A review on the domestication and breeding history of spinach (*Spinacia oleracea* L.)

The possible origin and spread of spinach

Arnau Ribera Tort

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Supervisors:

Prof. Dr. Yuling Bai – Laboratory of Plant Breeding
Dr. Chris Kik – Centre for Genetic Resources, the Netherlands
Dr. Robbert van Treuren – Centre for Genetic Resources, the Netherlands
Dr. Ir. Anne-Marie Wolters – Laboratory of Plant Breeding

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Summary

This thesis consists of two parts, namely "A review on the domestication and breeding history of spinach (*Spinacia oleracea* L.)" and "The possible origin and spread of spinach".

In the first part, an overview of the crop is presented. Characteristics and relevance of spinach are followed by a brief review of its two wild relatives, the state of its germplasm and its domestication. A subsequent section on spinach breeding history is presented, from what it is available in the first references of the 16th century to current breeding and its future perspectives.

In the second part, the phylogenetic relationship and population structure of a selection of spinach landraces and wild spinach accessions have been analysed. The goal of the study is to confirm the phylogenetic relationship between the 3 *Spinacia* species and study the centre of origin of spinach. Moreover, a selection of landraces from the Eastern Mediterranean and Eastern and Southern Asia has also been included in the analysis to examine if it is possible to relate phylogenetic differences to the hypothetical spread of spinach outside its centre of origin.

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Part I: A review on the domestication and breeding history of spinach (*Spinacia oleracea* L.)

Spinach [*Spinacia oleracea* L., 2n=2x=12] is one of the most nutritious consumed vegetables (Morelock and Correll 2008). It is a very versatile crop as it is eaten either raw or cooked, and it is a common ingredient in various cuisines. The history of spinach, both from a domestication and a breeding history perspective, is arguably little known and not many references are available on this topic. For this reason, this study intends to be a review of what is known about spinach and what are the current trends on the breeding of this vegetable.

I.1. Importance of spinach

The global production of spinach reached more than 26.7 million tonnes in 2016 (FAOSTAT 2018). Especially in China, which is the leading country in spinach production (Table 1), the spinach industry has had a steady growth during the last years. The rest of the top 10 spinach producing regions are presented in Table 1. At a global scale, in 2016 the spinach industry had a value of production of $18 billion and solely China accounted for approximately $15.4 billion of this amount (Table 1).

Spinach seed production is mainly based in Denmark (Morelock and Correll 2008; Deleuran 2010; Correll et al. 2011), as this country contains more than 70% of the hectares destined to spinach seed production. The second most important region is the Pacific Northwest of the USA, represented by the states of Washington and Oregon, which accounts for ~ 20% of the seed production land (Du Toit 2018). These two regions share a common mild marine climate with long days to favour flowering (Morelock and Correll 2008).

Table 1. Spinach production and value of production of the 10 regions with the largest spinach production.

Region	Global production (%, yr 2016)	Global production (%, yr 2000)	Value of production (millions of USD, yr 2016)
China	91.50	78.75	15,436.0
European Union	2.21	5.59	464.0
USA	1.36	3.45	371.8
Japan	0.92	3.32	1,172.8
Turkey	0.79	2.15	99.3
Iran	0.44	0.92	27.8
Pakistan	0.40	0.77	17.0
Kenya	0.28	0.14	11.6
South Korea	0.27	1.27	139.2
Malaysia	0.20	0.20	23.1
Global	**26.78 million tonnes**	**9.52 million tonnes**	**18,013.7 million USD**

Source: FAOSTAT (2018)

I.2. Description of spinach

Spinach is a leafy vegetable from the Amaranthaceae family (Hassler 2018). This family from the Caryophyllales order includes other important crops such as beet [*Beta vulgaris* L.], quinoa [*Chenopodium quinoa* Willd.] and amaranth [*Amaranthus spp.*]. Spinach is an annual plant with marked vegetative growth and reproductive phases. Typically, at the end of winter or during early spring seeds are sown and seedlings grow to form a rosette of leaves up to 15 to 30 cm tall. (Krarup and Moreira 1998; Van der Vossen 2004). Many cultivars exist with distinct leaf attributes, from round to hastate shape and from flat to crinkly [savoy] texture (Morelock and Correll 2008). Moreover, the petiole of the leaves is also variable, both its colour and length (Serpolay et al. 2011; Ma et al. 2016). The arrival of warmer and longer summer days induces bolting; hence spinach plants start their reproductive phase by growing a peduncle of about one metre tall, simple or branched

Figure 1. Schematic drawing of a spinach plant with pistillate flowers in the basal part of the stalk and staminate flowers in the apical one. Pistillate flowers are shown both in their smooth [upper] and spiny [lower] form.

(Krarup and Moreira 1998), and generally with terminal staminate flowers and/or pistillate flowers at bract axils (Uotila 1997). Nowadays, there are spinach varieties adapted to different climatic conditions and photoperiods. Some varieties possess bolting resistance to longer and warmer days, which make them compatible with summer cultivation. In a similar fashion, winter-hardy varieties can tolerate colder temperatures and are adapted to winter cultivation (Van der Vossen 2004). This makes spinach a very flexible crop that can be cultivated virtually all year round in many regions.

Spinach is a wind-pollinated dioecious species, even though monoecious plants with flowers of both sexes also exist (Khattak et al. 2006). Different classes of spinach have been described based on sex and morphology (Rosa 1925), ranging from extreme males with staminate flowers and degenerated apical bracts to female plants with pistillate flowers and developed apical bracts. However, some apparently dioecious plants can suffer a process of sex reversion and show gynomonoecy and andromonoecy in the case of female and male plants, respectively (Komai and Masuda 2004; Morelock and Correll 2008). Yamamoto et al. (2014) showed that monoecism and dioecism in spinach are controlled by two different genetically linked loci on the largest spinach chromosome. Furthermore, spinach sex chromosomes seem to be homomorphic at the cytological level (Deng et al. 2012).

Recently, a high-quality assembly of the spinach genome was published (Xu et al. 2017) and is publicly available in the database SpinachBase (http://www.spinachbase.org). Its genome has an estimated size of ~ 1,000 Mb and approximately 75% of it comprises repetitive DNA. Furthermore, it contains about 25,500 protein-coding genes (Xu et al. 2017).

I.3. Nutritional content and functional properties

Spinach is rich in mineral elements but also in vitamins (Table 2) (Morelock and Correll 2008; Roberts and Moreau 2016). When compared to other common leafy green vegetables, spinach mineral and vitamin content is substantially diverse (Roberts and Moreau 2016). Interestingly, it has a high content of Vitamin B9 [folate], one of the Essential Medicines listed by the WHO (2017) used as a supplement during pregnancy (Bibbins-Domingo et al. 2017) and to prevent anaemia (De Benoist 2008).

Spinach also has substantial levels of carotenoids [e.g. vitamin A, lutein and zeaxanthin] (Bunea et al. 2008), which are known to be good antioxidants and reactive oxygen species [ROS] scavengers (Issa et al. 2006; Fiedor and Burda 2014). Moreover, spinach contains other molecules with high antioxidant properties, like vitamin C and vitamin E (Chun et al. 2005).

Table 2. Average nutritional content per 100 g of raw spinach and its equivalent Dietary Reference Intake [DRI].

Nutritional profile	Content	% DRI[1]	Nutritional profile	Content	% DRI[1]
Energy	23 kcal	1.1	Potassium	558 mg	11.9
Macronutrients			Sodium	79 mg	5.3
Carbohydrates	3.63 g	2.8	Zinc	0.53 mg	6.6
Dietary fibre	2.2 g	8.8	***Vitamins***		
Sugars	0.42 g	0.8	Vitamin A[3]	469 µg	67.0
Lipids (fat)	0.39 g	1.4	Vitamin B9[4]	194 µg	48.5
Protein	2.86 g	6.7	Vitamin C	28.1 mg	37.5
Water	91.4 g	-	Vitamin E	2.21 mg	14.7
Minerals			Vitamin K	482.9 µg	536.6
Calcium	99 mg	9.9	***Other***		
Iron	2.7 mg	15.1[2]	Lutein + zeaxanthin	12.2 mg	-
Magnesium	79 mg	25.1	Total phenolics[5]	32.5 mg	-
Manganese	0.9 mg	49.8	Oxalate[6]	2.08 g	-
Phosphorus	49 mg	7.0	Nitrate[6]	146 mg	-

Source: United States Department of Agriculture [ÙSDA] (2018) and USDA & United States Department of Health and Human Services [HHS] (2015). [1] DRI based on a moderately active, 1.65 meters tall, average weight adult female. [2] The required iron for an average adult male is significantly lower, as 100 g of raw spinach account for 33.9 % of his DRI. [3] Expressed in retinol activity equivalents [RAE], which accounts for both vitamin A and provitamin A forms [e.g. β-carotene]. [4] Expressed in dietary folate equivalents [DFE], which accounts for differences in food folate and synthetic folic acid. [5] Expressed in gallic acid equivalents [GAE] and based on Chun et al. (2005) results. [6] Based on Wang et al. (2018) results.

Other phytochemicals, in the form of phenolic compounds, are notably present in the crop (Chu et al. 2002; Issa et al. 2006; Pandjaitan et al. 2005). Phenolics include compounds such as flavonoids and polyphenols and many of them have antioxidant properties (Lin et al. 2016). Several studies concerning spinach antioxidant activity have been performed in model animals with results showing anti-aging, antiproliferative and reduced oxidative-stress effects (Joseph et al. 1998; Nyska et al. 2003; Ko et al. 2014). In the case of humans, antioxidant effects of spinach consumption have also been shown multiple times (Pool-Zobel et al. 1997; Cao et al. 1998; Castenmiller et al. 1999; Porrini et al. 2002).

Furthermore, Roberts and Moreau (2016) compiled evidence for additional effects of spinach consumption, including anti-inflammatory, anti-obesity, hypoglycaemic and lipid-lowering effects in animal models and humans. In addition, anti-cancer effects in *in vitro* and murine studies were also compiled, which were principally related to an inhibition of cancer cell growth (Vogel et al. 2005; Maeda et al. 2008).

Potential risks of spinach consumption

Spinach is known for its higher content of oxalic acid when compared to other crops (Mou 2008). This acid can form insoluble salts when combined to calcium, magnesium, zinc and iron (Noonan and Savage 1999). Even though data seems to indicate that oxalic acid does not influence iron availability in humans (Gillooly et al. 1983; Bonsmann et al. 2008), it has been shown to reduce calcium, magnesium and zinc bioavailability (Kelsay 1983; Heaney et al. 1988; Noonan and Savage 1999; Bohn et al. 2004). Moreover, calcium oxalate can potentially deposit in kidneys and form kidney stones (Noonan and Savage 1999; Ermer et al. 2016) and it has been found to have a tumour-inducing effect on breast epithelial cells in humans (Castellaro et al. 2015). Besides oxalic acid, spinach is also rich in nitrate (Santamaria 2006), which can be converted in nitrite in the digestive system (Tiso and Schechter 2015). Nitrite can react with haemoglobin to form methaemoglobin, which impedes oxygen delivery and its accumulation can lead to a serious pathology called methaemoglobinaemia (Santamaria 2006). This disease is particularly dangerous in infants (Greer and Shannon 2005) and many severe cases have been related to spinach consumption (Hack et al. 1983; Sanchez-Echaniz et al. 2001; Chan 2011; Carlier et al. 2016).

I.4. Spinach wild relatives

Currently, 11[1] plant species from the genus *Blitum* and 2 plant species from the genus *Spinacia* are part of the gene pool of *S. oleracea* (Table 3). While the *Blitum* species are classified in the tertiary gene pool of *S. oleracea*, the *Spinacia* species [*S. tetrandra* Steven ex M. Bieb. and *S. turkestanica* Iljin.] are classified in its primary gene pool. These 2 *Spinacia* species, together with *S. oleracea*, are the only members of the *Spinacia* genus.

Table 3. List of spinach wild relatives.

Blitum asiaticum (Fisch. & C.A. Mey.) S. Fuentes et al.	*Blitum litwinowii* (Paulsen) S. Fuentes et al.
Blitum atriplicinum F. Müll.	*Blitum nuttallianum* Roem. & Schult.
Blitum bonus-henricus (L.) Rchb.	*Blitum spathulatum* (A. Gray) S. Fuentes et al.
Blitum californicum S. Wats.	*Blitum virgatum* L.
Blitum capitatum L.	*Spinacia tetrandra* Steven ex M. Bieb
Blitum hastatum Rydb.	*Spinacia turkestanica* Iljin
Blitum korshinskyi Litv.	

Source: Vincent et al. (2013)

Until recently, both *S. tetrandra* and *S. turkestanica* were considered to be wild relatives from which *S. oleracea* could have been domesticated (Andersen and Torp 2011). Recent studies indicate that *S. turkestanica* is phylogenetically closer to *S. oleracea* than *S. tetrandra* (Fujito et al. 2015; Xu et al. 2017). This suggests *S. turkestanica* is the ancestor of cultivated spinach. Additionally, it was also suggested that there was a high sexual compatibility between the cultivated spinach and the two wild relatives (Andersen and Torp 2011), thus supporting the idea of both wild species being part of the primary gene pool of *S. oleracea* (Harlan and Wet 1971). Fujito et al. (2015) found that hybrids of the cross *S. oleracea* x *S. tetrandra* and *S. tetrandra* x *S. oleracea* showed a greatly diminished pollen fertility. However, the authors only used one accession of *S. oleracea* and two accessions from *S. tetrandra* for test crossing. Considering Fujito et al. (2015) observations, *S. tetrandra* should probably not be included in the primary gene pool of *S. oleracea* but in the secondary gene pool of the species.

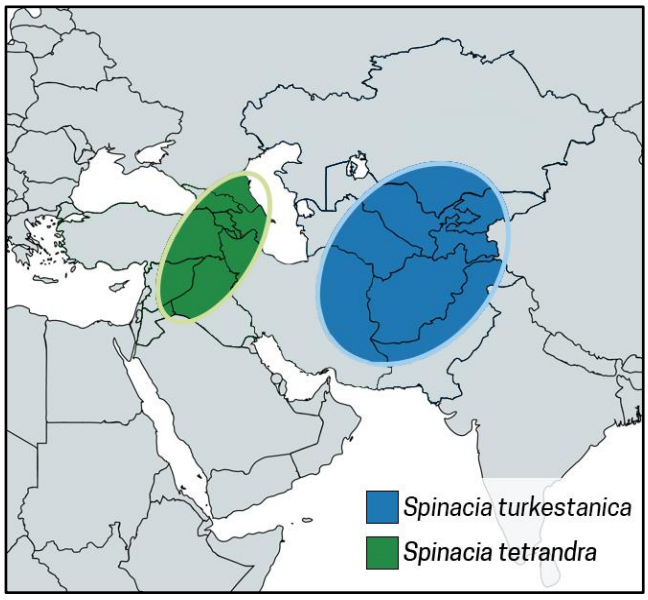

Figure 2. Hypothetical area of distribution of *S. tetrandra* (green) and *S. turkestanica* (blue).

The distribution of *S. tetrandra* and *S. turkestanica* is distinct (Figure 2). *S. tetrandra* is present in countries from the Middle East and Transcaucasia, while *S. turkestanica* is present in countries from Central and South Asia (Hassler 2018). Nevertheless, both species seem to be present in Iran although it is not mentioned if they cohabitate in this country.

During collecting expeditions of the Centre for Genetic Resources, the Netherlands [CGN], a difference in the habitat of both species was observed (Kik 2008; Van Treuren et al. submitted). In Uzbekistan and Tajikistan, *S. turkestanica* was mainly collected in the surroundings of dryland farming steppe consisting of loess soil. In Armenia and Azerbaijan, *S. tetrandra* was

[1] A 12th *Blitum* species is listed as a spinach wild relative [*Blitum petiolare* Link] (Vincent et al. 2013), but the species is considered a synonym for *Blitum capitatum* L. (Hassler 2018).

collected in a wider range of environments, predominantly on tertiary clay soils and often cohabiting with the plant *Seriphidium fragrans* (Willd.) Poljakov [syn. *Artemisia fragrans* Willd.].

In the regions where wild spinach is autochtonous, local inhabitants collect it for consumption. Wright (2011) mentions *S. tetrandra* being used in Anatolia and van Treuren et al. (submitted) indicate it is also collected in Azerbaijan. Moreover, Kik (2008) states that *S. turkestanica* is gathered in Tajikistan and subsequently traded at local markets. Wild spinach consumption seems to be done in parallel to cultivated spinach consumption, even though *S. oleracea* cultivation was found to be rare in Armenia, Azerbaijan, Uzbekistan and Tajikistan during CGN collecting missions (Van Treuren et al. submitted). The same authors also highlight that wild spinach is the first available plant after wintertime, which would help explain why locals of those countries gather them.

Differences between spinach wild relatives

At the morphological level, some differences between both wild species can be observed. While *S. turkestanica* shows only cauline clusters of flowers in the inflorescence and short-petioles in its bracts, *S. tetrandra* shows both cauline and basal clusters of flowers, and bracts lack a petiole (Uotila 1997). However, during the CGN collecting missions, Kik (personal communication) observed *S. tetrandra*'s bracts showed petioles on basal positions but not on apical ones. Other differences presented by Uotila include different plant size [*S. tetrandra* smaller] and a clearly bigger leaf terminal lobe in *S. turkestanica*, which is not differentiable in *S. tetrandra*. Details of flowers, developing fruits, bracts and leaves of both *S. turkestanica* and *S. tetrandra* are presented in Appendix 1. Furthermore, sexual dimorphism seems to be well marked in *S. tetrandra*, as male plants are considerably smaller than females (Figure 3).

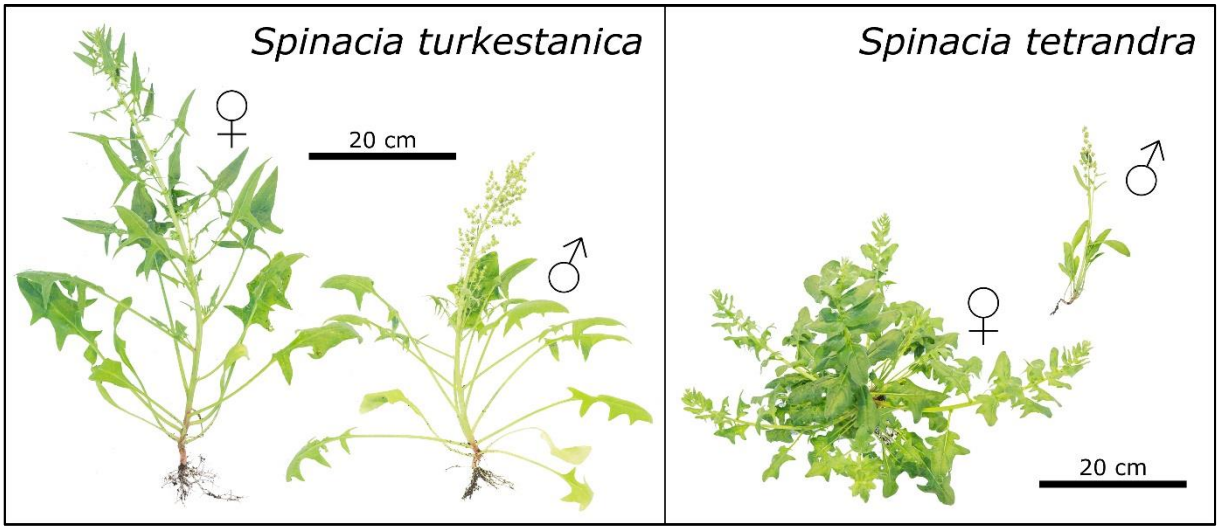

Figure 3. Wild spinach plants of both sexes and species.

Divergences between species have also been observed at a genetic level. Fujito et al. (2015) found the presence of heteromorphic sex chromosomes and a larger nuclear DNA content in *S. tetrandra*, in contrast with the homomorphic sex chromosomes and a lower nuclear DNA content present in *S. turkestanica,* and also *S. oleracea*. Previous studies involving karyotypic differences in *S. tetrandra* were conducted during the 1930s and concluded opposing results (Araratjan 1939; Lorz 1937). At the transcriptome level, Xu et al. (2015) found some significant differences between the wild species, although only 2 to 3 different accessions per species were used in the analysis.

Misclassifications of the wild species seem to have occurred with *S. tetrandra* accessions from the USDA collection (https://npgsweb.ars-grin.gov). These conflicting assignments were used in a range of studies (Hu et al. 2007; Fuentes-Bazan et al. 2012; Fujito et al. 2015; Xu et al. 2015, 2017), where apparently divergent results were obtained in phylogenetic classifications and omics data

concerning *Spinacia* species. Current results (Fujito et al. 2015; Xu et al. 2015, 2017) are based on only 3 *S. tetrandra* accessions [USDA accessions PI 647859, PI 647860 and PI 647861] which were originally gathered closely to each other.

I.5. State of spinach germplasm

Several gene banks maintain spinach accessions worldwide. Information extracted from the European germplasm database EURISCO (https://eurisco.ipk-gatersleben.de), the American germplasm database GRIN (https://npgsweb.ars-grin.gov) and the International Spinach Database [ISDB] (https://ecpgr.cgn.wur.nl/Ivintro/spinach) is presented in Appendix 2.

Even though the combined databases hold around 2100 accessions of the *Spinacia* genus*,* nearly 2000 are accessions classified as *S. oleracea*. Of these, 34% are labelled as landraces and 24% as modern cultivars. Most of these accessions are Asian and European (Appendix 2.1.), with an important amount of these being from Western Asia and Western Europe, respectively. The gene bank with the most accessions of cultivated spinach is the CGN in the Netherlands, with more than 400. Other gene banks with large numbers of *S. oleracea* accessions are located in the USA, Germany, Bulgaria and Turkey.

Availability of wild spinach accessions in germplasm banks is poor. Van Treuren et al. (2012) stated that *S. turkestanica* and *S. tetrandra* were insufficiently represented in germplasm collections as only 14 and 12 accessions of each species respectively were internationally available at that moment. In this sense, due to the lack of material and the interest of breeding companies, the CGN completed two different expeditions in 2008 and 2011 to collect wild *Spinacia* specimens (https://missions.cgn.wur.nl). In total, 66 accessions of *S. turkestanica* and 36 accessions of *S. tetrandra* were collected (Van Treuren et al. submitted).

At present, 89 accessions of *S. turkestanica* and 39 accessions of *S. tetrandra* are available in major gene banks based on the available information (Appendix 2.2.). However, 20 more accessions of *S. tetrandra* will become available in 2 different batches by the end of 2020 (Van Treuren et al. submitted). Only a few collecting missions took place in a limited number of countries, which make a few geographical regions overrepresented in germplasm collections and many others not represented at all (Appendix 2.2.). For this reason, current spinach collections could still be improved by introducing accessions from regions not represented, namely *S. tetrandra* accessions from the Middle East and *S. turkestanica* accessions from Southern Asia.

Both wild *Spinacia* species are of great interest for breeding companies as they are used as source material for agronomically important traits, especially resistance genes against spinach downy mildew, caused by the oomycete *Peronospora farinosa* f. sp. *spinaciae* [sometimes abbreviated as Pfs] (Correll et al. 1994; Qian et al. 2016). As new races of Pfs continuously appear (Feng et al. 2018), breeders benefit from potential new resistance sources present in wild spinach germplasm.

I.6. Spinach domestication

Domestication is a dynamic process in which humans take a species out of the wilderness and adapt it for cultivation in the case of crops or livestock or companionship in the case of animals (Gepts and Papa 2002; Larson et al. 2014). During the course of domestication, a range of traits, which will intrinsically define the future crop and will mark the divergence between the wild relative and the domesticate, are fixed (Meyer et al. 2012). These traits are designated as a whole as domestication syndrome (Hammer 1984; Harlan 1992) and are the product of different processes highly influenced by humans (Larson et al. 2014) but also dependent on natural selection and gene flow (Gepts and Papa 2002; Olsen and Wendel 2013).

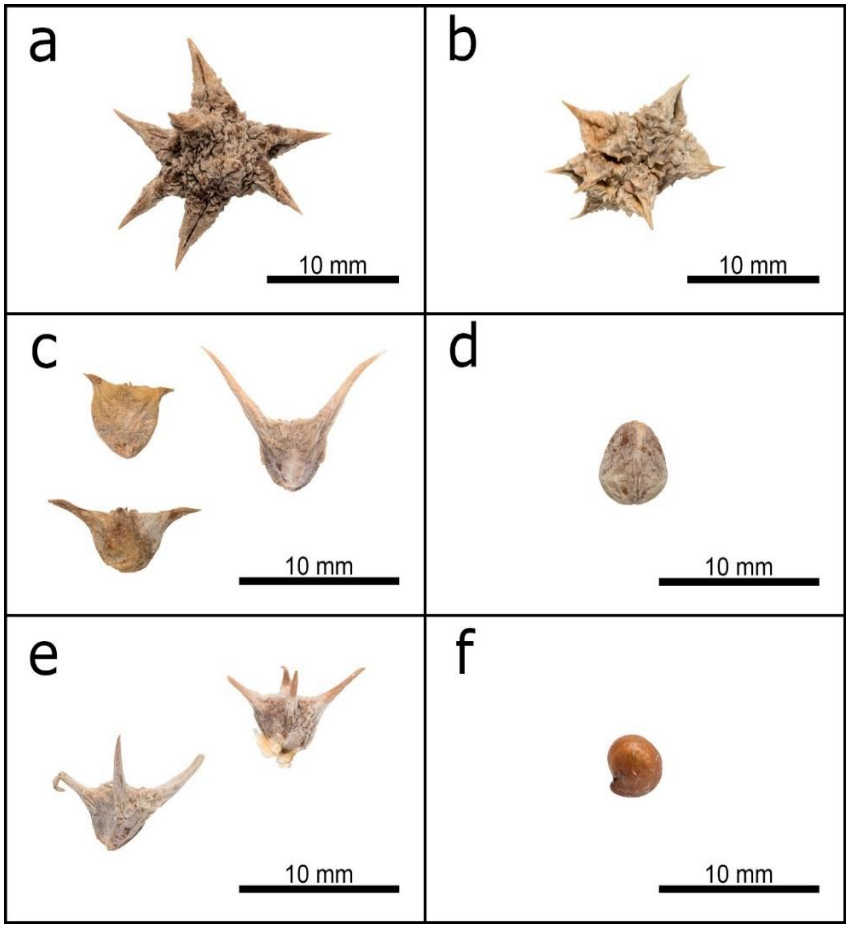

Figure 4. Spinach fruits and seed. (a) Aggregated fruit of *S. turkestanica*, (b) Aggregated fruit of *S. tetrandra*, (c) Typical spiny fruits of *S. oleracea*, (d) Round fruit of *S. oleracea*, (e) Atypical spiny fruits of *S. oleracea*, with three [left] and four [right] spines, (f) Seed of *S. oleracea*.

As domestication starts using a limited set of wild plants, genetic variation within a crop is limited by the subset of variation which the chosen wild plants contained (Eyre-Walker et al. 1998; Olsen and Wendel 2013). Only gene flow with other wild plants and mutation events increase the variation within the crop population (Gross and Olsen 2010; Meyer et al. 2012). In contrast, both artificial and natural selection reduce genetic diversity at genes under selection and genetically linked regions to those genes. In addition, genetic drift events further reduce genetic diversity across the genome.

After the initial domestication step, cultivation and human intervention [before modern breeding] on the domesticated plant influenced the fixation of more traits within the crop, which led to the appearance of different populations and varieties (Olsen and Wendel 2013). These traits were generally associated to changes in the quality of a crop [e.g. colour and flavour] (Gross and Olsen 2010).

In the case of spinach, domestication syndrome traits are not obvious because *S. oleracea* morphologically resembles very much both wild *Spinacia* species. The main difference can be found in the pistillate flower clusters. Wild species show clusters of multiple flowers fused between them, which progress into spiny aggregated fruits containing multiple seeds (Figure 4a, 4b). Astley and Ford-Lloyd (1981) pointed out the advantage of wild spinach regarding its dispersal, as the possibility of having both sexes enclosed in one single fruit is valuable to avoid isolation of the sexes and ensure reproduction in dioecious plants.

Cultivated spinach shows clusters of flowers not united with one another. Due to this reason, each flower develops into a separate round and slightly flattened fruit, with horns (Figure 4c, 4e) or without (Figure 4d). When and how [consciously or unconsciously] this trait was selected is not known. However, as the presence of separate fruits probably eases the sowing and seed collecting tasks, selection for this trait could have been desired in order to reduce manual labour. Seed morphology is visually similar in all three species, although *S. oleracea* seeds (Figure 4f) are noticeably larger compared to the ones of the wild species.

Some authors (e.g. van der Vossen 2004; Meng et al. 2017) consider two different taxonomic varieties of *S. oleracea* based on the spines of the spinach fruit: *S. oleracea* var. *inermis* for plants with smooth fruits (Figure 4d) and *S. oleracea* var. *spinosa* for plants with spiny fruits (Figure 4c,

4e). Even though clustered spiny fruits resemble the fruits of both spinach wild relatives, the smooth fruit trait seems to have appeared in Europe at the end of the Middle Ages [15th century] or beginning of the Modern History [16th century]. In fact, the first written evidence of smooth fruits dates back to 1539 (Bock 1539), thus this trait was not part of the domestication syndrome of spinach. Additionally, the presence or absence of spines follows a monogenic inheritance, with spines being dominant over smoothness (Sneep 1958; Pandey and Kalloo 1993). Considering the genetic inheritance of this trait, multiple emergences of this phenotype could have independently occurred as the smooth phenotype is also present in landraces from the Middle East (Sabaghnia et al. 2014; Mohebodini et al. 2017).

The oldest spinach drawings date back to the 14th and 15th centuries (Hallavant and Ruas 2014), such as the plant depicted in the manuscript "Liber de herbis et plantis" by the author Manfredus de Monte Imperiali, but do not offer great detail of the plants. It was not until the 16th century that more accurate descriptions and drawings were available with the works of Leonhart Fuchs, in his book "New Kreüterbuch" of 1543 (Figure 5a), and Hieronymus Bock, in his book "Kreüter Buch" of 1546 (Figure 5b) (Sneep 1957/1983). If one compares the drawings to the morphology of a wild *Spinacia* plant [especially *S. turkestanica*, Figure 5c], the similarity is clearly visible. Considering this evidence, leaf shape and plant morphology were probably not part of the domestication syndrome of spinach.

Both wild spinach species are dioecious (Astley and Ford-Lloyd 1981). Intriguingly, Fuchs' (1543) and Bock's (1546) drawings depict monoecious plants, which raises the question of whether monoecism was a potential domestication trait or not.

