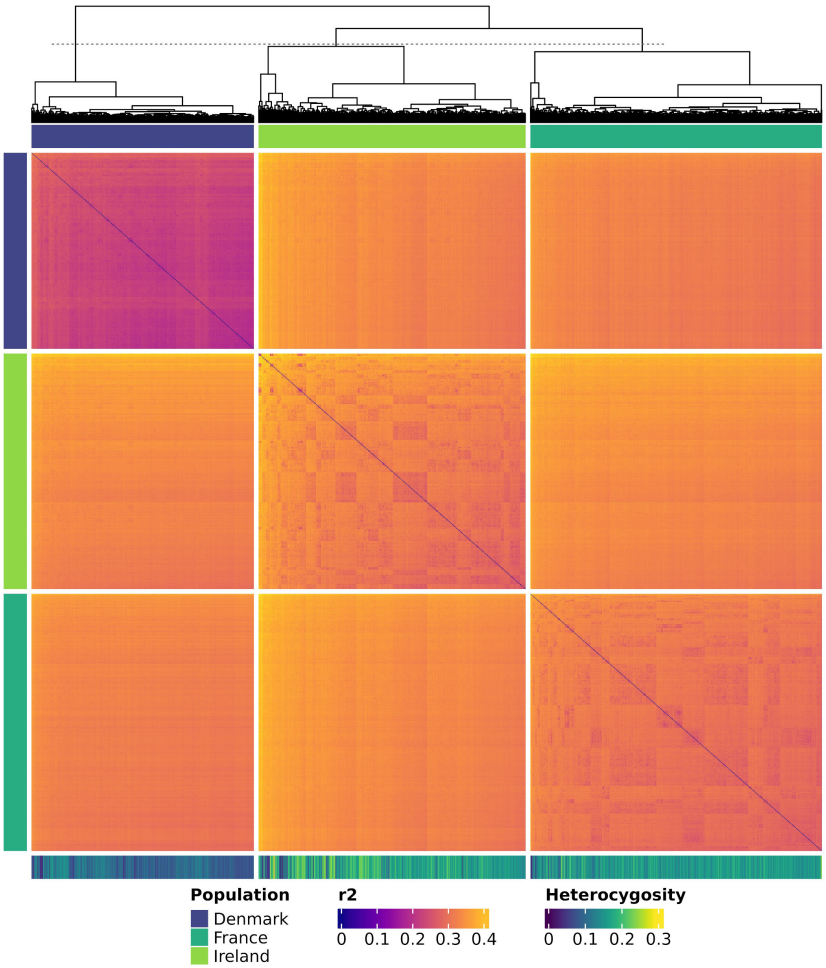

FIGURE 9
Heatmap showing genetic distances among all individuals. Genetic distances are calculated as modified Roger's Distance. Individuals are colored by population and clustered according to the dendrogram. Each row and each column represent a genotyped individual. The colored bars at the top and the left of the heatmap show the corresponding population of the individuals. The horizontal bar at the bottom shows the heterozygosity of each individual.

genome regions appear to be under selection within one population, with specific alleles unique to each population (Figure 5, Table 1). Further analyses would be required to determine the cause of selection within the populations, which is beyond the scope of this study. Nevertheless, the high expected heterozygosity indicates a substantial genetic diversity within these populations.

4.3 Minor allele frequency

The minor allele frequencies observed within the analyzed populations are low, which corresponds to the low heterozygosity observed in this study. The high genetic diversity of sea beet populations may also result in many alleles being present at low frequencies. Minor allele frequencies observed within the three analyzed populations on average lie between 7.0% in Denmark, 11.9% in France, and 12.9% in Ireland (Figure 6). Low minor allele frequencies can pose challenges for association mapping, as rare alleles are more likely to be observed in heterozygotes rather than homozygotes (Bernardo, 2010), reducing the statistical power to detect associations. Additionally, literature shows that low minor allele frequencies can lead to an increased rate of false positives (Tabangin et al., 2009). This occurs because the sample size of individuals carrying the rare allele is small, leading to higher variability and less reliable statistical inference.

4.4 Genetic diversity

Genetic diversity was overall highest in the Irish population, while the Danish population exhibited the lowest phenotypic and genetic variation. While high genetic diversity is beneficial, it also requires careful sampling and appropriate statistical analysis to ensure that the associations detected are accurate and not due to random chance and to effectively utilize high genetic diversity in association mapping studies. Firstly, to capture the full extent of genetic diversity within a population, it is crucial to sample a sufficiently large number of individuals within the population.

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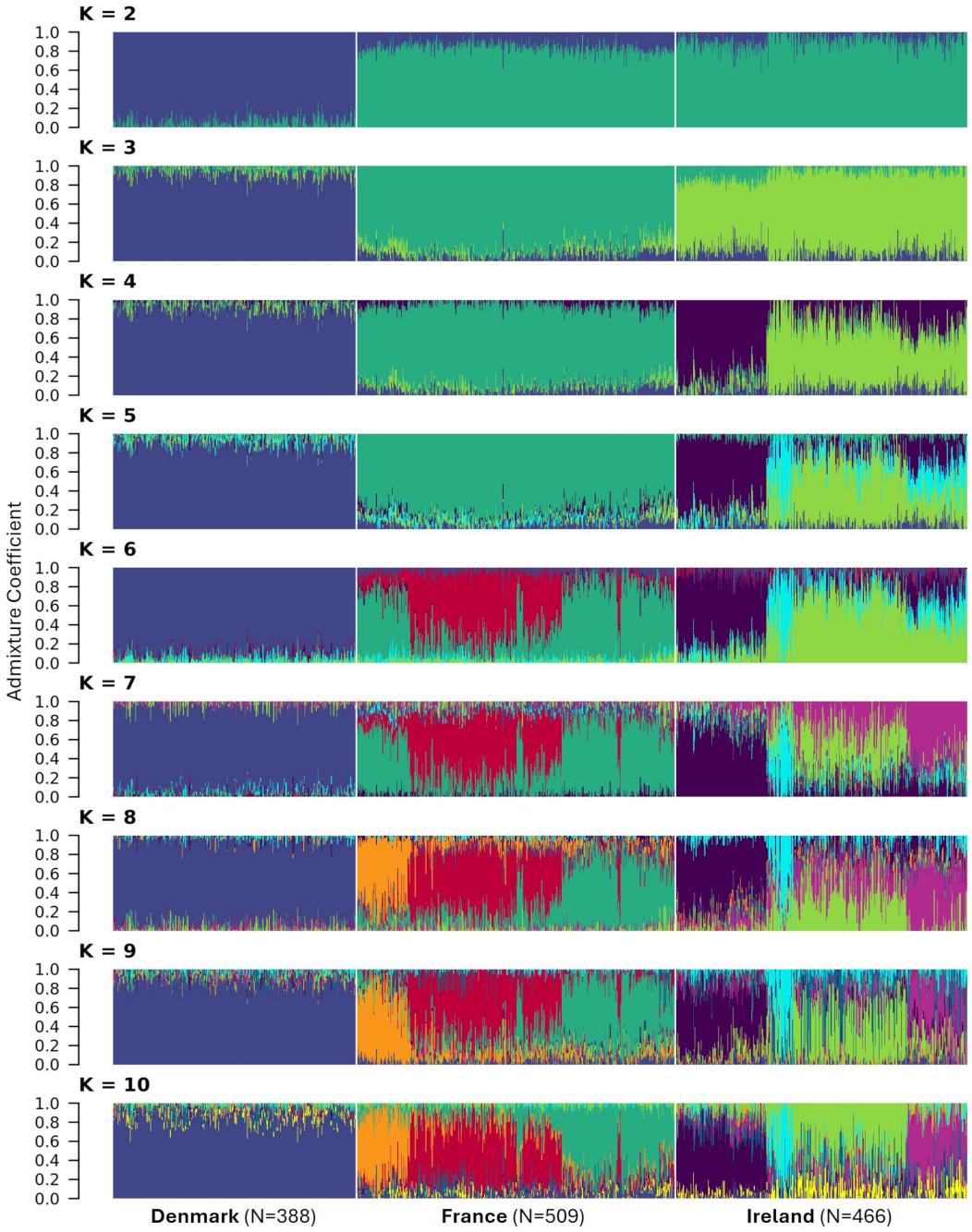

FIGURE 10
Assignment of 1,363 individuals from three sea beet populations to genetic clusters. Admixture coefficients for K = 2 to 10 were computed based on allelic data of 16,201 SNPs. Each individual is represented by a vertically stacked column indicating the proportions of ancestry in K constructed ancestral populations. Populations are separated by whilte vertical lines based on geographic origin.

Given the high genetic diversity of the French and especially Irish populations, ensuring adequate sampling sizes will be essential to comprehensively represent their genetic variation. On the other hand, studies should incorporate a substantial number of entries to ensure proper representation of low-frequency alleles within the analysis. This enhances the statistical power and reliability of the associations detected. Additionally, sophisticated statistical models are necessary to accurately detect true associations in populations with high genetic diversity (Santure and Garant, 2018; Otyama et al., 2019). These models need to account for the low frequency of rare alleles and help reduce the variability and improve the reliability of the results. Especially for the Danish population with the low minor allele frequency appropriate statistical models and sufficient study sizes will be crucial. Despite these challenges, sea beet populations can be valuable for association mapping studies when a sufficient sample size and appropriate statistical models for analysis are employed. Appropriate measures must be tailored to the genetic diversity present within each population. Based on the

observed high genetic diversity, the Irish population appears to have the most potential to directly map traits by association mapping and to contribute new variation to breeding programs.

4.5 Hardy-Weinberg equilibrium

Among the three analyzed sea beet populations, the population from Denmark shows the highest percentage of markers in Hardy-Weinberg equilibrium (81.53%; Figure 7). This corresponds to its low minor allele frequencies and low heterozygosity. The population from France has fewer markers in Hardy-Weinberg equilibrium (55.81%), while the population from Ireland has the lowest percentage of markers in Hardy-Weinberg equilibrium (44.64%). In both populations a heterozygote deficiency (or excess of homozygotes) can be observed. Other studies also found deviations from Hardy-Weinberg equilibrium caused by deficiency in heterozygotes within some sampled sea beet populations (Andersen et al., 2005; Fievet et al., 2007).

This deviation from Hardy-Weinberg equilibrium in the French and Irish populations suggests the presence of non-random mating or population substructure. This can occur, when individuals are more likely to mate with others that are genetically similar to them, also called positive assortative mating (Crow and Kimura, 1970; Garnier-Géré and Chikhi, 2013). Under these conditions a certain amount of inbreeding and, hence, more homozygote loci than expected under Hardy-Weinberg, will be observed (Garnier-Géré and Chikhi, 2013; Chen et al., 2017). Individuals may for example be more likely to mate, if they are located geographically close together or have a similar time of flowering (Breese, 1956; Fox, 2003). A large range in flowering time and in some areas even perennial growth habit has been observed during the sampling of populations within this study (Figure 2) as well as in others (Doney et al., 1990; Bartsch and Schmidt, 1997). This may be one explanation for the observed deviation from Hardy-Weinberg equilibrium in these populations.

Subpopulations between which there is partial or complete isolation may also occur due to other reasons such as geographic barriers or environmental factors. In such cases, each subpopulation may experience genetic drift, selection, or other evolutionary forces that cause their allele frequencies to diverge (Crow and Kimura, 1970; Chen et al., 2017). When these subpopulations are considered together, the overall heterozygosity is lower than expected under Hardy-Weinberg equilibrium. The combination of different allele frequencies reduces the proportion of heterozygotes, leading to a heterozygote deficit (Chen et al., 2017). This is also known as Wahlund effect (Crow and Kimura, 1970; Garnier-Géré and Chikhi, 2013).

As the efficiency of association mapping studies strongly depends on patterns and extent of linkage disequilibrium within in sea beet populations (Garnier-Géré and Chikhi, 2013), differences in allele frequencies between subpopulations can lead to false positives. This is caused by genetic variants appearing to be associated with a trait simply because of population stratification rather than a true causal relationship (Bernardo, 2010). Hence, deviations from Hardy-Weinberg equilibrium can reduce the statistical power to detect true associations and increase the likelihood of false positives. While this poses less of an issue within the Danish population, this may severely influence analysis of the populations from France and Ireland. Consequently, correcting for population structure is essential to avoid these confounding effects and to accurately identify genetic variants linked to traits of interest, especially in the latter populations (Bernardo, 2010; Garnier-Géré and Chikhi, 2013).

4.6 Genetic distances and population structure

Population structure and genetic distance of natural sea beet populations also play a crucial role in their useability for association mapping studies. The three evaluated populations from Denmark, France, and Ireland exhibit distinct genetic clusters, as evidenced by principal coordinate analysis (Figure 8), dendrogram and heat map (Figure 9), and admixture coefficients (Figure 10). Genetic diversity is much larger between the analyzed populations than within these according to the principal coordinate analysis (Figure 8). The Danish population exhibits strong genetic distinction from both the Irish and French populations, with the latter two appearing more closely related to each other within principal coordinate analysis and dendrogram.

This pattern aligns with findings from previous studies, where Danish populations show strong genetic similarity among themselves, with little population structure, and remain notably distinct from other European populations (Andersen et al., 2005; Felkel et al., 2023). This genetic distinctiveness may result from their geographic isolation at the Northern edge of the sea beet distribution range. Although Denmark is connected to other regions by waterways, physical distance likely may act as a barrier, limiting gene flow from other populations. This is also reflected in the population's high number of monomorphic markers and the limited presence of exclusive polymorphisms (Table 1). Interestingly, Danish populations exhibit closer genetic relatedness to Irish populations than to French populations (Figures 8, 9), a trend also reported in earlier research (Andersen et al., 2005; Andrello et al., 2016). Other studies have observed a decrease in genetic diversity from Southern to Northern Atlantic coastal regions (Leys et al., 2014; Veloso et al., 2021).

Little population structure and close genetic relatedness (estimated modified Roger's distance = 0.231) can be observed within the Danish population (Figures 8-10). This absence of subpopulations within the Danish population, even with K = 10 (Figure 10) and despite sampling across a large geographical distance, indicates a high level of gene flow within this population. In contrast, the populations from Ireland and France exhibit more pronounced substructure. This is consistent with findings from other studies, which also have observed more population structure in populations from France and Ireland than from Denmark (Felkel et al., 2023).

The French population shows substructure, despite being sampled along a short geographic stretch (Figure 11A). K-means

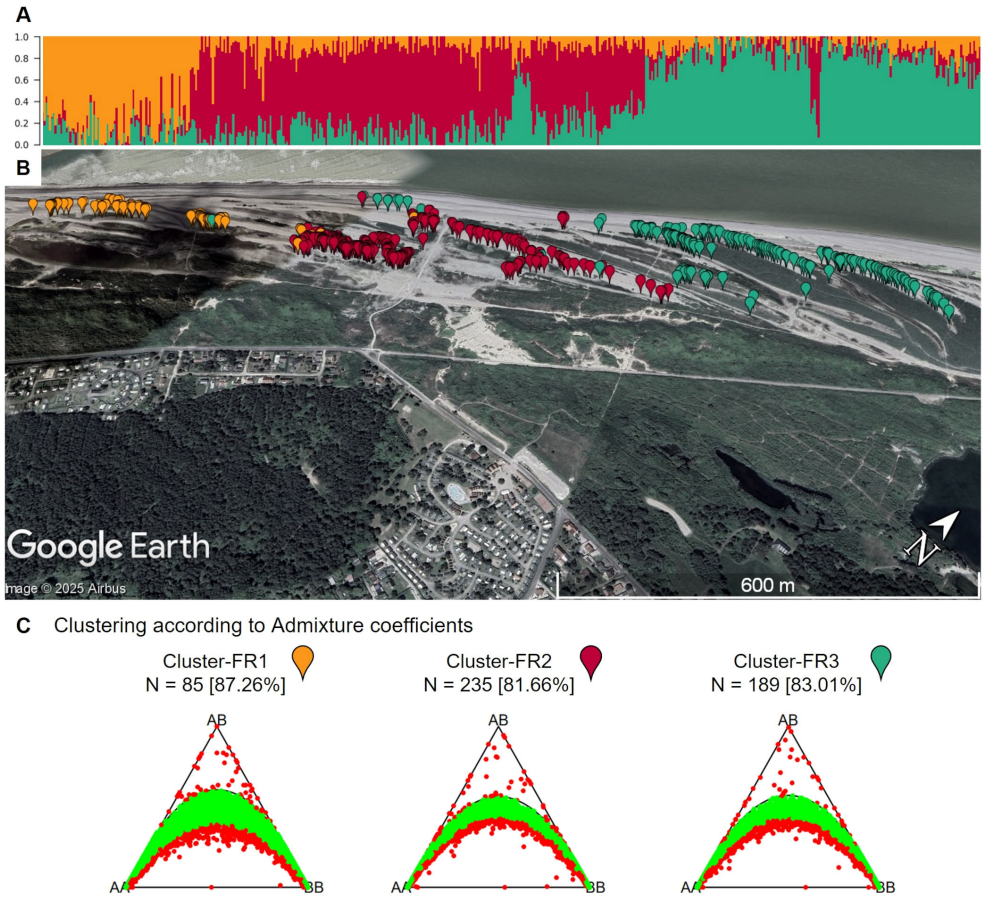

FIGURE 11
(A) Admixture coefficients for K = 3 for all 509 individuals from the French sea beet population. Each individual is represented by a vertically stacked column indicating the proportions of ancestry in K constructed ancestral populations. Individuals are ordered based on geographic origin **(B)** Map shows the geographic origin of the sea beets colored by assigned group according to admixture coefficients. Within the short geographic stretch, a gradient can be observed. The map was generated using GoogleEarth Pro. **(C)** Ternary plots for three-way genotypic compositions of all 16,201 SNP marker loci for the population from France divided into K = 3 clusters based on admixture coefficients. The parabolas within the plot represent the acceptance region corresponding to the Chi-square test for Hardy-Weinberg equilibrium. The (non-)significance of the test can be inferred from the position of the markers in the ternary plot. Significant markers are indicated by red points, non-significant markers by green points. Significance level is 0.05. The number of individuals within each subpopulation (N) is depicted above each plot. Values in brackets determine the percentage of markers in Hardy-Weinberg equilibrium.

cluster analysis estimated the optimal number of clusters to be three. A clear correlation between the geographic origin and the allocated clusters can be seen (Figure 11B). However, the reason for the emergence of these subpopulations is not clear and would require further investigation within another study. When these subpopulations are analyzed separately, the percentage of markers in Hardy-Weinberg increases (Figure 11C). This confirms the presence of subpopulations.