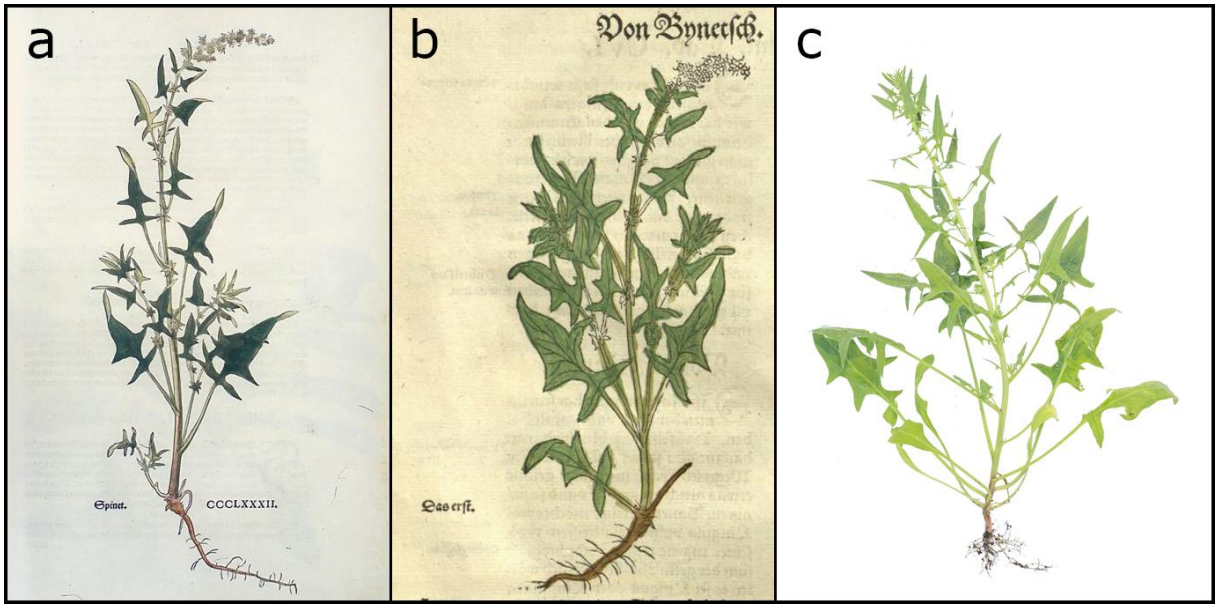

Figure 5. First botanical drawings of *S. oleracea*, in both cases depicting monoecious plants. (a) Spinach drawing in the book New Kreüterbuch (Fuchs 1543, p.668), (b) Spinach drawing in the book Kreüter Buch (Bock 1546, p.277), (c) Female plant of *S. turkestanica*, for comparison.

Another trait that was possibly domesticated was a decrease in seed dormancy, as this is high in the wild species but low in cultivated spinach (Van Treuren, personal communication). Loss of dormancy is a common domestication trait and it has apparently been selected in parallel in multiple crop families (Rendón-Anaya and Herrera-Estrella 2018; Wang et al. 2018). Dormancy is advantageous for the survival of the plant in the wild as it helps the species to avoid potentially unsuitable environmental conditions and optimise germination over time (Bentsink and Koornneef 2008). However, from a cultivation point of view, uniformity and reliability in germination are preferred (Finch-Savage and Bassel 2016).

Intriguingly, even though *S. oleracea* does not exist in the wild and only refers to cultivated spinach, its introduction and presence as a weed have been reported in countries from all six inhabited continents (Hassler 2018). After domestication and many years of cultivation, spinach has maintained, at least in the less advanced varieties, a certain weedy character which resembles the one of wild spinach (Astley and Ford-Lloyd 1981).

At the genome level, Xu et al. (2017) identified a total of 93 regions associated with selective sweeps that could be potentially related with the domestication process. In these regions, QTLs and markers associated with bolting, leaf number and stem length were present.

Origin and global spread of spinach

Currently, spinach is believed to have been first domesticated in the region of present Iran (Boswell 1949). Spinach seems to have spread to other regions late in history as no evidence to this plant from the ancient Greek and Roman civilizations has been found (Heine 2018). In fact, the oldest available written records about spinach indicate that it was consumed in Mesopotamia in the 4th century AD (El Faïz 1995, as cited in Hallavant and Ruas 2014). The earliest written evidence of cultivated spinach is from China and dates back to the 7th century (Laufer 1919). This evidence sets that spinach arrived in China via Nepal, even though it is not clear how spinach arrived in this latter country (Laufer 1919). Rolland and Sherman (2006) declare that the Saracens introduced spinach to Sicily in the 9th century. However, the first written records mentioning cultivation around the Mediterranean region date back to the 10th century in Muslim territories (Sneep 1957/1983) and the first written record in continental Europe dates back to the 12th century in Moorish Spain (El Faïz 2000). This latter document establishes spinach cultivation in the Iberian Peninsula since at least the 11th century. Following written evidence, first European archaeobotanical evidence was found in a French Pyrenean village and dates back to the end of the 12th century or beginning of the 13th (Hallavant and Ruas 2014). Considering the proximity of this location to Spain, it seems plausible to think that spinach spread to Europe from the Iberian Peninsula. Even though the diffusion routes throughout Europe remain unclear (Hallavant and Ruas 2014), archaeobotanical evidence from the 13th century has been found in Germany (Rösch 1991).

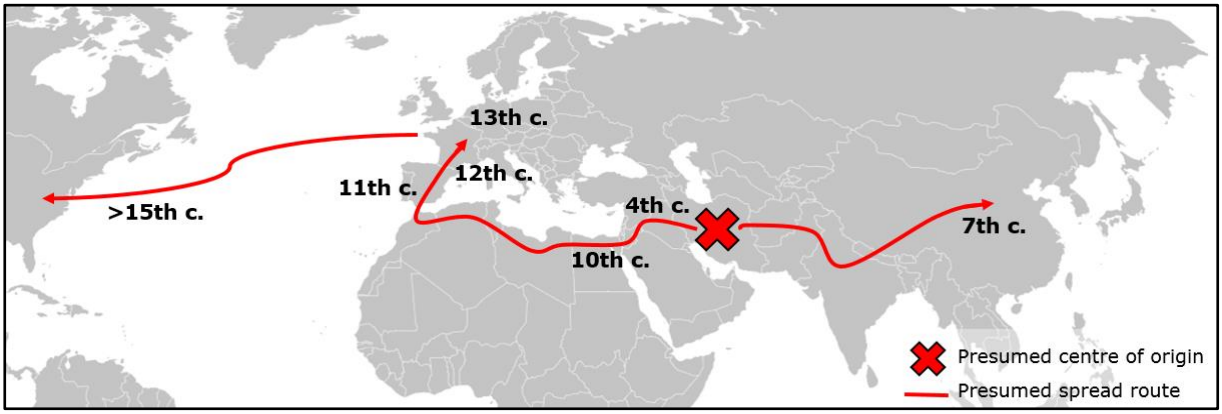

Figure 6. Presumed centre of origin and spread routes of spinach. The centuries in which evidence concerning spinach has been found in a specific region are presented along the spread routes. All the cited centuries are anno Domini [AD].

The above-mentioned historical evidence and the presence of caravan routes through Iran and its neighbouring countries (Elisseeff 2000) suggest that the spread of spinach followed two separate directions, one to Southern and Eastern Asia and another one to Africa, the Mediterranean and Northern Europe; which would later on continue to the Americas (Figure 6). This situation and the subsequent further selection of traits in each region probably led to the differences that still exist today and characterize traditional cultivars and landraces from each area.

Differences in Eastern and Western spinach

Even though there are many different landraces and varieties of spinach, each one with particular traits, several authors make a distinction between two main groups: Asian-type [mainly Chinese] and Western-type [European and American] varieties (Simoons 1990; Van der Vossen 2004). The differences between the groups have probably derived from the different selections applied in each region, which resulted in varieties having a set of common characteristics depending on where they were produced.

Asian varieties preserved part of the original narrow, pointy and hastate leaf shape of wild spinach, apart from maintaining smooth leaves and long and reddish petioles. Leaf colour depended on the territory, with a tendency to light green in China and to dark green in Japan, and fruits and seeds kept the spiny shape (Van der Vossen 2004). Varieties were generally adapted to the cold season thus they bolt quickly when exposed to relatively long photoperiods (Van der Vossen 2004). On the other hand, Western varieties changed from the hastate leaf shape to a round one and leaves were also enlarged. The colour changed to a generally darker green and petioles lost the reddish tone to a fully green one. In addition, in the West, savoy texture as well as summer spinach, which can stand long photoperiods without bolting, appeared (Sneep 1957/1983). However, both smooth leaves and winter varieties continued to be used so only a diversification and not a transition to the new types took place.

Previous phylogenetic studies seem to show there is an association between genetic relationship and geographical origin of spinach accessions. Despite Hu et al. (2007) found no correlation between these two aspects, subsequent studies (Wu et al. 2013; Shi et al. 2017; Xu et al. 2017) could genetically cluster spinach accessions in major groups in line with their geographical origin. Although Wu et al. (2013) did not present a major difference between Asian and Western varieties, Xu et al. (2017) and Shi et al. (2017) showed a consistent difference between the two groups of accessions.

I.7. Breeding spinach cultivars: past, present and future

It is not clear how breeding of new spinach varieties began in Europe. During the 1950s, J. Sneep studied and compiled evidence regarding the domestication and breeding history of spinach. In 1957, an article with his results was published as a communication from the IVT [Institute for Horticultural Plant Breeding, Wageningen, NL]. However, it was not until 1982 that Sneep's article was translated into English and published as a supplement of the 1983 edition of the journal Euphytica. Sneep's paper is probably the best and most complete source of information regarding the breeding history of this vegetable until the 1950s. In the current study, an adaptation of his work is presented in the form of a diagram, completed and expanded with additional information from IVT publications (Banga 1954, 1956, 1959, 1960; Banga and Koopmans 1962; Koopmans 1965; Banga et al. 1966) and the Oranje Lijst (deoerakker.cgn.wur.nl), a list that compiles old varieties cultivated before the Second World War in the Netherlands. As modern breeding history of spinach is diffuse due to companies being confidential about the breeding of their varieties, the diagram stops in the 1960s. The diagram based on all the mentioned information, which can be consulted in Appendix 3, comprises three different diagrams of the breeding history of spinach: one for spiny-seeded varieties (Appendix 3.1.), a second one for smooth-leaved round-seeded varieties (Appendix 3.2.) and a third one for savoy-leaved round-seeded varieties (Appendix 3.3.). Moreover, a general overview of the most relevant events of the breeding history of spinach is presented in Figure 7.

It is difficult to study how spinach looked like during previous times and how new varieties arose as evidence is scarce. Based on current evidence, Bock (1539) was the first author to differentiate between two varieties of spinach. While one had spiny seeds and leaves with hastate shape, the second variety had smooth seeds and broader leaves with a less pronounced hastate shape (Bock 1539; Sneep 1957/1983).

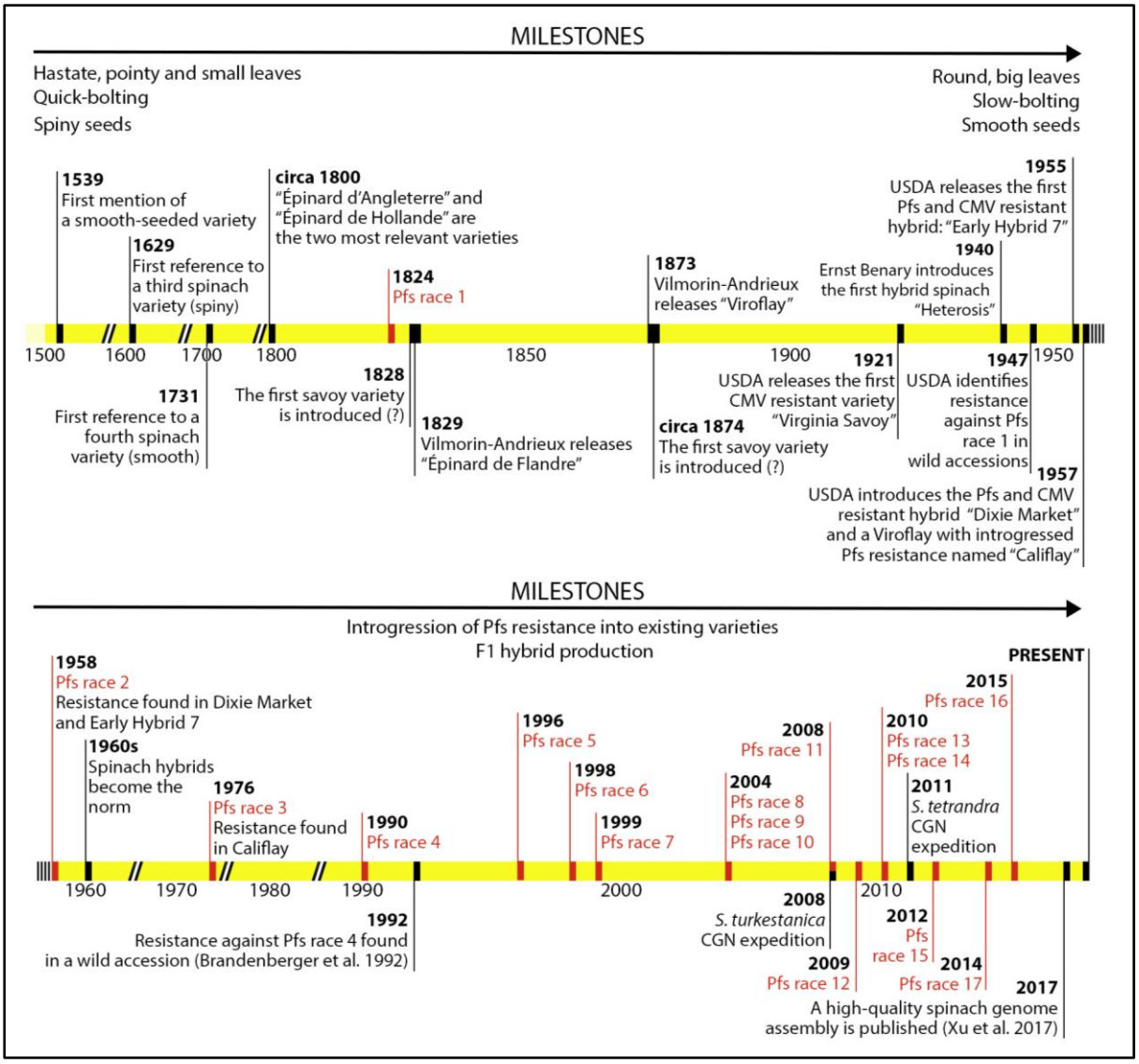

Figure 7. An overview of spinach breeding history. Red time points represent the years a new downy mildew [Pfs] race was observed as an isolate for the first time. Sources for Pfs occurrence: Brandenberger et al. (1991), Irish et al. (2007), Feng et al. (2014), Feng et al. (2018) and Naktuinbouw (2018).

John Parkinson in his book "Paradisi in Sole Paradisus Terrestris" (1629/1904) described a third variety of spinach, spiny-seeded and larger overall than the previous spiny-seeded spinach. Later references led to establish the presence of a fourth variety of spinach, smooth-seeded. It appeared in "The Gardeners Dictionary" by Philip Miller (1731), who described it as having very large round leaves. Subsequent editions of Miller's work (1768) described the presence of six varieties of spinach: three spiny-seeded varieties, differentiated by leaf morphology and the grade of seed prickliness, and three smooth-seeded varieties, which differed in the thickness, size and shape of the leaves.

As Sneep states in his 1957 article, it was not until around the 19th century that more information concerning spinach and many other crops became available. This is due to the fact that breeding companies and seed merchants started naming and adding a brief description to the varieties they sold, remarking their qualities and marketable traits. In this sense, the French seed company Vilmorin-Andrieux took a major role in spinach breeding during the 19th century and important information can be extracted from their price-lists, starting as early as 1771 (Sneep 1957/1983), and their publications [e.g. "Description des plantes potagères" (1855) and "Les plantes potagères" (1883)]. In these two publications, a spinach variety under the name of "Épinard ordinaire" and "Épinard commun" is stated to be the closest form to the wild plant, having narrow, sharp and very

sagittate [~ hastate] leaves; red petioles and spiny seeds. Additionally, it was remarked that it was a very unique variety, which utilization was explicitly not recommended. Vilmorin-Andrieux marketed improved varieties of both spiny and smooth-seeded spinach, namely "Épinard d'Angleterre" and "Épinard de Hollande", and both had larger leaves than previous varieties (Vilmorin-Andrieux 1855, 1883). Gibault (1912) considered that Épinard d'Angleterre was derived from Épinard ordinaire and Vilmorin-Andrieux (1883) mentioned that posterior smooth-seeded varieties derived from Épinard de Hollande. Gibault (1912) emphasized that the Épinard d'Angleterre and the Épinard de Hollande became the two most relevant varieties by the end of the 18th century and pointed out that both likely originated in the Netherlands. Furthermore, Sneep (1957/1983) argued that almost all existing spinach varieties probably derived from these two old varieties.

From the Épinard de Hollande, Vilmorin-Andrieux produced the smooth-seeded variety "Épinard de Flandre" in 1829 (Gibault 1912), which was called "Vlaamse" in the Netherlands (Sneep 1957/1983). This variety was described as the most common at that time and it was similar to the Épinard de Hollande but with larger dimensions and rounder leaves (Vilmorin-Andrieux 1883).

Since the end of the 19th century, Dutch breeders have had the leading role in spinach breeding (Sneep 1957/1983). In this respect, the role of Sluis & Groot [currently part of Syngenta] and Rijk Zwaan are especially significant in the history of this leafy vegetable.

Different improved varieties were selected from the spiny-seeded Épinard d'Angleterre and the smooth-seeded Épinard de Hollande and Épinard de Flandre. These selections showed the improved characteristics that defined the main breeding targets of spinach selections until mid-20th century: larger, fleshier and darker green leaves with a rounder shape and a decreased tendency to bolt. Moreover, many varieties were selected against leafless males, making most new cultivars markedly female or monoecious (Sneep 1957/1983). Even though spiny-seeded varieties were diversified, and new selections appeared during the 20th century, the general trend was focused on breeding for new smooth-seeded varieties instead of spiny-seeded ones.

It is worth-mentioning that there were two main directions in smooth-seeded spinach improvement: one to develop smooth-leaved varieties, possibly derived from the Épinard de Flandre, and a second one to develop savoy-leaved varieties, derived from a spinach called "Bloomsdale".

Smooth-leaved varieties were mainly derived from two supposedly selections of Spinach of Flanders: "Gaudry" [Figure 8a] and "Monstrueux de Viroflay", or simply "Viroflay" [Figure 8b]. Gaudry was released in 1843 and Viroflay was commercialized for the first time in 1873 by Vilmorin-Andrieux. Both had broader leaves than their predecessors but Viroflay's leaves were more triangular.

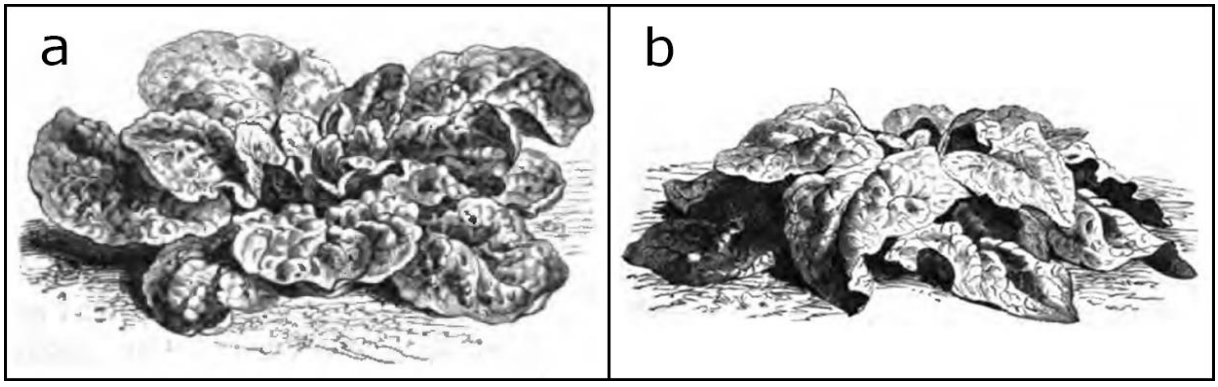

Figure 8. Drawings included in Vilmorin-Andrieux's ¨Les plantes potagères" (1883, p.205-206). (a) ¨Épinard à feuille de laitue¨, assumedly akin to Gaudry as the name is considered as a synonym by the publication. (b) Viroflay.

Gaudry and Viroflay were the starting point of many new selections and crossings, which interestingly resulted in both late-bolting summer varieties [e.g. "Nobel" in 1926 and "Viking" in 1933] and winter-hardy ones [e.g. "Géant d'Hiver" in 1927] (Sneep 1957/1983).

Savoy-leaved varieties, also called Bloomsdale-type spinach, followed a separated breeding history. Due to the shape of the leaves, these varieties were preferred over the smooth-leaved ones in regions where vegetables needed to be transported over long distances (Sneep 1957/1983; Rubatzky and Yamaguchi 1997). Crinkles helped spinach to stay less compact during packing and travelling, thus extending its shelf life.

The initial savoy variety was called "Savoy-leaved", "Norfolk" and "Bloomsdale" later on. It could have derived from the Épinard de Flandre or a similar cultivar (Kinney 1896) and its year of introduction is not precisely known. Evidence seems to suggest it was around 1874 (Kinney 1896; Sneep 1957/1983) but some sources cite 1828 as the year of introduction (Sneep 1957/1983; Decoteau 2000). Several selections were made from Bloomsdale, improving the colour to dark-green and delaying its bolting time.

I.7.1. Disease resistance breeding in spinach

In 1920, the spinach industry in the USA was threatened by a virulent outbreak of spinach blight [Cucumber Mosaic Virus, CMV] (Kaplan 1998). Smith (1920) found that Chinese spinach accessions from Manchuria [collected in a USDA expedition 20 years before] were distasteful to the aphids that transmitted the virus. The same author was able to transfer this desirable trait from Manchurian accessions to American varieties and selected "Virginia Savoy" in 1921 when he crossed CMV-resistant plants with Bloomsdale. This was the first documented case of resistance breeding in spinach breeding history.

Virginia Savoy was further used to select improved varieties of savoy spinach. Furthermore, Cook et al. (1947) reported the development of a wilt resistant line [against *Fusarium oxysporum*] by positive selection of Virginia Savoy plants. Even though the process started in 1936, the commercialization of a wilt resistant variety, called "Vates Wilt Resistant", did not take place until 1947 (Sherbakoff 1949).

As it had already happened with spinach blight, the spinach industry in the USA also had problems with downy mildew. The pathogen had already been identified as early as 1824 (Greville 1824) but it was not until 1946 that the USDA started screening germplasm for downy mildew resistance. One year later, resistance was found in two wild Persian-collected accessions: PI 140464 and PI 140467 (Smith and Zahara 1956). This finding led to the first use of wild germplasm in spinach breeding, as these two accessions were used as source material to develop resistant commercial varieties. On the one hand, savoy mildew-resistant varieties were produced. Parental lines [e.g. USDA line 99x95, using PI 140467 (Jones et al. 1956)] were derived from the resistant accessions and were later crossed to Virginia Savoy to create F1 hybrids with heterosis and resistance to both downy mildew and spinach blight. In this context, "Early Hybrid #7" was released in 1955 and "Dixie Market" in 1957, both having been developed by the USDA (Jones and Dainello 1982). It is also remarkable that these two varieties were the first major commercial hybrids present in the literature. However, they were possibly not the first spinach hybrid varieties as Sneep (1957/1983) stated hybrids had been discussed in the industry since the 1930s and mentioned the variety "Heterosis", released by Ernst Benary in 1940. On the other hand, smooth-leaved mildew-resistant varieties started being developed too. The first was "Califlay", a cross between the Persian accession PI 140467 and Viroflay and backcrossed four times to Viroflay again (Smith and Zahara 1956). Califlay was released by the USDA in 1957, the same year Dixie Market was released.

After the arrival of the USDA hybrids, F1 hybrid spinach became the norm in the spinach seed industry. At the beginning, production of hybrids was laborious due to the need to rogue male plants from the normally dioecious female seed-producing plants, as otherwise less hybrid seed would be generated. Nowadays, the process requires less work since the industry uses gynomonoecious and andromonoecious plants to be able to develop and maintain the parental lines used for hybrid production (Janick 1998; Morelock and Correll 2008). Moreover, as gynomonoecious plants perform as females unless they do not pollinate and suffer sex reversion, no rogueing of male plants is required for seed generation.

In 1958, some Califlay plants got infected by downy mildew in the USA and Europe because a second race of downy mildew appeared (Zink and Smith 1958; Smith et al. 1961). Interestingly, Early Hybrid #7 and Dixie Market were resistant to the new race [Pfs race 2] even though both hybrids and Califlay were developed with the same initial resistant Persian accession PI 140467 (Jones et al. 1956; Smith et al. 1961). Later it was found that the source of immunity to race 2 was a different Persian accession [PI 140462] that had also been used to develop the parental lines for Early Hybrid #7 and Dixie Market (Smith et al. 1962). At least two genes were hypothesized to be involved in the resistance: *M1*, present in Califlay and the hybrids, and *M2*, only present in the hybrids (Smith et al. 1962). Later, it was found that *M1* and *M2* were closely linked (Eenink 1976a). Additionally, Eenink (1976b) found that the genes involved in downy mildew resistance and the gene involved in spinach blight resistance were weakly linked.

After the start of downy mildew resistance breeding in the late 1950s, the whole industry focused its resources on introgressing both resistances into existing cultivars. A new Viroflay-derived variety was developed with both downy mildew resistances, which was called "Resistoflay" (Koopmans 1965). In the Netherlands, the spinach seed industry boomed during the 1960s and many new varieties were put on the market. The IVT annually published descriptive lists of vegetable crops [Beschrijvende rassenlijst voor groentegewassen] and one can see a meaningful transition to releasing resistant hybrid varieties from the beginning of the 1960s onwards. All these varieties were derived from Resistoflay or USDA resistant accessions (Banga 1960; Banga and Koopmans 1962). It is noteworthy that the later ones were seemingly available at the IVT, as in the IVT catalogues many varieties are stated to derive from IVT material (e.g. Koopmans 1965).

The downy mildew resistance lasted until 1976, when a third race appeared and an outbreak was observed in the Netherlands. However, Califlay and its derivatives were found resistant to this new race (Eenink 1976). This led to believe that a third gene [*M3*] was present in Califlay (Jones and Dainello 1982) and breeding efforts were focused on developing hybrid varieties with all three resistant genes *M1*, *M2* and *M3* (Jones and Dainello 1982; Brandenberger et al. 1991). In 1978, resistant spinach to the three races of downy mildew had already been put on the market (Morelock 1999).

It was not until 1990 that a fourth race of downy mildew, which could infect commercially available cultivars, appeared in California (Correll et al. 1990). Nevertheless, resistant cultivars were put on the market as early as in 1991 (Morelock 1999). Brandenberger et al. (1991) stated that the spinach breeding program in Arkansas adopted a new perspective, from single-gene qualitative resistance to polygenic quantitative resistance against downy mildew. In 1994, horizontal resistance was reported in several cultivars released by the University of Arkansas during the 1980s (Brandenberger et al. 1994; Morelock 1999). Brandenberger et al. (1994) emphasized that spinach varieties with horizontal resistance to downy mildew had been selected from lines that displayed horizontal resistance to white rust. Furthermore, Goode et al. (1988) had also previously reported resistance to soil borne and foliar pathogens in some of these cultivars.

At the same time efforts were focused on horizontal resistance breeding, screening of spinach germplasm was performed in order to find resistance genes against Pfs race 4 (Brandenberger et al.

1992). In their study, two spinach accessions were found to have high resistance against this race of downy mildew: *S. oleracea* SPI 82/87, from Iraq, and *S. turkestanica* CGN09546, from Uzbekistan. Not only both resistant sources were described as adequate material for spinach breeders but its variability in traits was highlighted by the authors. Furthermore, the two resistant accessions were multiplied and distributed to breeders (Morelock and Correll 2008). It is remarkable that the publication from Brandenberger et al. (1992) is the following reference to using spinach wild relatives for breeding purposes after nearly 50 years since the use of the USDA Persian accessions PI 140462, PI 140464 and PI 140467 (Smith and Zahara 1956; Smith et al. 1962).

I.7.2. Current and future trends in spinach breeding

Since the 1990s, with the advance of molecular and genomic techniques, quantitative breeding has been on the rise. Although downy mildew resistance has always been the priority in the spinach industry, new resistances and qualitative traits have also been considered during the breeding process.

Resistance breeding against downy mildew

Efforts to breed for new downy mildew resistant cultivars have never ceased as new races have uninterruptedly appeared. In fact, downy mildew is still the main and one of the most destructive diseases of spinach at the global scale (Correll et al. 1994, 2011). Breeders started being interested in using wild germplasm to find new resistance genes and be able to keep up with the pathogen's evolution, which resulted in CGN's 2008 and 2011 expeditions.

The International Working Group on Peronospora [IWGP] is a consortium of seed companies and the Netherlands Inspection Service for Horticulture [Naktuinbouw], which is also supported by the University of Arkansas and the University of California. The IWGP monitors the emergence and development of new spinach downy mildew races and it is the organisation that officially designates them (Plantum 2009). Currently, there are a total of 17 defined races and the last one [Pfs17] was denominated in 2018 (Naktuinbouw 2018).

The introgression of qualitative resistance genes [R-genes] from germplasm is the norm in breeding for downy mildew resistance in spinach (Correll et al. 2011). These R-genes, predominantly nucleotide-binding site leucine-rich repeats [NBS-LRR] genes (Marone et al. 2013), confer resistance to pathogens by encoding proteins specialized in recognizing pathogen-related molecules [i.e. pathogen effectors], which can trigger R-mediated defence (Gurunani et al. 2012). R-genes are located in so-called major resistance clusters [MRCs], in which recombination is suppressed and variation is induced via unequal recombination and gene conversion, resulting often in an array of paralogs of which some are involved in disease resistance (Michelmore and Meyers 1998). Therefore, it is often difficult to identify which paralog is responsible for resistance to a certain pathogen race.

Xu et al. (2017) identified a total of 139 different NBS-LRR R-genes in the spinach genome. It was hypothesized that in spinach 6 loci controlled known resistances against downy mildew [designated *RPF* loci], even though at present none have been identified nor cloned (She et al. 2018). Currently, in the literature up to 13 different *RPF* loci appear (Dijkstra 2015a, 2015b, 2016). The IWGP parties use a common differential set of cultivars with different *RPF* loci to characterize and identify new downy mildew isolates (Table 4). Some of these cultivars are near-isogenic lines [NILs] that contain a single *RPF* locus each [*RPF1-6*].

Recently, She et al. (2018) tried to locate a known *RPF* locus named *RPF1*. The authors could locate the *RPF1* region and further infer three potential candidate genes present in it by using a comparative genomics approach. All of the three suffered an amino acid substitution, which changed one of the protein domains differentiating resistant and susceptible plants.

Table 4. Differential set of spinach lines used for Pfs isolate identification.

Differential cultivar[1]	Parental resistance		Downy mildew race																
	Male	Female	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Viroflay	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Resistoflay	RPF5	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Califlay	-	RPF3	-	+	-	+	-	+	+	-	-	+	-	-	+	-	+	-	+
Clermont	RPF4	RPF5	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+
Campania	RPF6	RPF4	-	-	-	-	-	+	-	+	+	+	-	+	+/-	+	-	-	+
Boeing	RPF1	RPF5	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	+
Lion	RPF1	RPF3	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
Lazio	RPF2	RPF4	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+
Whale	-	RPF3	-	-	-	(-)	-	(-)	(-)	-	-	+	-	+	+	-	+	-	+
Pigeon	RPF2	RPF9	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
Caladonia	RPF3	RPF9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
Meerkat	RPF2	RPF10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	(-)
Hydrus	RPF11[2]	RPF11[2]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NIL1	RPF1		-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	-	+
NIL2	RPF2		-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+
NIL3	RPF3		-	+	-	+	-	+	+	-	-	+	-	-	+	-	+	-	+
NIL4	RPF4		-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+
NIL5	RPF5		-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NIL6	RPF6		-	+	-	-	-	+	-	+	+	+	-	+	(-)	+	-	-	+

Sources: International Seed Federation (2017) and Feng et al. (2018). Legend: + Susceptible reaction, - Resistant reaction, (-) Intermediate resistant reaction, +/- Variability in reaction. [1] The near-isogenic lines (NILs) can replace the Resistoflay to Lazio lines. [2] Dijkstra (2015a), which parental line gave the *RPF* locus is not specified in the source.

Further experiments with similar comparative approaches could help identify the position and sequence of more spinach *RPF* genes. As R-genes are normally clustered, there is no straightforward approach to precisely characterize which locus is the one contributing to the resistance. Moreover, additional insights in the structure of R proteins would be desirable to be able to better understand the mechanisms by which they interact with their ligands.

Resistance based on R-genes is explained by gene-for-gene interactions. For this reason, there is a rapid loss of their effectiveness when a new virulent pathogen race appears, as the latter has a large selective advantage and will spread quickly among [spinach] plants with the same genotype (Brown 2015). Even though there are methods to slow down the breakdown of R-genes, such as gene stacking and cultivating mixtures of varieties with different resistance genes (Brown 2015), the potential emergence of hyper-virulent races or races without a matching resistance locus could be an important obstacle for the industry (Kapos et al. 2019). As the current major approach to obtain downy-mildew resistance consists in temporary solutions, there is a major need to find new durable resistance approaches.

Two cases are known of durable resistance, namely quantitative resistance to powdery mildew caused by *Blumeria graminis* f. sp. *tritici* in wheat and resistance to powdery mildew [*Blumeria graminis* f. sp. *hordei*] in barley caused by loss-of-function alleles of the Mildew resistant Locus O (*MLO*) gene (Brown 2015). Some members of the MLO gene family are susceptibility genes [S-genes]. S-genes code for proteins that facilitate a compatible host-pathogen interaction and, therefore, enable the pathogen to infect the host (Van Schie and Takken 2014). For this reason, mutation or loss of a certain S-gene can lead to recessive resistance due to the inability of a compatible host-pathogen interaction. The most widely known S-genes are from the *MLO* gene family, which are functionally conserved in angiosperms (Appiano et al. 2015). Even though the original *MLO* gene confers resistance against powdery mildew to barley, several orthologues have been found in many other plants (Acedevo-Garcia et al. 2014).

Powdery mildew is not an important disease on spinach, although unusual cases have been found (Nakova 2012). However, it is a relevant disease on beet, one of spinach's closest crops (Neher and Gallian 2013). The *MLO* gene family is not the only known susceptibility gene family. Powdery Mildew Resistance [*PMR*] genes [*PMR4-6*] are also involved in powdery mildew susceptibility in *Arabidopsis*

thaliana and their function is associated with the cell wall composition of the plant (Vogel et al. 2002; Nishimura et al. 2003; Vogel et al. 2004). Huibers et al. (2013) silenced the *PMR4* ortholog [*SIPMR4*] in tomato [*Solanum lycopersicum*], resulting in plants with resistance to tomato powdery mildew.

On the other hand, Downy Mildew Resistance (*DMR*) genes [*DMR1-6*] are S-genes associated with downy mildew susceptibility in *A. thaliana*. Brewer et al. (2014) demonstrated the *DMR1* role on susceptibility to *Fusarium spp.* and Zeilmaker et al. (2015) demonstrated the role of *DMR6* concerning susceptibility to *Pseudomonas syringae* and *Phytophtora capsici*. Both Schouten et al. (2014) and Porterfield and Meru (2017) used *DMR1* and *DMR6* to look for novel *DMR* candidate genes in the Cucurbitaceae family. Huibers et al. (2013) studied the *DMR1* ortholog in *S. lycopersicum* [*SIDMR1*] and found that its silencing was associated with downy mildew resistance, although it also had a detrimental role on the plant growth.

Schouten et al. (2014) identified candidate S-genes to downy and powdery mildew in cucumber [*Cucumis sativus*] based on homology with functionally proven *A. thaliana* S-genes from the *MLO*, *PMR* and *DMR* gene families. Porterfield and Meru (2017) did a similar study using the same gene families in watermelon [*Citrullus lanatus*] and squash [*Cucurbita pepo, C. maxima and C. moschata*].

Even though no mention to S-genes in *Spinacia* have been found in the literature, breeding for mutant S-genes could be a viable approach to develop resistant spinach varieties to quickly evolving pathogens like downy mildew. Orthologues of the *MLO*, *PMR* and *DMR* families might be interesting targets to be studied and analysed in spinach.

Resistance breeding against white rust

Another relevant spinach pathogen is white rust, caused by the oomycete *Albugo occidentalis*. White rust presence is mainly localized in some parts of the USA (Correll et al. 1994; Koike et al. 2007), although outbreaks have happened in distinct countries such as Iran (Ebrahimi and Afzali, 2000), Greece (Vakalounakis and Doulis 2013), Mexico (Correll et al. 2016) and Turkey (Soylu et al. 2018). For this reason, the possible geographical expansion of this oomycete could represent an additional issue for spinach cultivation.

White rust was recognized as a serious disease during the 1930s and 1940s in the USA (Brandenberger et al. 1992). However, the first varieties with partial resistance to white rust were not released until 1975 by the USDA (Brandenberger et al. 1994). Contrary to downy mildew, no qualitative resistance against white rust is yet known (Correll et al. 2016) and quantitative approaches have been used to mitigate its effects. According to Bowers (1972, as cited in Morelock and Correll 2008), the initial plants to develop these varieties were the commercial hybrid "Hybrid 178" and the USDA breeding line "WRG 70-5". In 1987, an improved open-pollinated variety ["Fallgreen"] with a high level of resistance was released by the University of Arkansas (Morelock 1999). Even though new open-pollinated white rust resistant varieties were developed, the spinach breeding industry used the former ones to create white rust resistant hybrids (Morelock and Correll 2008), which can be commercially found in catalogues of several breeding companies.

Resistance breeding against other pathogens

Leaf spot diseases are also important in spinach. Ascomycete fungi are the main causal agents of this disease group, which is characterized by the emergence of spots on the leaves (Koike et al. 2007). The main ascomycetes that cause spinach leaf spots are *Colletotrichum dematium* [which causes anthracnose], *Stemphylium botryosum* and *Cladosporium variabile* (De Visser 2015; Liu et al. 2018). Minor leaf spot pathogens are *Cercospora beticola*, *Colletotrichum coccodes*, *Colletotrichum truncatum* and *Myrothecium verrucaria* (Liu et al. 2018). Different virulence of *C. dematium* isolates on different spinach varieties have been reported in literature (Correll et al. 1993), partial resistances to *S. botryosum* and *C. variabile* were found in spinach germplasm (Mou et al.

2008) and molecular markers associated with resistance against *S. botryosum* have been identified (Shi et al. 2016a). Even though it seems that conventional efforts to create leaf spot resistant varieties of spinach are still ongoing, the discovery and use of molecular markers linked to disease resistance genes are promising milestones for this goal.

Soilborne spinach diseases are another significant disease group and are mainly characterized by root rot and damping off at very early stages of the plant. The major causal agents of these diseases are fungi, such as *Fusarium oxysporum* [along with other *Fusarium* species] and *Rhizoctonia solani;* and oomycetes, such as *Aphanomyces cochlioides* and some *Pythium* species [e.g. *P. aphanidermatum* and *P. irregulare*] (Correll et al. 1994; Koike et al. 2007; De Visser 2015). However, a large proportion of damping off in spinach is caused by *Pythium spp.* (Magnée et al. 2017). In this context, there is currently an active research project in the Netherlands involving *Pythium* resistance and its variation in the available spinach germplasm (Magnée et al. 2017).

Moreover, wilt caused by *Fusarium oxysporum* and *Verticillium dahliae* [along with other *Verticillium* species] is also a problem that affects spinach cultivation (Correll et al. 1994; Koike et al. 2007). For *V. dahliae*, molecular markers associated with resistance to the fungus have been identified (Shi et al. 2016b).

Breeding for abiotic stress tolerance

Some authors have analysed spinach responses under water stress (Zuccarini and Savé 2016; Ors and Suarez 2017), salinity and osmotic stress (Bagheri et al. 2015; Ors and Suarez 2016; Ors and Suarez 2017; Ferreira et al. 2018), heavy metal stress (Fagioni et al. 2009; Bagheri et al. 2015) and temperature stress (Mogren et al. 2015; Chitwood 2016; Ors and Suarez 2016). Even though two spinach genes involved in osmotic tolerance are known and have been characterized (Weretilnyk and Hanson 1988; Burnet et al. 1995; Hibino et al. 2002), there seem to be no studies on the genetics of tolerance for any of these stresses.

Crop adaptation to low N availability is an important task to develop a sustainable and more efficient agriculture (Witcombe et al. 2008). In this sense, the 2 QTL regions related to growth in nitrogen-poor conditions identified by Chan-Navarrete et al. (2016) will help improve nitrogen use efficiency (NUE) in spinach.

Considering available literature, abiotic stress improvement in spinach currently remains an immature field. As climate change will offer serious challenges to agriculture (Pereira 2016), it seems likely to believe that spinach breeding foci will also include abiotic stress in the near future.

Breeding for quality

The chemical composition of the crop has been shown to differ significantly between varieties, including oxalic acid, nitrate, vitamin C, lutein, carotenoid and phenolic content (Murphy and Morelock 2000; Murphy 2001; Howard et al. 2002; Pandjaitan et al. 2005; Solberg et al. 2015; Wang et al. 2018). Even though the nutritional composition is also influenced by other factors like cultivation method and storage (Lester et al. 2010; Koh et al. 2012), large discrepancies between varieties indicate that breeding for these traits is possible, as it was already pointed out by Howard et al. (2002), Morelock and Correll (2008) and Wang et al. (2017).

Taking into consideration the existing chemical variation among spinach germplasm, maximizing valuable health-related compounds and minimizing oxalic acid and nitrate content is fundamental to increase the quality of spinach varieties. In this sense, Shi et al. (2016c) identified SNP markers associated with oxalate concentration in spinach and Qin et al. (2017) identified SNP markers associated with 13 different mineral elements. In both studies, the identified accessions with breeding potential were cultivated spinach accessions from diverse origin that included both landraces and

modern cultivars. Additionally, the 2 QTLs related to NUE (Chan-Navarrete et al. (2016) could be helpful to reduce nitrate levels in spinach leaves by minimizing the amount of applied fertilizer.

It is worth mentioning that Howard et al. (2002) detected differences in the concentration of phenolics and flavonoids between modern cultivars with downy mildew and white rust resistance and without, being the concentration of both compound families larger in resistant cultivars. Moreover, Pandjaitan et al. (2005) showed that mid-mature spinach leaves had much higher levels of the two compound groups when compared to baby and adult leaves.

Morphological traits are important for better appealing and easy processing of spinach plants. Recently, Cai et al. (2018) identified one major QTL and three candidate genes associated with leaf colour. Ma et al. (2016) identified SNP markers related to petiole colour, leaf texture and leaf edge shape and Chitwood et al. (2016) associated SNP markers to leaf erectness, plant tallness and bolting. Controlling and understanding bolting time is an important element in spinach breeding as it would allow to develop bolting-resistant varieties, thus extending the period of leaf production.

Moreover, controlling monoecism and dioecism might also be attractive for breeding purposes. Several markers associated with both conditions have been found (Khattak et al. 2006; Onodera et al. 2011; Yamamoto et al. 2014). Qian et al. (2017) identified two potential regions for the X/Y sex-determining gene. Nevertheless, as sex expression in spinach is flexible and still not well understood, further research will be needed in order to be able to manipulate this characteristic.

Over the past few years, an increasing number of studies about markers and loci associated with quality traits have been published. Moreover, high-quality genetic maps are available (Qian et al. 2017; Cai et al. 2018) and the sequences of the spinach nuclear and mitochondrial genomes have also been recently reported (Cai et al. 2017; Xu et al. 2017) and complement the chloroplast genome (Schmitz-Linneweber et al. 2001). This availability of new genomic information is expected to translate into the ease of cultivar development with selected characteristics. Despite this, further research is needed to have a better understanding of spinach and improve the breeding of this vegetable.

Part II: The possible origin and spread of spinach

The origin of spinach is uncertain although it is believed to have been first domesticated in the area of current Iran (Boswell 1949). No references to spinach from the Greek and Roman cultures have been found (Heine 2018) and the oldest written records mention spinach in the 4th century AD in Mesopotamia (El Faïz 1995, as cited in Hallavant and Ruas 2014), thus it seems that the spread of this vegetable happened late in history. However, how the spread of spinach was and which regions it went through from its centre of origin are still topics on which we do not have a clear explanation (Simoons 1990; Hallavant and Ruas 2014).

II.1. Introduction

Considering the assumed domestication area of cultivated spinach (Boswell 1949), the wild spinach native areas of distribution in Western and Central Asia (Hassler 2018), the differences between spinach accessions from the West and the East (Van der Vossen 2004) and the historical presence of trade routes in the Middle East (Elisseeff 2000), it is assumed that the spread of spinach followed two differentiated routes: one to the Middle East and Europe [the West], which would later on continue to the Americas, and a second one to Eastern Asia [the East].

Spread of spinach to the West possibly occurred through expansion in Muslim territories (Sneep 1957/1983). However, the spread of the crop could have occurred earlier with ancient trade or with the rise of the first Persian Empire [6th – 4th centuries BC], as this empire controlled an important part of the Eastern Mediterranean (Gates 2005; Shahbazi 2012). Nevertheless, both scenarios seem unlikely as no Greco-Roman evidence has been found and the first proof in the East dates back to the 7th century AD (Laufer 1919).

Considering the history of Islam and the hypothesis of spinach expansion in the West through Muslim territories, it seems plausible to think that the crop started its spread through the Mediterranean at the same time or after the expansion of Islam in this region, which happened during the 7th and 8th centuries with the Rashidun and the Umayyad Caliphates (Kennedy 1986). The first written evidence of spinach cultivation neighbouring the Mediterranean area dates back to the 10th century (Sneep 1957/1983), so it would be possible that spinach expansion did not occur during the initial spread of Islam but afterwards.

During the 20th century, three different but not mutually exclusive possibilities were accepted to explain how spinach entered Europe: through the Moors in Spain, via the several Crusades of the medieval period and via commerce in Venice (Sneep 1957/1983). Diverse evidence suggests the first scenario occurred. First, the earliest written record in continental Europe dates back to the 12th century in Moorish Spain (El Faïz 2000). The document sets the cultivation of spinach in the region since the 11th century. Hallavant and Ruas (2014) found physical archaeobotanical evidence from the end of the 12th century or beginning of the 13th in a French Pyrenean village, not far from the Spanish border. Furthermore, several written evidence from the 12th and 13th centuries in northern Spain and southern France mention the presence of spinach (Puig 2003), which is also the earliest Christian written evidence in Europe about this crop. Even though spinach seeds from the 13th century have been found in Germany (Rösch 1991), the first written documents with spinach references in England, Italy and northern France date back to the 14th century (Mulon 1971; Harvey 1981). Considering the mentioned evidence, it seems plausible to think that spinach spread to Europe from the Iberian Peninsula. However, the exact expansion routes throughout the continent remain uncertain (Hallavant and Ruas 2014).

Not much is known about the spread of spinach in the East. The oldest written records in the region mention spinach entered China for the first time via Nepal in the 7th century (Laufer 1919). Nepal is not part of the native range of the spinach wild relatives and the closest countries with presence of

wild spinach are Pakistan and Afghanistan (Hassler 2018). In this sense, how spinach was transported to Nepal is unknown. However, caravan routes already connected different points in Asia (Elisseeff 2000), which could help explain the spread of this vegetable in the region.

Humans and the spread of spinach

Since the spread of crops is closely linked to human activity, each way a certain crop was geographically spread could be conceivably linked to human history. In this sense, the phylogeny of spinach landraces could potentially relate to the spread route of spinach. Nevertheless, it is relevant to notice that important assumptions are made in such a study. There are many factors that could interfere in the relationship between a phylogenetic analysis and the spread of spinach. Commerce and trade, human migration and further conquests or wars could interfere in the study as spinach could have been exchanged, traded and mixed between communities. In this work, there is the assumption that each landrace was established in a certain area and had no contact with other existent spinach landraces and cultivars. No further crossings and mixtures are assumed to have happened, only the own changes landraces suffer due to the environment and human cultivation.

The CGN currently holds the largest available spinach collection worldwide with over 480 different spinach accessions. Within these, 75 different accessions of *S. turkestanica* and 19 of *S. tetrandra* are accessible to study. Moreover, the collection contains an important number of landraces from different parts of the world, especially from the Middle East and Central and South Asia.

Considering the gaps in spinach history and the availability of spinach accessions at the CGN, it would be interesting to study a selection of wild spinach and cultivated landraces from the same countries where the wild species occur and from where interesting historical gaps and questions arise. Comparing these accessions and studying how they cluster when a phylogenetic analysis is performed, it would be possible to [i] estimate the potential region where cultivated spinach was originally domesticated and [ii] evaluate a potential distribution route of this leafy vegetable.

Previous phylogenetic studies in spinach

Recently, two major studies (Shi et al. 2017; Xu et al. 2017) have analysed the phylogeny and population structure of spinach germplasm from the USDA collection (https://www.ars-grin.gov), in addition to spinach germplasm from breeding companies.

Shi et al. (2017) found that the USDA germplasm collection population structure was estimated to be divided in two [K = 2] or five [K = 5] subpopulations. The two subpopulations differentiated European and Asian spinach accessions; while the five subpopulations differentiated Asian, European, American and 2 Turkish groups of accessions. However, landraces were not the only type of accessions used, as many cultivars were also introduced in the analysis. Additionally, no wild spinach accessions were included. For these two reasons, it is difficult to draw conclusions regarding the domestication, the possible spread of spinach and the geographical relationships between accessions from different countries.

Xu et al. (2017) included several wild spinach accessions in their study, although most of the cultivated spinach accessions the authors used were modern cultivars. The authors found that *S. turkestanica* accessions were more closely related to cultivated spinach than *S. tetrandra* accessions. Moreover, the population structure of their accessions was estimated to be divided in two subpopulations [K = 2]. Even though European and American accessions predominantly belonged to one of these clusters, Asian and wild spinach accessions were divided among the two subpopulations.

Both studies lacked an emphasis on including landraces from geographically key areas in the history of spinach. For example, it would have been interesting to include more representation from areas where wild spinach grows, as well as from their contiguous areas in the East and in the West.

Hypothesis: The spread of spinach to the East

The current hypothesis of spinach entering China via Nepal is puzzling. Nepal and China are separated by the Himalayas, a major mountain system isolating the Tibetan plateau from South Asia and acting as a natural barrier between these two areas. Additionally, the current Xinjiang region in Western China contains natural corridors to Central Asia and Northwest South Asia, which have been part of the Silk Road route during millennia (Elisseeff 2000; Hansen 2012). Moreover, both mentioned regions are expected or known to be part of the *S. turkestanica* distribution range (Hassler 2018). Considering this evidence, the arrival of spinach into China could have occurred via Central and/or Southern Asia [Afghanistan, Kyrgyzstan, Pakistan or Tajikistan] instead of via Nepal.

When taking the phylogeny of spinach plants into account, if the Central and Southern Asia hypothesis is correct, Chinese landraces could be phylogenetically closer to Central and Southern Asian landraces than to Nepalese landraces. Such a relationship could potentially contradict current written evidence of spinach distribution in the East but would be compatible with what is known about trade and cultural disseminations in the Old World.

Hypothesis: The spread of spinach in the Eastern Mediterranean

Evidence seems to indicate that the arrival of spinach into Europe was via Southwestern Europe. However, on the other side of the Mediterranean, Anatolia and part of the Balkans were controlled by the Byzantine Empire previously to and during the years of the spread of Islam. Byzantines fought several wars with Persians between the 3rd and the 7th centuries (Shahbazi 2005) and with Muslim Arabs [Arab-Byzantine wars] between the 7th and the 11th centuries (Calabro 2016). One of the two connections between Europe and Anatolia [the Strait of the Dardanelles] belonged to the Byzantines until the second half of the 14th century, when it was conquered by the Ottoman Empire (Goffman 2002). In fact, even though after conquering the Dardanelles the Ottomans could enter Europe, the main connection to the Western continent [the Bosporus Strait] was not ultimately captured by the Ottomans until the fall of Constantinople [current Istanbul] in 1453 (Angold 2014; Philippides and Hanak 2017). Considering the history of this region, it seems reasonable to analyse if landraces from the Balkans cluster together with Anatolian ones or form a separate group. Distinct Empires in the region could have meant a cultural and trading separation of communities. In the case of disconnection, Balkan and Anatolian spinach landraces could form different phylogenetic clusters, potentially meaning Balkan spinach could have originally come from a different region [e.g. from Western Europe]. If, on the other hand, regions had had contact with each other, Balkan spinach and Anatolian spinach could form a single phylogenetic cluster.

II.2. Materials & methods

Selection of *Spinacia* accessions

A total of 95 Spinacia accessions were selected for this study (Figure 9, Appendix 4), including 16 *S. tetrandra*, 25 *S. turkestanica* and 54 *S. oleracea* accessions.

The set of spinach wild relatives was selected from the CGN spinach collection using the Core selection CGN tool (https://cgngenis.wur.nl), which selects accessions based on geographical origin to maximize the amount of genetic diversity (Hoekstra, personal communication). Accessions without a clear geographical origin were not considered for the selection. A posterior visual review of the geographical origin of the selected accessions was performed to eliminate accessions notably adjacent to one another. Of the 16 *S. tetrandra* accessions, 2 are from Georgia, 7 from Armenia and 7 from Azerbaijan; and of the 25 *S. turkestanica* accessions, 4 from Turkmenistan, 8 from Tajikistan and 13 from Uzbekistan.

The set of cultivated spinach landraces was first performed considering the countries in which *S. tetrandra* and *S. turkestanica* are autochthonous (Hassler 2018). The geographical origin of the totality of CGN spinach landraces from these countries was analysed by mapping their coordinates in a map. As with the spinach wild relatives, accessions without known geographical information were discarded from this selection with the exception of Iran, as this country is presumably the place where spinach was originally domesticated. Based on the location of these landraces, 39 of them were selected by maximizing the number of represented countries and visually filtering accessions within a country adjacent to one another. A second set of cultivated spinach landraces was randomly selected from the Eastern Mediterranean and the Southern and Eastern Asian regions where wild spinach is not autochthonous using the CGN Core selection tool, after reviewing their landrace status and their availability of origin coordinates. Five more landrace accessions from the Eastern Mediterranean [1 from Bulgaria, 1 from Greece, 1 from Macedonia and 2 from Western Turkey] and 7 more from Southern and Eastern Asia [3 from China, 2 from India and 2 from Nepal] were selected. Finally, 3 Western European spinach cultivars [Viroflay, Resistoflay and Viking] were included in the analysis as controls. Resistoflay is a Viroflay cultivar with an introgressed downy mildew resistance, and Viking is a cross of Viroflay and the old European variety King of Denmark.

The overall selection of *Spinacia* plants for the study, along with their corresponding species, country of origin and collecting site coordinates, are displayed in Appendix 4. More information about the accessions is available on the CGN database (https://cgngenis.wur.nl).

Figure 9. Geographical origin of the 95 *Spinacia* accessions used in this study. Interactive map available at: https://drive.google.com/open?id=17QiOw71a17Y-Pb3YGzrAA2VBvG-slSEa&usp=sharing

Pre-treatment and sowing of the seeds

Due to the high dormancy of *S. turkestanica* and *S. tetrandra* seeds, a slightly modified protocol from the one presented by van Treuren et al. (submitted) was performed for germinating wild spinach. In the case of *S. tetrandra*, prior to the pre-treatment fruits were broken with pliers without damaging the seeds. In the case of *S. turkestanica*, fruits were not broken prior to the pre-treatment of the seeds.

The pre-treatment of wild spinach fruits consisted in putting them under running tap water for 4 days, after which they were sown in trays with soil and vermiculite. Subsequently, a cold treatment at 4°C for 3 days was applied to the trays. Cultivation started in the greenhouse at 15°C with an 8-hour photoperiod and continued under these conditions until sampling.

Cultivated spinach seeds were directly sown on trays with soil and vermiculite. Cultivated spinach trays were kept in the greenhouse at 15 °C with a 16-hour photoperiod, until sampling.

Sampling and DNA isolation

Cotyledons from 20-day-old spinach seedlings were harvested and separately put in liquid nitrogen cold-tubes [8-strip tubes] prepared for subsequent grinding using metal bullets. Extraction of DNA from cotyledon samples was performed as described in Appendix 5. The quality of the purified DNA was assessed on a 1% (w/v) agarose gel electrophoresis and the DNA concentration was determined using the Qubit dsDNA BR assay kit (Invitrogen).

SNP selection

The SNP markers used in this study are a selection from the 419 SNP markers that Chan-Navarrete et al. (2016) used for constructing their spinach genetic map. Each SNP marker and its flanking sequence [50 bp upstream and downstream of the marker] was analysed in a BLASTn search using the BLAST tool available at the SpinachBase database (http://www.spinachbase.org), which uses the cultivated spinach genome as reference. Markers with multiple hits, no hits, indels and mismatches near the SNP position were discarded for further analysis. Furthermore, with the use of the BLASTn analysis, the genomic position of the selected markers was extracted. Following the BLASTn analysis, the presence of the SNP markers and of extra SNPs and indel elements surrounding the markers was analysed using the transcriptomic data available at SpinachBase. The SNPs were verified to be present in transcripts from accessions of the three *Spinacia* species, and the lack of extra SNPs and indels surrounding the SNPs of interest was also verified. After these two confirmatory steps, a total of 60 SNP markers were obtained and were finally selected for the KASP assay. The flanking sequences and alleles of the selected SNPs are presented in Appendix 6.

SNP genotyping

The genotype for the 60 SNP markers from all 95 accessions was scored by Dr. van Haeringen Laboratorium (Wageningen, The Netherlands). A microplate with 1200 ng of purified DNA [in 200 µL, concentration of 6 ng/ µL] from each accession and a negative control [empty well] was sent to Dr. van Haeringen Laboratorium, which scored the genotypes using a Competitive Allele Specific PCR [KASP] assay (Semagn et al. 2014). The primers required for the KASP assay were designed by Dr. van Haeringen Laboratorium based on each marker's flanking DNA sequences. The resulting SNP scores for each accession are presented in Appendix 7.

Phylogenetic and population structure analyses

SNP markers with missing data for all the accessions [a total of 4 SNPs] were discarded for further analysis. For the phylogenetic tree analysis, the colons separating the SNP alleles from each genotype (Appendix 7) of the remaining 56 SNPs were eliminated, and the missing data symbol '?' was substituted with the character 'N'. The resulting file was uploaded to the software Mesquite (Maddison and Maddison 2018), where it was converted to a Simplified NEXUS file [.nex]. The resulting NEXUS file was edited with a text editor to change the dimensions line to [NTAX = 95 NCHAR = 56] and the format line to [DATATYPE = DNA RESPECTCASE GAP = - MISSING = N]**.** The modified NEXUS file (Appendix 8) was used to construct a maximum likelihood phylogenetic tree using IQ-TREE (Trifinopoulos et al. 2016) [parameters "substitution model = auto, bootstrap = ultrafast"] with 1000 bootstrap replications. The display of the resulting phylogenetic tree was performed using iTOL (Ciccarelli et al. 2006).

For the population structure analysis, for each accession the text line with the diploid 56 SNP genotypes was separated into 2 lines, a first line for the first allele of each SNP genotype and a second one for the second allele of each SNP genotype. Following this change, each allele was substituted with the character '0' if it belonged to 'allele 1' of a certain SNP and to '1' if it belonged to 'allele 2' [based on 'allele 1' and 'allele 2' nomenclature in Appendix 6]. Missing data was

substituted with the character '999'. A column between the accession names and the markers was added to label each accession with its corresponding world region [Appendix 4]. The resulting file was saved as a text file and is available in a separate document called Input_Structure.txt. The software STRUCTURE v.2.3.4. (Pritchard et al. 2000) was used to assess the population structure of the 95 *Spinacia* accessions. The text file was uploaded to STRUCTURE [parameters individuals = 95, ploidy = 2, loci = 56, missing value = 999] and it was run 25 times on the 56 selected SNPs for each K value from 1 to 10 [parameters burn-in period = 500,000, MCMC repetitions = 750,000, ancestry model = admixture] with a correlated allele frequency model (Porras-Hurtado et al. 2013). CLUMPAK (Kopelman et al. 2015) was used to calculate the optimal K using the Evanno method (Evanno et al. 2005) and to obtain the population structure bar plots for the analysis.

II.3. Results

SNP scores

From the SNP score results (Appendix 7), it is evident the higher abundance of missing data for *S. tetrandra* [13 missing SNP markers] when compared to the *S. oleracea* and *S. turkestanica* scores [4 missing SNP markers, which are part of the 13 SNPs from *S. tetrandra*]. Moreover, *S. tetrandra* accessions also show the majority of loci have fixed alleles, with only 2 out of 60 SNP markers [SO037 and SO040] showing some heterozygous individuals.

Both *S. oleracea* and *S. turkestanica* show a high degree of heterozygosity. In fact, there are no fixed SNP alleles in the subset of analysed *S. oleracea* accessions. For *S. turkestanica*, only the marker SO032 is fixed.

Phylogenetic analysis

The relationship between the three species of the *Spinacia* genus indicates that *S. tetrandra* is a distant relative to *S. oleracea*, while *S. turkestanica* is the closest relative to cultivated spinach (Figure 10). The phylogenetic tree clearly differentiates between each species except for 4 *S. turkestanica* accessions that are positioned within the *S. oleracea* group ['turk TJK61', 'turk TKM90', 'turk TKM92' and 'turk TKM95']. The power of discrimination of accessions within each species is poor, especially concerning *S. tetrandra* accessions as only a few SNPs are available for the species.

When the geographical origin of the *S. oleracea* accessions is considered in the phylogenetic tree, several interesting patterns arise (Figure 11). First, there is a clear node separating accessions according to their geographical origin. This node separates a small clade of Eastern and Southern Asian cultivated spinach accessions from a larger clade of European and Southern and Western Asian accessions. From now on, the former clade is going to be referred as the Eastern clade or cluster, and the latter clade as the Western clade or cluster. The Eastern cluster comprises accessions from Afghanistan, Pakistan, Nepal, India and China, while the Western cluster comprises accessions from virtually the rest of the analysed countries, except for some accessions from Pakistan and Afghanistan that also group in this clade.

Western Asian accessions are scattered in the Western cluster and do not form a concrete distribution pattern. Several small clades are formed within the Western cluster of accessions. Accessions from the Caucasus region in Western Asia form a very defined clade, which is related to several other Western Asian accessions, especially from Iran and Turkey. The cultivar Viking 'oler NLD17', the Iranian landrace 'oler IRN19', the Bulgarian landrace 'oler BGR51' and the *S. turkestanica* accession 'turk TKM95' are also related to this cluster. Another defined small clade of 4 Afghan, 2 Iranian and 2 Turkish accessions is also formed. It might be appropriate to consider that Iran neighbours both countries, which are situated East and West of it, respectively.