The Irish population also shows clear substructure, with the most likely number of subpopulations estimated to be K = 4 (Figure 12). This population was sampled along a coastline stretch of ~11km, interrupted by cliffs and inaccessible stretches. The population therefore consists of four geographically separated sampling sites (Figures 1, 12). However, these do not constitute the four separate genetic clusters. Rather, the two sites geographically furthest apart seem to be related closest genetically (Figure 12). On the other hand, a strong presence of population substructure is detected in a very short stretch of coastline within one of the four sampling sites. When regarding subpopulations according to their admixture assignment, the number of markers found in Hardy-Weinberg equilibrium greatly increases (Figure 13). This suggests that population structure is in this case not caused by spatial distance. Strong population structure on a very local scale has also been observed in other studies and has been explained by founder events along with restricted gene flow (De Cauwer et al., 2012). The part of the Irish population with the most structure was sampled on a beach close to the town of Ardmore. Human activities and environmental constraints can have a major influence on the genetic variability of sea beet populations (Doney et al., 1990; Ascarini et al., 2021) and could have also impacted the structure of the population near Ardmore. However, this would require further research.

Population structure can significantly affect the outcomes of association mapping (Bernardo, 2010; Garnier-Géré and Chikhi, 2013). Differences in allele frequencies between subpopulations can

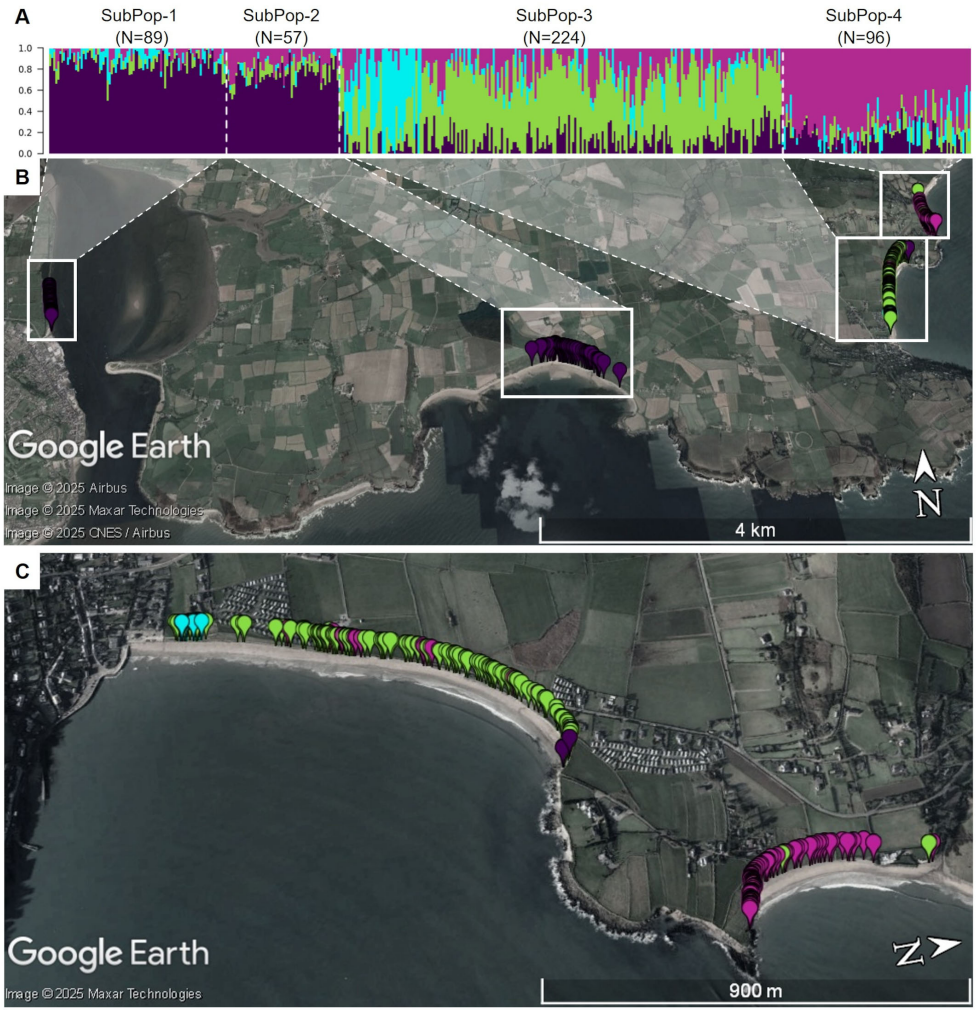

FIGURE 12
(A) Admixture coefficients for K = 4 for all 466 individuals from the Irish sea beet population. Each individual is represented by a vertically stacked column indicating the proportions of ancestry in K constructed ancestral populations. Populations are separated by dotted white vertical lines and ordered according to geographic origin **(B)** Map shows the geographic origin of the sea beets colored by assigned group. White dotted lines were added, to show where individuals from **(A)** are located geographically. Two of the subpopulations group together, despite being further away, whereas there is admixture within one group and within a short geographic stretch. **(C)** Shows an enlarged view of SubPop-3 and SubPop-4, turned by 90 degrees for better view. The maps were generated using GoogleEarth Pro.

lead to false positives caused by shared ancestry rather than actual genetic linkage (Bernardo, 2010). Hence, correcting for population structure in association studies is essential to avoid these confounding effects and to accurately identify genetic variants linked to traits of interest (Bernardo, 2010; Garnier-Géré and Chikhi, 2013). While this is a modest challenge in the population sampled in Denmark, the French and Irish population exhibit population structure that needs to be accounted for during analysis to ensure that detected associations reflect true genetic associations rather than population-related biases.

4.7 Geographic distances

The geographic distance across which populations were sampled did not correlate with their genetic diversity or their population structure in this study. For instance, the population from Denmark, sampled across the largest geographic distance (~16km), exhibited the lowest genetic diversity and little population structure. This population showed many monomorphic markers and few polymorphisms exclusive to it, with the lowest heterozygosity and closely related individuals. Conversely, the population from France, sampled along a shorter stretch of coastline (~2km), also had limited polymorphisms exclusive to it, but showed a higher minor allele frequency and average expected heterozygosity compared to the population from Denmark. The population from Ireland, sampled across an intermediate geographic distance (~11km), demonstrated the highest phenotypic and genetic diversity among all analyzed populations and the most pronounced population structure. Our results were in contrast to literature, where often a correlation between geographic distance and genetic distance is described (Raybould et al., 1998; Leys et al., 2014). However, these studies

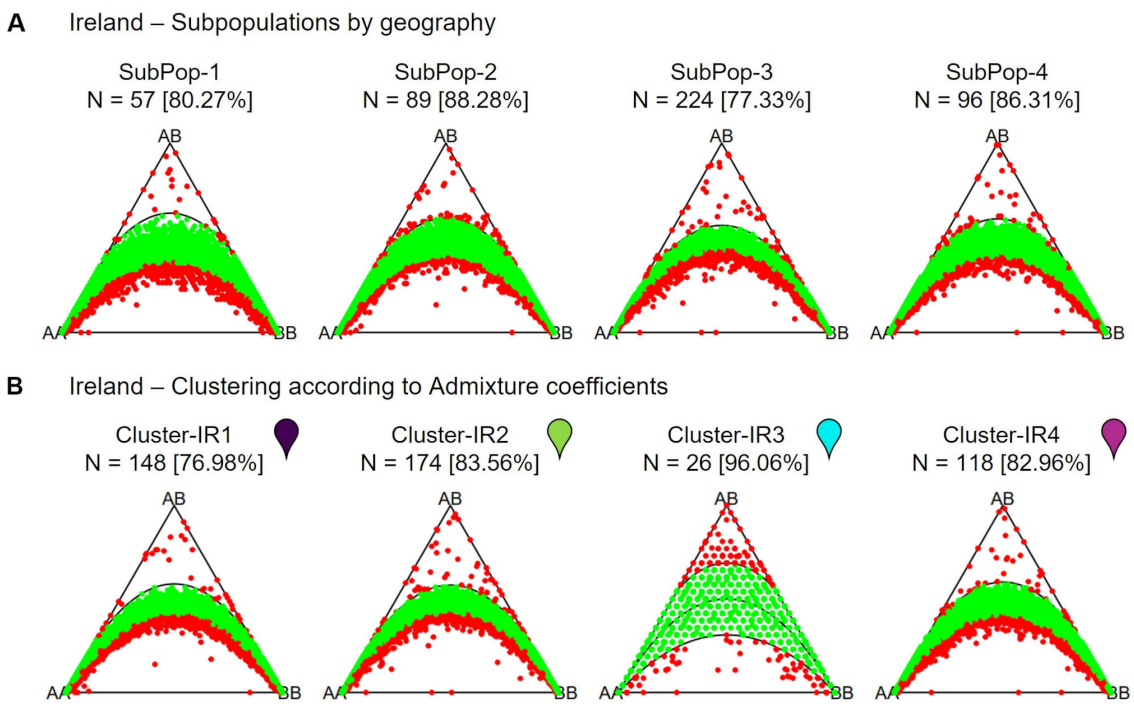

FIGURE 13
Ternary plots for three-way genotypic compositions (AA, AB, BB) of all 16,201 SNP marker loci. The parabolas within the plot represent the acceptance region corresponding to the Chi-square test for Hardy-Weinberg equilibrium. The (non-)significance of the test can be inferred from the position of the markers in the ternary plot. Significant markers are indicated by red points, non-significant markers by green points. Significance level is 0.05. The number of individuals within each subpopulation (N) is depicted above each plot. **(A)** divided into subpopulations based on the 4 geographic sampling regions, **(B)** divided into K = 4 clusters based on admixture coefficients. Colored pins represent the color of the cluster in corresponding admixture plot for K = 4 (Figure 12).

were based on data for few loci or pure morphological analysis. Other studies have also observed population structure on a very local scale and found populations extending over a larger geographical stretch (Doney et al., 1990; De Cauwer et al., 2012; Sandell et al., 2022) or described regions in which neighboring beets had lower genetic relatedness than beets from greater genetic distance (Raybould et al., 1996).

During the sampling of sea beet populations for their use in breeding, it is crucial to consider that genetic diversity and population structure are influenced by more than just geographic distance. Other factors, such as historical population dynamics, gene flow, and local environmental conditions, may play significant roles in shaping genetic diversity and population structure. A very localized sampling may not exclude population structure while broader sampling does not necessarily increase genetic diversity.

5 Conclusion

Genetic diversity and population structure of sea beet populations have implications for their use in sugar beet breeding. The populations from Denmark, France, and Ireland show different levels of genetic diversity and population structure, which present specific challenges for association mapping studies. While the Danish population's lack of substructure and the high amount of markers in Hardy-Weinberg equilibrium simplifies association analysis, this population shows the lowest genetic diversity with many monomorphic markers and few polymorphisms exclusive to this population, despite being sampled across the largest geographic distance. The low minor allele frequency observed within the population requires larger study sizes to ensure that rare alleles are adequately represented in the study and hence to assure statistical power and reliability of the associations detected. In contrast, the populations from France and Ireland, with their higher genetic diversity and higher minor allele frequencies offer greater potential for detecting new valuable genetic variation. However, this also requires larger sampling sizes to cover this genetic variation. Additionally, the presence of subpopulations in these populations necessitates careful consideration of population structure in genetic analyses to avoid false positives and misleading conclusions. This is even more pronounced for the population from Ireland, where a strong population structure was observed at a very local scale. Despite this, these populations offer great potential when analysis is carried out correctly and population structure is accounted for appropriately.

Overall, while all three populations show genetic diversity and hence have the potential to contribute genetic variation to breeding programs, their successful use for breeding within association studies requires careful consideration of their genetic structure and diversity. The analysis of the three sea beet populations revealed the population from Ireland to have the highest

phenotypic and genetic diversity among all analyzed populations. Based on this high genetic diversity, the Irish population appears to have to most potential for use to directly map traits by association mapping, provided that the challenges posed by the severe population structure can be adequately addressed within analysis.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository can be found below: https://github.com/lisabertram/Seabeetpopulations, 1363.

Author contributions

LB: Resources, Validation, Formal Analysis, Project administration, Writing – original draft, Conceptualization, Software, Data curation, Writing – review & editing, Visualization, Methodology. MG: Visualization, Methodology, Writing – review & editing, Software. FK: Resources, Conceptualization, Supervision, Writing – review & editing. MF: Conceptualization, Software, Writing – review & editing, Methodology, Supervision.

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Conflict of interest

The authors have the following interests: LB, MG and FK are employed by KWS SAAT SE & Co. KGaA, Einbeck, Germany.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

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Chapter 4

Crop Wild Relative Populations of *Beta Vulgaris* as Source for Genome-Wide Association Mapping of Complex Traits[1]

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ORIGINAL ARTICLE

Crop wild relative populations of *Beta vulgaris* as source for genome-wide association mapping of complex traits

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Abstract

Key message **Wild beet populations can be used for the detection of minor genes underlying quantitative traits**

Abstract Wild beet populations as valuable source for new genetic variation have so far not been used to detect minor genes underlying quantitative traits, such as drought tolerance or yield. These traits cannot be assessed in wild beets per se but require the development of a beet for phenotypic evaluation. Hence, crossing to elite genome is necessary. Our objective was to determine how QTL detection is affected by (1) the properties of the wild beet population, (2) the quantitative trait architecture, and (3) the structure of the mapping populations. Based on genotypic data of three wild beet populations, nine crossing designs to construct mapping populations were simulated and evaluated for their power to detect minor QTL and their false detection rate. Mapping populations containing 50% wild beet genome have the highest power in this study and can detect even QTL with allele frequencies of < 1% with reasonable power. However, to allow for reasonable phenotyping within field trials at least 75% elite genome in the mapping population is needed. We conclude that crossing designs based on elite x wild beet F1s are most suitable for genome-wide association mapping of complex traits in wild beet populations.

Introduction

Crop wild relatives harbor tremendous genetic diversity and carry many alleles for traits of agronomical importance, that were lost during domestication but allow them to adapt to diverse and rapidly changing environments. Hence, they are a valuable source of allelic variation for breeding programs (Fénart et al. 2008; Dempewolf et al. 2017). There are many successful examples of the use of crop wild relatives in breeding for disease and pest improvement, such as in wheat (*Triticum aestivum*), rice (*Oryza sativa*), chickpea (*Cicer arietinum*), potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*), cassava (*Manihot esculenta*), sunflower (*Helianthus annuus*), banana (*Musa acuminate*) or lettuce (*Lactuca sativa*, Hajjar & Hodgkin 2007, Kashyap et al. 2022).

Quantitative traits are generally more difficult to detect due to their more complex inheritance. Nevertheless, there have been examples also for the use of crop wild relatives for the improvement of such traits. In example for improving tolerance to abiotic stress such as drought and cold or salt tolerance through wild relatives in rice, tomato, barley (*Hordeum vulgare*), cowpea (*Vigna unguiculata*) or chickpea (Hajjar & Hodgkin 2007; Kashyap et al. 2022). Most crop wild relatives have a poor per se agronomic performance. Therefore, they are rarely sought for when targeting yield-related traits (Hajjar & Hodgkin 2007). Nevertheless, crop wild relatives may show favorable variation even at loci for yield components (Liu et al. 2016; Xu et al. 2022). The expression of these valuable alleles, however, may be masked and the performance of crop wild relatives per se might not appear beneficial in standard breeding trials (Dempewolf et al. 2017; Bohra et al. 2022).

Several approaches were successful in overcoming the challenges presented by wild relatives by crossing to elite germplasm (pre-breeding) to increase overall performance and to enable testing of material based on wild relatives also for yield-related traits (Bohra et al. 2022; Schulthess et al. 2022). Working with such segregating populations allows

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for the demasking of useful genomic regions by mapping. For example, Mace et al. (2020) have described a combination of nested association mapping and backcrossing of crop wild relatives with common elite parents in sorghum (*Sorghum bicolor*). Wild x elite F1s were used to estimate the contribution of wild relatives to yield in wheat (Schulthess et al. 2022). In wheat and banana breeding cryptic variation has been found to result in significant and often unexpected superior performance of the crosses between wild relatives and the domesticated crop (Dempewolf et al. 2017). For common bean (*Phaseolus vulgaris*) wild alleles were detected, that increased the yield over the domesticated parent in backcrossed-inbred-line populations (Berny Mier y Tera et al. 2020). Combs and Bernardo (2013) used genomic selection to increase yield in exotic x elite populations of maize (*Zea mays*). Crop wild relative-derived lines of durum wheat (*Triticum durum*), barley and lentil (*Lens culinaris*) were found to outperform the used elite checks (El Haddad et al. 2021). Yield-related QTL were detected in Advanced Backcross-Nested Association Mapping populations of crosses between wild and cultivated barley (Nice et al. 2016). By backcrossing introgression lines with a wild species of rice, F2-populations with improved yield were created (Beerelli et al. 2022). In backcross populations of tomato, the respective wild relative, despite an overall inferior appearance, carried alleles capable of enhancing most traits important to tomato production, including yield-related traits such as fruit size and shape (Tanksley et al. 1996).

Crop wild relative populations of outcrossing species conserved in their natural habitat have usually undergone many generations of outcrossing. They do not require complex statistical modeling to account for population structure (Capistrano-Gossmann et al. 2017). At the same time, the expected low linkage disequilibrium allows for high resolution mapping (Hansen et al. 2001; Capistrano-Gossmann et al. 2017). Genome-wide association studies scan markers across the genome to find genetic variants associated with phenotypic traits by exploiting pre-existing recombination events within genetically diverse natural populations (Santure & Garant 2018; Arora et al. 2019). While the potential of outcrossing crop wild relative populations for the discovery of monogenic traits relevant for crop improvement was demonstrated (Capistrano-Gossmann et al. 2017), many agronomically important traits are controlled by more than one locus. However, the advantages of these populations have so far not been used for the detection of minor genes underlying quantitative traits.

Sea beets or wild beets [*Beta vulgaris* ssp. *maritima* (L.) Arcang.] are the wild relative of sugar beet. They have a high level of genetic diversity (Renzi et al. 2022; Sandell et al. 2022) and carry many alleles for traits of agronomical importance that were lost during domestication (Panella et al. 2020). The use of wild beets in commercial sugar beet breeding programs is accompanied by several challenges, such as linkage drag, poor agronomic performance, annuality, sterility as well as a wide range of phenotyping challenges. In sugar beet breeding, wild beet populations have been mainly used for the discovery of resistance traits (Capistrano-Gossmann et al. 2017) or discovery of bolting genes so far (Hansen et al. 2001). While few studies evaluated crosses between elite sugar beet material to detect yield QTL (Würschum et al. 2011; Wang et al. 2019), so far, no reports have been made about yield QTL that have been identified and introgressed from wild beet to sugar beet. Most traits that are assessed in yield trials, such as drought tolerance or yield, cannot be properly assessed directly in the crop wild relative (wild beets per se), but require the development of a beet for phenotypic evaluation. This can be achieved by developing mapping populations which contain some amount of elite genome. The question of the optimum amount of elite genome in mapping populations for the detection of quantitative traits within wild beet populations was not yet investigated.

Within this study, we simulated nine different crossing designs to construct mapping populations based on the genotypic data of three different crop wild relative populations. These crossing designs were evaluated for their power to detect phenotype-trait associations for minor genes of complex traits and their false detection rate to explore the optimum amount of elite genome in such mapping populations. Our objective was to determine how QTL detection rate and false positive rate in wild beet populations are affected by (1) the properties of the originating wild beet population (2) the quantitative trait architecture, and (3) the structure of the population used for QTL detection.

Materials and methods

Plant material

We used three wild beet populations sampled across the coastal areas of Europe in their natural habitat in Denmark, France and Ireland (Fig. 1). Populations from these regions have already been described to some extent in other studies (Andersen et al. 2005; Fénart et al. 2008; Capistrano-Gossmann et al. 2017). All populations were chosen due to their location north of the 50th latitude, assuming mainly biannual lifeforms which would facilitate yield trials (Van Dijk et al. 1997).

Genetic data

Genotyping was carried out for about 1,000 individuals per wild beet population. Additionally genotypic data of one elite line and a tester, both from the sugar beet breeding

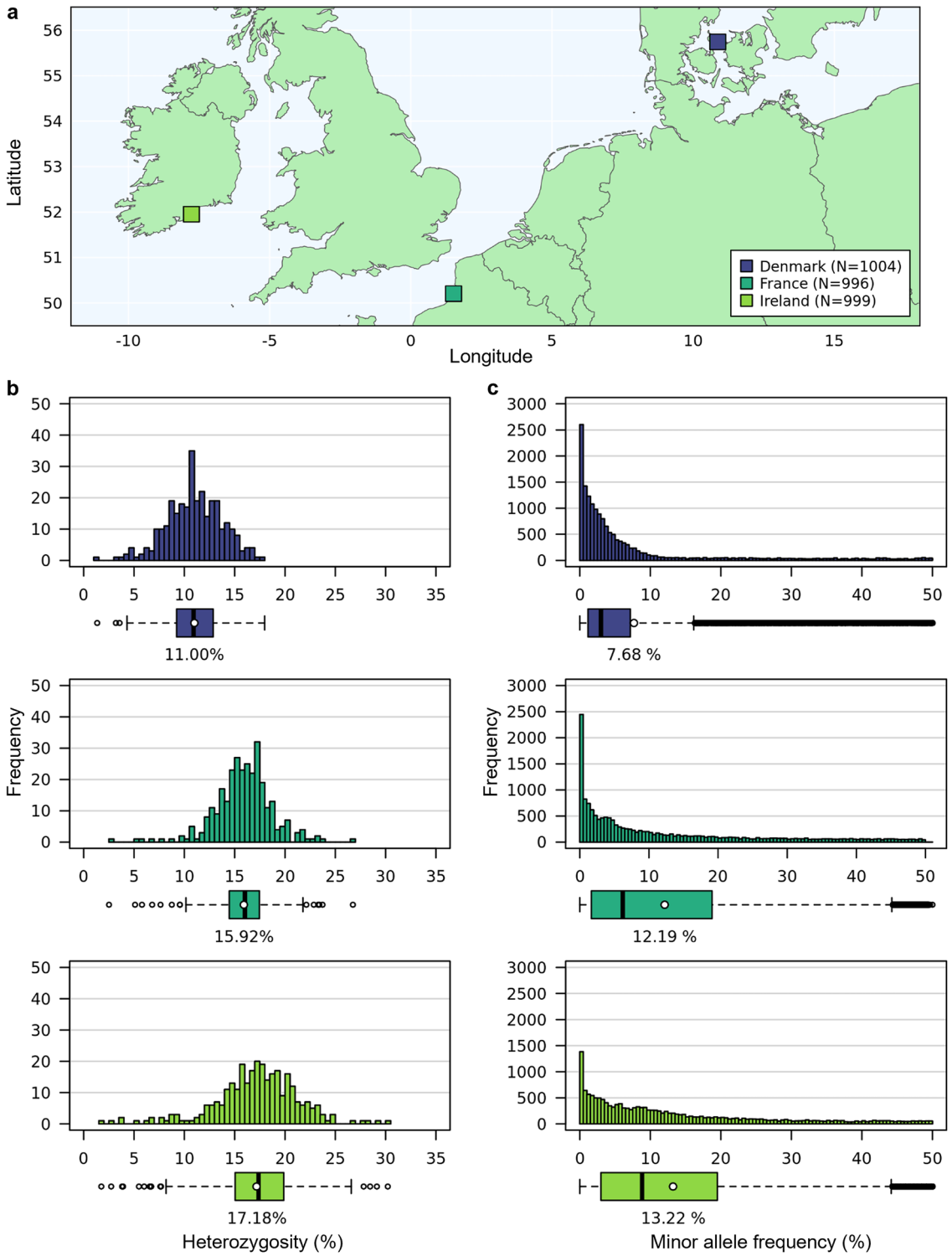

Fig. 1 **a** Map showing the locations of the collected crop wild relative populations in Denmark (Kalundborg), France (Brighton), and Ireland (Ardmore). The map was created using the R package "rnaturalearth" (version 1.0.1, Massicotte & South 2023) and "rnaturalearthdata" (version 1.0.0, South et al. 2024). **b** Histograms showing the percentage of heterozygous individuals and **c** the distribution of minor allele frequencies among the selected individuals of all three populations. The colors used for the populations are the same across all plots

program of the company KWS SAAT SE & Co. KGaA, were used for the simulation. The elite line was almost fully inbred with ≤ 1% of heterozygous markers and the tester was a CMS sterile within-pool 2-way hybrid with 24.44% heterozygous markers.

All analyses were conducted using R version 3.5.1 (R Core Team 2018). A principal coordinate analysis based on Modified Roger's Distance was conducted using the R package SelectionTools (v 19.4; https://population-genetics.uni-giessen.de/~software/) which contains the simulation routines of software Plabsoft (Maurer et al. 2008). Genetic outliers were visually identified. A total of nine outliers (Denmark: 3, France: 5, and Ireland: 1) were excluded from the final analysis. Markers with more than two alleles and more than 1% missing values were excluded. Markers that were monomorphic across all three populations and the elite and tester genotypes were also excluded from the analysis. This resulted in the same 16,076 SNPs being used for the simulations across all datasets.

Phasing

SNP array data generally does not show to which of the two parental chromosomes an allele belongs. For a proper simulation of identical by decent, phasing of the data is necessary to identify alleles co-located on the same gamete. We used BEAGLE (v5.0, Browning & Browning 2007) for phasing. BEAGLE is more accurate than other programs for sample sizes of about 1,000 individuals and high marker density (Browning & Browning 2011). BEAGLE further allows for phasing of data without a phased reference (Browning & Browning 2009). Each wild beet population was phased individually using the remaining individuals of the population as reference. During this process, missing data was imputed by BEAGLE (Browning et al. 2018). The elite and tester were phased separately from the wild beet populations. PLINK (v1.9; Purcell et al. 2007) was used to create the required vcf files.

Covering the genetic variation of wild beet populations with a subset of size N = 300

A subset of $N = 300$ individuals was chosen from each of the wild beet populations for the simulation. The aim was to cover as much available genetic variation by the chosen individuals. The same set of $N = 300$ individuals was used across all simulation runs.

The individuals were chosen based on haplotypes. For this purpose, haploblocks were build based on a defined window size of five non-overlapping markers using the functions st.def.hblocks and st.recode.hil of the R package SelectionTools.

Individuals were chosen in a two-step process: A subset of 25 individuals was first chosen based on the rarity of their haplotypes (rarity score = 1 / number occurrences of the haplotype; the sum across all markers was calculated per individual and the individuals with the highest values were selected). In a next step, individuals were added to the set one by one, that contained the largest number of alleles not yet represented in the previously chosen individuals. For this, only haplotypes present at least twice within the population were considered. With this approach, most of the variation for haplotypes present more than once within the populations was covered in the chosen sets of $N = 300$ individuals. The chosen $N = 300$ individuals of each population represent the diversity of the original wild beet populations (Fig. 2).

Quantitative genetic model

QTL were simulated based on 16,076 SNP markers spread across nine chromosomes for a total length of 625 cM using the package SelectionTools. The average genetic distance between markers was 0.0389 cM with a maximum of 1.838 cM.

We compared three genetic models, simulating either one, two or five QTL with the positive effect assigned to wild beet alleles. A total of 1000 independent simulation runs were performed for each combination of genetic model and wild beet population for all crossing designs. The 1000 simulation runs were conducted to avoid spurious associations with markers. Within each run the QTL were newly assigned.

The total effect sizes of wild beet and elite alleles, as well as their ratio was determined in preceding simulations. These analyses assumed that over time, selection has incorporated numerous small yield-enhancing alleles into elite breeding material (Fulton et al. 1997). Hence, most beneficial alleles originated from the elite genome resulting in its superior performance. As a result, the total elite effects were defined to be twice those of the wild beet effects, which also reflects the average performance observed in field experiments. To differentiate the impact of wild beet and elite alleles, alleles were coded differently for the assignment of effects and during the estimation of genotypic values. A total of three effect files were generated for the analysis.

First, the effect file for the predefined number of QTL ($n = 1$, 2, or 5) originating from the wild beet was created. Previous studies suggest that QTL for complex traits such as yield with larger positive effects exist (Schneider et al. 2002; Reif et al. 2010) and can originate from wild material (Tanksley & Nelson 1996; Reif et al. 2010). However, studies also suggest that most quantitative traits are influenced by a few major-effect QTL, along with additional minor QTL that contribute smaller effects (Tanksley 1993). Hence, a limited number of QTL were simulated, to reflect

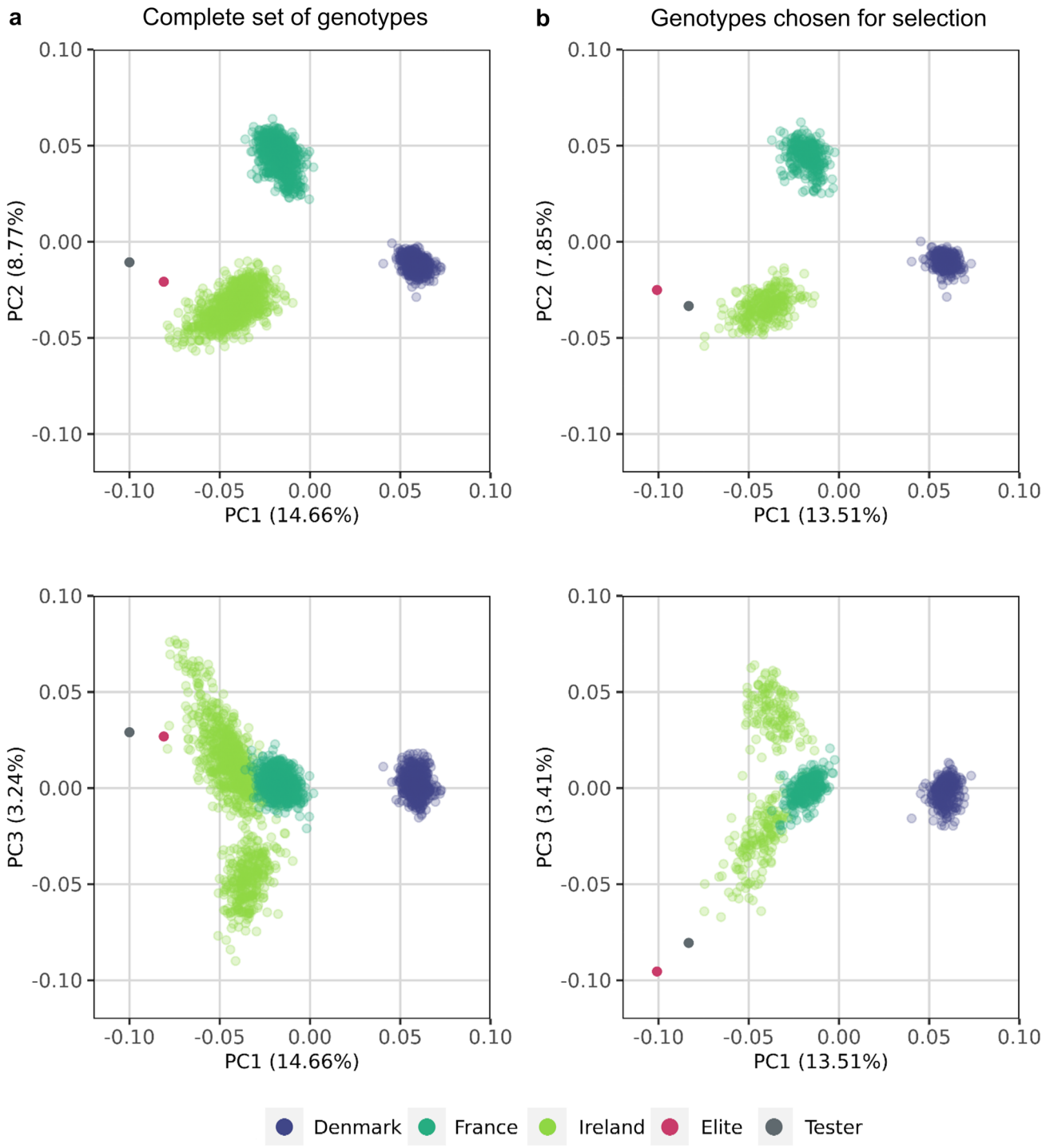

Fig. 2 Principal coordinate analysis plot of **a** the complete set of genotypes and **b** the genotypes chosen for simulation. Principal coordinates were calculated as Roger's distance based on 16,076 SNP loci. The diversity of the genotypes chosen for simulation is representing the diversity present in the complete set of genotypes. The values in parentheses refer to the percentage of variation explained by the principal coordinate

the expectation that beneficial QTL from wild relative populations are relatively rare (Fulton et al. 1997). For each QTL a random marker was selected from all polymorphic markers within the wild beet population. The allele which the effect was assigned to was determined randomly from both wild alleles present at this locus. The effect of the other wild beet allele was set to 0. All QTL allelic effects were simulated to be additive and of unequal size (Wu et al. 2007), following

a geometric series (Lande & Thompson 1990), with each additional QTL having an effect half the size of the previous QTL (eQTL = emax * 1/2n-1). The effect size of the largest simulated QTL (emax) was defined as 5% of the sum of the positive effects of the elite material.

Second, the effect file simulating background noise from the wild beet genome was generated, assuming that a low yield is produced by the wild beets also in the absence of QTL. The total effect size for the remaining markers was set to half the total effects of the elite genome, minus the sum of effects already assigned to QTL.

Third, the effect file for elite alleles was generated. For the elite genome, according to the infinitesimal model (Hayes & Goddard 2001), it was assumed that yield is not based on a few single large QTL but is due to the enrichment with small positive effects over a long period of time. The additive genetic variance was therefore simulated as polygenic variation caused by numerous loci with small effects spread throughout the elite genome. Hence, all markers were assigned equally small effects, with the total elite effects equaling twice the amount of the wild beet effects. The three effect files were combined and used to calculate genotypic values.