As expected, the cultivars Viroflay 'oler FRA18' and Resistoflay 'oler NLD16' cluster together in the phylogenetic tree. Three other accessions form a clade with these cultivars, a landrace from Greece ['oler GRC27'], a landrace from Afghanistan ['oler AFG26'] and a *S. turkestanica* accession from Turkmenistan ['turk TKM92']. Interestingly, Viking 'oler NLD17' separates from the two European cultivars and closely clusters with the Western Asian accessions from the Caucasus.

Finally, a heterogeneous clade is formed by 3 Turkish, 2 Iranian, 2 Uzbek, 1 Chinese and 1 Macedonian accession. Even though Macedonia is relatively close to Turkey, which neighbours Iran, both China and Uzbekistan are far from them.

Population structure analysis

The CLUMPAK analysis showed that the largest delta K was observed at K = 2 (Appendix 9), inferring that the analysed population consists of two main subpopulations (Figure 12, Appendix 10). For this K, one of the subpopulations consists of the accessions of *S. tetrandra* and *S. turkestanica*, as well some Southern and Eastern Asian accessions, which coincide with the Eastern cluster of spinach accessions already shown in the phylogenetic tree. The second subpopulation consists of accessions from Central Asia, Western Asia [Caucasus], Southern Europe [Balkans] and the 3 European cultivars. Accessions from Western and Southern Asia seem to be admixtures of both subpopulations (Figure 12, Appendix 10), with the Southern Asian accessions being closer to the first subpopulation than Western Asian accessions. Interestingly, two accessions of *S. turkestanica* ['turk TKM92' and 'turk TKM95'] are also shown to be admixtures of both subpopulations (Appendix 10).

The second largest delta K was observed at K = 3 (Appendix 9). In this case, the accessions of *S. tetrandra* separate from the previous two subpopulations and form a third one, well differentiated from the rest of accessions.

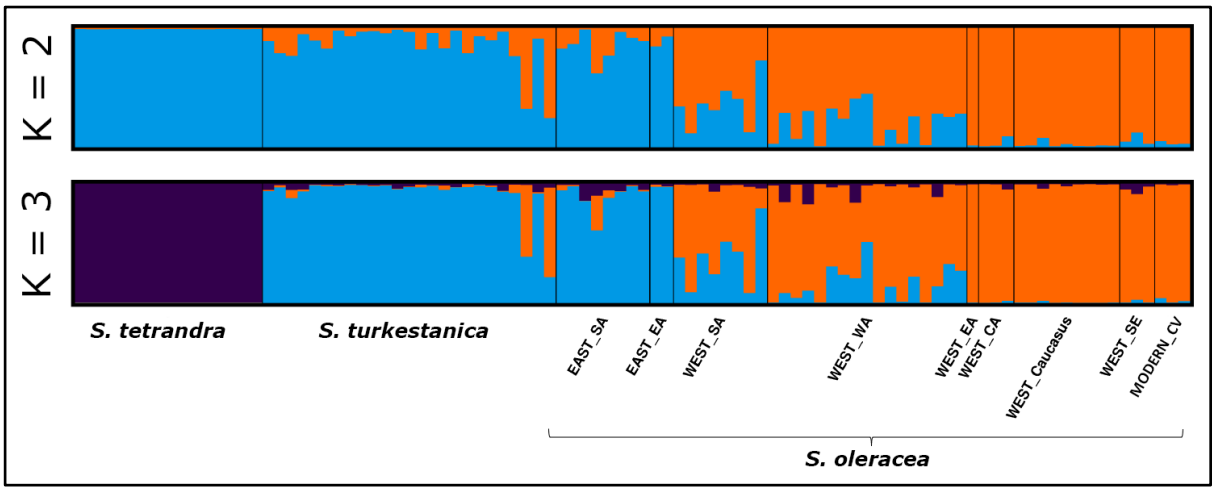

Figure 12. Classification of the 95 *Spinacia* accessions into two (K = 2) and three (K=3) populations. The y-axis represents the subgroup membership and the x-axis the different accessions. Geographical labels have been given to *S. oleracea* accessions. EAST groups cultivars from the Eastern cluster in the phylogenetic tree while WEST groups the cultivars from the Western cluster. SA: Southern Asia; EA: Eastern Asia; WA: Western Asia; CA: Central Asia; Caucasus: Western Asia (Caucasus region); SE: Southern Europe (Balkans); MODERN_CV: modern cultivars from Western Europe.

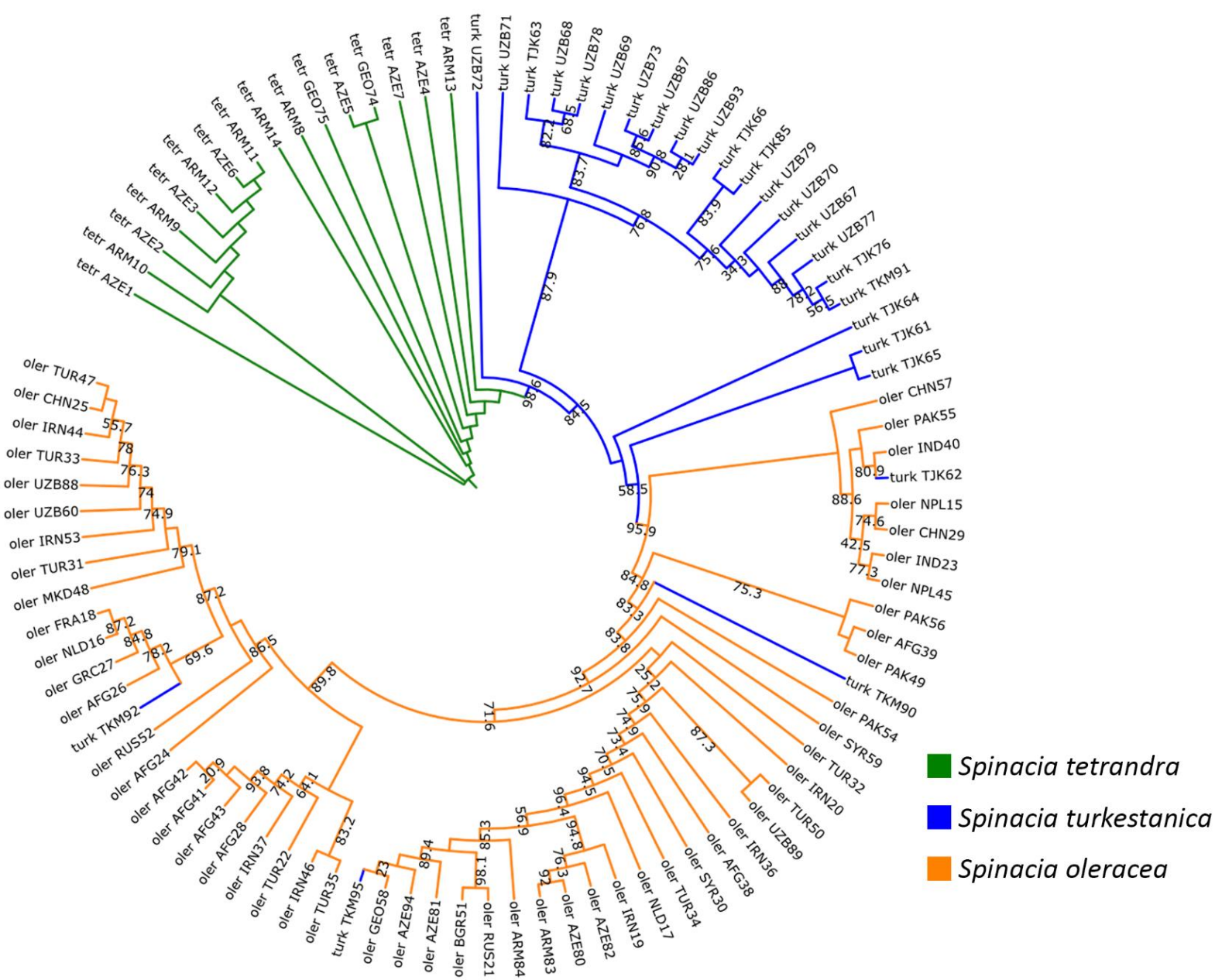

Figure 10. Maximum Likelihood (ML) phylogenetic tree of the *Spinacia* accessions, coloured by species. The nomenclature used for the accessions is available in Appendix 4. Bootstrap values presented in the tree branches.

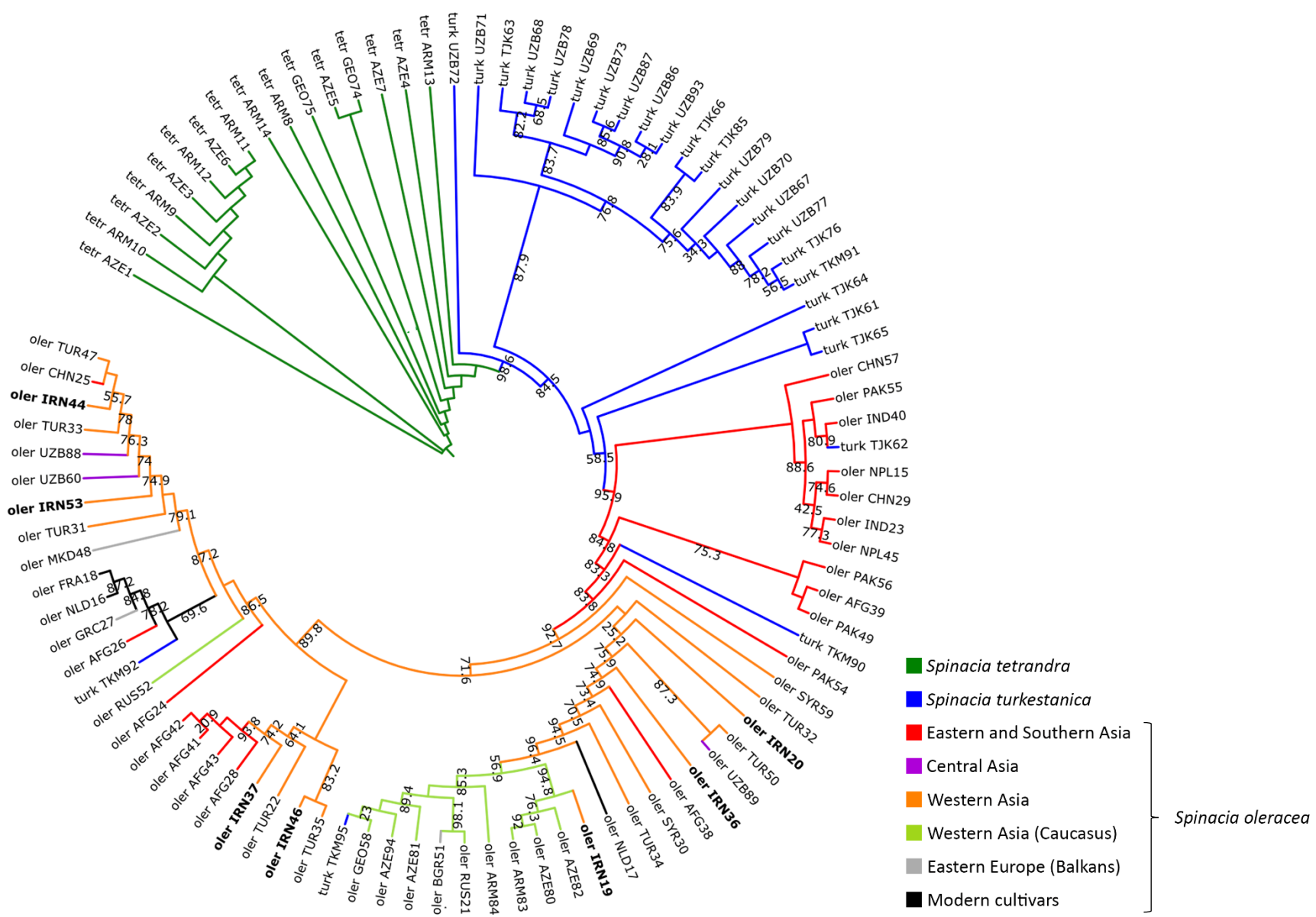

Figure 11. Maximum likelihood (ML) phylogenetic tree of the *Spinacia* accessions, coloured by species and geographical origin. In bold, accessions from Iran, the country where spinach domestication is assumed to have occurred. The nomenclature used for the accessions is available in Appendix 4. Bootstrap values presented in the tree branches.

II.4. Discussion

The *Spinacia* genus

Previous phylogenetic studies (Fujito et al. 2015; Xu et al. 2017) indicated that *S. oleracea* descended from *S. turkestanica*. In this study, this relationship is maintained. The high number of missing markers' data for *S. tetrandra* in the KASP results already suggests that the sequences used to design the KASP primers, which were based on *S. oleracea*, do not hybridize properly in the *S. tetrandra* genome. A larger evolutionary distance between *S. tetrandra* and *S. oleracea* could explain this situation as more differences would exist between the sequences of the two species. Moreover, the high rate of fixed alleles found in *S. tetrandra* accessions may also indicate that the SNP is not present in the species, meaning that the SNP could have appeared after the speciation event between the ancestors of *S. oleracea* and *S. tetrandra*. For *S. turkestanica*, the SNP scores obtained from the KASP analysis seem to follow a similar trend to the ones of *S. oleracea*, as shown by their similar high heterozygosity and number of missing markers. The lesser amount of differences between *S. turkestanica* and *S. oleracea* could indicate a shorter evolutionary distance between the species, thus indicating that *S. turkestanica* is the ancestor of *S. oleracea*.

Both the phylogenetic tree and the population structure analyses support the phylogenetic relationship between the 3 *Spinacia* species, with *S. turkestanica* being the ancestor of *S. oleracea*, and *S. tetrandra* a distant relative to it. Furthermore, *S. oleracea* accessions from Southern and Eastern Asia are the closest ones to *S. turkestanica* in the phylogenetic tree. Considering the native distribution range of *S. turkestanica* is Central and Southern Asia (Hassler 2018), the linkage between the species seems also discernible from a geographical perspective. Interestingly, the population structure analysis does not make a distinction between *S. tetrandra*, *S. turkestanica* and the Eastern cluster of *S. oleracea* at K = 2, as they are all grouped in the same subpopulation. However, for K = 3 *S. tetrandra* is placed in a different cluster than *S. turkestanica* and the Eastern cluster of *S. oleracea*, which remain together in the analysis.

Dubious *S. turkestanica* accessions

The 'turk TJK61', 'turk TKM90', 'turk TKM92' and 'turk TKM95' *S. turkestanica* accessions cluster with cultivated spinach in the phylogenetic tree. Interestingly, 3 of these accessions ['turk TKM90', 'turk TKM92' and 'turk TKM95'] originally come from the USDA collection (https://www.ars-grin.gov). Even though the 3 American accessions are classified as *S. turkestanica* in the database, 'turk TKM92' and 'turk TKM95' are stated to show mixed traits of *S. turkestanica* and *S. oleracea*. This classification casts doubts on the validity of the species' label for these 2 accessions, as well as for the other remaining 2 *S. turkestanica* accessions.

Based on STRUCTURE results, for K = 2 and K = 3 the hybrid nature of accessions 'turk TKM92' and 'turk TKM95' is supported by the analysis, as these two accessions are the only *S. turkestanica* accessions that belong to the Western *S. oleracea* cluster. For K = 2 and K =3, the hybrid nature of 'turk TJK61' and 'turk TKM90' is not shown, although K ≥ 4 (Appendix 10) show 'turk TJK61' being a potential hybrid of *S. turkestanica* and the Eastern group of *S. oleracea*.

Interestingly, if the 4 dubious accessions are not considered as *S. turkestanica*, the number of markers with a fixed allele for *S. turkestanica* increases from 1 [SO032] to 7 [SO014, SO019, SO032, SO240, SO381, SO382 and SO419]. This could mean that a lesser amount of selected SNP markers is present in *S. turkestanica*, compared to *S. oleracea*.

The Viroflay clade

A Greek and an Afghan spinach landrace form a clade with the 'turk TKM92' *S. turkestanica* from Turkmenistan, together with the Viroflay 'oler FRA18' and the Resistoflay 'oler NLD16' cultivars. This

disparity of geographical origins [Western Europe, Eastern Europe, Southern Asia and Central Asia] suggests there might be a relationship between the 5 accessions. It is possible that the Greek and the Afghan accessions were originally crossed with or derived from Viroflay, as this latter is a popular cultivar released already in 1873 (Vilmorin-Andrieux 1883). Concerning the possible hybrid 'turk TKM92', it is possible that the original *S. turkestanica* was crossed with a Viroflay or a related cultivar, thus leading to the accession clustering together with Viroflay and Resistoflay. Additionally, the cultivar Viking 'oler NLD17' does not belong to this clade even though it was selected from a cross with Viroflay. This situation also adds uncertainty on the other parental cultivar, King of Denmark, as it could have a phylogenetic relationship with the Caucasian accessions that Viking clusters with.

The Caucasian clade

Even though the Caucasian accessions seem to be related to accessions from Western Asia, the fact that they cluster closely together in the phylogenetic tree seems to suggest the close relationship between spinach landraces in the Caucasian countries. This contrasts with the high diversity found in Western Asian landraces from the neighbouring countries of Iran and Turkey. Moreover, the relatedness of the Caucasian clade with the dubious *S. turkestanica* 'turk TKM95' and the cultivar Viking 'oler NLD17' might suggest that this group of accessions might be related to modern spinach cultivars.

The heterogeneous clade

The presence of a heterogeneous clade grouping accessions from distinct geographical origins [5 from Western Asia, 2 from Central Asia, 1 from Eastern Asia and 1 from Eastern Europe (Balkans)] complicates a logical interpretation of the clade. Even though Western Asia and the Balkans are geographically close, both Eastern Asia and Central Asia are far apart from the former regions and from each other. Considering the relationship of the accessions, it is possible that contemporary trade of spinach material helped in shaping this situation. Alternatively, these accessions could have been selected from a common spinach cultivar not represented in the analysis.

The origin of spinach

Iran is assumed to be the spinach centre of origin (Boswell 1949). However, results from the phylogenetic tree and the population structure analyses indicate that the seven studied spinach accessions from Iran are not as close to *S. turkestanica* as accessions from the Eastern spinach cluster, which are from Southern and Eastern Asian countries. In this sense, Afghanistan and Pakistan are the only countries with accessions in the Eastern cluster that are also part of the native distribution range of *S. turkestanica* (Hassler 2018). Moreover, accessions from both countries are also closer to the Western spinach cluster than accessions from the rest of the analysed countries in Southern and Eastern Asia. The obtained results suggest that Pakistan or Afghanistan, which also belong to one of the primary centres of origin of cultivated plants (Ladizinsky 1998), could also be potential regions where the domestication of spinach occurred.

The Afghan-Iranian-Turkish clade

Four different Afghan accessions ['oler AFG28', 'oler AFG41', 'oler AFG42' and 'oler AFG43'] form a clade together with Iranian and Turkish accessions within the Western cluster of cultivated spinach. Considering that Afghanistan could have been the centre of origin of spinach, it is conceivable to think that these four accessions were derived from spinach that initially spread from Southern Asia to Western Asia and subsequently returned to Southern Asia. After returning, divergence into the four different accessions could have happened. This might explain why these four Afghan spinach landraces form a cluster separated from most Southern Asian accessions and are in turn related to Western Asian landraces.

The spread of spinach

The presence of the Eastern and the Western cluster of spinach landraces supports the idea of cultivated spinach spreading to the East [Southern and Eastern Asia] and to the West [Western Asia and Europe]. Interestingly, the population structure analysis shows that the Eastern cluster belongs to the same genetic population as *S. turkestanica*. In the Western cluster, there is a gradual trend to showing less admixture with the genetic population of the Eastern cluster the more geographically separated the accessions are from Southern and Eastern Asia. In this sense, the bar plot from the population structure analysis at K = 2 (Appendix 10) shows that Southern Asian accessions in the Western cluster have a higher group membership to the genetic population of the Eastern cluster than Western Asian accessions. Caucasian, Eastern European [Balkans], European spinach cultivars and Central Asian accessions belong to the second genetic population, thus they are less related to the Eastern cluster than Southern and Western Asian landraces in the Western cluster.

The spread of spinach to the East

The Eastern cluster of spinach accessions shows that Chinese spinach landraces are related to Nepalese and Indian ones, therefore adding value to the written evidence pointing at spinach entering China via Nepal. Nevertheless, as Nepal is not a country where *S. turkestanica* grows, spinach should have arrived there from a different region. In this sense, Afghanistan and Pakistan have been shown to have accessions grouping in the same Eastern cluster and are also potential candidates for being the centre of origin of cultivated spinach.

The spread of spinach in the Eastern Mediterranean

The 3 analysed landrace accessions from the Balkan region in Eastern Europe do not show a close phylogenetic relationship with each other. As already mentioned, the Greek landrace 'oler GRC27' clusters with the cultivars Viroflay 'oler FRA18' and Resistoflay 'oler NLD16', suggesting that these accessions could have been possibly derived from Viroflay, Resistoflay or a related cultivar. Interestingly, the Bulgarian landrace 'oler BGR51' belongs to the Caucasian clade. As the cultivar Viking 'oler NLD17' seems to be partly related to this clade, both the Bulgarian and the Caucasian landraces could have been derived from modern cultivars. Alternatively, Bulgaria was a close ally to the Soviet Union. Trade and exchanges between the two countries could have originated the close relationship between the accessions from the Caucasus countries, which are former Soviet Republics, and Bulgaria. The Macedonian landrace 'oler MKD48' clusters in the heterogeneous clade. In this case, a genetic relationship between the Macedonian and three Turkish landraces is suggested. Of these Turkish accessions ['oler TUR31', 'oler TUR33' and 'oler TUR47'], the landrace 'oler TUR33' was collected in the Turkish Mediterranean, showing some geographical proximity between this landrace and the Bulgarian one. Nevertheless, the grouping in this clade of a Chinese as well as two Central Asian landraces from Uzbekistan diffuses this relationship. On the whole, the obtained results do not help in clarifying the relationship between the Balkan and the Turkish spinach landraces. A larger set of landraces from these two regions should be used to analyse the possible relationship between them. Furthermore, landraces from the rest of the Mediterranean could also be added to the analysis in order to study if the current hypothesis of spinach entering Europe via Spain through spread in Northern Africa is verified.

II.5. Conclusion

This study supports previous evidence regarding the phylogenetic relationship within the *Spinacia* genus. *Spinacia turkestanica* has been shown to be the closest spinach wild relative to cultivated spinach. Furthermore, differences cited in the literature between Eastern and Western spinach cultivars have been supported by the existence of two separate clades in the cultivated spinach phylogeny. A clade formed by Southern and Eastern Asian spinach landraces [the Eastern spinach

cluster] is differentiated from a second clade predominantly formed by Western Asian and European accessions [the Western spinach cluster].

The population structure analysis showed the presence of two main subpopulations: a first genetic population formed by the spinach wild relatives *S. tetrandra*, *S. turkestanica* and the Eastern spinach cluster; and a second genetic population formed by the Western spinach cluster. In this analysis, the Eastern spinach cluster has been shown to be phylogenetically closer to *S. turkestanica* than the Western spinach cluster. Moreover, a geographical trend within landraces can be appreciated, with less admixture from the former genetic population the more distant the accession is from Southern and Eastern Asia.

Even though Iran was previously assumed to be the centre of domestication of spinach, results from this study suggest Afghanistan and Pakistan might also be suitable candidates for it. Both Afghanistan and Pakistan belong to the native distribution range of *S. turkestanica.* In this study, landraces from the two countries are closer to *S. turkestanica* than landraces from Iran. Moreover, Afghan and Pakistani landraces are present in both the Eastern and Western spinach clusters, while Iranian landraces are only present in the Western spinach cluster.

In addition to this, a close phylogenetic relationship between Indian, Nepalese and Chinese landraces has been shown, thus supporting historical evidence pointing at spinach entering China via Nepal in the 7th century. On the other hand, a phylogenetic relationship between Turkish and Balkan spinach landraces has not been demonstrated. Further research using a larger set of Eastern Mediterranean landraces, as well as landraces from Northern Africa and the Western Mediterranean, might shed light upon the spread of spinach in the region.

The inclusion of spinach cultivars in the analysis showed that some spinach accessions classified as landraces might be related to modern cultivars, thus hindering a potential linkage between geographical origin of the accessions and their phylogeny. Moreover, the selection of SNPs in this study, based on the genome of *S. oleracea*, has been shown to be inadequate to discriminate within wild spinach accessions [especially for *S. tetrandra*, as most of the SNP alleles were fixed for this species]. Furthermore, the high heterozygosity of both *S. turkestanica* and *S. oleracea* is also problematic as it makes the genetic analysis of the accessions more complex. Future studies using species-specific SNP markers would be useful to properly discriminate within accessions of *S. tetrandra* and *S. turkestanica*, as well as it could facilitate the detection of introgressions in cultivated spinach. A potential source of these SNP markers could be the database SpinachBase, as it contains information of transcripts from the 3 *Spinacia* species, including SNP variants referenced to the *S. oleracea* genome available in the database. The sequencing of the genomes of *S. tetrandra* and *S. turkestanica* might also be helpful to find and locate variation within and among both species. Next-generation sequencing (NGS) allows both *de novo* and reference-based SNP discovery (Kumar et al. 2012), and it has already been used to get most of the available genomic data from spinach (e.g. Xu et al. 2017). Furthermore, the availability of the genomic sequences from the three *Spinacia* species would also be interesting to study the shared synteny and the chromosomal rearrangements between the species, as well as the regions in the genome potentially linked to the domestication process.

Future studies using a large set of SNP markers, spread throughout the genome and including species-specific markers, could allow for more accurate discrimination between accessions. Additionally, using a larger set of spinach accessions might also help to identify a more accurate link between the phylogenetic relationship between spinach accessions and the history of this vegetable.

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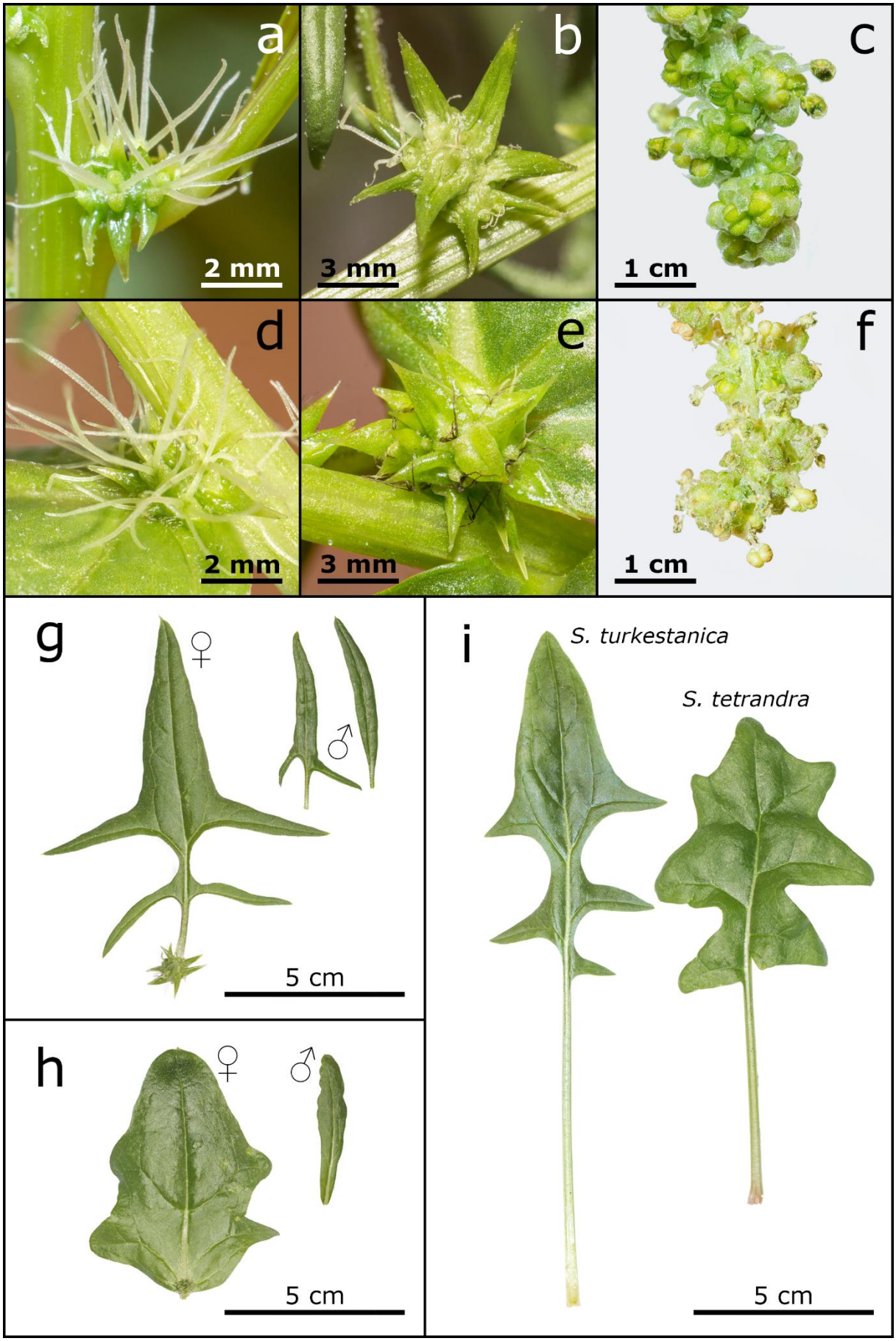
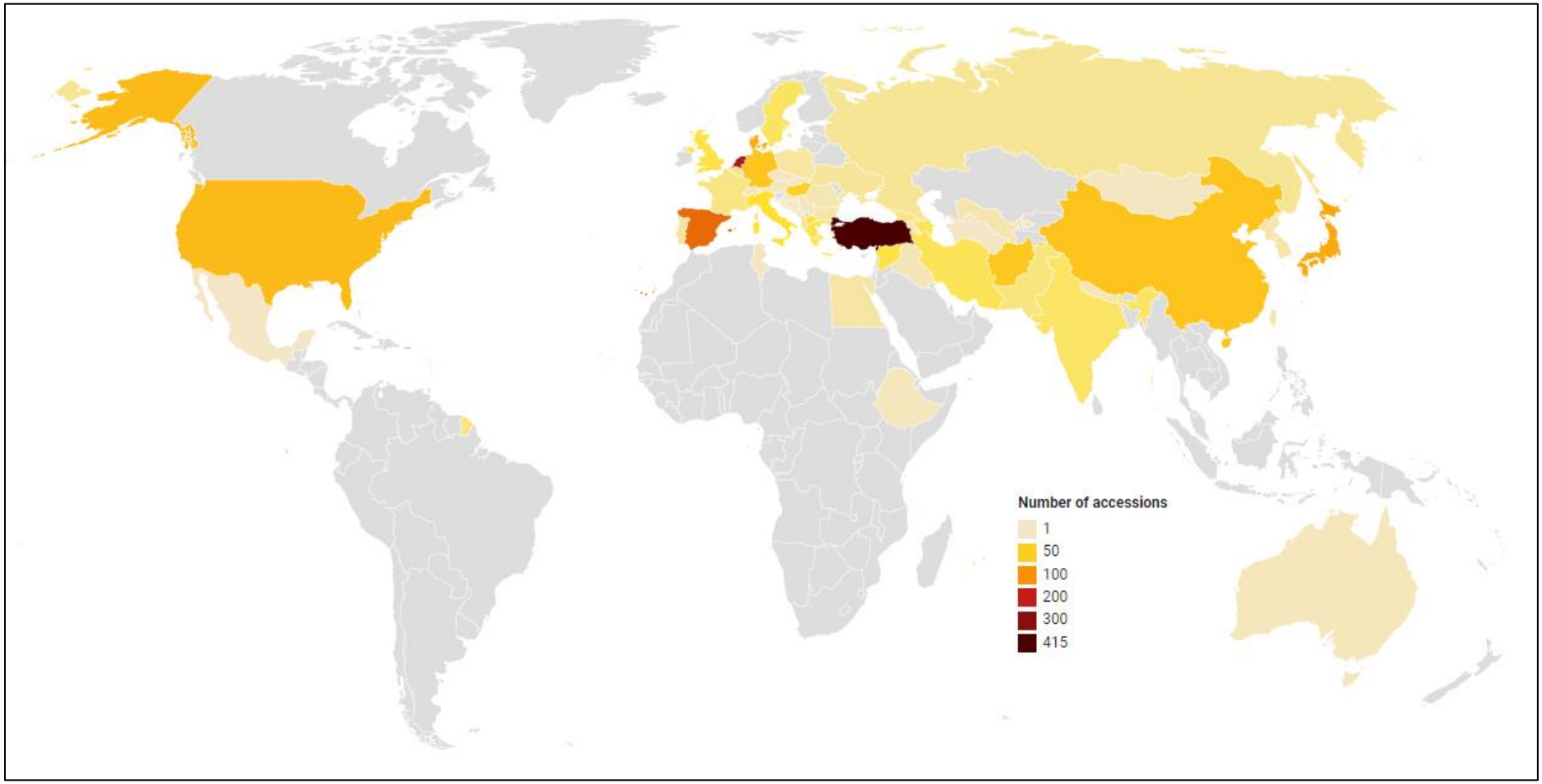
Appendix 1. Morphological details of spinach wild relatives



Morphological details of spinach wild relatives. Pistillate flowers (a), developing fruit (b) and staminate flowers (c) of *S. turkestanica*. Pistillate flowers (d), developing fruit (e) and staminate flowers (f) of *S. tetrandra*. Bracts of *S. turkestanica* (g) and *S. tetrandra* (h). From left to right (i), leaves from a female plant of *S. turkestanica* and from a female plant of *S. tetrandra.*

Appendix 2. Overview of spinach germplasm

Appendix 2.1. Germplasm of cultivated spinach



Geographical origin of cultivated spinach accessions. The data used to create this map is presented in the tables below. Accessions with unknown origin have not been represented. Created with Datawrapper; interactive map available at: https://datawrapper.dwcdn.net/VPYjR/5/.