Crossing designs to construct mapping populations

The detection of QTL was evaluated for crossing designs differing in their percentage of elite genome, the amount of wild beet haplotype and the wild beet allele frequency in the mapping population (Fig. 3). Furthermore, the crossing designs differ in the amount of time necessary to develop the mapping populations. For developing the mapping populations, we simulated the following crossing designs M1 to M9 (Fig. 3).

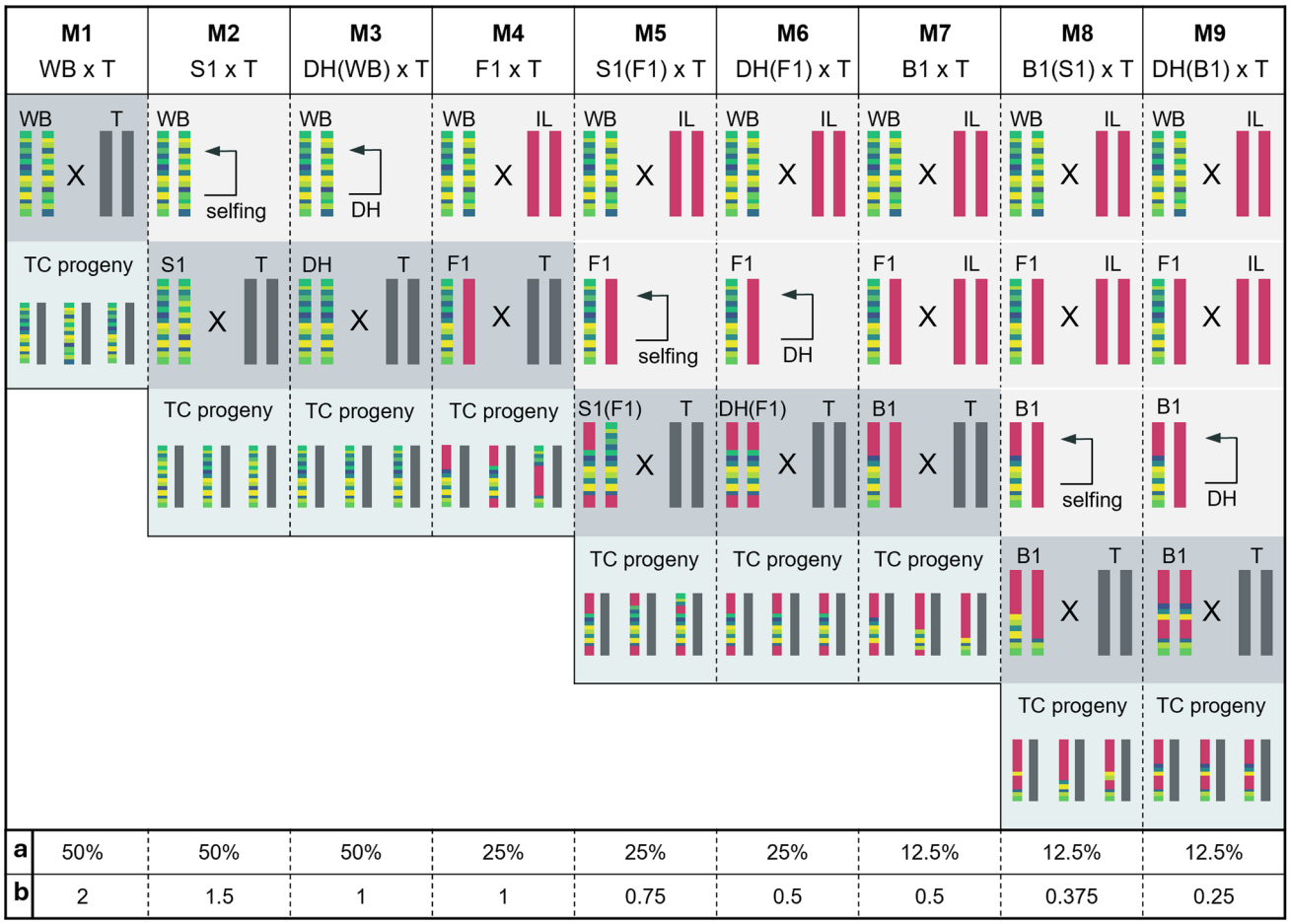

Fig. 3 Overview of the nine simulated crossing designs to construct mapping populations (M1 to M9). One exemplary chromosome with a possible segregation of the wild beet genome throughout the cycles of each crossing design is shown. Per wild beet, one random offspring plant per generation is continued. For each entry of the mapping population **a** is giving the average percentage of wild beet genome represented and **b** the average wild beet haplotypes represented. Abbreviations. DH: double haploid, WB: wild beet, T: tester, IL: inbred line, F1S1: selfing of an F1, B1: backcross of an F1 with the elite line, B1(S1): selfing of a backcross

M1: The wild beet genotypes were crossed to a common tester. Every testcross was analyzed as one entry of the mapping population.

M2: The wild beet genotypes were selfed. One random progeny of each selfing was crossed with the common tester.

M3: DH lines were produced from the wild beet genotypes. One random DH line per progeny was chosen and crossed to the common tester.

M4: The wild beet genotypes were crossed to a common elite line. The resulting F1s were crossed with the common tester.

M5: The wild beet genotypes were crossed to a common elite line. The F1s were selfed. One random progeny from each selfing was crossed with the common tester.

M6: The wild beet genotypes were crossed to a common elite line. DH lines were produced for every F1. One DH line per progeny was randomly chosen and crossed to the common tester.

M7: The wild beet genotypes were crossed to a common elite line. The resulting F1s were backcrossed to the same elite to generate a B1. From every progeny, one random B1 was chosen and crossed to the common tester.

M8: The wild beet genotypes were crossed to a common elite line. The resulting F1s were backcrossed to the same elite to generate a B1. From every progeny, one random B1 was chosen and selfed. One random progeny from each selfing was crossed with the common tester.

M9: The wild beet genotypes were crossed to a common elite line. The resulting F1s were backcrossed to the same elite to generate a B1. From every progeny, one random B1 was chosen to produce DH lines. One random DH line per progeny was crossed with the common tester.

All of these crossing designs effectively represent different forms of multi-parent populations with each of the previously selected $N = 300$ individuals from the wild beet population used as parents for one entry of the mapping population. For every test cross progeny, a total of 594 offspring genotypes were simulated.

In total, per wild beet population three different QTL models ($n = 1$, 2 or 5) were simulated for each of the nine crossing designs and 1,000 independent simulation runs were conducted each. The populations were analyzed separately to have independent populations for cross validation of the results.

Phenotypic values

The genotypic values of the resulting mapping population for each crossing design were determined using SelectionTools. The genotypic value was calculated for each individual of the testcross progeny, by summing up QTL and background effects of the alleles present in the individual based on the previously generated effect files. The mean of the genotypic values of all individuals was used as the mean genotypic value of the entry. The phenotypic values were simulated by adding random nongenetic effects to the known genotypic values. The nongenetic effects followed a normal distribution with a mean of zero and a variance scaled according to the set heritability. The heritability was calculated as broad-sense heritability with $h^2 = v_g / (v_g + v_e)$ with v_e being the nongenetic (masking) variance (Bernardo 2004). Based on previous studies on yield QTL in sugar beet (Reif et al. 2010; Würschum et al. 2011) the heritability was set to either 0.5, 0.6, 0.7, 0.8 or 0.9.

Association mapping

Association mapping was performed for detection of QTL using the R package GenABEL in a two stage process (version 1.8–0; Aulchenko et al. 2007b). In an initial stage, the function polygenic was used to estimate the polygenic model with covariates by maximizing likelihood, providing residuals adjusted for family effects and the inverse of the variance–covariance matrix. For this, the effect of the polygenic term was fitted to the previously simulated phenotypic values.

The mixed model for the initial step of the analysis is given by

$$y_i = \mu + \sum_j \beta_j c_{ji} + G_i + e_i$$

where y_i is the phenotype of the ith individual, c_{ji} is the value of the jth covariate or fixed effect for the individual i, βj is an estimate of the jth fixed effect or covariate, and G_i and e_i are random additive polygenic and residual effects, respectively (Aulchenko et al. 2007a). The random effects are assumed to follow a multivariate normal distribution with mean zero (Aulchenko et al. 2007a).

To adjust for relatedness among individuals a genetic pairwise kinship matrix based on the shared alleles between individuals at all 16.076 SNPs was utilized. Marker to which QTL effects were assigned was excluded from the analysis.

The variance for the polygenic effects is defined as $\Phi^{\sigma^2_G}$, where Φ is the relationship matrix and σ^2_G is the additive genetic variance due to polygenes (Aulchenko et al. 2007a). For the residual random effects, the variance is defined as $I^{\sigma^2_e}$, where I is the identity matrix and σ^2_e is the residual variance (Aulchenko et al. 2007a). The residuals from this analysis are given by

$$y_i^* = y - \left(\hat{\mu} + \sum_j \hat{\beta}_j c_{ji} + \hat{G}_i \right) = \hat{e}_i$$

where $\hat{\beta}_j$ is the estimate of the jth fixed effect and $\hat{G}_i$ is the estimated contribution from the polygene (Aulchenko et al. 2007a).

In a second step, a score test for association was performed with the function mmscore, using a linear mixed-effects model approach. The score test is performed using the formula

$$\frac{\left((G - E[G])V^{-1}\text{residual}Y\right)^2}{(G - E[G])V^{-1}(G - E[G])}$$

where G is the vector of genotypes and E[G] is a vector of mean genotypic values, V^{-1} the previously estimated inverse covariance-matrix and *residualY* the residuals from the polygenic model, which were now adjusted for population structure (Chen & Abecasis 2007).

Evaluation of the simulated crossing designs

The different crossing designs were evaluated for their power to detect phenotype-trait associations for minor genes and their frequency of falsely declaring QTL.

The QTL detection power was evaluated by calculating the true positive rate (TPR) as the number of QTL correctly detected divided by the total number of simulated QTL (in %; TPR = N simulated QTL detected / N simulated QTL). A detection window size around the QTL of ± 2.5 cM was assumed.

The false detection rate (FD) was calculated as percentage of incorrectly detected QTL among all QTL detected (FD = N falsely detected QTL / N detected QTL). If no QTL were detected despite the fact that QTL were simulated, the FD rate was set to 100%. *P*-values were adjusted to control the FD for multiple testing with the fdr method (Benjamini & Hochberg 1995). A threshold of $-\log10\,(p) > 3$ was used. An area was identified as a QTL region, if five or more significant markers were located in an area of five cM. Significant markers located next to each other on the chromosome were identified as two separate QTL regions, if there was a stretch of five or more cM without significant markers between these.

Results

In general, TPR decreases with the percentage of wild beet genome and the amount of wild beet haplotypes represented in the mapping population. TPR is highest for M1 and one simulated QTL (up to 97.60% for $h^2 = 0.9$; see. Figure 4a). As we move from M1 to M3, reducing the average wild beet haplotypes represented within the mapping population from two to one (Fig. 3), the TPR decreases slightly (i.e. to 93.97% for M3 and $h^2 = 0.9$, Fig. 4a). In M4, despite containing only 25% wild beet genome on average, TPR is still reaching 89.87% for $h^2 = 0.9$. As soon as less than one wild beet haplotype is represented in the mapping population (M5 to M9; Fig. 3), TPR drops more severely. While M5 to M7 range between 53.90 and 72.93%, M9 with 12.5% wild beet genome and only 0.25 wild beet haplotypes in the mapping population only reaches a TPR of 37.27% for $h^2 = 0.9$.

While TPR also decreases with heritability, crossing designs are affected differently. Reducing heritability only has a small effect on M1 to M3 and TPR is still > 90%, even for $h^2 = 0.5$. M4 still has a TPR of 83.70% for $h^2 = 0.5$. However, with less than one wild beet haplotype represented within the mapping population (M5 to M9), for $h^2 = 0.5$ the TPR is reduced to below 10% (M4, M5 to M9) and only with M6, the TPR is still at 56.7%.

Simulating (several) small QTL next to the 5% QTL also affects QTL discovery of the latter differently across the crossing designs (Fig. 4b, c). For M1 to M4 the TPR is reduced by between 3.50–9.87 percent points and by 9.17–11.83 percent points in M6. When five QTL are simulated in total, there is almost no effect on the TPR of the 5% QTL in M5 as well as M7 to M9.

For M1 to M4 the reduction in TPR for decreasing heritability and simulating multiple smaller background QTL on the TPR of the 5% QTL is of similar size. Heritability affects TPR of the designs M5 and M7 to M9 more severely, while there is almost no effect on TPR when simulating multiple smaller QTL.

FD decreases with the percentage of wild beet genome represented in the mapping population. M1 to M3 with 50% wild beet genome (Fig. 3) show the overall lowest FD while M7 to M9 with 12.5% wild beet genome show the highest FD (Fig. 4d). M4 and M6 are intermediate, while M5 is being an exception and is ranging on a similarly high level as M7 to M9. For M5 and M7 to M9 in many cases no QTL are detected correctly, resulting in very high FD rates of up to 97.63%. In general, the FD decreases with an increasing number of simulated QTL. Especially in M5 and M7 to M9 heritability has a strong effect not only on TPR but also on FD, which increases with decreasing heritability (Fig. 4e, f).

The larger the effect size of the QTL, the higher the TPR (Fig. 5). For a QTL with 5% effect, TPR is overall highest, independent of the number of smaller QTL and heritability in these simulations. For QTL with smaller effect sizes, observations are similar to the observations made for QTL with 5% effect. The TPR is reduced with increasing percentage of wild beet genome and represented wild beet haplotypes within the mapping population from M1 to M9. TPR of M5 is breaking down compared to M4 and M6 for all effect sizes. TPR is also reduced with heritability. QTL with 2.5% can still be found with the crossing designs M1 to M4 with a probability of 62.53–70.30% when heritability is at 90%. However, when heritability is reduced, the TPR is reduced to less than 52% for these crossing designs. With all other crossing designs, TPR of a 2.5% QTL reaches 35.03% at best for M6 with $h^2 = 0.9$. TPR is reduced to almost 0% for

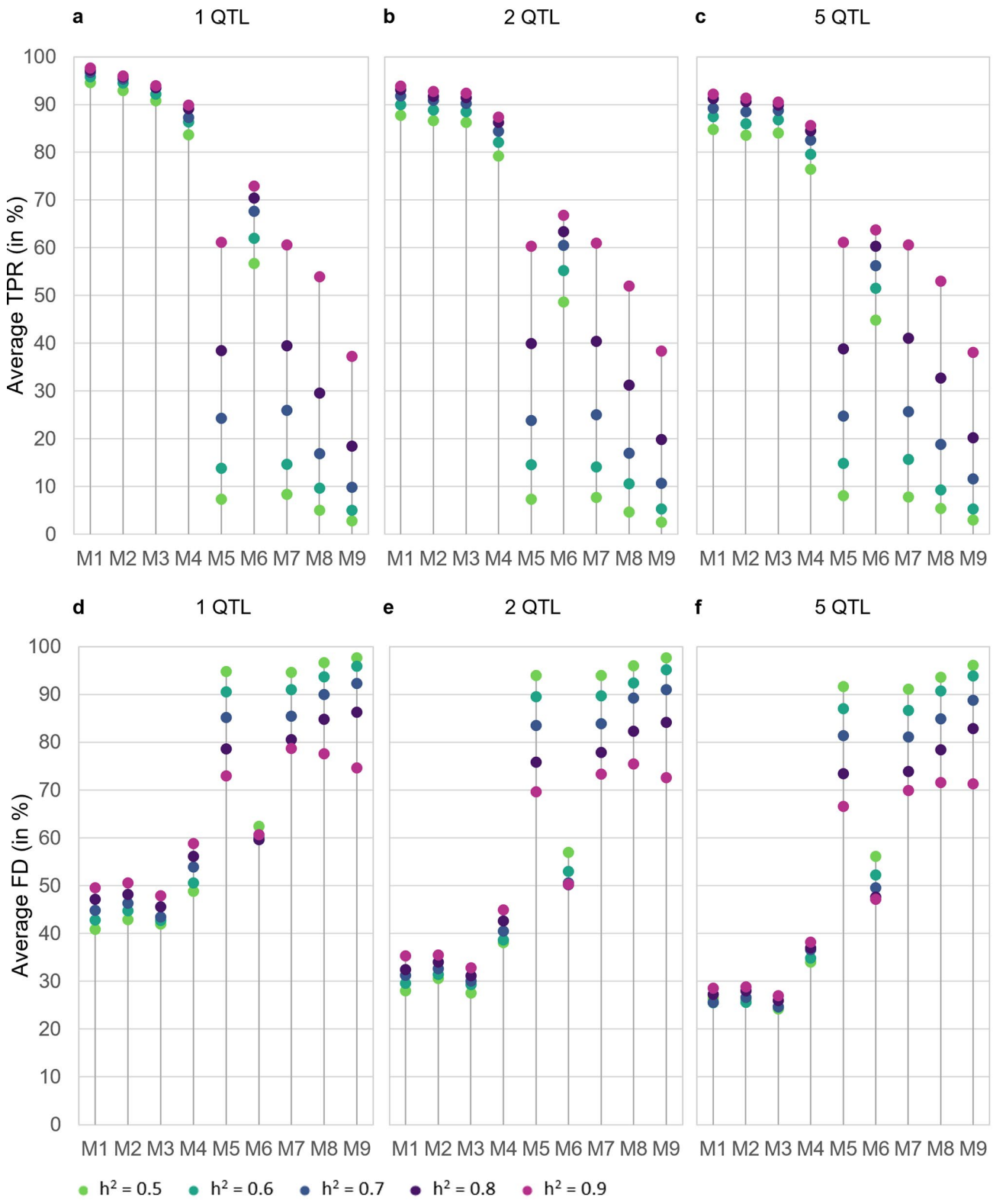

Fig. 4 **a–c** Average true positive rate (TPR) and **d–f** average false detection rate (FD) across all three wild beet populations by crossing design (M1 to M9), number of simulated QTL (1, 2 or 5) and heritability (0.5, 0.6, 0.7, 0.8 or 0.9) in percent

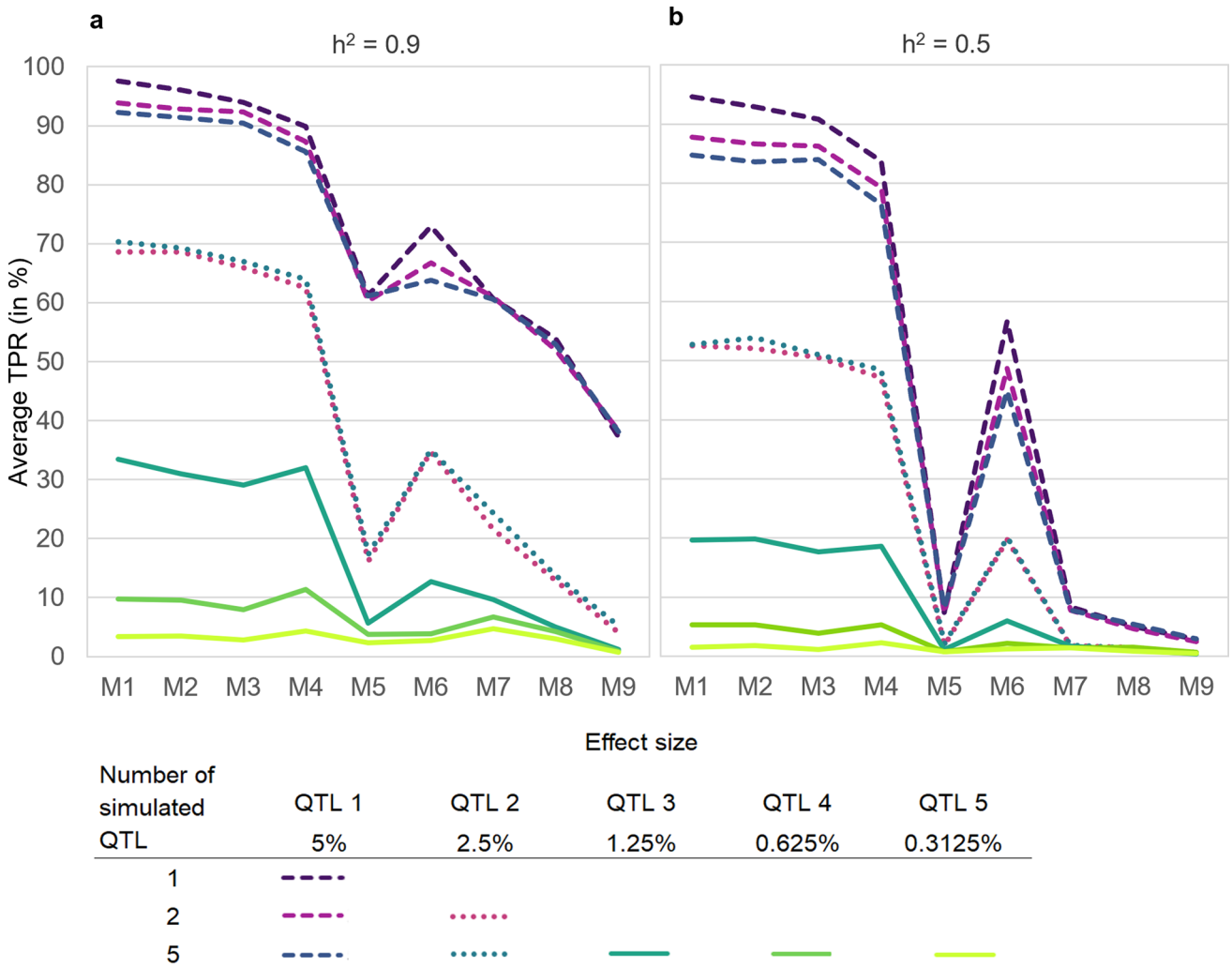

Fig. 5 Average true positive rate (TPR) in percent per number of simulated QTL (1, 2 or 5) and corresponding effect size across all three wild beet populations by crossing design (M1 to M9) for a heritability of **a** 0.9 and **b** 0.5

other crossing designs and lower heritability. The effect of simulating additional smaller QTL on the TPR of the 2.5% QTL is very low. TPR of the 2.5% QTL when simulating two QTL or five QTL are very similar.

QTL with smaller effect sizes will most likely not be detected, independent of heritability and crossing design of the mapping population. All QTL with an effect size of ≤ 1.25% have a TPR of 33.47% at best (M1 at $h^2 = 0.9$) and close to 0% for smaller effect sizes. With a lower heritability (i.e., $h^2 = 0.5$), TPR is reduced to below 20% and goes down to almost 0 in many cases.

Depending on the simulated crossing design, QTL can be discovered, even if the QTL allele has a low frequency within the wild beet individuals chosen for simulation (Fig. 6). In general, the TPR is higher if the frequencies of both wild beet alleles are balanced. If the allele carrying the QTL effect is present with a low frequency within the wild beet individuals, TPR decreases. At which allele frequency the TPR starts to decrease and how quickly it drops down how far, depends on the simulated crossing design. In M1, with 50% of wild beet genome and both wild beet haplotypes represented in the mapping population, the TPR only decreases slightly. Even in the most extreme cases simulated within this study with an allele frequency of the QTL allele of 0.17% (equal to only one allele present in all 300 individuals), the TPR is still by 90.11% on average (Fig. 6a, b). The required frequency of the QTL allele within the chosen wild beet individuals to reach a TPR of ≥ 90% increases with an increasing percentage of elite genome in the mapping population. In M4 with one wild beet haplotype and around 25% wild beet genome represented (Fig. 3) an allele frequency of 1.67% (M2: 0.50%, M3: 1.00%) or higher is required to achieve a TPR of about 90%. In M9 the TPR does not exceed 73.24% in any case, even with high frequencies of the QTL allele. When the QTL allele has a frequency of ≥ 5% within the

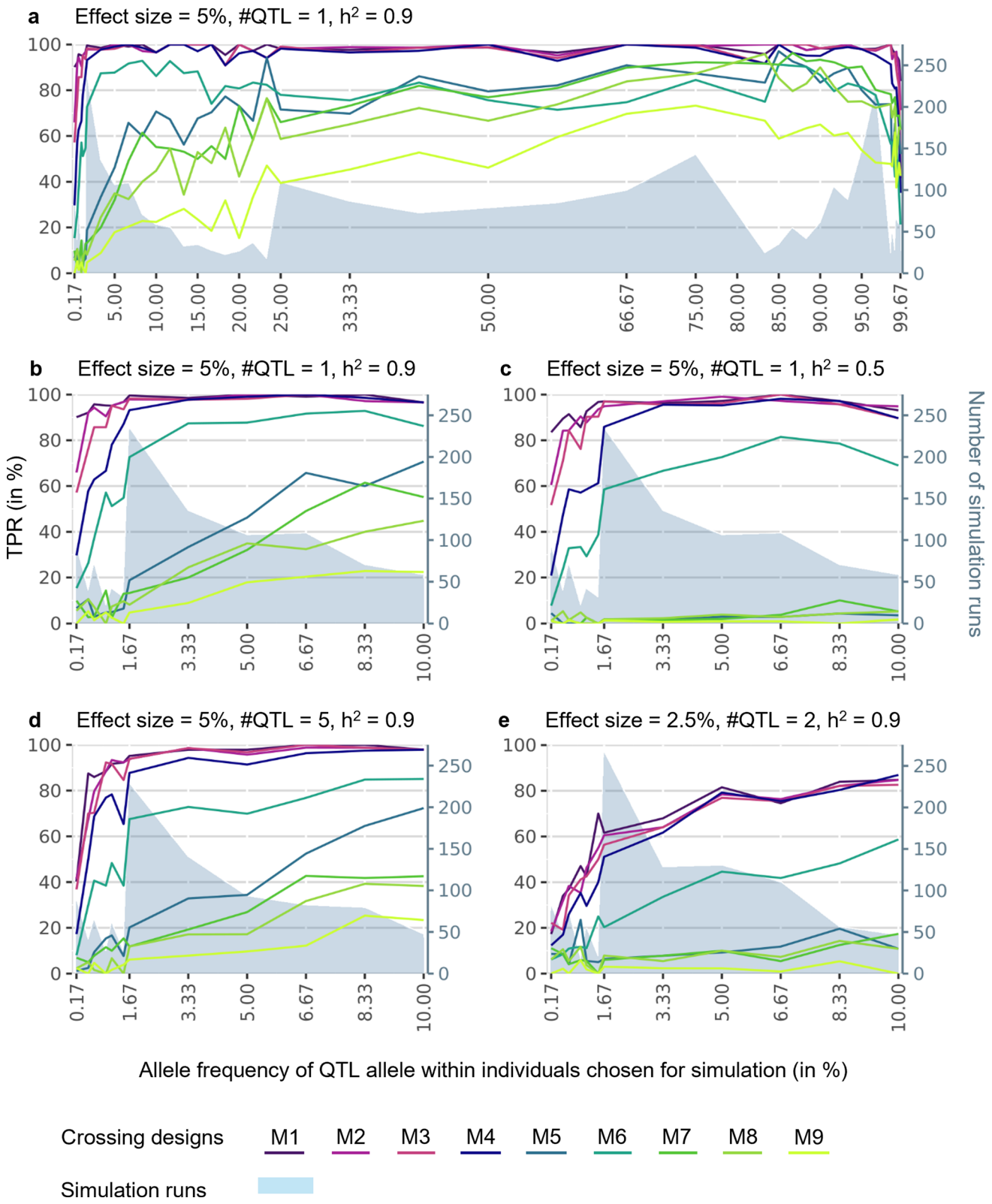

Fig. 6 Number of simulation runs and corresponding true positive rates (TPR) of different classes of allele frequencies of the QTL allele across all simulation runs and wild beet populations for different heritabilities (h^2), effect sizes and number of QTL simulated (#QTL). Allele frequencies shown are those of the individuals chosen for simulation

wild beet individuals chosen for simulation, TPR of crossing designs M1 to M4 are similar (~ 100%).

If heritability is reduced, in all crossing designs a higher frequency of the QTL allele is required to reach a certain TPR (Fig. 6c). A TPR of 90% requires at least an allele frequency of 0.67% in M1, 1.00% in M2, and 1.17% in M3. With an allele frequency of the QTL allele of 3.33%, a TPR of 95% can be achieved with M4. TPR does not exceed 10% for M5 or M7 to M9 for allele frequencies of the QTL of 10% or below.

Increasing the number of simulated QTL increases the QTL allele frequency required for QTL discovery (Fig. 6d). Even with a heritability of 0.9, in M1 an allele frequency of 1.17% is required to reach a TPR of 90%. With an allele frequency of 0.17%, only a TPR of 40.23% is reached with M1.

The described trends hold true for an effect size of 5%. If QTL are smaller, the general trend is similar, however on an overall lower level. Also, maximum TPR reached are lower. With smaller effect sizes, the required QTL allele frequencies increase severely (Fig. 6e). If two QTL are simulated with a heritability of 0.9, even with a frequency of the QTL allele of 10%, only a TPR of 84.78% is reached with M1.

In general, decreasing heritability, increasing number of simulated QTL, increasing amount of introduced elite genome and a decreasing effect size of the QTL allele are all factors that cause for an increasing allele frequency of QTL allele required to reach desired TPR. However, an increase in number of simulated QTL appears to affect TPR less than decreasing heritability or increasing the elite genome. All trends described above are observed across all three populations independently.

Discussion

The main objective of this paper was to compare different crossing designs concerning their power to detect minor QTL of quantitative traits in wild beet populations, which cannot be evaluated in the wild beet per se, such as yield or drought stress.

Population structure

For wild beet populations increasing homogeneity within the material is not found to be beneficial in terms of increasing power (Fig. 4). *Beta vulgaris* ssp. *maritima* (L.) Arcang. is a naturally outcrossing species with a high degree of self-incompatibility and hence usually high levels of heterozygosity within the populations (Hautekeete et al. 2020; Felkel et al. 2023). Contrary to that, the individuals of all the populations we used are homozygous at 69.78–98.66% of all loci under evaluation. Between on average 11.00% of the markers in the population from Denmark and 17.18% in France are heterozygous (Fig. 1). Selfing reduces the heterozygosity by half. In this case this would correspond to an increase from 11–17% to only ~ 5–9% on average in homozygosity. Double haploid production leads to a homozygous state within one generation. However, increase in homozygote loci by only 5–17 percent points at most appears to not improve homogeneity of the mapping population and therefore phenotyping accuracy enough to have a major impact on QTL detection power. The increase in homozygosity via selfing or DH production adds additional steps to material development, which increases development time, but does not increase TPR. This is independent of the percentage of elite genome within the mapping population.

With a decrease in the percentage of wild beet genome and wild beet haplotypes present in the mapping population, there is a trend for TPR to decrease. M6 is an exception to this trend. M1 to M3, which contain 50% wild beet genome in the mapping population, have the highest TPR. All crossing designs in which elite genome was introduced, except for M4, have severely lower TPR. As soon as less than one full wild beet haplotype is represented within the mapping population (M5 to M9), TPR drops severely to below 20% for $h^2 = 0.9$ and down to 0% with $h^2 = 0.5$ (Fig. 4).

One reason for this may be that the probability to lose wild beet alleles during material development increases as the proportion of the wild beet genome in the mapping population decreases. This also increases the risk of reducing the frequency of the QTL alleles or losing them completely and hence lowers the TPR. Wild beet genome may be masked by increasing the percentage of elite genome, since in association mapping alleles that are identical by descent cannot be differentiated. SNP array data generally does not show to which of the two parental chromosomes an allele belongs. Hence, while the SNP allele coming from the wild beet may cause the effect, the same SNP allele coming from the elite genome may not, which may cause problems with the associations. With M4 to M9, by crossing wild beet to elite, genomes are "mixed" and cannot necessarily be differentiated in genotyping. Clear associations of alleles to either wild beet or elite parent could improve power.

Further, there may also be variation between entries caused by the elite genome represented in the entries of the mapping populations. Whereas in M4, there is only one wild beet and one elite haplotype present in the mapping population, this does not hold true for crossing designs M5 to M9. In these crossing designs, different percentage and parts of the wild beet and elite haplotypes may be represented within the genotypes of each entry of the mapping population. This may cause for additional variation that further reduces the precision of the phenotyping and hence power. This does not hold true for the crossing designs involving DH production. In these crossing designs, the only variation remaining in the mapping population is caused by the tester genome.

On the other hand, the crossing designs involving selfing steps have unbalanced allele frequencies within the mapping populations. While some of the genotypes within one entry are fixed for the wild allele, some are heterozygous, and others are fixed for the elite allele. Hence, some parts of the wild beet genome will not be represented at all in some of the genotypes. This segregation causes for a higher phenotypic variation and results in a lower power to detect QTL, especially in M5. In M8, the drop in TPR is not as extreme compared to the corresponding designs M7 and M9. This may be due to the fact, that there is a higher percentage of elite genome present in M8. The elite line contains less than 1% heterozygous markers, so a higher percentage of elite genome causes for a higher homogeneity compared to M5.

Heritability in this paper is simulated in the range of 0.5–0.9. This is similar to the range of heritability for yield related traits in sugar beet observed in field trials (Schneider et al. 2002; Reif et al. 2010). With decrease in heritability, a decrease in power to detect QTL can be observed (Figs. 4, 5). This trend has also been observed in other studies (Reif et al. 2010). Heritability affects the power of crossing designs M5 and M7 to M9 quite strongly, whereas crossing designs M1 to M4 and M6 are a lot less affected. This may also be due to the higher phenotypic variation caused by the elite genome in M5 and M7 to M9.

Despite the fact, that in M4 the percentage of wild beet genome is reduced by 50% compared to M1 to M3, the TPR is still on a similar level as for those designs, especially, when multiple QTL are simulated (Fig. 4). While the TPR in M6 is overall lower, it is still high compared to M5 and M7 to M9, especially for lower heritabilities. In M4 and M6 the variation caused by differing haplotypes and percentage of the elite genome represented within different entries is limited compared to M5 and M7 to M9. Further, in M6, due to DH production, the entries of the mapping population are homogenous. The only remaining variation within the entries of the mapping population is caused by the tester genome. Overall, this may increase phenotyping accuracy and hence power in M4 and especially in M6. Therefore, this may be an explanation for the limited influence of heritability on TPR for these crossing designs. However, the additional step of DH production in M6 entails a higher risk for loss of the QTL allele during material development. This seems to outweigh the advantages of homogeneous entries, less variation and increased phenotyping accuracy in the mapping population in M6 and may explain the reduced TPR compared to M4.

Overall, the TRP in wild beet populations is influenced by several factors. Crossing designs M1 to M4 with a higher percentage of wild beet haplotypes seem to be preferable and have the highest TPR. Additional steps to increase homogeneity within the mapping population in M2 or M3 have no beneficial effect on TPR compared to M1.