Appendix 2. Overview of spinach germplasm

Appendix 2.1. Germplasm of cultivated spinach

Availability of cultivated spinach accessions ordered by gene bank and country of origin [Table 1/3].

Country of origin[1]	Total number of accessions	Gene bank[5]										
		AUT 046	AUT 047	AZE 015	BEL 002	BGR 001	CHE 001	CZE 122	DEU 146	ESP 026	ESP 027	GBR 006
AFG	**54**	0	0	0	0	0	0	0	9	0	0	0
ALB	**12**	0	0	0	0	0	0	0	12	0	0	0
ARM	**4**	0	0	0	0	0	0	0	0	0	0	0
AUS	**3**	1	0	0	0	0	0	0	0	0	0	0
AUT	**2**	0	2	0	0	0	0	0	0	0	0	0
AZE	**35**	0	0	22	0	0	0	0	1	0	0	0
BEL	**14**	0	0	0	0	0	0	0	0	0	0	0
BGR	**12**	0	0	0	0	9	0	0	2	0	0	0
BIH	**1**	0	0	0	0	0	0	0	0	0	0	0
CHE	**3**	2	0	0	0	0	0	0	0	0	0	0
CHN	**58**	0	0	0	0	1	0	0	15	0	0	2
CSK[2]	**10**	0	0	0	0	0	0	3	0	0	0	3
CZE	**3**	0	0	0	0	0	0	0	0	0	0	0
DEU	**57**	7	2	0	0	0	0	0	33	0	0	6
DNK	**78**	0	0	0	0	2	0	0	1	0	0	1
EGY	**8**	1	0	0	0	0	0	0	0	0	0	0
ESP	**132**	0	0	0	0	0	0	0	2	30	79	0
ETH	**3**	0	0	0	0	0	0	0	0	0	0	0
FRA	**15**	1	0	0	0	0	0	1	0	0	0	2
GBR	**28**	2	0	0	0	0	0	0	0	0	0	20
GEO	**15**	0	0	0	0	0	0	0	5	0	0	0
GRC	**26**	1	0	0	0	0	0	0	6	0	0	1
HKG	**1**	0	0	0	0	0	0	0	0	0	0	0
HRV	**2**	1	0	0	0	0	0	0	1	0	0	0
HUN	**48**	0	0	0	0	1	0	0	2	0	0	0
IND	**21**	0	0	0	0	0	0	0	0	0	0	0
IRN	**24**	0	0	1	0	1	0	0	0	0	0	0
IRQ	**4**	0	0	0	0	0	0	0	2	0	0	0
ISR	**2**	0	0	0	0	0	0	0	0	0	0	0
ITA	**35**	1	0	0	0	0	0	0	11	0	0	19
JPN	**80**	0	0	0	0	22	0	3	4	0	0	0
KOR	**7**	0	0	0	0	0	0	0	1	0	0	0
MEX	**1**	0	0	0	0	0	0	0	0	0	0	0
MKD	**34**	0	0	0	0	0	0	0	0	0	0	0
MNE	**1**	0	0	0	0	0	0	0	0	0	0	0
MNG	**2**	0	0	0	0	0	0	0	1	0	0	0
NLD	**241**	1	0	0	0	96	0	8	5	0	0	0
NPL	**5**	0	0	0	0	0	0	0	1	0	0	1
PAK	**16**	0	0	0	0	6	0	0	0	0	0	0
POL	**9**	0	0	0	0	0	0	0	0	0	0	0
PRK	**6**	0	0	0	0	0	0	0	6	0	0	0
PRT	**9**	0	0	0	0	0	0	0	0	0	0	2
ROU	**5**	0	0	0	0	0	0	0	1	0	0	0
RUS	**11**	0	0	0	0	5	0	0	0	0	0	0
SRB	**3**	0	0	0	0	0	0	0	0	0	0	0
SUN[3]	**8**	0	0	0	0	1	0	0	3	0	0	1
SVK	**2**	0	0	0	0	0	0	0	1	0	0	0
SWE	**22**	3	0	0	0	0	0	0	1	0	0	0
SYR	**28**	0	0	0	0	0	0	0	0	0	0	14
TKM	**1**	0	0	0	0	0	0	0	0	0	0	0
TUN	**2**	0	0	0	0	0	0	0	2	0	0	0
TUR	**415**	0	0	0	0	1	0	0	8	0	0	48
TWN	**6**	0	0	0	0	0	0	0	0	0	0	0
UKR	**10**	0	0	0	0	0	0	0	0	0	0	0
USA	**65**	1	0	0	0	0	0	1	3	0	0	1
UZB	**6**	0	0	0	0	0	0	0	0	0	0	0
YUG[4]	**1**	0	0	0	0	0	0	0	0	0	0	0
Unknown	**253**	1	1	0	2	59	8	1	69	1	1	1
TOTAL	**1959**	**23**	**5**	**23**	**2**	**204**	**8**	**17**	**208**	**31**	**80**	**122**

Information extracted from EURISCO (https://eurisco.ipk-gatersleben.de), GRIN (https://npgsweb.ars-grin.gov) and the International Spinach Database (https://ecpgr.cgn.wur.nl/LVintro/spinach/). [1] Countries represented by their 3-letter code [ISO 3166-1 alpha-3]. [2] Former Czechoslovakia. [3] Former USSR. [4] Former Yugoslavia. [5] Gene banks represented by their WIEWS Code (https://www.genesys-pgr.org/wiews/active).

Appendix 2. Overview of spinach germplasm

Appendix 2.1. Germplasm of cultivated spinach

Availability of cultivated spinach accessions ordered by gene bank and country of origin [Table 2/3].

Country of origin[1]	Total number of accessions	Gene bank[5]										
		GBR 017	GEO 013	GRC 005	HUN 003	ISR 002	ITA 363	MKD 001	NLD 037	POL 030	PRT 001	ROM 007
AFG	**54**	0	0	0	0	0	0	0	21	0	0	0
ALB	**12**	0	0	0	0	0	0	0	0	0	0	0
ARM	**4**	0	0	0	0	0	0	0	4	0	0	0
AUS	**3**	0	0	0	0	0	0	0	0	0	0	0
AUT	**2**	0	0	0	0	0	0	0	0	0	0	0
AZE	**35**	0	0	0	0	0	0	0	9	0	0	0
BEL	**14**	0	0	0	0	0	0	0	1	0	0	0
BGR	**12**	0	0	0	0	0	0	0	1	0	0	0
BIH	**1**	0	0	0	0	0	0	0	1	0	0	0
CHE	**3**	0	0	0	0	0	0	0	0	0	0	1
CHN	**58**	0	0	0	0	0	0	0	19	0	0	0
CSK[2]	**10**	0	0	0	4	0	0	0	0	0	0	0
CZE	**3**	0	0	0	0	0	0	0	1	0	0	0
DEU	**57**	0	0	0	0	0	0	0	7	0	0	0
DNK	**78**	1	0	0	1	0	0	0	4	0	0	0
EGY	**8**	0	0	0	0	0	0	0	3	0	0	0
ESP	**132**	0	0	0	0	0	0	0	2	0	0	0
ETH	**3**	0	0	0	0	0	0	0	1	0	0	0
FRA	**15**	0	0	0	0	0	0	0	4	0	0	0
GBR	**28**	0	0	0	0	0	0	0	3	0	0	0
GEO	**15**	0	3	0	0	0	0	0	1	0	0	0
GRC	**26**	0	0	4	0	0	0	0	10	1	0	0
HKG	**1**	0	0	0	0	0	0	0	0	0	0	0
HRV	**2**	0	0	0	0	0	0	0	0	0	0	0
HUN	**48**	0	0	0	37	0	0	0	1	0	0	0
IND	**21**	0	0	0	0	0	0	0	9	0	0	0
IRN	**24**	0	0	0	0	0	0	0	7	0	0	0
IRQ	**4**	0	0	0	0	0	0	0	0	0	0	0
ISR	**2**	0	0	0	1	1	0	0	0	0	0	0
ITA	**35**	0	0	0	0	0	1	0	1	0	0	0
JPN	**80**	0	0	0	0	0	0	0	25	12	0	0
KOR	**7**	0	0	0	0	0	0	0	4	0	0	0
MEX	**1**	0	0	0	0	0	0	0	0	0	0	0
MKD	**34**	0	0	0	0	0	0	4	11	0	0	0
MNE	**1**	0	0	0	0	0	0	0	0	0	0	0
MNG	**2**	0	0	0	0	0	0	0	0	0	0	0
NLD	**241**	0	0	0	2	0	0	0	104	0	0	0
NPL	**5**	0	0	0	0	0	0	0	2	0	0	0
PAK	**16**	0	0	0	0	0	0	0	8	0	0	0
POL	**9**	0	0	0	0	0	0	0	1	7	0	0
PRK	**6**	0	0	0	0	0	0	0	0	0	0	0
PRT	**9**	0	0	0	0	0	0	0	0	0	7	0
ROU	**5**	0	0	0	1	0	0	0	0	0	0	2
RUS	**11**	0	0	0	0	0	0	0	5	0	0	0
SRB	**3**	0	0	0	2	0	0	0	0	0	0	0
SUN[3]	**8**	0	0	0	1	0	0	0	1	0	0	0
SVK	**2**	0	0	0	0	0	0	0	0	1	0	0
SWE	**22**	0	0	0	0	0	0	0	2	0	0	0
SYR	**28**	0	0	0	0	0	0	0	3	1	0	0
TKM	**1**	0	0	0	0	0	0	0	0	0	0	0
TUN	**2**	0	0	0	0	0	0	0	0	0	0	0
TUR	**415**	0	0	0	0	0	0	0	86	2	0	0
TWN	**6**	0	0	0	0	0	0	0	5	0	0	0
UKR	**10**	0	0	0	0	0	0	0	1	1	0	0
USA	**65**	2	0	0	0	0	0	0	2	0	0	0
UZB	**6**	0	0	0	0	0	0	0	5	0	0	0
YUG[4]	**1**	0	0	0	0	0	0	0	0	0	0	0
Unknown	**253**	0	0	0	48	0	0	0	33	0	0	0
TOTAL	**1959**	**3**	**3**	**4**	**97**	**1**	**1**	**4**	**408**	**25**	**7**	**3**

Information extracted from EURISCO (https://eurisco.ipk-gatersleben.de), GRIN (https://npgsweb.ars-grin.gov) and the International Spinach Database (https://ecpgr.cgn.wur.nl/LVintro/spinach/). [1] Countries represented by their 3-letter code [ISO 3166-1 alpha-3]. [2] Former Czechoslovakia. [3] Former USSR. [4] Former Yugoslavia. [5] Gene banks represented by their WIEWS Code (https://www.genesys-pgr.org/wiews/active).

Appendix 2. Overview of spinach germplasm

Appendix 2.1. Germplasm of cultivated spinach

Availability of cultivated spinach accessions ordered by gene bank and country of origin [Table 3/3].

Country of origin[1]	Total number of accessions	Gene bank[5]					
		ROM 023	SWE 054	TUR 001	UKR 008	UKR 021	USA 020
AFG	**54**	0	0	0	2	0	22
ALB	**12**	0	0	0	0	0	0
ARM	**4**	0	0	0	0	0	0
AUS	**3**	0	0	0	2	0	0
AUT	**2**	0	0	0	0	0	0
AZE	**35**	0	0	0	3	0	0
BEL	**14**	0	0	0	2	0	11
BGR	**12**	0	0	0	0	0	0
BIH	**1**	0	0	0	0	0	0
CHE	**3**	0	0	0	0	0	0
CHN	**58**	0	0	0	1	0	20
CSK[2]	**10**	0	0	0	0	0	0
CZE	**3**	0	0	0	2	0	0
DEU	**57**	0	0	0	1	0	1
DNK	**78**	0	65	0	1	0	2
EGY	**8**	0	0	0	1	0	3
ESP	**132**	0	0	0	16	0	3
ETH	**3**	0	0	0	0	0	2
FRA	**15**	0	0	0	2	0	5
GBR	**28**	0	0	0	1	0	2
GEO	**15**	0	0	0	0	0	6
GRC	**26**	0	0	0	0	0	3
HKG	**1**	0	0	0	0	0	1
HRV	**2**	0	0	0	0	0	0
HUN	**48**	0	0	0	1	0	6
IND	**21**	0	0	0	0	0	12
IRN	**24**	0	0	0	0	0	15
IRQ	**4**	0	0	0	1	0	1
ISR	**2**	0	0	0	0	0	0
ITA	**35**	0	0	0	0	0	2
JPN	**80**	0	0	0	9	0	5
KOR	**7**	0	0	0	0	0	2
MEX	**1**	0	0	0	1	0	0
MKD	**34**	0	0	0	0	0	19
MNE	**1**	0	0	0	0	0	1
MNG	**2**	0	0	0	0	0	1
NLD	**241**	0	0	0	21	0	4
NPL	**5**	0	0	0	0	0	1
PAK	**16**	0	0	0	0	0	2
POL	**9**	0	0	0	0	0	1
PRK	**6**	0	0	0	0	0	0
PRT	**9**	0	0	0	0	0	0
ROU	**5**	1	0	0	0	0	0
RUS	**11**	0	0	0	1	0	0
SRB	**3**	0	0	0	0	0	1
SUN[3]	**8**	0	0	0	0	0	1
SVK	**2**	0	0	0	0	0	0
SWE	**22**	0	14	0	1	0	1
SYR	**28**	0	0	0	2	0	8
TKM	**1**	0	0	0	1	0	0
TUN	**2**	0	0	0	0	0	0
TUR	**415**	0	0	168	4	0	98
TWN	**6**	0	0	0	0	0	1
UKR	**10**	0	0	0	1	7	0
USA	**65**	0	0	0	5	0	50
UZB	**6**	0	0	0	1	0	0
YUG[4]	**1**	0	0	0	1	0	0
Unknown	**253**	0	1	0	0	0	27
TOTAL	**1959**	1	80	168	84	7	340

Information extracted from EURISCO (https://eurisco.ipk-gatersleben.de), GRIN (https://npgsweb.ars-grin.gov) and the International Spinach Database (https://ecpgr.cgn.wur.nl/LVintro/spinach/). [1] Countries represented by the 3-letter code [ISO 3166-1 alpha-3]. [2] Former Czechoslovakia. [3] Former USSR. [4] Former Yugoslavia. [5] Gene banks represented by their WIEWS Code (https://www.genesys-pgr.org/wiews/active).

Appendix 2. Overview of spinach germplasm

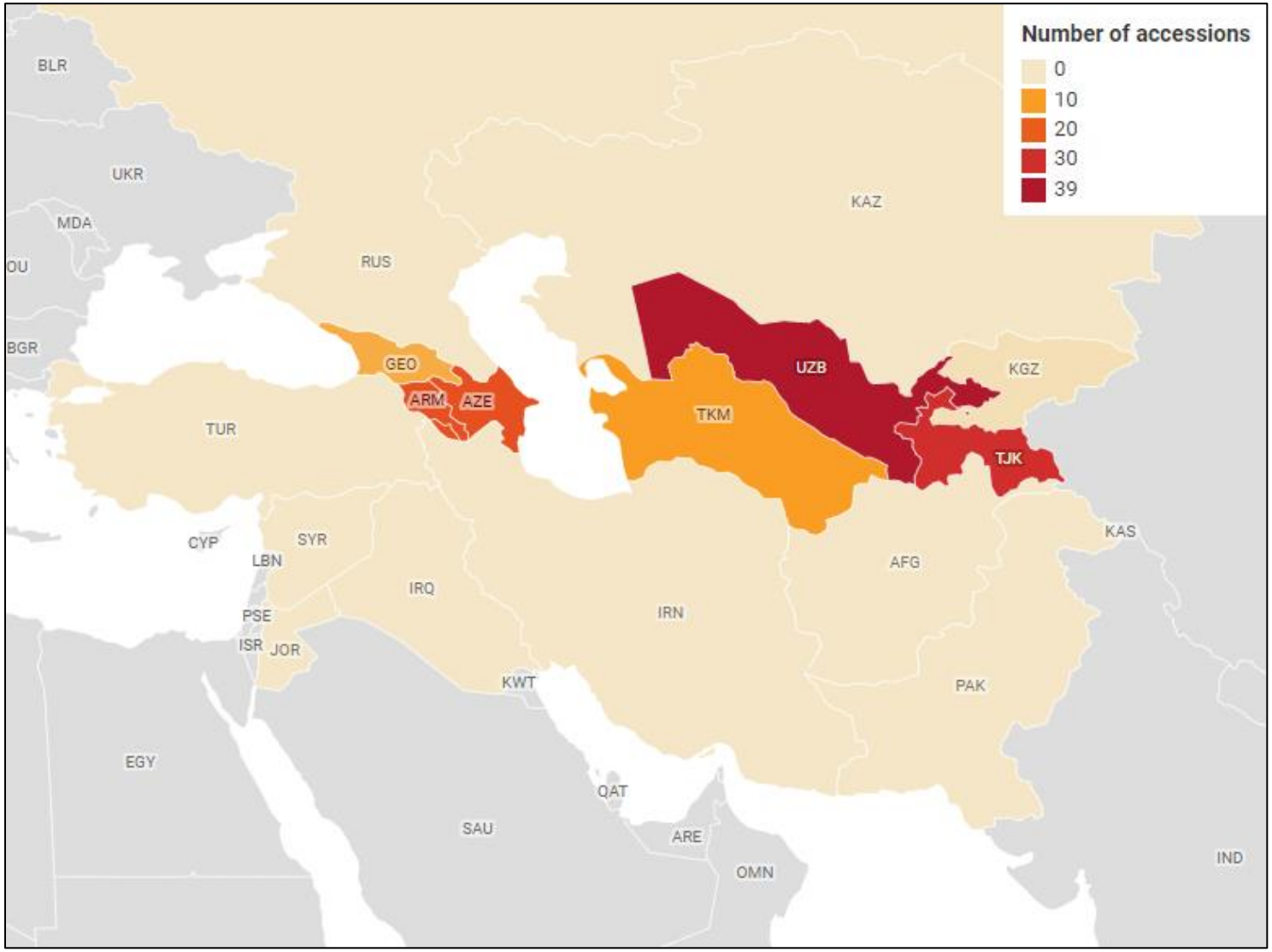
Appendix 2.1. Germplasm of cultivated spinach

Cultivated spinach accessions ordered by gene bank and accession type.

Gene bank[1]	Total number of accessions	Number of landraces	Number of modern cultivars	Other types of accessions
AUT046	**23**	10	8	5
AUT047	**5**	0	5	0
AZE015	**23**	0	0	23
BEL002	**2**	0	2	0
BGR001	**204**	12	33	159
CHE001	**8**	8	0	0
CZE122	**17**	0	17	0
DEU146	**208**	102	100	6
ESP026	**31**	31	0	0
ESP027	**80**	79	0	1
GBR006	**122**	55	55	12
GBR017	**3**	0	3	0
GEO013	**3**	2	1	0
GRC005	**4**	3	0	1
HUN003	**97**	31	4	62
ISR002	**1**	0	0	1
ITA363	**1**	1	0	0
MKD001	**4**	4	0	0
NLD037	**408**	122	145	141
POL030	**25**	7	18	0
PRT001	**7**	7	0	0
ROM007	**3**	0	3	0
ROM023	**1**	1	0	0
SWE054	**80**	0	0	80
TUR001	**168**	167	1	0
UKR008	**84**	6	66	12
UKR021	**7**	3	0	4
USA020	**340**	20	9	311
TOTAL	**1959**	**671**	**470**	**818**

Information extracted from EURISCO (https://eurisco.ipk-gatersleben.de), GRIN (https://npgsweb.ars-grin.gov) and the International Spinach Database (https://ecpgr.cgn.wur.nl/LVintro/spinach/). [1] Gene banks represented by their WIEWS Code (https://www.genesys-pgr.org/wiews/active).

Appendix 2.2. Germplasm of spinach wild relatives



Geographical origin of *S. tetrandra* and *S. turkestanica* accessions. The data used to create this map is presented in the tables below. Accessions with unknown origin have not been represented. Created with Datawrapper; interactive map available at: https://datawrapper.dwcdn.net/thgWB/6/.

Appendix 2.2. Germplasm of spinach wild relatives

Availability of *S. tetrandra* accessions ordered by country of origin.

Country of origin[1]	Number of *S. tetrandra* accessions
ARM	23
AZE	22
GEO	8
Unknown	6
TOTAL	**59[2]**

Information extracted from EURISCO (https://eurisco.ipk-gatersleben.de), GRIN (https://npgsweb.ars-grin.gov) and van Treuren et al. (submitted).
[1] Countries represented by their 3-letter code [ISO 3166-1 alpha-3]. [2] Of these accessions, 20 are still not publicly available and will become available to the general public in 2 different batches by the end of 2020 (Van Treuren et al. submitted).

Availability of *S. turkestanica* accessions ordered by country of origin.

Country of origin[1]	Number of *S. turkestanica* accessions
KGZ	1
TJK	30
TKM	10
UZB	39
SUN[2]	1
Unknown	8
TOTAL	**89**

Information extracted from EURISCO (https://eurisco.ipk-gatersleben.de) and GRIN (https://npgsweb.ars-grin.gov).
[1] Countries represented by their 3-letter code [ISO 3166-1 alpha-3]. [2] Former USSR.

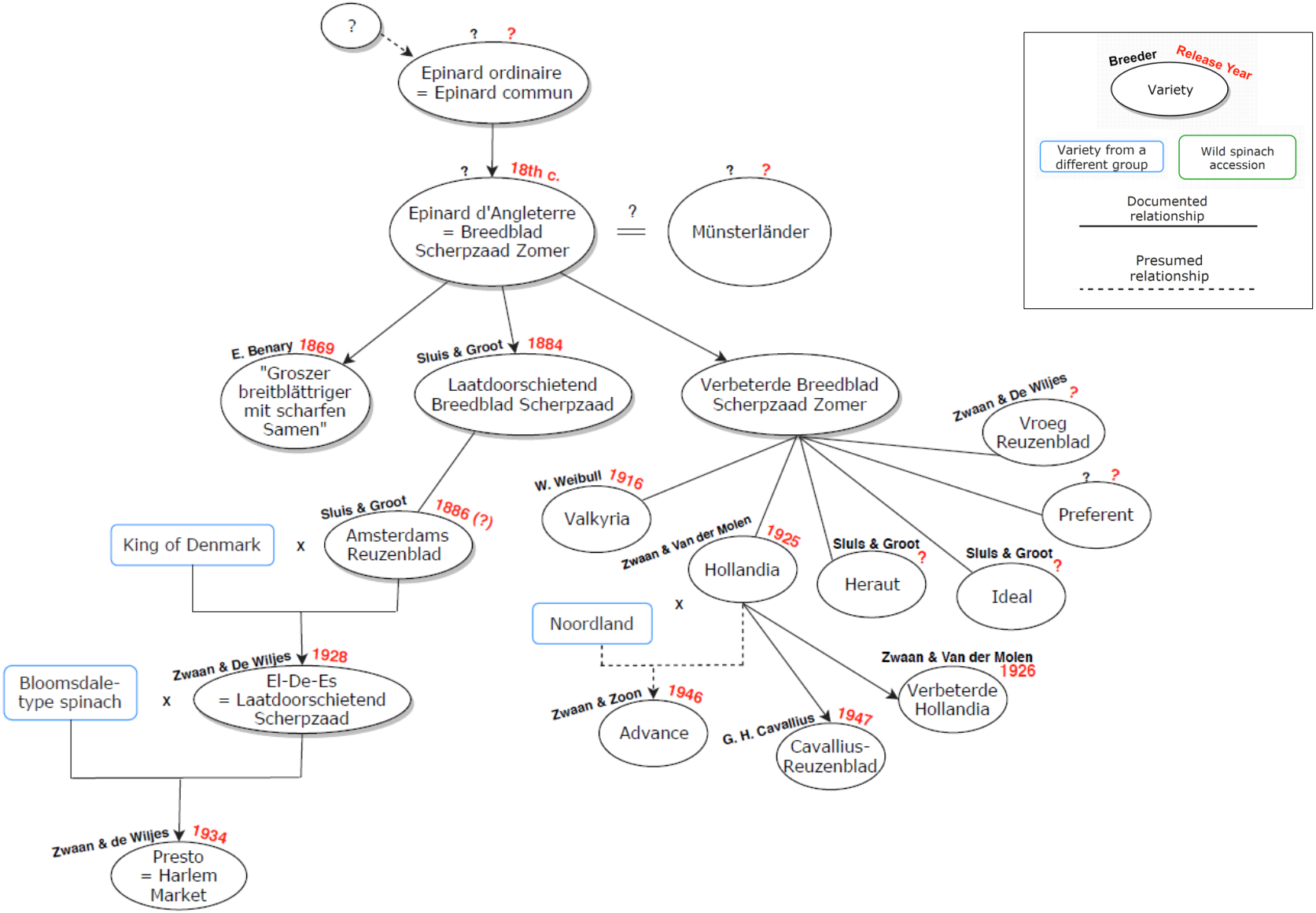
Availability of *S. tetrandra* and S. *turkestanica* accessions in international gene banks

Gene bank[1]	Number of *S. turkestanica* accessions	Number of *S. tetrandra* accessions	Total number of accessions
ARM002	0	1	1
ARM035	0	5	5
AZE014	0	2	2
DEU146	3	3	6
GBR004	1	2	3
GBR006	2	1	3
NLD037	75	39[2]	114
USA020	8	6	14
TOTAL	**89**	**59**	**148**

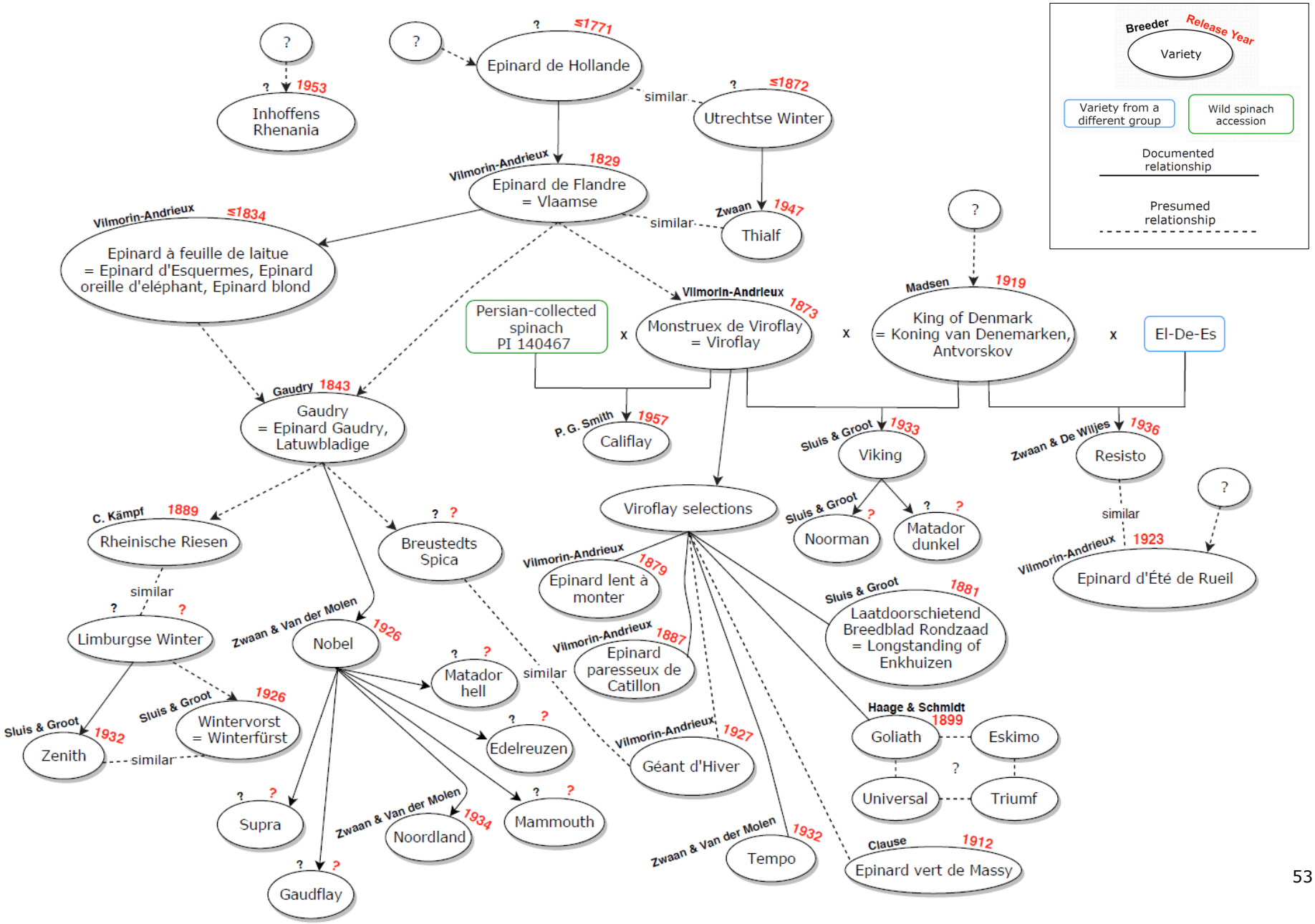
Information extracted from EURISCO (https://eurisco.ipk-gatersleben.de), GRIN (https://npgsweb.ars-grin.gov) and van Treuren et al. (submitted).
[1] Gene banks represented by their WIEWS Code (https://www.genesys-pgr.org/wiews/active). [2] Of these accessions, 20 are still not publicly available and will become available to the general public in 2 different batches by the end of 2020 (Van Treuren et al. submitted).