In addition to the low TPR, M5 to M9 also show high FD rates. The high FD rates of these crossing designs result mainly from not identifying any QTL at all, despite QTL were simulated, less from falsely identifying QTL. M1 to M4 are hence also favorable in terms of low FD rates compared to M5 to M9.

Trait architecture

No negative QTL were simulated for the wild beet genome, but an overall inferior performance. Falke and Frisch (2011) showed in simulations with a donor carrying positive as well as negative alleles for the trait under consideration, that unless the loci carrying the alleles are closely linked, the presence of negative alleles does not hinder the detection of positive alleles for the trait under consideration. Since linkage disequilibrium in wild beet populations is very low (Capistrano-Gossmann et al. 2017), this was not regarded in these simulations.

QTL explaining 5% of the sum of the genotypic effects can be detected with at least some of the crossing designs such as M1 to M4 and with a lower probability also with M6. QTL with an effect of about 2.5% can only be detected with M1 to M4 with a probability of 60–70% and a high heritability. With a low heritability or any other crossing design, QTL with these effect sizes are most likely not detected under the simulated conditions. QTL with effects smaller than 2.5% are most likely not detected, regardless of the simulated type of material development and heritability as the results of this study show (Fig. 5). This also corresponds to other studies, which found that for smaller effect sizes, the required heritability (Falke & Frisch 2011) or sample size to reach sufficient power increases (Spencer et al. 2009; Visscher et al. 2017).

QTL with a small effect of $\leq 2.5\%$ will most likely not be pursued further or used within practical breeding programs per se. Nevertheless, it is important to evaluate the influence of their presence on the detection of larger QTL. The presence of smaller QTL has an influence on the power to detect QTL in some of the simulated crossing designs. For M1 to M4 and M6, a reduction of up to ten percent points in TPR can be observed, if smaller QTL are simulated additionally to the 5% effect QTL (Fig. 5).

Within this study, QTL are assigned at random and therefore more than one QTL may by chance be assigned to the same chromosome or even to a similar position. If multiple QTL are assigned to a similar region on a chromosome, the variance explained by this interval is increased and thus the power to detect a QTL in this interval (Stich et al. 2005). Hence, the presence of a smaller QTL may increase the chance of finding a larger QTL. However, while the chance to identify this region may be increased, it may not be possible to determine the exact number of contributing QTL

(Stich et al. 2005). Consequently, the number of QTL associated with a trait influences the power of QTL detection and the overall power decreases with an increasing number of QTL. These effects appear to be more pronounced when heritability decreases. This has also been observed and described in other studies (Bernardo 2004).

Characteristics of wild beet populations

The wild beet populations used in this study all exibit a low heterozygosity (on average 11.00–17.18%), low minor allele frequencies (between 7.68% and 13.22% on average, Fig. 1) and due to many generations of outcrossing a low linkage disequilibrium.

Due to the already low heterozygosity observed within these populations, increasing homozygosity within the material further is not found to be beneficial in terms of increasing power. Rather, this increases the risk of losing the QTL alleles. Considering the low average minor allele frequencies within these populations, most alleles are not represented in many individuals. The less frequent alleles are, the more likely they are lost during material development (Reif et al. 2010; Lou et al. 2020). Only if the QTL allele is still present in the mapping population, the QTL can be detected. With crossing designs M1 to M4, QTL can be discovered, even if the QTL allele has a frequency of 0.17% (equal to 1 allele in 300 genotypes) within the wild beet individuals chosen for simulation. The risk to lose alleles is higher, the less wild beet genome and the less wild beet haplotypes are represented within the mapping population, especially when no further steps for optimization are carried out.

The power to identify a true association between a SNP and trait with association mapping depends on the phenotypic variance within the population explained by the SNP (Korte & Farlow 2013). The phenotypic variance is determined by how strongly the two allelic variants differ in their phenotypic effect size, and their frequency. Therefore, rare variants and small effect size both present problems for association mapping. Rare alleles are present in only few individuals, which reduces power. When the QTL allele is rare, the power to detect the QTL is low, unless the effect and sample sizes are large (Spencer et al. 2009; Wang & Xu 2019). The smaller the effect, the larger the required sample size to reach sufficient power, especially when the QTL allele is rare within the population (Zondervan & Cardon 2004; Spencer et al. 2009; Visscher et al. 2017).

The use of genetic resources such as wild relative populations with a low extent of linkage disequilibrium between markers (Capistrano-Gossmann et al. 2017) can increase mapping resolution, also in chromosomal regions with an otherwise high degree of linkage disequilibrium (Hansen et al. 2001). By taking advantage of the recombination events accumulated over a long period of time within the wild beet population, the number of samples necessary to achieve a given resolution is greatly reduced (Hansen et al. 2001). This may explain why alleles present only in very few individuals of the population can still be found in the crossing designs that cover a large percentage of the wild beet haplotypes in the resulting mapping populations.

Practical aspects of breeding with crop wild relatives

Crossing designs containing a large percentage of wild beet genome may face some practical issues, that are not considered within the simulations of this paper. Traits from wild beets, such as annuality or a tendency towards bolting, forked beets and so forth, can mask yield potential by causing problems within field trials and hence with measurement of yield related traits. Especially when considering lethal alleles carried within the wild beets, crossing designs where wild alleles are present only in a heterozygous state in the mapping population (M4 and M7) may be beneficial.

Practical experience shows that at least 75% elite genome in the mapping population is needed, to allow for reasonable phenotyping within yield trials. Material containing more than 25% wild beet genome does not produce proper beets, but often forked beets or has a tendency to bolting. Bolting plants use most of their energy to produce seed rather than the desired beet. While the extent of this depends also on the frequency of annuality within the wild beet population, populations from Northern Europe, as are the ones regarded within this study, in general have a lower tendency for bolting. They are located north of the 50th latitude and hence annual bolting behavior is assumed to not be the dominating life form (Van Dijk et al. 1997). Populations from more Southern regions on the other hand can contain extremely high frequencies of annual genotypes. On the other hand, forked beets are extremely difficult to harvest mechanically. While a manual harvest is theoretically possible, this will largely increase trial cost. Both, forked beets and bolting, hence impact yield trials and make phenotyping for yield in practice very difficult.

Overall, a minimum of 75% elite genome is therefore recommended to enable proper yield trial with this type of material. This is also found in studies with other crops such as wheat, where lodging can mask yield potential (Schulthess et al. 2022). Producing F1s with elite lines reduces this risk and allows for phenotyping in the field (Schulthess et al. 2022). Therefore, despite the fact that M1 to M3 have the overall highest TPR, they cannot be recommended for practical application in traits which require proper beet development for phenotyping and when frequency for bolting and forked beets within the material is high.

Further issues from working with wild beets may arise and impact the feasibility of some of the simulated crossing

designs. Wild beets do not generally allow for selfing due to various reasons, one of them being self-incompatibility (Hautekeete et al. 2020). This can lead to strong problems with seed production in crossing designs, that involve selfing steps, especially, if no elite genome is introduced as in M2. Also, low pollen production, sterility, problems with bolting or with the flowering time adjustment can pose strong difficulties on material development. These issues may impact seed production and seed availability and may be more pronounced in designs with a small percentage of elite genome. Within this study, for every entry of the mapping population a total of 594 genotypes were simulated, representing six plots with 99 beets per plot. This represents a normal plot size in practical breeding and is similar to what has been done in other studies for yield related traits in sugar beet (Schneider et al. 2002; Würschum et al. 2011). More plots would most likely improve the accuracy of estimation of yield parameters. However, even the amount of seed required for six plots may be difficult to obtain based on only one plant as pollen parent, especially from wild plants without any elite background. With those crossing designs in which the percentage of elite genome is higher, these problems will be reduced, nevertheless the available seed will be limited by being based on one plant as a pollen parent. Especially within the crossing designs involving DH production the number of progeny could be larger, but this would also require more input. Production of DH lines based on wild material may also be difficult.

In summary, various practical factors influence different approaches to material development. Since these aspects are not accounted for in the simulations, they are not directly reflected in the results but should be taken into account for interpreting the findings. Although it requires one additional material development cycle compared to M1, M4 offers the best balance between maintaining wild allele representation and ensuring proper phenotyping, as it includes a sufficient percentage of elite genome to reduce undesirable traits while still preserving rare wild alleles for QTL detection. Further, the potential challenges of selfing during seed production strongly support selecting a crossing design like M4.

Methodological aspects

The power to detect QTL is also influenced by sample size (Spencer et al. 2009; Wang & Xu 2019)**.** The simulations in this study are based on 300 genotypes per wild beet population. Developing a mapping population based on all 1,000 genotypes would be very costly and labor intensive. Since not all genotypes provide new genetic variation due to relatedness, a subset of N = 300 genotypes was chosen from each of the wild beet populations for the simulation. This number is based on population sizes used for association mapping in other studies in sugar beet (Reif et al. 2010) or studies based on wild beet populations (Capistrano-Gossmann 2017). The genotypes were chosen based on haplotypes rather than on allelic diversity, since the choice of SNPs might be biassed and inhibit capturing all the variation existing in the exotic material. Using a defined number of markers rather than haploblocks based on linkage disequilibrium allows for applying the same procedure across populations and better comparison of results. Haplotypes that were present only once within the entire population have a high possibility of being errors, such as genotyping errors, imputation errors or phasing errors. These haplotypes were not discarded because they might also constitute rare alleles. However, they were not specifically considered in the process of choosing genotypes for simulation, which was restricted to haplotypes present at least twice in the population.

Effects caused by alleles contributed by the tester are not considered in the genotyping step, since genotyping in this simulation is carried out prior to crossing with the tester. However, the alleles from the tester also affect the phenotype. This discrepancy causes variation which is unaccounted for and hence adds to the error variance and reduces power. However, this issue applies to all simulated crossing designs similarly.

In this study, the QTL allele is removed from the analysis to simulate that the loci carrying the QTL allele is usually not covered by a SNP and hence not genotyped. However, it may be linked to another marker that is indeed genotyped (Zondervan & Cardon 2004). Indirect association studies make use of the principle, that markers which are close to a QTL allele on the same chromosome will be more often co-inherited than expected under independent assortment (Zondervan & Cardon 2004). Therefore, the power of detection is influenced by the linkage disequilibrium between the genotyped markers and the locus carrying the QTL allele as well as the marker density. Especially with a low linkage disequilibrium, the marker density needs to be sufficiently high to achieve reasonable power to detect association between genotyped markers and the QTL allele (Santure & Garant 2018).

In this study, an area was identified as QTL if five or more significant markers were located in a stretch of five cM. Two such regions were identified as different QTL, when a stretch of five or more cM without significant markers separated these. This approach is based on practical experience and represents a minimum requirement of what would be followed up in practical breeding programs.

QTL discovery can be influenced by the marker density of the region of the chromosome in which the QTL allele is assigned. In this study, the average genetic distance between markers was below 0.05 cM on all chromosomes. Hence, the impact of this is assumed to be limited.

In this paper a few assumptions were made for simplicity, which not necessarily always hold true in practical

application. For one, a purely additive model was used. Dominance or epistatic effects were not regarded.

A significant effect of dominance variance in the inheritance of root yield and sugar content with a less significant effect of the additive effects was observed in some studies (Stancic et al. 2014). However, other studies found additive genetic variances to be considerably larger than epistatic and dominance variances for root yield in sugar (Kristensen et al. 2023). If parental lines are not fully inbred or when regarding three-way hybrids, additive genetic variance explains the largest portion of the variance (Kristensen et al. 2023).

The power to detect epistasis with testcross performance is low due to masking effects from the tester (Gallais & Rives 1993). Further, Reif et al. (2010) argue that in cases where the genetic contribution of the genotypes to the testcross progenies is 25%, the relative contribution of additive variance to the genetic variance among testcross progenies is eightfold larger than the additive-by-additive variance. This results in a lower power to detect epistatic effects compared to main effects. These results are in accordance with the results reported for testcross performance of complex traits in other cross-pollinating species such as maize (Mihaljevic et al. 2005). Reif et al. (2010) therefore conclude, that epistasis may be ignored in sugar beet breeding. In this study, in crossing design M1 to M3, the contribution of the wild beet genome to the test cross progeny is 50%. For all other crossing designs the contribution of the wild beet genome within the test cross progeny is 25% or below. Therefore, epistatic effects were not considered in this study either.

Within this study, the costs of different approaches for material development were not regarded. These do not only differ strongly for different organizations, but also may change rapidly over time with new technology evolving. That said, crossing designs including DH production, such as M3, M6, and M9, are likely to lead to higher material development cost, assuming that DH development is possible. The different crossing designs also vary in the time required to create a mapping population. It can also be assumed that crossing designs which require multiple steps of material development also produce higher cost than crossing designs based on fewer development steps.

Conclusion

QTL originating from the wild beet genome can be found with some of the simulated crossing designs, while others are not suitable for QTL detection under the simulated conditions within wild beet populations. Due to the low minor allele frequency, the largest risk to detection power is the loss of QTL alleles during material development. Under the assumptions within this study, QTL detection is most effective when assessing wild beet directly in testcrosses, making crossing design M1 preferable for minimal resource investment.

However, field trials require at least 75% elite genome to mitigate unfavorable wild traits like bolting and forked beets, which could obscure trait evaluation. Increasing elite genome improves phenotyping but lowers wild allele frequencies, reducing association mapping power. The number of QTL affects detection power, with slight reductions when multiple QTL with small effects are present. M1 is best for detecting rare alleles (< 1%), while M4 can still identify those with ~ 1.5% frequency, even under lower heritability. Overall, M4 offers the best balance for detecting yield-related QTL, providing sufficient elite genome for proper beet development while maintaining representation of wild beet haplotypes to preserve low-frequency alleles for QTL detection.

These findings offer valuable insights into optimizing QTL detection and crossing designs based on wild beet populations. This also highlights the need to protect and conserve these populations in their natural habitats to ensure their availability for future breeding efforts. A similar approach could be applied to other predominantly outcrossing species such as rye or sunflower, where allogamous flowering promotes the accumulation of recombination events over successive generations within crop wild relative populations. Further research is required to refine these strategies for different species and assess their feasibility in practical breeding programs.

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Author's contribution FK, LB, and MF have planned the studies. LB and FK collected the plant material. LB generated the experimental data. LB has carried out the simulations and the data analysis. LB wrote the manuscript. FK and MF contributed to writing the manuscript and to the data analysis. All authors read and approved the final manuscript.

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Data availability The genetic data and code are available from the authors upon reasonable request.

Declarations

Conflict of interest The authors have the following interests: LB and FK are employed by KWS SAAT SE & Co. KGaA, Einbeck, Germany. The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Chapter 5

General Discussion

5.1 Comparing Literature-Based Results and Empirical Findings

The remarkable adaptability to diverse and challenging environments, including tolerance to salinity, drought, and pathogen pressure, has made *Beta maritima* populations a focus of both morphological and genetic investigations. Studies across European and North African populations have consistently highlighted its diversity, using both phenotypic assessments and molecular analyses (Doney et al., 1990; Frese et al., 1990; Desplanque et al., 1999; Andersen et al., 2005; Leys et al., 2014; Abdelhameed et al., 2024). These investigations revealed that morphological differentiation often exceeds genetic variation, with environmental factors playing a substantial role in shaping population-level differences (Ribeiro et al., 2016; Ascarini et al., 2021; Abdelhameed et al., 2024). This highlights the importance of genetic analyses to complement morphological observations and deepen our understanding of population structure.

However, many early genetic studies have been constrained by limited sample sizes and low-resolution marker sets, which restrict the ability to fully characterize the genetic architecture of sea beet populations (Bertram & Frisch, 2026). This study hence provides novel insights into the genetic characterization of sea beet populations. Using high-density SNP genotyping with 16,201 markers spanning the entire genome and large sample sizes with a total of 1,363 individuals from Denmark, France, and Ireland, the authors provide a detailed assessment of genetic diversity and population structure of these Northern European sea beet populations (Bertram et al., 2025a).

Consistent with existing literature, genetic diversity observed between populations is substantially greater than within populations (Bertram et al., 2025a). The Danish population shows clear genetic separation from other European populations (Driessen et al., 2001;

Driessen, 2003; Andersen et al., 2005), whereas the French and Irish populations exhibit closer genetic relatedness (Bertram et al., 2025a).

One of the key findings of this study is that fine-scale population structure can emerge even across short geographic distances, sometimes independent of spatial separation. This was observed in the Irish population, where population structure was detected along a short stretch of coastline (Bertram et al., 2025a). These results challenge assumptions about the correlation between geographic distance and genetic differentiation. Within the literature, there is no consensus on the relationship between diversity and geographic separation (Bertram & Frisch, 2026). While some studies report a clear correlation between genetic and spatial distance (Raybould et al., 1998; Leys et al., 2014), others suggest more complex dispersal dynamics than simple geographic patterns (Richards et al., 2014). This points to active gene flow, local selection pressures and complex dispersal dynamics shaping the genetic structure of sea beet populations. The pronounced substructure observed in some populations, particularly at local scales, emphasizes the importance of *in situ* conservation and broad sampling strategies to capture the full spectrum of genetic variation.

The low linkage disequilibrium observed across all populations within this study (Bertram et al., 2025a) reflects the outcrossing mating system of *Beta maritima* and aligns with findings from previous research (Fievet et al., 2007; Capistrano-Gossmann et al., 2017). This characteristic enhances the suitability of such populations for association mapping, enabling detection of trait-associated loci with high resolution (Visscher et al., 2017).

A novel contribution of this thesis lies in the detailed analysis of minor allele frequencies, an aspect not sufficiently explored in prior literature. Minor allele frequencies averaged between 7% (Denmark) and 13% (Ireland) within the studied populations (Bertram et al., 2025a). These findings were made possible only by the large sample sizes and high-density SNP dataset. While contrary to earlier reports (Desplanque et al., 1999; Veloso et al., 2021), observed heterozygosity was low across all populations (none exceeding 30%), expected heterozygosity was notably higher (Bertram et al., 2025a). This indicates substantial underlying genetic diversity, likely induced by local selection pressures. The low minor allele frequencies and the presence of exclusive polymorphisms further support previous findings that sea beet populations harbor a wealth of genetic variation (Bertram et al., 2025a). This

population-specific genetic insight offers valuable guidance for designing targeted breeding strategies that effectively harness the diversity within sea beet populations.

In conclusion, while previous research has laid a strong foundation for understanding the diversity of sea beet populations, this study advances the field by providing robust population-level genetic data. It challenges conventional assumptions about spatial-genetic relationships and underscores the need for population-specific analysis. Sea beet populations clearly represent valuable genetic resources and unlocking their full potential will require careful consideration of population structure and allele frequency dynamics.

5.2 Genotyping Strategies for Unlocking Sea Beet Diversity

Morphological variation alone does not reliably represent genetic relationships within sea beet populations (Ribeiro et al., 2016; Ascarini et al., 2021; Abdelhameed et al., 2024). Accurate genotyping is hence essential for understanding their genetic diversity and for unlocking their potential for breeding. Over the past decades, various molecular marker systems have been employed to characterize *Beta maritima* populations (Bertram & Frisch, 2026). These include RFLPs (Restriction Fragment Length Polymorphisms), AFLPs (Amplified Fragment Length Polymorphisms), and SSRs (Simple Sequence Repeats). The respective studies have provided valuable insights on genetic diversity, gene flow, and population structure. However, most studies have relied on either a small number of markers, a small number of individuals per population, or both (Bertram & Frisch, 2026). They are hence limited in resolution, scalability, and reproducibility. This, in turn, further restricts the ability to detect rare alleles, assess linkage disequilibrium, or uncover fine-scale population structure. This study therefore marks a considerable advancement by employing a high-density SNP array with 16,201 markers to analyze 1,363 individuals across three Northern Atlantic populations (Bertram et al., 2025a).

SNPs are highly valuable for a wide range of genetic analyses. They allow for the evaluation of numerous loci distributed relatively evenly across the genome, exhibit low mutation rates, and demonstrate high heritability (Garrido-Cardenas et al., 2018). Additionally, the bi-allelic nature of SNPs enables efficient reading and consistent comparison across datasets (Andrello et al., 2017). This allows for high-throughput genotyping and ensures reproducible

results, making them particularly well-suited for large-scale diversity studies and breeding applications (Andrello et al., 2017; Garrido-Cardenas et al., 2018). The use of 16,201 SNP markers covering the entire genome within this study provided a level of resolution that enabled the detection of population-specific substructures and minor allele frequencies that were previously inaccessible (Bertram et al., 2025a).

Nonetheless, analyses based on SNPs have limitations. Being typically bi-allelic, they may underrepresent the allelic richness which can be observed with other markers, such as SSRs for example, which can show multiple alleles at the same locus (Andrello et al., 2017; Qian et al., 2017; Garrido-Cardenas, 2018). SNP-based diversity metrics can also be biased by ascertainment and marker selection. SNP panels are often developed from cultivated material or limited diversity samples, potentially underrepresenting rare or novel alleles in wild populations (Andrello et al., 2016; Andrello et al., 2017; Santure & Garant, 2018). One way to mitigate this issue is to develop and select markers based on a large and diverse germplasm set that includes, or even exclusively consists of, sea beet material. A notable example of this approach is provided by Andrello et al. (2016), who constructed a library of 15,360 DArT (Diversity Array Technology) markers using a comprehensive panel of 1,054 *Beta maritima* accessions sourced from global germplasm banks. By basing marker development on the full spectrum of available genotypes, they successfully minimized ascertainment bias and ensured a broader representation of allelic diversity.

While SNPs are excellent for detecting common variants and estimating genome-wide diversity, they may miss rare alleles or structural variants (Deschamps et al., 2012; Poland & Rife, 2012; Qian et al., 2017). In sea beet populations characterized by low linkage disequilibrium and low minor allele frequencies, as observed in this study (Bertram et al., 2025a), single-marker analyses may fail to capture the full extent of genetic variation. By grouping adjacent SNPs into haploblocks, the limitations of their bi-allelic nature may be overcome. Due to their higher heterozygosity and multiallelic structure, haplotypes are generally more informative than individual bi-allelic SNPs (Stephens et al., 2001). Hence, haplotype-based approaches offer a more accurate reflection of recombination history and genetic linkage, thereby enhancing the resolution of diversity estimates and improving the power of association mapping. In outcrossing species like sea beet, where recombination is frequent and linkage disequilibrium is generally low, haplotype analysis can be of limited

utility. When linkage disequilibrium is sparse, many markers may not form well-defined haplotype blocks or may only cluster into very small ones, reducing the effectiveness of this approach (Wall & Pritchard, 2003).

Looking ahead, genotyping-by-sequencing presents a powerful alternative for characterizing wild beet germplasm (Monteiro et al., 2013). Unlike predefined SNP arrays, genotyping-by-sequencing enables de novo SNP discovery by identifying polymorphisms directly from sequencing data (Bohra et al., 2022). One of its major advantages is the significant reduction of ascertainment bias, as it does not rely on preselected markers from cultivated material (Deschamps et al., 2012). Genotyping-by-sequencing can also detect rare variants and indels that are often missed by array-based approaches, making it particularly valuable for exploring new germplasm collections and the typically less characterized wild relatives of crops (Poland & Rife, 2012).

Although sequencing technologies were historically expensive, their costs are rapidly declining, making large-scale diversity studies increasingly feasible (Poland & Rife, 2012; Monteiro et al., 2013; Bohra et al., 2022). Initial studies have begun to generate sequence data for *Beta maritima*, but these have so far focused on *ex situ* accessions from germplasm banks, typically based on single plants per accession (Sandell et al., 2022; Felkel et al., 2023). While these studies provide a broad overview of variation within the genus *Beta* and inter-accession relationships, they offer limited insight into within-population diversity, population structure, and minor allele frequencies.

In breeding contexts, the choice of genotyping method should align with program objectives. For large-scale diversity assessments and initial screenings, SNP arrays offer a practical and efficient solution, especially when combined with haplotype analysis. For allele discovery in populations with high structural variation, sequencing may be more appropriate. Future research should explore sequencing-based methods to enhance diversity analysis and support targeted breeding strategies. Ultimately, integrating multiple genotyping approaches, alongside phenotypic and environmental data, will yield the most comprehensive understanding of wild beet diversity and its utility in crop improvement.

5.3 Challenges in Utilizing Wild Beets for Breeding

While accurate genotyping is essential for characterizing the genetic diversity of sea beet populations, it is only one part of the equation. Equally important is robust phenotyping, which enables the identification and utilization of alleles associated with desirable traits. However, agronomic traits such as yield are often difficult to evaluate under field conditions, and issues like linkage drag, poor performance of sea beet-derived lines, and phenotyping complexity can obscure the expression of favorable alleles (Dempewolf et al., 2017; Bohra et al., 2022; Flint-Garcia et al., 2023). Although literature often emphasizes the value of sea beet for introgression beneficial alleles, it rarely addresses the biological and agronomic hurdles that complicate its integration into elite breeding programs.

Sea beet populations retain traits that were deliberately eliminated during domestication, such as fanged root morphology, dormancy, seed shattering, self-incompatibility and a strong tendency toward bolting (Biancardi & de Biaggi, 2020; Hautekeete et al., 2020; Panella et al., 2020). These characteristics reintroduce inefficiencies that pose significant challenges to modern breeding systems, especially in the context of yield trials. Not only do these traits complicate agronomic evaluations, but they can also mask the expression of beneficial alleles and undermine the reliability of phenotypic data, ultimately hindering breeding progress (Dempewolf et al., 2017; Panella et al., 2020; Bohra et al., 2022; Flint-Garcia et al., 2023).

The fanged roots of sea beet are difficult to harvest mechanically and complicate both yield estimation and post-harvest processing. Although manual harvesting is theoretically possible, it dramatically increases labor and costs, making it impractical for large-scale evaluations. Bolting meanwhile diverts energy from root development to reproductive growth, leading to reduced root biomass and distorted yield data (Hautekeete et al., 2020). Notably, sea beet populations from Northern Europe, such as those analyzed in this study, tend to exhibit lower frequencies of annual bolting behavior (Van Dijk et al., 1997). Bolting in trials is especially problematic due to its variability, as sea beet populations display a range of life cycles influenced by genetic and environmental factors such as latitude and climate (van Dijk et al., 1997; Boudry et al., 2002). This heterogeneity complicates the establishment of uniform trial conditions, introduces noise into yield assessments and hence reduces the accuracy of phenotypic evaluations.

The strong self-incompatibility and irregular flowering behavior of sea beet (Hautekeete et al., 2020) pose challenges on its use in breeding by complicating the development of inbred lines or double haploids. Additionally, aligning flowering times of sea beets with those of elite breeding material to facilitate crosses may be difficult. In some cases, sterility and low pollen viability, particularly under environmental stress, may further reduce the availability of usable progeny (Biancardi & de Biaggi, 2020). These biological constraints complicate the scalability of breeding efforts and increase the time and resources required to develop viable mapping populations or conduct replicated trials, where uniformity and sufficient seed availability are critical.

Germination and dormancy issues also affect trial reliability. Sea beet seeds frequently show low germination rates and variable emergence (Hautekeete et al., 2020), leading to uneven stands in field trials and hence reducing the accuracy of phenotypic evaluations.

In conclusion, while material with a high proportion of wild genome content may offer valuable genetic diversity, it is often unsuitable for direct evaluation of agronomic traits. These limitations underscore the importance of reducing wild genome content in trial populations by integrating elite material to enhance testability and selecting appropriate crossing designs. Ultimately, successful breeding with sea beets requires a strategic balance between maximizing genetic diversity and ensuring practical agronomic feasibility.

5.4 Leveraging Natural Variation of Wild Populations for Breeding

Ultimately, the value of sea beet for crop improvement depends not only on the presence of genetic diversity but also on the ability to identify and utilize alleles associated with desirable traits. While recent studies have shown that resistance genes can sometimes be identified directly within wild populations (Capistrano-Gossmann et al., 2017), leveraging this diversity for traits like yield remains difficult, as such complex traits cannot be reliably assessed in sea beets due to poor agronomic performance and confounding factors like annuality or bolting (Bohra et al., 2022; Bertram et al., 2025b). To make wild beet variation accessible for breeding, it is often necessary to develop plant material with a substantial proportion of elite genome, allowing phenotypic evaluation under agronomically relevant conditions. For traits measured in field trials, such as yield or drought tolerance, at least 75% elite genome

content is typically required to ensure proper beet development and minimize interference from wild traits (Schulthess et al., 2022; Bertram et al., 2025b). Material with higher wild genome content often produces malformed or bolting plants, making reliable trait assessment in breeding trials difficult.

This trade-off between genetic diversity and phenotypic evaluability is a central theme when employing sea beets in breeding. On one hand, retaining a higher proportion of wild genome increases the likelihood of capturing beneficial alleles for desired traits. On the other hand, it compromises the ability to conduct reliable field trials, which are essential for validating the agronomic value of these alleles. A key challenge in this context is, how to evaluate the breeding potential of sea beet populations without extensive, resource-intensive material development?

Historically, breeders have relied on backcrossing to introduce segments of wild genomes into elite backgrounds. In this approach, individual wild plants or accessions are crossed with an elite parent, and the progeny are repeatedly backcrossed to the elite line. At each generation, selection is applied to reduce the frequency of undesirable wild alleles (Tanksley & Nelson, 1996). QTL mapping is then performed in advanced backcross generations, typically BC2 or BC3, where the genetic background is predominantly elite (Tanksley & Nelson, 1996; Bohra et al., 2022; Flint-Garcia et al., 2023). This method has been successful in identifying and transferring major-effect QTL, particularly for disease resistance, and allows for the development of nearly isogenic lines carrying specific wild segments (Tanksley et al., 1996; Tanksley & Nelson, 1996; Bohra et al., 2022).

Despite its successes, advanced backcrossing has significant limitations. A major drawback is its reliance on a single selected wild donor for the development of a mapping population, which inherently restricts the genetic diversity captured. This becomes particularly problematic when working with sea beet populations, which are characterized by low minor allele frequencies and a high number of unique polymorphisms (Bertram et al., 2025a). Such alleles are likely to be lost during material development and may remain undetected in the resulting mapping population (Bertram et al., 2025b). Advanced backcrossing is also resource intensive. The time required to progress from the initial cross to QTL mapping and validation can be considerable, requiring several generations of backcrossing and large

population sizes to recover desirable recombinants and minimize linkage drag (Tanksley & Nelson, 1996; Flint-Garcia et al., 2023).

Advanced backcross nested association mapping (AB-NAM), combines the advanced backcross QTL method with a nested association mapping population (Madhusudhana, 2015). In AB-NAM, diverse wild accessions are crossed to a common elite cultivar to develop a large set of related mapping progenies (Madhusudhana, 2015; Bohra et al., 2022). While AB-NAM enhances the potential to detect QTL from wild germplasm, its development remains resource- and time-intensive. Moreover, it still faces challenges in capturing the full spectrum of genetic diversity present in sea beet populations.

To address these limitations the simulation-based work presented in this thesis (Bertram et al., 2025b) has further refined these development schemes, specifically for the exploitation of sea beet populations. The results demonstrate that even complex traits like yield can be evaluated in sea beet populations, provided that the sampling strategy and crossing design are optimized to maintain rare alleles and minimize the loss of diversity during material development. One particularly promising approach is the use of F1/testcross designs (Bertram et al., 2025b). In this scheme, diverse sea beet genotypes are crossed with a common elite line to produce F1s, which are then testcrossed with a common tester. By leveraging the low linkage disequilibrium found in natural sea beet resulting from many generations of outcrossing (Capistrano-Gossmann et al., 2017), the approach minimizes the need for extensive population development. Instead, it includes only as much elite genome as necessary, preserving rare alleles and unique polymorphisms that are often lost in conventional mapping populations. The resulting mapping populations typically contain about 25% wild genome and 75% elite genome, striking a balance that enables reliable field phenotyping while retaining a broad spectrum of wild haplotypes. While populations with 50% wild genome content offer high power for QTL detection, they are unsuitable for yield trials due to poor agronomic performance. Conversely, populations with 75% or more elite genome are more suitable for phenotyping but have reduced power to detect rare alleles (Kashyap et al., 2022; Schulthess et al., 2022; Bertram et al., 2025b).

This F1/testcross design offers several advantages over advanced backcrossing populations. It is rapid, cost-effective and produces material suitable for field trials due to the high elite genome content. Sampling a large number of individuals from a sea beet

population enables a broader coverage of wild haplotypes and rare alleles. Simulation results presented in this thesis (Bertram et al., 2025b) demonstrate that optimized F1/testcross designs can detect alleles present at very low frequencies of approximately 0.17%, when associated with effect sizes of 5%. Furthermore, alleles occurring at around 1.5% frequency within the mapping population can still be identified under low heritability conditions. These findings highlight the potential of such designs to uncover valuable genetic variation that would likely remain undetected in more conventional mapping approaches.

Nevertheless, even with 75% elite genome, some wild traits may persist, particularly if present at high frequency in the wild population. Additionally, sea beet populations often exhibit population structure, which complicate association mapping and trait discovery. For example, deviations from Hardy-Weinberg equilibrium may reflect non-random mating or geographic isolation, causing population structure and hence inflating false positives in GWAS and reducing power to detect true associations (Egan et al., 2018). Also, rare alleles and private polymorphisms are often confined to specific populations or subgroups, and their representation in mapping populations depends heavily on sampling strategy and crossing design (Dempewolf et al., 2017; Tirnaz et al., 2022). Larger population sizes can improve overall detection power, especially for minor QTL (Egan et al., 2018; Schulthess et al., 2022).

Efficient utilization of sea beet variation for breeding requires strategies that balance the need for elite genome content to enable reliable phenotyping with the goal of capturing as much wild diversity as possible. While advanced backcrossing approaches have laid the groundwork for QTL discovery and transfer, their limitations in coverage, efficiency, and power to detect minor QTL have prompted the development of new approaches to leverage the genetic variation of sea beet populations. The F1/testcross design proposed in this study offers a practical and powerful alternative, enabling the comprehensive testing of this variation with reduced time and cost. Supported by advances in genotyping and statistical analysis, this strategy helps pave the way for the systematic exploitation of sea beet diversity in modern breeding programs, ultimately enhancing the genetic base and resilience of cultivated sugar beet.

5.5 The Role of Simulation Studies: Advantages, Limitations, and Future Directions

While empirical trials remain essential for validating outcomes, simulations offer a scalable and cost-effective way to test hypotheses, compare crossing designs, and prioritize strategies. One of key advantages of simulation studies lies in saving time and resources, especially valuable given the complexity, extended timelines, and high costs associated with field experiments. They enable the exploration, prediction, and optimization of breeding strategies in a virtual environment, allowing informed decisions before committing resources to real-world trials (Li et al., 2012; Li et al., 2025). By modeling breeding programs in silico, breeders also can test many more scenarios than would be feasible in the field (Li et al., 2012; Li et al., 2025).