Appendix 3. Breeding history of spinach

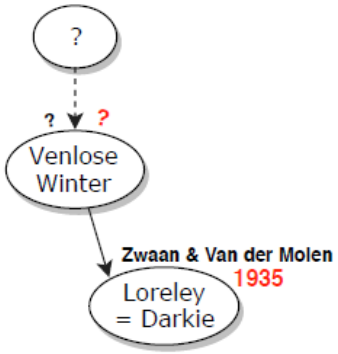
Appendix 3.1. Spiny-seeded cultivars

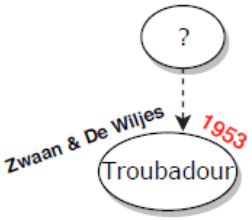


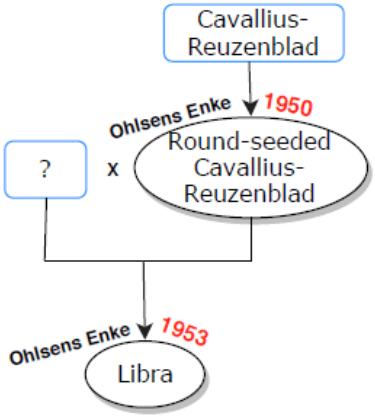
Appendix 3.2. Smooth-leaved smooth-seeded cultivars

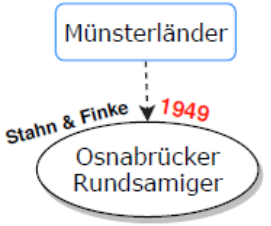


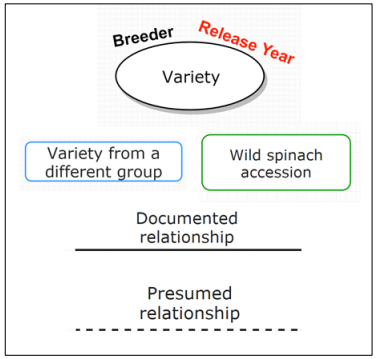
Appendix 3.2. Smooth-leaved smooth-seeded cultivars



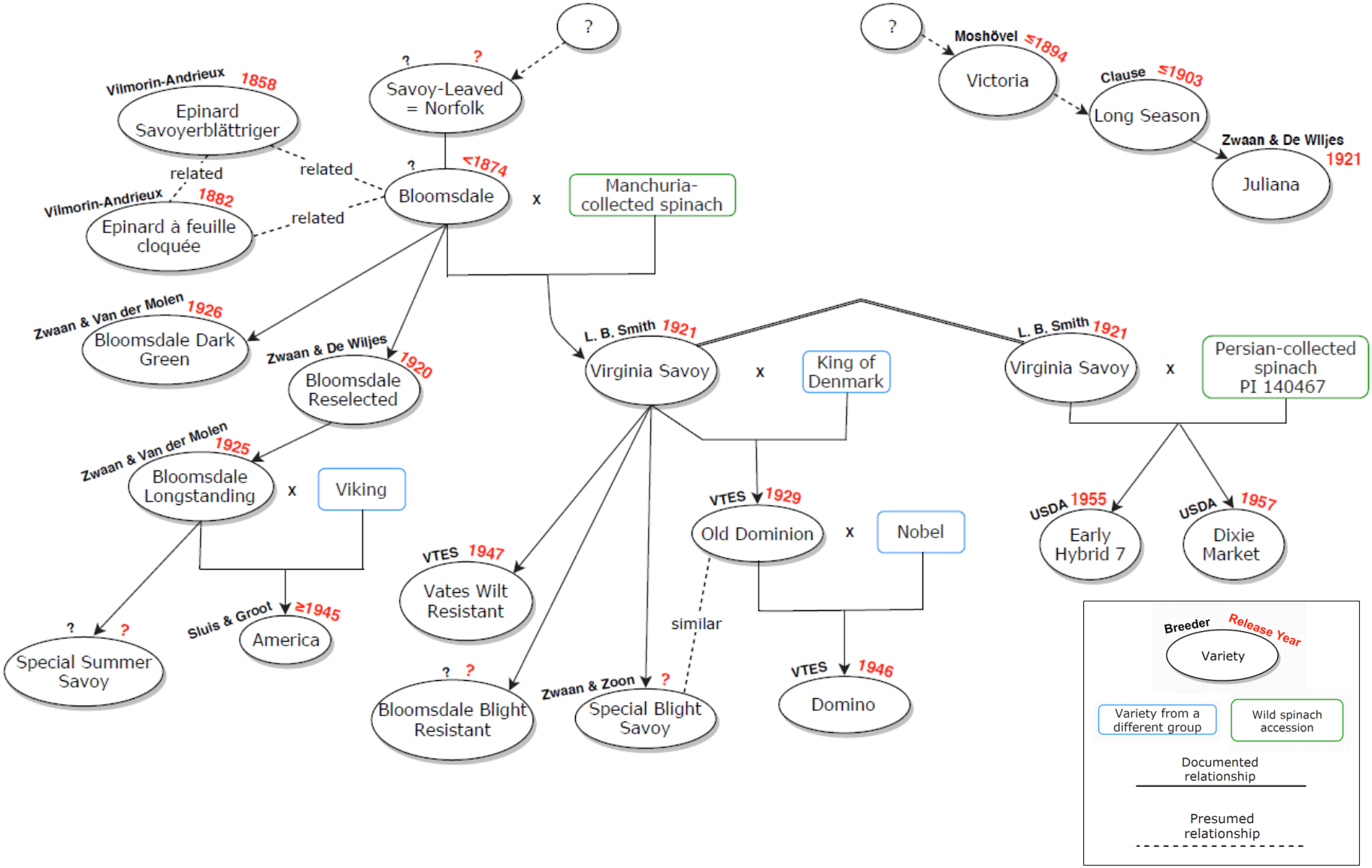








Appendix 3.3. Savoy-leaved smooth-seeded cultivars



Appendix 4. *Spinacia* accessions used in the study

Accession number, species and geographical origin of the *Spinacia* accessions used in this study [Table 1/2].

Accession number[1]	*Spinacia* species	Phylogeny number[2]	Country of origin[3]	World region[4]	Latitude	Longitude	Note
C25459	tetrandra	tetr AZE1	AZE	WA_C	40.97142	45.98253	
C25460	tetrandra	tetr AZE2	AZE	WA_C	40.91658	46.15855	
C25461	tetrandra	tetr AZE3	AZE	WA_C	40.92768	46.0903	
C25462	tetrandra	tetr AZE4	AZE	WA_C	39.39507	45.31957	
C25463	tetrandra	tetr AZE5	AZE	WA_C	39.24662	45.5779	
C25464	tetrandra	tetr AZE6	AZE	WA_C	40.40367	47.25708	
C25465	tetrandra	tetr AZE7	AZE	WA_C	40.49217	49.02003	
C25466	tetrandra	tetr ARM8	ARM	WA_C	39.73282	45.18015	
C25468	tetrandra	tetr ARM9	ARM	WA_C	39.84002	44.68265	
C25469	tetrandra	tetr ARM10	ARM	WA_C	40.10802	44.56088	
C25470	tetrandra	tetr ARM11	ARM	WA_C	40.13267	44.57372	
C25472	tetrandra	tetr ARM12	ARM	WA_C	39.87835	44.56933	
C25473	tetrandra	tetr ARM13	ARM	WA_C	39.87843	44.5773	
C25474	tetrandra	tetr ARM14	ARM	WA_C	40.07622	44.54882	
C09408	oleracea	oler NPL15	NPL	SA	28.16888	84.24316	
C09442	oleracea	oler NLD16	NLD	WE	-	-	cv. Resistoflay
C09463	oleracea	oler NLD17	NLD	WE	-	-	cv. Viking
C09464	oleracea	oler FRA18	FRA	WE	-	-	cv. Viroflay
C09477	oleracea	oler IRN19	IRN	WA	32	53	
C09478	oleracea	oler IRN20	IRN	WA	32	53	
C09492	oleracea	oler RUS21	RUS	WA_C	43.0769	46.85199	
C09504	oleracea	oler TUR22	TUR	WA	37.91667	40.23333	
C09505	oleracea	oler IND23	IND	SA	30.38333	78.48333	
C09519	oleracea	oler AFG24	AFG	SA	37.1	68.9	
C09524	oleracea	oler CHN25	CHN	EA	39.89972	116.4125	
C09528	oleracea	oler AFG26	AFG	SA	33.92625	67.6995	
C09529	oleracea	oler GRC27	GRC	SE	39.38306	22.75	
C09531	oleracea	oler AFG28	AFG	SA	33.92625	67.6995	
C09536	oleracea	oler CHN29	CHN	EA	45.75	126.65	
C09611	oleracea	oler SYR30	SYR	WA	35.13333	36.75	
C09618	oleracea	oler TUR31	TUR	WA	39.45	37.03333	
C09622	oleracea	oler TUR32	TUR	WA	38.35	38.31667	
C09623	oleracea	oler TUR33	TUR	WA	38.413	27.192	
C09626	oleracea	oler TUR34	TUR	WA	40.98333	39.71667	
C09627	oleracea	oler TUR35	TUR	WA	40.73333	31.61667	
C09631	oleracea	oler IRN36	IRN	WA	32	53	
C09633	oleracea	oler IRN37	IRN	WA	32	53	
C09635	oleracea	oler AFG38	AFG	SA	36.7	67.1	
C09637	oleracea	oler AFG39	AFG	SA	34.33333	62.2	
C09638	oleracea	oler IND40	IND	SA	22.3	73.2	
C09640	oleracea	oler AFG41	AFG	SA	31.58333	65.75	
C09641	oleracea	oler AFG42	AFG	SA	35.94	68.71	
C09642	oleracea	oler AFG43	AFG	SA	34.51667	69.2	
C09643	oleracea	oler IRN44	IRN	WA	35.66667	51.43333	
C09661	oleracea	oler NPL45	NPL	SA	27.71667	85.31667	
C09662	oleracea	oler IRN46	IRN	WA	32	53	
C09668	oleracea	oler TUR47	TUR	WA	36.73333	37.08333	
C09669	oleracea	oler MKD48	MKD	SE	41.46667	22.03333	
C09676	oleracea	oler PAK49	PAK	SA	30.18333	67.75	
C14166	oleracea	oler TUR50	TUR	WA	36.73333	37.08333	
C14168	oleracea	oler BGR51	BGR	SE	42.16667	23.05	
C14169	oleracea	oler RUS52	RUS	WA_C	42.05	48.3	
C14177	oleracea	oler IRN53	IRN	WA	32	53	
C14193	oleracea	oler PAK54	PAK	SA	29.1	66.75	
C14194	oleracea	oler PAK55	PAK	SA	28.31667	66.31667	
C14195	oleracea	oler PAK56	PAK	SA	30.33333	68.68333	
C14213	oleracea	oler CHN57	CHN	EA	23.08702	113.2157	
C15784	oleracea	oler GEO58	GEO	WA	41.455	45.004	
C21754	oleracea	oler SYR59	SYR	WA	33.5	36.25	
C23301	oleracea	oler UZB60	UZB	CA	40.53333	70.93333	

Information extracted from the CGN Database (https://cgngenis.wur.nl). [1] Nomenclature adapted from the CGN accession number. To get the CGN accession number, the "C" at the beginning of each accession should be replaced by "CGN". E.g. "C09477" is "CGN09477". [2] Nomenclature used in the phylogenetic and population structure analyses. [3] Countries represented by their 3-letter code [ISO 3166-1 alpha-3]. [4] World regions based on the United Nations Geoscheme [CA – Central Asia, EA – Eastern Asia, SA – Southern Asia, SE – Southern Europe (Balkans), WA – Western Asia, WA_C – Western Asia (Caucasus) and WE – Western Europe (modern cultivars)].

Appendix 4. *Spinacia* accessions used in the study

Accession number, species and geographical origin of the *Spinacia* accessions used in this study [Table 2/2].

Accession number[1]	*Spinacia* species	Phylogeny number[2]	Country of origin[3]	World region[4]	Latitude	Longitude	Note
C24954	turkestanica	turk TJK61	TJK	CA	38.17665	68.52482	
C24955	turkestanica	turk TJK62	TJK	CA	38.20503	68.53882	
C24957	turkestanica	turk TJK63	TJK	CA	38.19798	68.55287	
C24993	turkestanica	turk TJK64	TJK	CA	38.1847	68.48227	
C24998	turkestanica	turk TJK65	TJK	CA	38.33085	68.60532	
C24999	turkestanica	turk TJK66	TJK	CA	38.10793	68.4639	
C25002	turkestanica	turk UZB67	UZB	CA	38.1949	67.16722	
C25004	turkestanica	turk UZB68	UZB	CA	38.83818	67.12917	
C25005	turkestanica	turk UZB69	UZB	CA	39.99503	68.02248	
C25008	turkestanica	turk UZB70	UZB	CA	40.0533	68.45637	
C25010	turkestanica	turk UZB71	UZB	CA	39.9438	67.62033	
C25016	turkestanica	turk UZB72	UZB	CA	40.0819	67.58663	
C25083	turkestanica	turk UZB73	UZB	CA	41.0667	69.4795	
C25084	tetrandra	tetr GEO74	GEO	WA_C	41.36056	45.05028	
C25085	tetrandra	tetr GEO75	GEO	WA_C	41.35139	45.04861	
C25091	turkestanica	turk TJK76	TJK	CA	38.4145	68.546	
C25093	turkestanica	turk UZB77	UZB	CA	38.72435	66.39427	
C25095	turkestanica	turk UZB78	UZB	CA	39.84505	67.45718	
C25097	turkestanica	turk UZB79	UZB	CA	39.78033	67.86045	
C25119	oleracea	oler AZE80	AZE	WA_C	39.20613	45.42573	
C25120	oleracea	oler AZE81	AZE	WA_C	40.73852	46.39487	
C25121	oleracea	oler AZE82	AZE	WA_C	40.64222	47.47013	
C25123	oleracea	oler ARM83	ARM	WA_C	40.1092	44.27538	
C25125	oleracea	oler ARM84	ARM	WA_C	40.11142	44.27003	
C25130	turkestanica	turk TJK85	TJK	CA	38.40997	68.57283	
C25134	turkestanica	turk UZB86	UZB	CA	40.17998	67.20157	
C25135	turkestanica	turk UZB87	UZB	CA	40.96322	69.55753	
C25137	oleracea	oler UZB88	UZB	CA	38.27505	67.88927	
C25139	oleracea	oler UZB89	UZB	CA	41.32712	69.23467	
C25141	turkestanica	turk TKM90	TKM	CA	38.40194	56.99083	
C25142	turkestanica	turk TKM91	TKM	CA	38.43528	57.03861	
C25143	turkestanica	turk TKM92	TKM	CA	38.08888	58.05861	
C25259	turkestanica	turk UZB93	UZB	CA	40.23012	67.05917	
C25272	oleracea	oler AZE94	AZE	WA_C	40.64237	48.6363	
C25274	turkestanica	turk TKM95	TKM	CA	38.38888	57.02556	

Information extracted from the CGN Database (https://cgngenis.wur.nl). [1] Nomenclature adapted from the CGN accession number. To get the CGN accession number, the "C" at the beginning of each accession should be replaced by "CGN". E.g. "C09477" is "CGN09477". [2] Nomenclature used in the phylogenetic and population structure analyses. [3] Countries represented by their 3-letter code [ISO 3166-1 alpha-3]. [4] World regions based on the United Nations Geoscheme [CA – Central Asia, EA – Eastern Asia, SA – Southern Asia, SE – Southern Europe (Balkans), WA – Western Asia, WA_C – Western Asia (Caucasus) and WE – Western Europe (modern cultivars)].

Appendix 5. Protocol for DNA isolation using a CTAB buffer

Step	Protocol
1	Fresh spinach cotyledons are collected in 8-tube strips. Two metallic grinding balls have been previously placed in each tube. After harvesting, each sample is immediately frozen in liquid nitrogen.
2	Leaf material is grinded in the shaker for 60 seconds.
3	Add 2x 250 µl CTAB extraction buffer with RNase (per 1 ml CTAB 1 µl RNase (2 mg/ml) to each tube with sample. Close the tubes with caps.
4	Mix in the shaker for 60 seconds.
5	Place the tubes and holder in a press and tight the nuts to prevent the caps from popping off. Incubate in a 65 ºC water bath for 1 hour.
6	Cool the tubes in ice for 30 minutes, keeping the samples in the press to prevent the caps from popping off.
7	**From here on, steps must be done in a fume hood.** Add 250 µl of chloroform isoamyl alcohol (24:1) and mix by inversion, at least 40 times.
8	Separate phases by centrifuging the tubes at 4000 RPM for 15 minutes. Pipette 350 µl of the water phase into new 8-tube strips.
9	Add 175 µl of isopropanol, close with caps and mix by inversion during a few seconds.
10	Pellet the DNA by centrifuging the tubes at 4000 RPM for 15 minutes. After centrifuging, throw away the suspension in the tubes so only the pellet remains.
11	Wash the pellet by adding 300 µl of 70% ethanol and centrifuge the tubes at 4000 RPM for 15 minutes.
12	Dry pellets for 2-3 hours until there is no ethanol in the tubes.
13	Dissolve DNA in 50 µl of MQ water.

Composition of the CTAB buffer:

- 100 ml 1 M TRIS pH 7.5
- 140 ml 5 M NaCl
- 20 ml 0.5 M EDTA pH 8.0
- 740 ml MQ H_2O
- Add 2% CTAB (dissolve in a 65°C water bath)

Appendix 6. SNP information

List of the 60 SNP markers used in the KASP assay [Table 1/2].

SNP ID	Sequence[1]
SO001	TTGAGTCGTTCCCCGTTCTACCGGAGCAGCGTTCTCCTTCAATTAGCGAC[G/A]ACGGCGAGCCTTCAATCTAGTCGTCTGCTTGTCGTGGTTGGTGGTTGGTG
SO002	AAAGARTTGAATTTCCCAAATGCCATCATTCATTTCCATCCTTTGGACTT[G/C]TAACAACCTTTGCCTCCTTTTCTTGATGATGATGAACACACAAAGGGTGA
SO014	TCTGAACGCCCAGCAGCAAAGCCAACAGGAACACAGAAATTAGTTTGTCT[A/G]TTACTTTTTATCTCTGAACTTTCTGAATCAGCACCCTTTAAATATTCCAA
SO017	GTTATTTGGAGTCCTTGTGGGAAATTTTGGGTGTTGAATGAATAGGGTAC[C/G]AAGAGTTGAAGATTATATCATGCAAATGAAGGTGTGAGCTCTGTTATCCA
SO019	GAAGAATAAGCTCGGCCAGGGTTAACCACAGACAACTCACTAGGGTTTCT[C/T]GAGGAAGGATATCTCCCTAATATGCTGGCATCCCTGGGTTCGAGAATGGG
SO021	GGATGAATGGTGGAGACTTGCGTTGATGGACAATTCCAACCCACAGATAG[G/C]TTTGCTGCCCTTGCATCCYGATGTGCGTGCAAAATTCAACTCTACTGCTG
SO025	TTGGGTGGTGGTCTTCTTCACCTTAAGACATCTTCAGCCCACAGTTATAC[C/A]TGTATGGATCTTTATGTTTTTGCAACTCCCTACAGGGTTACTTGGGATTA
SO032	GGTTGAACCTGTTTAGCAATGCGCTCGAGAATTTGCTTCGCTTCATCTTG[T/C]CGCGGCTTTCGTTTCAGAGCTCTGATTTCCCATACTTTGTTCAGGTCACC
SO036	TAATTGTAGTAATATATCATTATAACCGTTTAATACATACAATTCACGGA[G/T]AGTAGAAAGTTAGACGATTACATACAACCACGACCGCCTACAATAATAAC
SO040	AGCRGTATCAGTTGAGATGTCAAATCACCAGGAACGGCATTGAATTCTTT[A/G]AAATTGCAACTTCAGACAAGTTGTAAGTTACCAGTGAAGAACCTGTTGAT
SO043	GGGTCGAGGGATTTGGGTGAAGCTTTGAGGAGAATTCGAGAAGGAGCTGC[T/C]ATGATTCGTACCCAAGGGGATTTATCCGGGTCAGGTAATATTGCTCAAAC
SO058	GATTGAGGAGCTTGGTCTTATGCTTTGCAATTTAGTGACTGCAGTCCCTG[A/G]AGGAATAGTTGTATTCTTCTCTTCATTTGATTATGAAGACAAGGTCTATG
SO066	ATGGGCTCCTCATCAATGGGCTTAAACACAGCTACAATCTTCTGACCTGA[T/G]GGGTTGAACATGAAATAAGCACCACCAGTACCATCTGTTGATCTAATTGG
SO071	GTACATTTATTCATCAATGTCTCCTCAAACTCAACCATCCCATTTGAAAC[C/A]CTTGAAGGAAGAGTCTTAAGGACATTATCCTTCCTCCTCCAATGCACACT
SO079	CCACAAATTATAGTTCAATGGTAAAATCTGATACTAAATTACCTTCGGTT[T/C]GTGGTATGAATTCCCTGGATAACTCTATAGCTGGAGTTGAGGATCAAGGA
SO091	GACGGTGGTCATACACATTGGAATTAGTGAAAATTCAAATCGTCCAAAAG[G/A]GGAAAAGCTTAAAGGAATTGGTGGAACCTCTGTAGTTGTGGGATTGAGCT
SO105	GAAAAAAATAAGTAATGATTAGGTGAATAGAACTTCACACAAAAGCAACA[T/A]ACAACAATTAGCAGACATTGGAAAAGCTGAAAAAACAATAGATTTGAAAA
SO115	CAAATCACCATAGTAATCTTAGCTAAGCCAATTACTTTTTTGGTGCTAAG[A/T]GCTTACCTTTAAAGTTGGATGCACAGCTGTAGCCTGYAGGACAGTTTTAT
SO125	TTTTTCCCSTGCTTCTTGACATGGTTTTGCAAGAATCCAATCCCCTTGAA[T/A]GCCTTTGAAATCTTCATCCCCATACTTAAATTCATCTTTCCAGCAGACAA
SO126	GGTTCTAAACCGAGGATGAGAAAATTGTGGGGAGGATGAGGGGTTTATAT[A/T]CCCAAAATAGTAAAGGATCCAAAGGAAAGTGGTAGGAGTAGTTTGAAAAA
SO129	CTCTCGGCTATGATCATAAACCACTATAAAATGAGGGGTAATGTGTTGAG[C/T]TACAACCTTGGTGGAATGGGGTGTAGCGCCAGTGTTATATCTATTGACTT
SO158	GCGGCCCTTTGAGGTCTTGAAGCCTTCGAAGACTTCTTCTTWGACCGTTT[T/A]CGTCCATTTCTTGATTTTCTCTTTTGGTCGACTCTGTGGGTACTACCATC
SO159	ATACTGTCTTCATCTTGGCCTGTTCCAGCAGACCACTGGCTGACGCTTCG[G/A]ATAATCTGACAATGACATGAAGTACGGGGACCAGTTTGTGAAGGTAGCCT
SO176	TGCAGCAAGATCCTCATTTGCTGCTGAGTTTCCAGCTGTAGAAGCTGAAA[C/T]TGGACCGTAACCAACTGGTGAGGCCCCATATGGTCCATAACCTCCACCAA
SO177	ACTTTCTGAGAACCCAAAGCTTGCTGTGACCGACTACCATAGTTAGAGGA[A/T]GGTCGACTAGAGCTTCCATGACTYAGTGATGAAGATTGCAAAAATTCTGC
SO178	TGCTGACCTTGAGGAGGAAGATTATAGAAAGAGTTCGCTGGTAAATTGGC[A/T]ATGTCACGTCCTGGTGCAGGAATCCAGACTGCTGGACCTTCGCTCTGTTG
SO198	CATGTAAGACGAGACATTTGATAGATGTCATATTAACCGGAGATTGTACG[G/C]CTTGGAATGTGAAACCAATCAAATTAAATGTATGAGAAAAGATATTAAGG
SO203	TTCATATTCAATTGATTAACGATTAATCCCCCTCCGAATCTTCTTTCATG[T/C]ACATCTGAGATTACCTGAAATACTACCCCAATGGGAATAGCGAGTTGCAC
SO205	AGCGTCTTCTATGTTTGTTATCACCTTTGCTTTTTCCATCACCCCGTCTA[C/A]GTGCACCTCAAAATTAACTGATAMAGTATCCAGAAATTCATCCTTTACCA
SO206	ATCTTCCTACTAAGTTGTATGTCGTAGGTCCTTTTTTAGGAATTGGGGTT[G/C]GCTACTTGGCTCAAGTGACGAGTTCTCCCTTCTTCCCTAGCATTAATTCC

[1] Each sequence contains the SNP marker with 50 bp flanking each side of the marker. The SNP is shown in the centre of the sequence separated by square brackets, with each of the SNP alleles separated by a slash: [allele1/allele2]. For ambiguous nucleotides, the IUPAC ambiguity codes have been used.

Appendix 6. SNP information

List of the 60 SNP markers used in the KASP assay [Table 2/2].

SNP ID	Sequence[1]
SO214	CAATGGTTAACGACAATAGCAATAACATTGTCGTTAAACAACGAAAATCT[G/A]AAATTTTAACAAATGGAGCGAGGCCTTTTTTAACTTTCTTGTCATCGTTA
SO237	TACTGACTTACAACCTTACTGTTCAACGAAGTAGAAGCAGACTTTACTAA[C/A]GATTCCCGATCTGAAAGCTCCAGAGGTACGGCCATSGAAGTGAGAACATG
SO240	ATTGAAAAGTAAATAAAAAGAAGGGGAAATCGGGAGGGAGAGATGAGTTT[C/T]CAAGATCTCGAAGCGGGTCGTCGGCCGCTGGCTTCGAGACGGGATTTAAT
SO244	CAAGCAATTTGGTATTAGAATAGGGAAAGTTTGCCAGTCTGCCGCGAATC[T/G]CAAAGTAGAGGACAATGAGACCCCCCTATTCACAAATAAACTCATTGGCC
SO247	TTTATTTGAGAACTAAGTACTGCTTAATTAGTTACCCTTTTGTTCTTCAG[C/T]AGTCTTGGTTTGTGGTGGGTAGTGACCATGACCACCGTGTYCATAGCCAC
SO264	TGAAGGTGCTATGAAACGTGGTGACTGGCATAGAACAAAGGACTTGGTTG[A/T]GAAGGGTGCTGACTGGATTGTCAAYGAAATGAAGAAGTCTGGTCTCCGTG
SO267	TTCCTGAAACTCTGACGGCTCACGAACATCATGCAGTTGCAGAACTGCTT[C/T]TGCTTCAAGCAGCTGCTTAAGTAAGCTTCCATTTTCTTYCTCTCTAGCCA
SO270	GCAAAGAGYAATCTCAAACCTGTGACTTTGGAGCTTGGTGGGAAATCTCC[A/G]TTTATAATATGTGAAGATGCAGATATAGACCAAGCAGTGGAGACCGCTCA
SO272	TGCAGCATCAGGGAAAACATTTCACACAGTGGACCCTCGGACTGGGAAGG[T/C]GATAGCTGATGTTGCTGAAGGAGATTCCGAAGATGTGAACAGAGCAGTCT
SO280	AAACCCTCAACAAAGAACAACAGGACGTTCTCCGCTCTAAATCCTCCGTT[C/G]TCGCTCTYATCGATGAGTTTGAGAAGCTTCGCGCTCCTCTCTCCTCCGCC
SO281	GCCTTCTTCRGCGGAGGAACAACAACCTTCATCGGAGAATAACGATGAAA[G/T]TTGTAACAACGATGAAGATGTTGCTGTGAAGGATCTGCTGGATTTGATGT
SO290	ACAATACAGAGTAAGCAAAAAGAACTGTACAGAACAAATTCCAGGTCAAG[G/A]CAAGTAAAAGGATGGAGAGACGAACTATGTTCAAACTTAGCAGATCATTT
SO292	TTAGTAACACTGTAACATGGTGACATAGTGAATTGGTAATAAATTTTTAA[C/A]CTCCTCTGTTTTCCTCCTCTCCCCATTTTTTTTCTTTTCAAATTATTCTG
SO304	TTTGAGCTCACCTGTTATGTTCTCATTTGGAGCATCTTTGGTCACGATTT[G/C]TTGGTGTTTTGGTATGCAAGAACTGGTTGCGGTGTATAGTACTGTTTTTC
SO314	ATATTTCAAGAATTGTGTTATTGGGATTCTACCCTGACTTCCGTTTTAGT[G/A]TATCCTGCAGCTAATTTCAGTTTGTCATGCTTTTTACAGTGTGGCACACG
SO334	ACMACAGCTGCACCCAACACACTACAACAGCAGTCCATCTAAACCAAGCA[T/C]AACCACTGCCAGATTCAAACCCAACAATAAATAATGATGCAAAATTTGCC
SO336	GACAGGTCTTTCGGGTTTGTTGGGTTGCCGCCGGGGAACCCGAACCCAAG[G/T]TCACAGGTTTAACAGATGCAGCTAAGTTTTTTCATGATGAAGCAAAATAAT
SO337	ACTGATCTTGGTCTTGCCTTAACACTACCAGTTGAGTCATCAACCTCATT[T/C]TTCTGTTTTCTATCATCATTTGTATCATTATTTGATACAATATAAGGAGA
SO346	AGCAGCATCAGGGTTCTCTAGGAGCATCTTCGATGTTAGATACCCAGCAA[C/T]AGTCCAAGTTTGATACAGMCGTGCCTGTTTACCAATAAACCGTCCCTTTT
SO360	GGTCTGTGCACCGGGGTTAAACACCAACGATATGTACATATGTTTGGCAA[C/T]GTACGAGTATTATTCGGTTAAACACCAAATACCAAGGTTATCGACCTTAA
SO366	GCAAACGTTGTTGGACGGCTGTGATGCGGACCATCTTTGTRGTTTTCTCC[A/G]TAGTTGTGCCAAGGATTTTGGTCGGATTGCTATAGATAGGTGTGGTTCTC
SO373	ATCTATTGATATAGCTCACTATGAGCCTGTATATATGGACTTTCTGACGC[T/A]GCTGGATGGTTAAAAGCCTCATTTTGCTTATTTCATCATCTTTGTTGTAA
SO374	TTGGATGAAGAGAGGTCCCTTGCAATGGATTTAGCCATGTCTTCTATGGA[C/T]GAATTGCTTAAGATGTGCCATGTAAATGAGCCACTTTGGGGTAGGAATAG
SO381	ATTCTTGTGACGGGGTTTCTGAAATAAAAGCATTTCATATCTTGCATAAT[A/G]TTCACCAATCTGGTGTCAATTGTCTGCACCTTTGTTGTCGAAATCTTGGA
SO382	AACTGTTTAGCTTGCGCTTTCAGCTGGGGTCTCTAGATAAACCAGGTGTT[G/A]GAACAGACCTAACTTTTTTACAGCTGTTGCCAAGATTTTCTCACTGGGTC
SO398	CTAGCTCTTTTCCTTGTCGTACCCTAACCATACCATCATTTTTCCCTTTA[A/C]ATTGATCACGGCACCAAGAGCATGTTTATCAAACACCTATCTTTGGTGTT
SO405	TTCATGTTCCAAACCCTAATCTTATGATCCTTGTGTGATGTGAAGAGGGT[A/G]TCCCCATGTGCCACCATGGCTCGAACCTCGCCGGAGCTTGCATGTATGTA
SO408	AACATCAGAGCCCCAAAATCAAACAATGCCTGACAATGATTGGCATTTTG[G/T]TGCAATTAGCAATGCATTTTAGTAACTGAATCAAAATGCATAACTATAGC
SO409	ATGGTCAGTCCTAAGAATGGTGGGACCCTATATGACTCCAGGGGAACAAC[T/G]CGATGCTCAACCCAACATTGGTTCAACCAATTAATAGTGACAATATTCTT
SO419	TTCAACAACCACAACAAGTAATCATTTGAAGAGGAAAGCTATGGATTTGG[A/C]TCTCTCCCTTGCCCTAGCTCATCCAAAAGATATCGACAACAACGATCCAT

[1] Each sequence contains the SNP marker with 50 bp flanking each side of the marker. The SNP is shown in the centre of the sequence separated by square brackets, with each of the SNP alleles separated by a slash: [allele1/allele2]. For ambiguous nucleotides, the IUPAC ambiguity codes have been used.

Appendix 7. KASP results

SNP marker scores from the KASP assay [Table 1/4]

Accession	Marker[1]														
	SO159	SO203	SO205	SO001	SO002	SO014	SO017	SO019	SO021	SO025	SO032	SO036	SO040	SO043	SO058
C25459	?	C:C	C:C	G:G	G:G	A:A	G:G	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C25460	?	?	C:C	G:G	G:G	A:A	G:G	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C25461	?	C:C	C:C	G:G	G:G	A:A	?	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C25462	?	C:C	C:C	G:G	G:G	A:A	G:G	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C25463	?	C:C	C:C	G:G	G:G	A:A	G:G	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C25464	?	?	C:C	G:G	G:G	A:A	G:G	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C25465	?	C:C	C:C	G:G	G:G	A:A	G:G	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C25466	?	C:C	C:C	G:G	G:G	A:A	G:G	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C25468	?	C:C	C:C	G:G	G:G	A:A	G:G	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C25469	?	C:C	C:C	G:G	G:G	A:A	G:G	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C25470	?	?	C:C	G:G	G:G	A:A	G:G	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C25472	?	C:C	C:C	G:G	G:G	A:A	G:G	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C25473	?	C:C	C:C	G:G	G:G	A:A	?	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C25474	?	C:C	C:C	G:G	G:G	A:A	G:G	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C09408	?	C:C	C:C	G:G	G:G	A:A	C:C	C:C	C:C	A:C	T:T	T:T	?	T:T	G:G
C09442	?	T:T	C:C	A:A	G:G	G:G	C:C	C:C	C:G	A:C	T:T	T:G	?	C:T	G:A
C09463	?	T:T	C:C	G:G	C:G	G:G	G:C	T:T	C:C	A:C	T:T	T:T	?	T:T	A:A
C09464	?	T:T	C:C	A:G	G:G	A:A	G:C	C:C	C:C	A:C	T:T	T:G	?	T:T	G:A
C09477	?	T:T	C:C	G:G	G:G	?	G:G	T:C	C:C	A:C	T:T	T:T	?	T:T	A:A
C09478	?	T:T	A:A	A:G	G:G	G:G	G:G	T:C	C:C	A:C	T:T	T:T	?	T:T	G:G
C09492	?	T:T	A:C	A:A	G:G	G:A	G:G	C:C	C:C	A:C	C:T	T:G	?	C:T	G:A
C09504	?	T:T	A:C	G:G	G:G	G:G	G:G	T:C	C:C	A:C	T:T	G:G	?	T:T	G:A
C09505	?	C:C	C:C	G:G	C:G	A:A	G:C	T:T	C:C	A:C	T:T	T:T	?	T:T	G:G
C09519	?	T:T	C:C	G:G	G:G	G:G	G:C	C:C	C:C	A:C	T:T	T:G	?	C:T	G:A
C09524	?	T:T	A:A	G:G	G:G	G:G	G:G	T:C	C:C	A:C	T:T	T:G	?	T:T	G:G
C09528	?	T:T	A:A	A:G	G:G	A:A	G:C	T:C	C:G	A:C	C:T	T:G	?	T:T	A:A
C09529	?	T:T	A:C	A:G	G:G	G:G	G:G	T:C	C:C	A:C	T:T	T:G	?	T:T	A:A
C09531	?	T:T	A:C	G:G	C:C	G:G	C:C	C:C	C:C	A:A	C:T	T:G	?	C:T	G:A
C09536	?	C:C	A:C	G:G	C:G	A:A	C:C	T:T	C:G	C:C	T:T	T:T	?	T:T	G:G
C09611	?	T:T	A:C	G:G	G:G	A:A	G:G	T:T	C:G	A:C	T:T	T:T	?	C:T	G:G
C09618	?	T:T	A:A	A:A	G:G	G:G	G:G	C:C	C:G	A:C	T:T	T:G	?	C:T	G:G
C09622	?	T:T	A:A	G:G	C:G	G:A	G:G	T:C	C:C	A:C	T:T	T:T	?	C:T	G:G
C09623	?	T:T	C:C	A:G	G:G	G:A	G:C	T:C	C:G	A:C	T:T	T:T	?	C:T	G:A
C09626	?	C:T	C:C	G:G	G:G	G:A	G:C	T:C	C:C	A:C	T:T	T:T	?	C:T	G:A
C09627	?	C:T	C:C	A:G	C:G	A:A	G:G	T:T	C:C	A:C	T:T	T:T	?	C:T	G:A
C09631	?	T:T	C:C	A:G	G:G	G:G	G:G	T:T	C:G	A:C	C:C	T:T	?	T:T	G:G
C09633	?	C:T	A:C	A:A	G:G	G:A	G:G	T:T	C:G	A:C	T:T	G:G	?	C:T	G:G
C09635	?	C:T	A:C	A:G	C:G	G:A	G:G	T:T	C:C	A:C	T:T	T:G	?	C:T	G:G
C09637	?	C:C	C:C	G:G	G:G	A:A	G:C	T:T	C:C	A:C	T:T	T:T	?	T:T	G:G
C09638	?	C:T	C:C	A:G	G:G	A:A	G:C	T:T	C:C	A:C	T:T	T:T	?	C:T	G:A
C09640	?	T:T	A:C	G:G	C:C	G:A	G:G	C:C	C:C	A:C	C:T	G:G	?	T:T	G:G
C09641	?	T:T	A:A	G:G	C:C	G:G	G:G	T:C	C:C	A:C	T:T	G:G	?	T:T	G:A
C09642	?	T:T	A:A	A:G	C:C	G:G	G:G	T:C	C:C	A:C	C:T	T:G	?	C:T	G:A
C09643	?	T:T	A:C	G:G	G:G	G:G	G:G	T:C	C:C	A:C	C:T	T:G	?	C:C	G:A
C09661	?	C:C	C:C	G:G	G:G	A:A	C:C	T:T	C:C	A:C	T:T	T:T	?	T:T	G:G
C09662	?	T:T	A:C	A:G	G:G	G:G	G:C	T:C	C:C	A:C	T:T	T:T	?	T:T	G:A
C09668	?	T:T	A:A	A:G	G:G	G:A	G:G	C:C	C:C	A:C	T:T	T:T	?	T:T	G:A
C09669	?	C:T	A:C	A:G	C:G	G:G	G:G	C:C	C:G	A:C	T:T	T:G	?	T:T	G:A
C09676	?	C:C	C:C	G:G	G:G	A:A	G:G	T:T	C:C	A:C	C:T	T:T	?	C:T	G:G
C14166	?	T:T	C:C	A:G	C:C	G:A	G:C	C:C	C:G	A:C	T:T	T:G	?	C:T	G:A
C14168	?	T:T	C:C	A:A	G:G	G:G	G:G	C:C	C:C	A:C	T:T	G:G	?	T:T	G:A
C14169	?	T:T	A:A	G:G	G:G	G:G	G:G	T:C	C:C	A:C	C:T	T:G	?	T:T	G:G
C14177	?	C:T	A:A	A:A	G:G	A:A	G:G	C:C	C:C	A:C	T:T	T:T	?	C:T	G:G
C14193	?	T:T	A:A	A:G	C:G	G:A	G:G	T:T	C:C	A:C	T:T	T:G	?	C:C	G:A
C14194	?	C:C	C:C	G:G	G:G	A:A	G:C	T:T	C:C	A:C	T:T	T:T	?	C:T	G:G
C14195	?	C:C	C:C	G:G	C:C	A:A	G:G	T:T	C:C	A:C	C:T	T:G	?	C:T	G:G
C14213	?	C:C	C:C	G:G	C:G	A:A	C:C	T:T	C:C	A:C	T:T	T:T	?	T:T	G:A
C15784	?	T:T	C:C	A:G	C:G	G:A	G:G	C:C	C:C	A:C	T:T	G:G	?	T:T	G:G
C21754	?	T:T	A:A	G:G	G:G	G:G	G:G	T:C	C:C	A:C	T:T	T:T	?	T:T	G:G
C23301	?	T:T	A:A	A:A	G:G	G:G	G:G	T:T	C:G	A:C	T:T	T:G	?	T:T	G:G
C24954	?	C:C	A:C	G:G	C:C	A:A	C:C	T:T	C:G	A:C	T:T	T:T	?	T:T	G:G
C24955	?	?	C:C	A:G	C:G	A:A	G:C	T:T	C:C	C:C	T:T	T:G	?	C:T	G:G
C24957	?	?	C:C	G:G	C:G	A:A	G:C	T:T	C:C	C:C	T:T	T:G	?	T:T	G:G
C24993	?	?	C:C	A:G	C:C	A:A	C:C	T:T	C:C	A:C	T:T	T:T	?	T:T	G:A
C24998	?	C:C	A:C	G:G	C:C	A:A	G:C	T:T	C:G	C:C	T:T	T:T	?	C:T	G:G
C24999	?	?	C:C	G:G	G:G	A:A	G:C	T:T	C:G	A:C	T:T	G:G	?	T:T	G:G
C25002	?	?	C:C	A:G	C:C	A:A	C:C	T:T	C:C	A:C	T:T	T:G	?	T:T	A:A
C25004	?	T:T	C:C	G:G	C:C	A:A	G:C	T:T	C:C	A:C	T:T	T:T	?	T:T	G:A
C25005	?	C:C	C:C	G:G	C:G	A:A	C:C	T:T	C:G	C:C	T:T	T:T	?	C:T	G:G
C25008	?	C:T	A:C	G:G	C:G	A:A	C:C	T:T	C:G	C:C	T:T	T:T	?	T:T	A:A
C25010	?	C:T	A:C	A:G	C:C	A:A	C:C	T:T	C:G	A:C	T:T	T:T	?	T:T	G:G
C25016	?	C:T	A:C	G:G	C:C	A:A	G:G	T:T	C:C	A:C	T:T	T:T	?	T:T	G:G
C25083	?	C:C	A:C	G:G	C:C	A:A	G:G	T:T	C:C	A:C	T:T	T:G	?	T:T	G:A
C25084	?	C:C	C:C	G:G	G:G	A:A	G:G	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C25085	?	C:C	C:C	G:G	G:G	A:A	G:G	T:T	C:C	C:C	T:T	T:T	?	T:T	A:A
C25091	?	?	C:C	G:G	C:G	A:A	C:C	T:T	C:C	A:C	T:T	G:G	?	T:T	G:G
C25093	?	C:T	A:C	G:G	C:C	A:A	G:C	T:T	C:G	A:C	T:T	T:T	?	T:T	G:G
C25095	?	T:T	C:C	G:G	C:C	A:A	C:C	T:T	C:C	A:C	T:T	T:T	?	T:T	G:G
C25097	?	T:T	C:C	A:G	C:G	A:A	G:C	T:T	C:G	C:C	T:T	T:T	?	T:T	G:A
C25119	?	T:T	A:C	A:G	C:G	G:G	G:G	T:C	C:C	A:C	T:T	T:T	?	T:T	G:A
C25120	?	T:T	A:C	A:G	C:G	G:G	G:G	T:C	C:C	A:C	T:T	T:G	?	T:T	A:A
C25121	?	T:T	A:A	G:G	C:G	G:A	G:G	T:T	C:C	A:C	T:T	T:G	?	T:T	A:A
C25123	?	T:T	C:C	G:G	C:G	G:A	G:C	C:C	C:C	A:A	T:T	T:T	?	T:T	G:G
C25125	?	T:T	A:C	G:G	G:G	G:A	G:G	C:C	C:C	A:C	C:T	T:G	?	T:T	A:A
C25130	?	?	C:C	A:G	C:C	A:A	G:C	T:T	C:G	A:C	T:T	T:G	?	T:T	G:A
C25134	?	C:C	A:C	G:G	C:G	A:A	G:G	T:T	C:G	A:C	T:T	T:G	?	T:T	G:A
C25135	?	C:C	A:C	A:G	C:C	A:A	G:C	T:T	C:C	A:C	T:T	G:G	?	T:T	A:A
C25137	?	T:T	A:C	A:A	G:G	G:G	G:G	C:C	C:C	A:C	C:T	T:G	?	T:T	G:A
C25139	?	T:T	C:C	G:G	G:G	G:A	G:G	T:C	C:C	A:C	C:T	T:T	?	T:T	G:A
C25141	?	T:T	C:C	G:G	C:G	A:A	G:G	T:T	C:C	A:C	T:T	T:T	?	T:T	A:A
C25142	?	T:T	C:C	G:G	C:C	A:A	C:C	T:T	C:G	C:C	T:T	T:G	?	T:T	G:G
C25143	?	T:T	A:A	A:A	C:G	G:G	G:C	T:T	C:G	C:C	T:T	T:T	?	T:T	G:G
C25259	?	C:T	A:C	G:G	C:C	A:A	G:G	T:T	C:G	C:C	T:T	T:T	?	T:T	A:A
C25272	?	T:T	A:C	G:G	C:C	G:G	G:G	C:C	C:C	A:C	T:T	G:G	?	C:T	G:A
C25274	?	T:T	C:C	G:G	C:G	A:A	G:G	C:C	C:C	A:C	T:T	G:G	?	T:T	G:G

[1] The two alleles for each marker are separated by a colon. The genotype of an accession for a certain marker can be homozygous [e.g. A:A], heterozygous [e.g. A:G] or missing [?].

Appendix 7. KASP results

SNP marker scores from the KASP assay [Table 2/4]

	Marker[1]														
Accession	**SO066**	**SO071**	**SO079**	**SO091**	**SO105**	**SO115**	**SO125**	**SO126**	**SO129**	**SO158**	**SO176**	**SO177**	**SO178**	**SO198**	**SO206**
C25459	G:G	C:C	C:C	?	T:T	?	A:A	?	T:T	T:T	C:C	A:A	A:A	?	?
C25460	?	C:C	C:C	?	T:T	?	A:A	?	T:T	T:T	C:C	A:A	A:A	?	?
C25461	G:G	C:C	C:C	?	T:T	?	A:A	?	T:T	T:T	C:C	A:A	A:A	?	?
C25462	?	C:C	C:C	?	T:T	?	A:A	?	T:T	T:T	C:C	A:A	A:A	?	?
C25463	G:G	C:C	C:C	?	T:T	?	A:A	?	T:T	T:T	C:C	A:A	A:A	?	?
C25464	?	C:C	C:C	?	T:T	?	A:A	?	T:T	T:T	C:C	A:A	A:A	?	?
C25465	G:G	C:C	C:C	?	T:T	?	A:A	?	T:T	T:T	C:C	A:A	A:A	?	?
C25466	G:G	C:C	C:C	?	T:T	?	A:A	A:A	T:T	T:T	C:C	A:A	A:A	?	?
C25468	G:G	C:C	C:C	?	T:T	?	A:A	?	T:T	T:T	C:C	A:A	A:A	?	?
C25469	G:G	C:C	C:C	?	T:T	?	A:A	?	T:T	T:T	C:C	A:A	A:A	?	?
C25470	?	C:C	C:C	?	T:T	?	A:A	?	T:T	T:T	C:C	A:A	A:A	?	?
C25472	?	C:C	C:C	?	T:T	?	A:A	?	T:T	T:T	C:C	A:A	A:A	?	?
C25473	G:G	C:C	C:C	?	T:T	?	A:A	?	T:T	T:T	C:C	A:A	A:A	?	?
C25474	G:G	C:C	C:C	?	T:T	?	A:A	?	T:T	T:T	C:C	A:A	A:A	?	?
C09408	G:T	C:C	C:C	A:A	T:T	A:A	T:T	A:A	T:T	T:T	C:C	T:T	A:A	G:G	?
C09442	T:T	A:C	C:C	G:G	T:T	T:A	T:T	A:A	C:C	A:A	T:T	T:T	A:A	C:C	C:C
C09463	G:G	A:A	C:C	G:G	A:A	A:A	A:A	T:T	C:C	A:A	C:C	A:A	T:T	C:G	C:C
C09464	T:T	C:C	C:C	A:G	T:T	T:A	T:T	A:A	C:C	A:A	T:T	T:T	A:A	C:G	C:C
C09477	G:T	A:A	C:C	G:G	A:A	T:A	A:A	T:T	C:C	A:A	T:T	T:T	A:A	C:G	C:C
C09478	G:T	C:C	C:C	G:G	T:T	T:T	A:T	T:T	T:T	T:T	T:C	T:A	T:A	C:C	?
C09492	G:T	A:C	C:C	A:A	A:T	T:A	A:T	A:A	C:C	A:A	C:C	A:A	T:T	C:G	?
C09504	G:G	C:C	C:C	G:G	A:T	T:A	A:T	T:T	C:C	A:A	C:C	T:A	T:A	C:C	C:C
C09505	G:G	A:C	C:C	G:G	T:T	T:T	T:T	T:A	C:C	T:T	C:C	T:T	A:A	C:G	?
C09519	G:G	A:C	C:T	A:G	A:T	A:A	A:A	T:A	C:C	A:T	T:T	T:T	A:A	G:G	?
C09524	G:T	A:A	C:C	G:G	T:T	T:A	A:T	T:A	T:T	A:A	T:T	T:T	A:A	C:G	C:C
C09528	G:T	A:A	C:C	G:G	T:T	A:A	A:T	A:A	C:C	A:A	T:T	T:T	A:A	C:C	C:G
C09529	G:T	C:C	C:C	G:G	A:T	A:A	T:T	T:A	T:T	A:A	T:T	T:T	A:A	C:G	C:C
C09531	G:G	A:C	C:T	G:G	T:T	A:A	A:A	T:T	T:T	A:A	C:C	T:A	T:A	C:G	G:G
C09536	G:G	C:C	C:T	A:A	T:T	T:A	T:T	T:A	T:T	T:T	T:C	T:T	A:A	G:G	?
C09611	G:T	C:C	C:C	G:G	A:A	A:A	A:A	T:T	C:C	A:T	T:C	T:T	A:A	C:C	C:C
C09618	G:T	A:C	C:C	G:G	T:T	A:A	A:T	A:A	C:C	A:A	T:T	T:T	A:A	C:G	C:C
C09622	G:G	A:C	C:C	A:G	A:T	T:A	A:A	T:A	T:C	A:A	T:C	T:A	T:A	C:G	C:C
C09623	G:T	A:C	C:C	A:G	T:T	T:A	A:T	A:A	T:T	A:A	T:C	T:A	T:A	C:G	C:C
C09626	G:G	A:C	C:C	G:G	A:T	T:A	A:A	T:T	C:C	A:T	C:C	A:A	T:T	C:G	C:C
C09627	G:G	A:C	C:C	G:G	T:T	T:T	A:A	T:A	T:C	A:A	T:C	T:T	A:A	C:C	C:C
C09631	G:G	C:C	C:C	G:G	T:T	A:A	A:T	T:T	C:C	A:A	C:C	T:T	A:A	C:C	C:C
C09633	G:T	A:C	C:C	A:A	A:T	A:A	A:T	T:T	C:C	T:T	C:C	T:A	T:A	C:C	C:C
C09635	G:T	A:C	C:C	A:G	T:T	A:A	A:A	T:A	C:C	A:T	T:T	T:T	A:A	C:C	?
C09637	G:G	A:C	C:C	A:A	T:T	T:A	A:T	T:A	T:C	T:T	C:C	T:T	A:A	C:G	C:G
C09638	G:G	C:C	C:C	A:G	T:T	T:A	A:T	A:A	T:T	T:T	T:C	T:A	T:A	G:G	C:C
C09640	G:G	A:A	C:C	A:G	T:T	T:T	T:T	T:A	T:T	A:T	C:C	T:A	T:A	C:C	G:G
C09641	G:T	C:C	C:C	G:G	T:T	A:A	A:T	T:T	T:C	A:T	T:C	T:A	T:A	G:G	G:G
C09642	G:G	C:C	C:C	G:G	A:T	T:A	A:T	A:A	T:T	T:T	C:C	T:T	A:A	G:G	?
C09643	T:T	C:C	C:C	A:G	T:T	T:A	A:T	T:A	T:T	A:A	T:T	T:T	A:A	C:G	C:C
C09661	G:G	A:C	C:C	G:G	T:T	T:T	T:T	A:A	T:T	T:T	C:C	T:T	A:A	G:G	?
C09662	G:T	C:C	C:C	G:G	T:T	T:T	A:A	T:A	T:C	A:T	C:C	T:T	A:A	C:C	C:C
C09668	T:T	A:C	C:C	G:G	T:T	T:T	A:A	A:A	T:C	A:A	T:C	T:T	A:A	C:G	?
C09669	T:T	C:C	C:C	G:G	A:A	T:T	A:A	T:A	T:C	A:A	T:T	T:T	A:A	C:C	C:C
C09676	G:G	A:C	C:C	A:G	T:T	A:A	T:T	T:T	T:T	T:T	C:C	T:T	A:A	G:G	C:G
C14166	T:T	A:C	C:C	G:G	A:T	T:A	A:A	A:A	T:C	A:A	C:C	T:T	A:A	C:G	C:C
C14168	G:T	A:A	C:C	A:G	A:A	A:A	A:A	A:A	T:C	A:A	T:C	T:A	T:A	C:G	?
C14169	G:T	C:C	C:C	A:G	A:T	T:A	A:A	T:A	C:C	A:A	T:C	T:A	T:A	C:G	?
C14177	G:T	A:A	C:C	G:G	T:T	T:A	T:T	A:A	T:C	T:T	C:C	T:A	T:A	C:C	G:G
C14193	G:T	A:C	C:C	A:G	T:T	T:A	A:T	T:A	T:C	T:T	C:C	T:A	T:A	C:G	G:G
C14194	G:G	A:A	C:C	A:G	T:T	A:A	T:T	A:A	T:C	T:T	C:C	T:A	T:A	C:G	G:G
C14195	G:G	C:C	C:C	A:A	T:T	A:A	T:T	T:A	T:T	T:T	C:C	T:T	A:A	G:G	C:C
C14213	G:G	C:C	C:C	A:G	T:T	A:A	T:T	T:A	T:C	T:T	C:C	T:T	A:A	G:G	G:G
C15784	G:G	A:C	C:C	G:G	A:T	A:A	A:A	T:A	T:C	A:A	T:C	T:A	T:A	C:C	C:C
C21754	G:T	C:C	C:C	G:G	A:T	T:A	A:T	T:T	C:C	A:A	T:C	T:T	A:A	C:C	?
C23301	G:T	A:C	C:C	G:G	T:T	T:A	A:A	A:A	T:T	A:A	T:C	T:T	A:A	C:G	?
C24954	G:T	A:C	C:C	A:G	T:T	T:A	A:T	T:A	T:C	T:T	C:C	T:A	T:A	C:G	?
C24955	G:T	C:C	C:C	G:G	T:T	T:A	T:T	A:A	T:T	T:T	C:C	T:T	A:A	C:G	C:C
C24957	G:G	A:C	C:C	G:G	T:T	T:T	A:A	T:A	T:T	T:T	C:C	T:A	T:A	G:G	C:C
C24993	G:T	A:C	C:C	G:G	T:T	T:T	A:T	A:A	T:C	T:T	C:C	T:A	T:A	C:G	C:G
C24998	G:T	A:C	C:C	G:G	T:T	T:A	T:T	T:A	T:T	T:T	C:C	T:A	T:A	G:G	?
C24999	G:G	C:C	C:T	G:G	T:T	T:A	A:T	A:A	T:C	T:T	C:C	A:A	T:T	G:G	?
C25002	G:T	A:A	C:C	G:G	T:T	T:T	T:T	T:A	C:C	T:T	C:C	A:A	T:T	C:C	?
C25004	G:T	C:C	C:C	A:G	T:T	T:A	A:T	T:T	C:C	A:T	C:C	T:A	T:A	G:G	G:G
C25005	G:G	A:C	C:C	G:G	T:T	T:T	A:T	T:T	T:C	T:T	C:C	T:A	T:A	C:G	?
C25008	G:T	A:C	C:C	G:G	T:T	T:A	A:A	A:A	T:C	T:T	C:C	A:A	T:T	C:G	G:G
C25010	G:G	C:C	C:C	A:A	T:T	T:A	A:T	T:A	C:C	T:T	C:C	T:A	T:A	G:G	G:G
C25016	G:G	A:C	C:C	G:G	T:T	T:A	A:A	T:A	T:T	T:T	C:C	T:A	T:A	G:G	G:G
C25083	G:G	A:A	C:T	A:G	T:T	T:A	A:A	T:A	T:C	T:T	C:C	T:A	T:A	G:G	G:G
C25084	G:G	C:C	C:C	?	T:T	?	A:A	?	T:T	T:T	C:C	A:A	A:A	?	?
C25085	G:G	C:C	C:C	?	T:T	?	A:A	A:A	T:T	T:T	C:C	A:A	A:A	?	?
C25091	G:T	A:A	C:T	G:G	T:T	T:T	T:T	T:A	C:C	T:T	C:C	T:A	T:A	C:G	?
C25093	T:T	A:A	C:T	G:G	T:T	T:A	A:T	T:A	C:C	T:T	C:C	T:A	T:A	C:C	G:G
C25095	G:G	A:C	C:C	G:G	T:T	T:A	A:A	T:T	T:C	A:A	C:C	T:A	T:A	C:G	?
C25097	G:G	A:C	C:C	G:G	T:T	T:A	A:A	T:A	T:C	T:T	C:C	T:A	T:A	G:G	?
C25119	T:T	A:C	C:C	G:G	A:T	T:T	A:A	T:A	C:C	A:A	T:T	T:T	A:A	C:G	C:C
C25120	G:G	A:A	C:C	G:G	A:T	A:A	A:T	T:A	T:C	A:A	C:C	A:A	T:T	C:C	C:C
C25121	G:G	A:A	C:C	G:G	A:T	A:A	A:T	T:A	C:C	A:A	T:T	T:T	A:A	C:C	C:C
C25123	T:T	A:A	C:C	G:G	A:A	A:A	A:A	T:T	C:C	A:A	T:T	T:T	A:A	C:C	?
C25125	G:G	A:A	C:C	G:G	A:A	A:A	A:T	T:A	C:C	A:T	C:C	A:A	T:T	C:C	?
C25130	G:T	C:C	C:T	G:G	A:T	T:T	A:T	T:A	C:C	T:T	C:C	T:A	T:A	G:G	G:G
C25134	G:G	A:C	C:T	G:G	T:T	A:A	T:T	T:T	T:C	T:T	C:C	T:T	A:A	C:G	G:G
C25135	G:G	A:C	C:T	A:G	T:T	T:A	A:A	T:A	C:C	T:T	C:C	T:T	A:A	G:G	G:G
C25137	T:T	A:A	C:C	A:G	T:T	A:A	A:T	T:T	T:T	A:T	T:C	T:A	T:A	C:C	?
C25139	T:T	A:A	C:C	A:G	T:T	A:A	A:A	T:T	C:C	A:T	T:C	T:A	T:A	C:G	C:C
C25141	G:G	A:C	C:C	G:G	T:T	T:A	T:T	T:T	C:C	T:T	C:C	T:A	T:A	C:G	G:G
C25142	T:T	A:C	C:C	G:G	A:A	T:A	T:T	T:A	T:C	T:T	T:C	T:T	A:A	C:C	G:G
C25143	T:T	C:C	C:C	G:G	T:T	T:A	T:T	A:A	T:C	A:A	C:C	A:A	T:T	C:C	?
C25259	G:G	C:C	C:C	G:G	T:T	A:A	A:A	T:A	C:C	T:T	C:C	T:T	A:A	C:C	?
C25272	G:T	A:A	C:C	G:G	A:A	A:A	A:A	A:A	C:C	A:A	C:C	T:A	T:A	C:C	?
C25274	G:T	A:A	C:C	G:G	A:T	T:T	A:A	A:A	T:C	A:T	C:C	A:A	T:T	C:C	?

[1] The two alleles for each marker are separated by a colon. The genotype of an accession for a certain marker can be homozygous [e.g. A:A], heterozygous [e.g. A:G] or missing [?].