Simulations also allow for the exploration of complex genetic architectures, including the effects of rare alleles, epistasis, and genotype-by-environment interactions, which are often difficult or sometimes even impossible to test empirically (Li et al., 2012; Stock et al., 2024). This makes these studies particularly valuable when working with wild germplasm, which often presents challenges such as poor agronomic performance, complex population structure, and low minor allele frequencies. In the context of sea beet, where minor allele frequencies can be low and population structure pronounced (Bertram et al., 2025a), simulations can help determine the optimal mapping population sizes required for reliable QTL detection, thus avoiding both underpowered studies and unnecessary expenditure (Santure & Garant, 2018).

Another strength of simulation studies is their scalability. Relying solely on field trials to compare breeding strategies carries significant risk. Each field trial is a random sample of all possible outcomes, meaning that random effects can misrepresent the actual situation (Li et al., 2012). In contrast, computer simulations offer a more reliable approach by enabling a large number of independent replications to account for stochastic variation and to generate statistically robust conclusions (Li et al., 2012). For example, in this study, 1,000 simulation runs were performed for each combination of genetic model and crossing design, enabling the estimation of true positive rates, false detection rates, and the influence of trait architecture on mapping success. This level of replication would be virtually impossible to

achieve in field trials, especially when working with wild populations that are difficult to propagate and evaluate (Bertram et al., 2025b).

Simulations are also invaluable for hypothesis testing, particularly when introgressing favorable alleles from wild germplasm. In many cases, the absence of detected effects in empirical trials raises the question of whether beneficial alleles are truly missing or simply undetectable due to limitations in the mapping strategy. Simulation studies help breeders to assess the sensitivity and specificity of their mapping designs before committing to large-scale experiments. This proactive approach enables the identification of optimal strategies for trait detection and helps avoid costly misinterpretations in practical breeding efforts.

Despite these advantages, simulation studies have important limitations. The accuracy of simulation results depends on the quality of the models and assumptions on which they are based (Viceconti et al., 2024). If these are not accurately represented, simulation results may be misleading. In this study for example, the pronounced population structure in the Irish and French sea beet populations introduces complexity that must be carefully modeled in simulations to avoid spurious associations (Bernardo, 2010; Garnier-Géré & Chikhi, 2013; Santure & Garant, 2018; Bertram et al., 2025a). Therefore, to address population structure within the association analysis, real genetic data and kinship matrices were incorporated, ensuring that underlying relatedness among individuals was appropriately accounted for (Bertram et al. 2025b). Many parameters required for simulations, such as heritability, genetic correlations, or the effects of specific alleles, however, may be poorly known, especially for wild germplasm, and are often based on assumptions.

Since it is not feasible to account for every influencing factor in complex biological systems (Viceconti et al., 2024), a further constraint is often the reliance on simplified genetic models. In the study presented here, additive genetic models were used, excluding dominance and epistatic interactions. While this approach is supported by previous studies in sugar beet (Reif et al., 2010; Kristensen et al., 2023), it may overlook important genetic mechanisms, particularly in hybrid breeding contexts where non-additive effects can play a significant role (Stancic et al., 2014). Simulations also typically assume idealized conditions, such as uniform environments and perfect phenotyping, which rarely occur in practice. This can lead to overestimation of mapping power and underestimation of confounding factors such as genotype by environment interactions.

Furthermore, simulations also often cannot fully capture the influence of human decision-making, organizational constraints, or unforeseen practical issues that arise in real breeding programs, such as issues with seed availability, reproductive barriers, or phenotyping difficulties. For example, sea beet's self-incompatibility and erratic flowering behavior pose significant challenges for material development, which are mostly not reflected in simulation models. These biological realities must be considered alongside simulation results when designing breeding strategies (Bertram et al., 2025b). For example, simulation results in this study indicated that mapping populations with 50% wild genome content (direct testcrosses) offer the highest power for detecting rare alleles (Bertram et al., 2025b). However, such designs are impractical for yield trials due to poor agronomic performance whereas designs based on F1/testcrosses strike a favorable balance between power and phenotypic evaluability (Bertram et al., 2025b).

The simulation study presented in this thesis is the first to systematically evaluate how different crossing designs and trait architectures influence the discovery of quantitative trait loci in sea beet populations. It provides a foundational framework for future research in this genetically complex species. Moving forward, simulation studies on sea beet populations can be enhanced by integrating empirical data, environmental variables, or more sophisticated genetic models. Incorporating genotype-by-environment interactions would allow for the assessment of trait stability across diverse conditions, while modeling epistasis and dominance effects could improve trait prediction accuracy.

Exploring QTL with larger effect sizes, i.e. exceeding 5%, may also be valuable. Although such large-effect QTL are generally considered unrealistic for complex traits like yield, they could be plausible for stress-related traits such as drought tolerance or resistance to diseases like Syndrome Basses Richesses and Rubbery Taproot Disease, which are often evaluated in yield trials. The 5% effect assumed in this study is therefore conservative and may underestimate the impact of stronger alleles. Nonetheless, focusing on smaller effect sizes ensures that subtle genetic signals are captured, offering a robust assessment of the mapping approach's sensitivity and limitations (Schulthess et al., 2022).

Future simulations could also investigate scenarios involving multiple large-effect QTL or model the negative consequences of wild beet genome introgression, such as linkage drag. Additionally, simulations can help determine optimal mapping population sizes tailored to

the specific minor allele frequencies found in sea beet populations. Advances in computational biology and machine learning may further expand the possibilities for simulating complex genetic scenarios and optimizing breeding strategies.

In conclusion, simulation studies provide a robust framework for evaluating breeding strategies, mapping potential, and genetic trade-offs. While they cannot replace empirical trials, they offer a powerful complement that enables hypothesis testing, scenario planning, and decision support. To fully realize their potential, simulations should be used in conjunction with high-resolution genotyping, robust phenotyping, and careful consideration of biological and logistical constraints. By following best practices and remaining mindful of their limitations, simulations can greatly enhance the efficiency and effectiveness of breeding programs aimed at harnessing wild diversity for crop improvement (Viceconti et al., 2024; Bertram et al., 2025b; Li et al., 2025).

5.6 Implications and Outlook

The approaches developed in this thesis extends beyond *Beta maritima* and sugar beet breeding, offering a framework that can be applied to other outcrossing crop species with wild relatives, such as rye, sunflower, sorghum, and maize, whose allogamous reproductive systems promote elevated recombination rates (Hariprasanna & Patil, 2015; Monteiro et al., 2016; Capistrano-Gossmann et al., 2017).

A key insight from this work is the importance of balancing the introduction of wild genome content with the need for agronomic performance – a trade-off not unique to sugar beet. In many crops, wild relatives harbor valuable alleles for stress resilience, disease resistance, or nutrient use efficiency, but also possess less favorable traits, such as small seed size, seed shattering, low biomass, or asynchronous flowering, that can mask beneficial effects in standard breeding trials (Bohra et al., 2022; Flint-Garcia et al., 2023). The agronomic inferiority of wild relatives, however, should not discourage their use, as they may contain critical genetic variation needed for future crop improvement (Bohra et al., 2022). Instead, breeders must adopt strategic evaluation procedures and crossing designs that maximize the retention of useful diversity while ensuring practical feasibility in breeding programs. By combining high-density marker or sequence-based characterization of genetic diversity and

population structure with simulation studies, this framework supports the evaluation and selection of optimal crossing designs and mapping population strategies. For sea beet populations, the F1/testcross design proposed in this study offers a practical and powerful approach to leverage their genetic variation, enabling the comprehensive testing of the material with reduced time and cost.

Looking ahead, future research should explore the scalability of these methods across different crops and environments. As sequencing costs decline and computational tools become more accessible (Monteiro et al., 2013; Bohra et al., 2022), the integration of simulation modeling into breeding pipelines is becoming increasingly powerful. Simulations help breeders to prioritize strategies and avoid unproductive paths, accelerating the translation of wild diversity into breeding gains.

While this thesis has advanced our understanding of the genetic diversity and breeding potential of Northern Atlantic sea beet populations, it remains unclear which specific alleles hold practical value for breeding. Moreover, it is often impossible to predict in advance which alleles or traits may become valuable in the future, underscoring the importance of *in situ* conservation to preserve unique genetic variants and the need for broad sampling and comprehensive testing to uncover useful diversity across regions.

In summary, this thesis presents a robust and scalable framework for integrating wild genetic diversity into modern breeding programs. By combining high-resolution genotyping with simulation-based design, it offers practical solutions for leveraging the adaptive potential of *Beta maritima* and other outcrossing crop species. The proposed F1/testcross strategy demonstrates how breeders can balance genetic diversity with agronomic feasibility, enabling efficient trait discovery and material development. As breeding challenges intensify under global change, the insights and approaches developed here provide a valuable foundation for future research and innovation in crop improvement.

Chapter 6

Summary

Sea beet (*Beta maritima*) populations are of critical importance for sugar beet breeding because they represent a rich reservoir of genetic diversity and adaptive traits that have been lost during domestication. Their ability to thrive in diverse and challenging environments makes them invaluable for improving the resilience of cultivated sugar beet. This thesis investigates the genetic diversity of sea beet populations and their practical relevance for sugar beet breeding, combining a broad literature review, empirical population studies, and simulation-based breeding design. The work addresses the urgent need to broaden the genetic base of cultivated sugar beet, which has been narrowed by intensive breeding, by leveraging the rich genetic resources found in sea beet populations.

The first objective was to assess the current state of research on *Beta maritima* diversity. The literature review reveals extensive morphological and genetic variation across European and North African populations, shaped by geography and local environmental pressures. Phenotypic diversity often exceeds genetic differentiation, emphasizing the influence of environmental factors and the need for genetic analyses to complement morphological studies. The review also highlights significant gaps in the genetic characterization of Mediterranean populations.

The second objective was to characterize the genetic diversity and population structure of three Northern Atlantic *Beta maritima* populations using high-density SNP markers. Within this study, a total of 1,363 genotypes across three populations from Denmark, France, and Ireland were analyzed using 16,201 SNP markers. The findings reveal genetic variation among the populations, with the Irish population exhibiting the highest genetic diversity and pronounced population structure. The Danish population showed low genetic diversity and minimal population structure, while the French population displayed intermediate levels of both. In the Irish population, a pronounced population structure was detected even within a

very small geographic area, illustrating that genetic diversity and population structure are shaped by more than just geographical distance. Also, all populations exhibit unique polymorphisms. This highlights the importance of *in situ* conservation to preserve unique genetic variants, as well as the need for broad sampling and comprehensive testing to fully uncover useful genetic diversity regions.

The third objective was to evaluate how population characteristics influence the power and accuracy of genome-wide association studies for detecting quantitative trait loci (QTL). The study found that the low minor allele frequencies within sea beet populations require larger sample sizes to ensure rare alleles are adequately represented and statistical power is maintained. Populations with greater genetic diversity, such as those from France and Ireland, offer more potential for trait mapping, but strong substructure poses analytical challenges that must be addressed to avoid confounding effects. Careful consideration of population structure is essential to avoid false positives and misleading conclusions. Nevertheless, these populations offer great potential when analysis is carried out correctly and population structure is accounted for appropriately.

The final objective was to simulate and compare crossing strategies between wild and elite genotypes to optimize mapping populations based on sea beet populations for QTL discovery, especially for complex traits like yield and drought tolerance. Simulation-based studies showed that mapping populations containing a high proportion of sea beet genome (up to 50%) have the greatest power to detect rare alleles, even those present at frequencies below 1%. However, practical breeding requires at least 75% elite genome content for reliable phenotyping, as wild traits can hinder trait evaluation. Crossing designs based on elite × wild beet F1s, followed by testcrossing, strike the best balance between maintaining wild allele representation and enabling proper phenotyping. This approach offers a practical solution for integrating wild genetic diversity into sugar beet breeding programs and can be adapted for other outcrossing crop species.

By combining empirical population studies with simulation-based breeding design, this thesis provides a robust framework for leveraging wild genetic resources in breeding. The insights gained in this thesis not only contribute to sugar beet breeding but also serve as a model for utilizing crop wild relatives in other breeding programs, emphasizing the importance of *in situ* conservation, broad sampling, and integrative approaches.

Chapter 7

Zusammenfassung

Wildrüben- (*Beta maritima*) Populationen sind von entscheidender Bedeutung für die Zuckerrübenzüchtung, da sie ein reichhaltiges Reservoir an genetischer Variation und Merkmalen bieten, die während der Domestikation verloren gegangen sind. Ihre Fähigkeit, unter verschiedensten und oft herausfordernden Umweltbedingungen zu gedeihen, macht sie wertvoll für die Verbesserung der Widerstandsfähigkeit, Krankheitsresistenz und Stresstoleranz von kultivierten Zuckerrüben. Diese Dissertation untersucht die genetische Vielfalt von Wildrübenpopulationen und ihre praktische Bedeutung für die Zuckerrübenzüchtung durch die Kombination einer umfassenden Literaturübersicht, empirischer Populationsstudien und Simulationen verschiedener Züchtungsansätze. Damit greift diese Arbeit die dringende Notwendigkeit auf, die durch intensive Züchtung stark verengte genetische Basis der kultivierten Zuckerrübe gezielt durch die Nutzung der reichen genetischen Vielfalt, die in Wildrübenpopulationen zu finden ist, zu erweitern.

Die Übersicht über vorhandenen Studien zeigt, dass es in europäischen und nordafrikanischen Populationen eine große morphologische und genetische Vielfalt gibt. Die phänotypische Vielfalt übersteigt oft die genetische Variation, was den starken Einfluss von Umweltfaktoren unterstreicht und die Notwendigkeit genetischer Analysen zur Ergänzung morphologischer Studien verdeutlicht. Zudem werden erhebliche Forschungslücken bei der genetischen Charakterisierung mediterraner Populationen aufgezeigt.

Die genetische Variation und Populationsstruktur von drei *Beta maritima* Populationen aus der Nordatlantikregion wurden mithilfe eines umfangreichen SNP-Markersets charakterisiert. In dieser Studie wurden insgesamt 1.363 Genotypen aus Populationen in Dänemark, Frankreich und Irland mit 16.201 SNP-Markern analysiert. Die Ergebnisse zeigen deutliche Unterschiede zwischen den Populationen: Die irische Population weist die höchste genetische Variation und eine ausgeprägte Populationsstruktur auf, während die

dänische Population eine geringe genetische Variation und kaum Struktur zeigt. Die französische Population verhält sich in beiden Punkten intermediär. Insbesondere in Irland wurde in einem sehr begrenzten geografischen Raum eine ausgeprägte Populationsstruktur festgestellt. Das verdeutlicht, dass genetische Variation und Populationsstruktur von mehr als nur geografischer Distanz beeinflusst werden. Alle Populationen weisen zudem spezifische Polymorphismen auf. Dies unterstreicht zum einen die Bedeutung der *in-situ*-Erhaltung von Wildrübenpopulationen, um einzigartige genetische Varianten zu bewahren, zum anderen die Notwendigkeit breiter Probenahmen und umfassender Tests, um die vorhandene genetische Vielfalt vollständig zu erfassen.

Wildrübenpopulationen mit ihren beschriebenen charakteristischen Merkmalen wurden in Bezug auf ihre Eignung für die Nutzung in genomweiten Assoziationskartierungen (GWAS) zur Identifizierung von quantitativen Merkmalen (QTL) bewertet. Die Ergebnisse zeigen, dass die niedrigen Allelfrequenzen in Wildrübenpopulationen größere Stichproben erforderlich machen, um seltene Allele ausreichend zu repräsentieren. Populationen mit größerer genetischer Variation, wie jene aus Frankreich und Irland, bieten zwar mehr Potenzial für die Kartierung von Merkmalen, aber ihre starke Substruktur muss adäquate Berücksichtigung in den Analysen finden, um falsch-positive und irreführende Ergebnisse zu vermeiden.

Verschiedene Kreuzungsstrategien zwischen Wild- und Elite-Zuckerrüben wurden verglichen, um identifizieren welche auf Wildrübenpopulationen basierenden Kartierungspopulationen sich besonders für die QTL-Kartierung eignen, insbesondere für komplexe Merkmale wie Ertrag oder Trockenheitstoleranz. Simulationsbasierte Studien zeigen, dass Kartierungspopulationen mit einem hohen Anteil an Wildrübengenom (bis zu 50%) die größte Power zur Entdeckung seltener Allele haben, selbst für solche Allele mit Frequenzen unter 1%. Für die praktische Züchtung ist jedoch ein Anteil von mindestens 75% Elitegenom erforderlich, um eine zuverlässige Phänotypisierung zu gewährleisten, da Wildmerkmale die Merkmalsbewertung erschweren können. Kreuzungsdesigns basierend auf Elite × Wildrüben-F1en mit anschließenden Testkreuzungen, bieten den besten Kompromiss zwischen dem Erhalt der Wildrübenvariation und zuverlässiger Phänotypisierung. Dieser Ansatz stellt eine praktikable Lösung für die Integration

genetischer Vielfalt aus Wildrüben in Zuckerrübenzüchtungsprogramme dar. Dieser Ansatz kann auch auf andere Fremdbestäubende Kulturarten angewendet werden.

Durch die Kombination empirischer Populationsstudien und Simulationen diverser Strategien zur Materialentwicklung bietet diese Arbeit einen robusten Rahmen zur Nutzung von Wildrüben in der Pflanzenzüchtung und balanciert dabei den Bedarf an neuer Variation mit den praktischen Anforderungen der Pflanzenzüchtung. Die gewonnenen Erkenntnisse sind nicht nur für die Zuckerrübenzüchtung wertvoll, sondern können auch als Modell für die Nutzung von wilden Verwandten in anderen Züchtungsprogrammen dienen.

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Eidesstattliche Erklärung

Ich erkläre:

Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe.

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Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.

Lisa Bertram

Gießen, 04.11.2025