Appendix 7. KASP results

SNP marker scores from the KASP assay [Table 3/4]

	Marker[1]														
Accession	**SO214**	**SO237**	**SO240**	**SO244**	**SO247**	**SO264**	**SO267**	**SO270**	**SO272**	**SO280**	**SO281**	**SO290**	**SO292**	**SO304**	**SO314**
C25459	?	C:C	T:T	T:T	C:C	T:T	?	A:A	T:T	C:C	G:G	G:G	?	?	?
C25460	?	C:C	T:T	?	C:C	T:T	?	A:A	T:T	C:C	G:G	G:G	?	?	?
C25461	?	C:C	T:T	T:T	C:C	T:T	?	A:A	T:T	C:C	G:G	G:G	?	?	?
C25462	?	C:C	T:T	T:T	C:C	T:T	?	A:A	T:T	C:C	G:G	G:G	?	?	?
C25463	?	C:C	T:T	T:T	C:C	T:T	?	A:A	T:T	C:C	G:G	G:G	?	?	?
C25464	?	C:C	T:T	T:T	C:C	T:T	?	A:A	T:T	C:C	G:G	G:G	?	?	?
C25465	?	C:C	T:T	T:T	C:C	T:T	?	A:A	T:T	C:C	G:G	G:G	?	?	?
C25466	?	C:C	T:T	T:T	C:C	T:T	?	A:A	T:T	C:C	G:G	G:G	?	?	?
C25468	?	C:C	T:T	?	C:C	T:T	?	A:A	T:T	C:C	G:G	G:G	?	?	?
C25469	?	C:C	T:T	T:T	C:C	T:T	?	A:A	T:T	C:C	G:G	G:G	?	?	?
C25470	?	C:C	T:T	?	C:C	T:T	?	A:A	T:T	C:C	G:G	G:G	?	?	?
C25472	?	C:C	T:T	T:T	C:C	T:T	?	A:A	T:T	C:C	G:G	G:G	?	?	?
C25473	?	C:C	T:T	T:T	C:C	T:T	?	A:A	T:T	C:C	G:G	G:G	?	?	?
C25474	?	C:C	T:T	T:T	C:C	T:T	?	A:A	T:T	C:C	G:G	G:G	?	?	?
C09408	?	C:C	T:T	G:G	T:T	T:T	?	G:A	T:T	C:C	G:G	G:G	A:A	G:G	G:G
C09442	?	A:C	C:C	T:T	T:T	T:T	?	G:A	C:T	G:C	T:T	G:G	C:C	G:G	G:G
C09463	?	A:C	C:C	G:G	T:C	T:T	?	G:G	C:C	G:G	T:T	G:G	A:A	C:C	G:G
C09464	?	A:C	C:C	G:T	T:T	T:T	?	G:G	C:C	G:G	T:T	A:G	C:C	G:G	G:G
C09477	?	A:C	T:C	G:T	T:T	T:A	?	G:A	C:T	G:G	T:T	A:G	A:A	C:C	A:G
C09478	?	A:C	T:T	T:T	T:T	T:T	?	G:A	C:T	C:C	T:G	G:G	A:A	G:G	G:G
C09492	?	A:C	T:C	T:T	T:T	A:A	?	G:G	C:C	G:G	T:T	A:G	A:C	C:G	A:G
C09504	?	C:C	T:T	T:T	T:T	T:A	?	G:G	C:C	G:C	T:T	G:G	A:C	C:G	A:G
C09505	?	C:C	T:T	G:G	T:T	T:T	?	A:A	T:T	C:C	G:G	G:G	A:A	G:G	G:G
C09519	?	A:C	T:C	T:T	T:T	T:A	?	G:A	C:T	G:G	T:T	A:G	A:C	C:G	G:G
C09524	?	A:C	T:C	G:G	T:T	T:T	?	G:G	C:C	G:G	T:T	G:G	A:C	C:G	G:G
C09528	?	A:C	T:T	G:T	T:T	T:T	?	G:G	C:C	G:G	T:T	G:G	C:C	G:G	G:G
C09529	?	A:C	T:T	T:T	T:C	T:T	?	G:G	C:T	C:C	?	G:G	A:C	C:G	A:A
C09531	?	A:C	T:C	G:T	T:T	T:T	?	G:A	C:T	G:G	T:T	G:G	A:A	C:G	G:G
C09536	?	C:C	T:T	G:G	T:T	A:A	?	A:A	T:T	C:C	G:G	G:G	A:C	C:G	A:G
C09611	?	A:C	T:T	G:G	T:T	T:A	?	G:G	C:T	C:C	G:G	A:G	A:C	G:G	G:G
C09618	?	A:C	T:T	G:T	T:T	A:A	?	G:G	C:C	G:G	T:T	G:G	A:A	C:G	A:A
C09622	?	A:C	T:T	T:T	T:T	A:A	?	G:G	C:T	C:C	G:G	G:G	A:A	G:G	A:A
C09623	?	A:C	T:T	G:G	T:T	T:T	?	G:G	C:C	G:C	T:T	G:G	C:C	C:G	A:G
C09626	?	A:C	T:C	G:G	T:T	T:T	?	G:A	C:T	C:C	G:G	G:G	A:A	C:C	G:G
C09627	?	A:C	T:T	T:T	T:T	T:A	?	G:A	C:T	G:G	T:T	G:G	A:C	C:G	G:G
C09631	?	A:C	T:C	G:G	T:T	T:A	?	G:G	C:C	G:C	T:T	A:A	A:A	C:G	G:G
C09633	?	A:C	T:T	G:G	T:T	T:A	?	G:G	C:C	G:C	T:T	?	A:C	C:G	A:G
C09635	?	A:C	T:T	G:G	T:T	T:T	?	G:A	C:T	C:C	G:G	A:G	A:C	G:G	G:G
C09637	?	C:C	T:T	G:T	T:T	T:T	?	A:A	T:T	C:C	G:G	G:G	A:A	G:G	G:G
C09638	?	A:C	T:T	G:T	T:T	T:T	?	G:A	C:T	G:C	T:T	G:G	A:A	G:G	G:G
C09640	?	A:C	C:C	G:G	T:T	T:T	?	G:A	C:T	C:C	G:G	G:G	A:C	C:G	G:G
C09641	?	A:C	T:T	G:G	T:T	T:T	?	G:A	C:T	C:C	?	G:G	A:A	G:G	A:A
C09642	?	A:C	T:T	G:G	T:T	T:T	?	G:G	C:C	G:G	T:T	G:G	A:A	G:G	A:G
C09643	?	A:C	T:C	G:T	T:T	T:T	?	G:G	C:C	G:C	T:T	A:G	A:C	C:G	A:G
C09661	?	C:C	T:T	G:G	T:T	T:T	?	A:A	T:T	C:C	G:G	G:G	A:A	G:G	G:G
C09662	?	A:C	T:C	G:T	T:T	T:T	?	G:G	C:C	G:C	T:T	G:G	A:A	C:G	A:G
C09668	?	A:C	C:C	G:G	T:T	T:T	?	G:G	C:C	G:C	T:T	G:G	A:C	G:G	G:G
C09669	?	A:C	T:C	T:T	T:T	T:A	?	G:G	T:T	G:G	T:T	A:G	A:A	G:G	G:G
C09676	?	C:C	T:T	T:T	T:T	T:T	?	A:A	C:T	C:C	G:G	G:G	A:A	G:G	G:G
C14166	?	A:C	T:T	T:T	T:T	T:T	?	G:A	C:T	C:C	G:G	A:G	A:A	C:G	A:G
C14168	?	A:C	C:C	T:T	C:C	T:A	?	G:G	C:C	G:G	T:T	G:G	A:C	G:G	A:G
C14169	?	A:C	T:T	G:T	T:T	A:A	?	G:G	C:C	G:C	T:T	A:G	A:A	C:G	G:G
C14177	?	A:C	T:T	G:G	T:T	T:A	?	G:G	C:C	G:G	T:T	G:G	A:A	G:G	A:G
C14193	?	A:C	T:T	G:T	T:T	T:T	?	A:A	T:T	C:C	G:G	G:G	A:A	G:G	G:G
C14194	?	C:C	T:T	T:T	T:T	T:T	?	A:A	T:T	C:C	T:G	G:G	A:C	G:G	G:G
C14195	?	C:C	T:T	T:T	T:T	T:T	?	A:A	T:T	C:C	T:G	G:G	A:A	G:G	G:G
C14213	?	C:C	T:T	T:T	T:T	T:T	?	A:A	T:T	C:C	G:G	G:G	A:C	G:G	G:G
C15784	?	A:C	C:C	G:T	T:T	T:A	?	A:A	T:T	G:G	T:T	A:A	A:A	C:G	G:G
C21754	?	A:C	T:C	T:T	T:T	T:T	?	G:A	C:T	C:C	G:G	G:G	A:A	G:G	A:G
C23301	?	A:C	C:C	G:G	T:T	A:A	?	G:G	C:C	G:G	T:T	G:G	A:C	G:G	G:G
C24954	?	A:C	T:T	G:T	T:C	T:T	?	G:A	C:T	C:C	G:G	G:G	?	C:G	A:G
C24955	?	A:C	T:T	T:T	T:T	T:A	?	A:A	T:T	G:C	T:T	G:G	A:A	C:G	G:G
C24957	?	C:C	T:T	G:T	T:C	T:T	?	G:A	C:C	G:G	T:T	G:G	?	C:G	G:G
C24993	?	C:C	T:T	T:T	T:T	T:T	?	A:A	T:T	C:C	G:G	A:G	C:C	G:G	G:G
C24998	?	A:C	T:T	T:T	T:T	T:T	?	A:A	C:C	C:C	T:G	G:G	C:C	C:G	G:G
C24999	?	C:C	T:T	T:T	T:T	A:A	?	A:A	C:T	G:C	T:T	G:G	C:C	G:G	G:G
C25002	?	C:C	T:T	T:T	T:T	T:T	?	A:A	T:T	C:C	G:G	G:G	A:A	G:G	A:G
C25004	?	C:C	T:T	T:T	T:T	T:T	?	A:A	C:T	G:C	T:T	G:G	C:C	G:G	G:G
C25005	?	C:C	T:T	T:T	T:T	T:T	?	A:A	C:T	G:G	T:T	G:G	C:C	C:G	G:G
C25008	?	C:C	T:T	T:T	T:T	T:T	?	A:A	C:T	G:C	T:T	G:G	C:C	G:G	A:G
C25010	?	C:C	T:T	T:T	T:T	T:T	?	A:A	C:T	G:C	T:T	G:G	A:C	G:G	G:G
C25016	?	C:C	T:T	G:T	T:T	T:T	?	A:A	T:T	C:C	T:G	G:G	C:C	C:G	G:G
C25083	?	C:C	T:T	T:T	T:T	T:T	?	A:A	T:T	G:G	T:T	G:G	A:C	C:G	G:G
C25084	?	C:C	T:T	?	C:C	T:T	?	A:A	T:T	C:C	?	G:G	?	?	?
C25085	?	C:C	T:T	T:T	C:C	T:T	?	A:A	T:T	C:C	G:G	G:G	?	?	?
C25091	?	C:C	T:T	G:T	?	T:T	?	G:A	C:T	C:C	G:G	G:G	C:C	G:G	A:G
C25093	?	C:C	T:T	G:T	T:C	T:T	?	A:A	C:T	C:C	?	G:G	C:C	C:G	G:G
C25095	?	A:C	T:T	T:T	T:C	T:T	?	A:A	C:T	G:C	T:T	G:G	C:C	G:G	G:G
C25097	?	C:C	T:T	G:T	T:T	T:T	?	A:A	T:T	C:C	T:G	G:G	A:C	C:G	G:G
C25119	?	A:C	C:C	G:G	T:T	T:T	?	G:G	C:C	G:G	T:T	G:G	A:C	C:G	G:G
C25120	?	A:C	C:C	G:G	T:C	A:A	?	G:G	C:C	G:C	T:T	A:A	A:A	C:G	G:G
C25121	?	A:C	C:C	G:T	T:T	A:A	?	G:G	C:C	G:C	T:T	A:G	A:A	G:G	G:G
C25123	?	A:C	C:C	G:G	T:T	T:T	?	G:G	C:C	G:G	T:T	G:G	A:A	G:G	G:G
C25125	?	A:C	C:C	G:T	T:C	A:A	?	G:G	C:C	G:G	T:T	G:G	A:C	C:G	A:G
C25130	?	C:C	T:T	G:T	T:T	T:A	?	G:A	C:T	G:C	T:T	G:G	C:C	G:G	G:G
C25134	?	C:C	T:T	G:T	T:T	T:T	?	A:A	T:T	G:C	T:T	G:G	C:C	C:G	G:G
C25135	?	C:C	T:T	G:T	T:T	T:T	?	A:A	C:T	G:C	T:T	G:G	C:C	C:G	A:G
C25137	?	A:C	T:T	G:T	T:T	A:A	?	G:G	C:C	G:G	T:T	G:G	C:C	C:G	G:G
C25139	?	A:C	T:T	T:T	T:C	T:A	?	G:G	C:C	C:C	?	A:G	A:A	C:G	A:G
C25141	?	C:C	T:T	T:T	T:T	T:T	?	A:A	T:T	C:C	G:G	G:G	C:C	C:G	A:G
C25142	?	C:C	T:T	T:T	T:T	T:A	?	A:A	T:T	C:C	?	G:G	C:C	C:G	G:G
C25143	?	A:C	T:T	T:T	T:T	A:A	?	G:A	C:T	G:C	T:T	G:G	C:C	C:C	G:G
C25259	?	C:C	T:T	T:T	T:C	T:T	?	G:A	C:T	G:C	T:T	G:G	C:C	G:G	A:G
C25272	?	A:C	T:C	G:G	T:C	A:A	?	G:G	C:C	G:G	T:T	A:A	C:C	C:G	G:G
C25274	?	A:C	T:C	G:T	C:C	T:A	?	G:G	C:C	G:C	T:T	A:G	A:A	C:C	A:G

[1] The two alleles for each marker are separated by a colon. The genotype of an accession for a certain marker can be homozygous [e.g. A:A], heterozygous [e.g. A:G] or missing [?].

Appendix 7. KASP results

SNP marker scores from the KASP assay [Table 4/4]

	Marker[1]														
Accession	SO334	SO336	SO337	SO346	SO360	SO366	SO373	SO374	SO381	SO382	SO398	SO405	SO408	SO409	SO419
C25459	C:C	G:G	?	T:T	?	G:G	T:T	T:C	G:G	G:G	C:C	?	T:G	G:G	A:A
C25460	C:C	G:G	C:C	T:T	?	G:G	T:T	T:C	G:G	G:G	C:C	?	T:G	G:G	A:A
C25461	C:C	G:G	C:C	T:T	?	G:G	T:T	C:C	G:G	G:G	C:C	?	T:G	G:G	A:A
C25462	C:C	G:G	?	T:T	?	G:G	T:T	T:T	G:G	G:G	C:C	?	T:G	G:G	A:A
C25463	C:C	?	C:C	T:T	?	G:G	T:T	T:T	G:G	G:G	C:C	?	T:G	G:G	A:A
C25464	C:C	?	C:C	T:T	?	G:G	T:T	C:C	G:G	G:G	C:C	?	T:G	G:G	A:A
C25465	C:C	G:G	?	T:T	?	G:G	T:T	T:T	G:G	G:G	C:C	?	T:T	G:G	A:A
C25466	C:C	G:G	C:C	T:T	?	G:G	?	T:C	G:G	G:G	C:C	?	T:G	G:G	A:A
C25468	C:C	G:G	?	T:T	?	G:G	?	T:C	G:G	G:G	C:C	?	T:T	G:G	A:A
C25469	C:C	?	?	T:T	?	G:G	T:T	T:C	G:G	G:G	C:C	?	T:G	G:G	A:A
C25470	C:C	?	C:C	T:T	?	G:G	?	C:C	G:G	G:G	C:C	?	T:G	G:G	A:A
C25472	C:C	?	C:C	T:T	?	G:G	T:T	C:C	G:G	G:G	C:C	?	T:G	G:G	A:A
C25473	C:C	?	?	T:T	?	G:G	?	T:T	G:G	G:G	C:C	?	T:T	G:G	A:A
C25474	C:C	G:G	?	T:T	?	G:G	?	T:C	G:G	G:G	C:C	?	T:T	G:G	A:A
C09408	T:T	T:T	T:T	C:C	T:T	G:G	A:A	T:T	G:G	G:G	A:A	G:A	T:T	G:G	A:A
C09442	C:C	G:G	T:T	T:T	T:T	A:A	A:T	T:T	A:A	A:A	A:A	G:G	T:T	G:G	A:A
C09463	C:C	G:G	C:C	T:T	T:T	G:G	T:T	C:C	G:A	A:G	A:A	G:A	T:G	T:T	A:A
C09464	C:C	G:G	C:C	C:C	T:T	A:A	T:T	T:T	G:A	A:G	A:A	G:G	T:T	G:G	A:A
C09477	C:C	G:G	C:C	T:T	T:C	G:A	T:T	C:C	G:A	A:G	A:A	G:G	T:G	G:T	A:A
C09478	C:C	T:G	C:T	T:T	T:C	G:A	T:T	C:C	A:A	A:A	A:A	G:A	T:T	G:G	A:A
C09492	C:C	T:G	C:T	T:T	T:T	G:A	T:T	T:T	A:A	A:A	A:A	A:A	T:G	T:T	A:A
C09504	C:T	T:G	C:T	T:C	C:C	G:A	T:T	C:C	G:A	A:G	A:A	A:A	T:G	T:T	A:A
C09505	T:T	T:G	C:T	T:C	T:C	G:G	A:A	T:T	G:A	A:G	A:A	A:A	T:T	G:G	A:A
C09519	C:T	T:G	C:T	T:T	T:T	G:G	A:A	T:T	G:G	G:G	C:A	G:A	T:T	G:G	A:A
C09524	C:C	T:G	C:C	T:T	T:T	G:A	T:T	C:C	G:G	G:G	A:A	A:A	T:G	G:T	A:A
C09528	C:C	G:G	C:C	T:T	T:C	G:G	A:T	C:C	G:G	G:G	A:A	G:A	T:T	G:G	A:A
C09529	C:C	T:G	C:C	T:T	T:C	G:G	T:T	T:C	A:A	A:A	A:A	G:G	T:G	G:T	A:A
C09531	C:C	G:G	T:T	T:T	T:C	G:G	A:T	C:C	G:G	G:G	A:A	A:A	T:T	G:G	A:A
C09536	C:C	T:T	T:T	C:C	T:C	G:G	A:A	T:T	G:A	A:G	C:C	A:A	T:T	G:G	A:A
C09611	C:C	G:G	C:C	T:C	T:T	G:A	T:T	C:C	G:G	G:G	C:A	G:A	T:T	G:G	A:A
C09618	C:C	G:G	C:T	T:T	T:T	G:G	A:T	T:C	G:G	G:G	A:A	G:A	T:G	T:T	A:A
C09622	C:T	T:G	T:T	T:C	T:C	G:A	T:T	C:C	G:A	A:G	C:A	A:A	T:T	G:G	A:A
C09623	C:T	T:T	C:T	T:C	T:C	G:A	T:T	C:C	G:A	A:G	C:C	G:A	T:G	G:T	A:A
C09626	C:C	G:G	C:T	T:C	T:C	G:A	T:T	C:C	G:A	A:G	A:A	A:A	T:T	G:G	A:A
C09627	C:T	T:G	T:T	C:C	C:C	G:A	T:T	T:T	G:G	G:G	A:A	G:A	T:T	G:G	A:A
C09631	C:C	T:T	T:T	T:T	T:T	A:A	T:T	T:C	A:A	A:A	A:A	A:A	T:T	G:G	A:A
C09633	C:C	T:G	T:T	T:T	T:C	G:A	T:T	C:C	G:A	A:G	A:A	A:A	T:T	G:G	A:A
C09635	C:C	G:G	C:C	T:T	T:T	G:G	A:A	T:C	A:A	A:A	A:A	A:A	T:G	G:T	A:A
C09637	T:T	G:G	C:T	T:C	T:T	G:G	A:T	T:T	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C09638	C:T	T:G	C:C	T:C	T:T	G:A	A:T	T:T	G:G	G:G	A:A	A:A	T:T	G:G	A:A
C09640	C:C	T:G	C:T	T:C	T:T	G:G	T:T	C:C	G:G	G:G	C:A	A:A	T:T	G:G	A:A
C09641	C:T	G:G	C:T	T:T	T:C	G:A	A:T	T:C	G:G	G:G	A:A	A:A	T:G	G:T	A:A
C09642	C:C	T:T	C:C	T:T	T:C	G:G	T:T	T:C	G:A	A:G	A:A	A:A	T:T	G:G	A:A
C09643	C:C	T:T	T:T	T:T	T:T	A:A	A:A	C:C	G:G	G:G	A:A	G:A	T:G	G:T	A:A
C09661	C:C	G:G	C:T	T:C	T:T	G:G	A:A	T:T	A:A	A:A	A:A	G:G	T:T	G:G	A:A
C09662	C:T	T:G	C:T	T:T	T:C	G:A	A:T	T:C	G:G	G:G	C:A	G:A	T:T	G:G	A:A
C09668	C:C	T:T	C:C	T:C	T:C	G:A	T:T	C:C	G:A	A:G	A:A	G:A	T:G	G:T	A:A
C09669	C:C	G:G	C:C	T:T	T:T	G:G	A:T	T:C	G:A	A:G	C:C	G:G	T:G	T:T	A:A
C09676	T:T	G:G	C:C	C:C	T:C	G:G	A:A	T:T	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C14166	C:C	G:G	C:T	T:T	T:C	G:A	T:T	T:C	G:A	A:G	C:A	G:A	T:G	G:T	C:A
C14168	C:C	G:G	C:C	T:T	T:T	G:G	A:T	T:C	G:G	G:G	A:A	A:A	T:G	G:T	A:A
C14169	C:C	G:G	C:C	T:C	T:T	A:A	T:T	C:C	G:A	A:G	A:A	A:A	T:T	G:G	A:A
C14177	C:T	T:G	C:T	T:C	T:C	G:G	A:T	T:C	G:A	A:G	A:A	G:G	T:G	G:T	A:A
C14193	T:T	G:G	T:T	T:T	T:T	G:G	T:T	T:C	G:G	G:G	C:C	A:A	T:G	G:T	A:A
C14194	T:T	T:T	C:T	C:C	T:C	G:G	A:A	T:T	G:G	G:G	A:A	A:A	T:G	G:T	A:A
C14195	T:T	T:G	T:T	C:C	T:T	G:G	A:T	T:T	G:G	G:G	C:A	A:A	T:G	G:T	A:A
C14213	T:T	G:G	C:T	C:C	T:T	G:G	A:A	T:C	G:A	A:G	C:A	G:A	T:T	G:G	A:A
C15784	C:C	T:G	C:C	T:T	T:C	G:A	T:T	T:C	G:A	A:G	A:A	G:A	T:G	G:T	A:A
C21754	T:T	G:G	T:T	T:T	T:C	A:A	A:T	T:C	G:A	A:G	A:A	A:A	T:G	T:T	A:A
C23301	C:C	G:G	C:C	T:T	T:T	A:A	T:T	C:C	G:A	A:G	A:A	G:A	T:G	T:T	A:A
C24954	C:T	G:G	T:T	T:C	T:T	G:G	T:T	T:T	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C24955	T:T	T:T	T:T	C:C	C:C	G:A	A:T	T:T	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C24957	T:T	G:G	T:T	C:C	T:C	G:A	A:T	T:T	G:G	G:G	C:C	A:A	T:G	G:T	A:A
C24993	C:T	T:G	T:T	T:T	T:C	G:G	A:T	C:C	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C24998	T:T	T:G	T:T	T:T	T:C	G:A	A:T	T:T	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C24999	T:T	T:G	T:T	T:C	T:C	G:A	A:T	T:T	G:G	G:G	C:C	G:A	T:T	G:G	A:A
C25002	T:T	G:G	T:T	T:T	C:C	G:G	A:A	T:T	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C25004	T:T	T:G	T:T	C:C	T:C	G:G	A:A	T:T	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C25005	C:T	G:G	T:T	T:T	T:C	G:G	A:A	T:C	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C25008	T:T	G:G	T:T	T:C	C:C	G:G	A:A	T:C	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C25010	C:T	T:G	T:T	T:T	C:C	G:G	T:T	T:C	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C25016	C:T	T:G	T:T	T:T	C:C	G:G	T:T	T:C	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C25083	C:T	T:G	C:C	T:C	C:C	G:G	A:A	T:C	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C25084	C:C	G:G	C:C	T:T	?	G:G	T:T	T:T	G:G	G:G	C:C	?	T:G	G:G	A:A
C25085	C:C	G:G	C:C	T:T	?	G:G	T:T	T:T	G:G	G:G	C:C	?	T:G	G:G	A:A
C25091	C:C	T:G	T:T	T:T	C:C	G:A	A:A	T:T	G:G	G:G	C:C	A:A	T:G	G:T	A:A
C25093	C:T	T:G	C:T	T:C	T:C	G:G	A:A	T:T	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C25095	T:T	G:G	C:T	T:C	T:T	G:G	A:A	T:T	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C25097	T:T	G:G	C:T	T:C	T:C	G:G	A:A	T:T	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C25119	C:C	T:G	C:C	T:T	T:C	G:A	A:T	T:T	A:A	A:A	A:A	G:A	T:G	T:T	A:A
C25120	C:C	G:G	C:C	T:T	T:T	G:A	T:T	C:C	G:A	A:G	C:A	G:A	T:T	G:G	C:A
C25121	C:C	G:G	C:C	T:T	T:C	G:A	T:T	C:C	G:A	A:G	A:A	G:A	T:G	G:T	A:A
C25123	C:C	G:G	C:C	T:T	C:C	G:A	T:T	T:T	A:A	A:A	A:A	G:G	T:G	G:T	A:A
C25125	C:C	G:G	C:C	T:C	T:C	G:A	T:T	T:C	A:A	A:A	A:A	G:G	T:G	T:T	A:A
C25130	T:T	T:T	T:T	T:C	C:C	G:G	A:A	T:C	G:G	G:G	C:C	G:G	T:T	G:G	A:A
C25134	C:C	G:G	T:T	T:C	C:C	G:G	A:A	T:T	G:G	G:G	C:A	G:A	T:T	G:G	A:A
C25135	C:C	T:G	C:T	T:C	T:T	G:G	A:A	C:C	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C25137	C:C	T:T	C:C	T:T	T:T	G:A	T:T	T:C	G:A	A:G	A:A	G:G	T:G	T:T	A:A
C25139	C:C	T:G	T:T	T:T	T:T	G:G	T:T	T:C	?	A:A	A:A	G:G	T:T	G:G	A:A
C25141	C:T	T:G	C:T	T:C	T:T	G:G	A:A	C:C	G:G	G:G	C:C	G:G	T:T	G:G	A:A
C25142	C:T	T:T	C:C	T:T	C:C	G:G	A:A	T:T	G:G	G:G	C:C	G:G	T:T	G:G	A:A
C25143	C:C	T:T	T:T	T:T	C:C	G:G	T:T	T:C	G:G	G:G	A:A	A:A	T:T	G:G	A:A
C25259	C:T	G:G	T:T	T:C	T:C	G:G	A:A	T:T	G:G	G:G	C:C	A:A	T:T	G:G	A:A
C25272	C:C	G:G	C:T	T:T	T:T	A:A	T:T	T:C	A:A	A:A	C:A	G:A	T:G	G:T	A:A
C25274	C:T	T:T	C:C	C:C	T:T	G:G	A:T	T:C	A:A	A:A	C:C	G:G	T:G	G:T	C:A

[1] The two alleles for each marker are separated by a colon. The genotype of an accession for a certain marker can be homozygous [e.g. A:A], heterozygous [e.g. A:G] or missing [?].

Appendix 8. NEXUS file

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#NEXUS

BEGIN DATA;
	DIMENSIONS  NTAX=95 NCHAR=56;
	FORMAT DATATYPE = DNA RESPECTCASE GAP = - MISSING = N ;
	MATRIX
	'tetr_AZE1' CCGGAGTCCTTTAGCCNTNANTTCAANNCTTCTATCGGNNNCGNTNGT(C T)GGCN(G T)GA
	'tetr_AZE2' NCGGAGTCCTTTANCCNTNANTTCAANNCTNCTATCGGNNNCGCTNGT(C T)GGCN(G T)GA
	'tetr_AZE3' CCGGANTCCTTTAGCCNTNANTTCAANNCTTCTATCGGNNNCGCTNGTCGGCN(G T)GA
	'tetr_AZE4' CCGGAGTCCTTTANCCNTNANTTCAANNCTTCTATCGGNNNCGNTNGTTGGCN(G T)GA
	'tetr_AZE5' CCGGAGTCCTTTAGCCNTNANTTCAANNCTTCTATCGGNNNCNCTNGTTGGCN(G T)GA
	'tetr_AZE6' NCGGAGTCCTTTANCCNTNANTTCAANNCTTCTATCGGNNNCNCTNGTCGGCN(G T)GA
	'tetr_AZE7' CCGGAGTCCTTTAGCCNTNANTTCAANNCTTCTATCGGNNNCGNTNGTTGGCNTGA
	'tetr_ARM8' CCGGAGTCCTTTAGCCNTNAATTCAANNCTTCTATCGGNNNCGCTNGN(C T)GGCN(G T)GA
	'tetr_ARM9' CCGGAGTCCTTTAGCCNTNANTTCAANNCTNCTATCGGNNNCGNTNGN(C T)GGCNTGA
	'tetr_ARM10'CCGGAGTCCTTTAGCCNTNANTTCAANNCTTCTATCGGNNNCNNTNGT(C T)GGCN(G T)GA
	'tetr_ARM11'NCGGAGTCCTTTANCCNTNANTTCAANNCTNCTATCGGNNNCNCTNGNCGGCN(G T)GA
	'tetr_ARM12'CCGGAGTCCTTTANCCNTNANTTCAANNCTTCTATCGGNNNCNCTNGTCGGCN(G T)GA
	'tetr_ARM13'CCGGANTCCTTTAGCCNTNANTTCAANNCTTCTATCGGNNNCNNTNGNTGGCNTGA
	'tetr_ARM14'CCGGAGTCCTTTAGCCNTNANTTCAANNCTTCTATCGGNNNCGNTNGN(C T)GGCNTGA
	'oler_NPL15'CCGGACCC(A C)TTTG(G T)CCATATATTCTAGNCTGTT(A G)TCGGAGGTTTCTGATGGA(A G)TGA
	'oler_NLD16'TCAGGCC(C G)(A C)T(G T)(C T)(A G)T(A C)CGT(A T)TACATTACC(A C)CTTT(A G)(C T)(C G)TGCGGCGTTTA(A T)TAAAGTGA
	'oler_NLD17'TCG(C G)G(C G)TC(A C)TTTAGACGAAATCACAT(C G)C(A C)CG(C T)TGCGTGACGCGCTTGTC(A G)(A G)A(A G)(G T)TA
	'oler_FRA18'TC(A G)GA(C G)CC(A C)T(G T)T(A G)TCC(A G)T(A T)TACATTA(C G)C(A C)C(G T)TTGCGT(A G)CGGCGCCTATT(A G)(A G)AGTGA
	'oler_IRN19'TCGGNG(C T)C(A C)TTTA(G T)ACGA(A T)ATCATTA(C G)C(A C)(C T)(G T)T(A T)(A G)(C T)GT(A G)AC(A G)CGCT(C T)(A G)TC(A
	G)(A G)AG(G T)(G T)A
	'oler_IRN20'TA(A G)GGG(C T)C(A C)TTTG(G T)CCGTT(A T)TTT(C T)(A T)(A T)CN(A C)TTTT(A G)(C T)C(G T)GAGGC(G T)(C T)T(C T)(A
	G)TCAAA(A G)TGA
	'oler_RUS21'T(A C)AG(A G)GCC(A C)(C T)(G T)(C T)(A G)(G T)(A C)CA(A T)(A T)(A T)ACACAT(C G)N(A C)(C T)TTAGCGT(A G)(A C)(C G)(A
	G)C(G T)(C T)TT(A G)TTAAAA(G T)TA
	'oler_TUR22'T(A C)GGGG(C T)C(A C)TGT(A G)GCCG(A T)(A T)(A T)TCAC(A T)(A T)CCCTTT(A T)GC(C G)TG(A C)(C G)(A G)(C T)(G T)(C T)(C
	T)C(A G)TC(A G)(A G)AA(G T)TA
	'oler_IND23'CCG(C G)A(C G)TC(A C)TTTGG(A C)CGTTT(A T)CTCTA(C G)NCTGTTATCGGAGGT(G T)(C T)(C T)(C T)GAT(A G)(A G)AATGA
	'oler_AFG24'TCGGG(C G)CC(A C)T(G T)(C T)(A G)G(A C)(C T)(A G)(A T)AA(A T)C(A T)TTAGN(A C)(C T)TT(A T)(A G)(C T)GT(A G)(A C)(C
	G)G(C T)(G T)(C T)TTGATGG(A C)(A G)TGA
	'oler_CHN25'TAGGGG(C T)C(A C)T(G T)TG(G T)ACGT(A T)(A T)(A T)TATTA(C G)C(A C)(C T)GTTGCGTG(A C)(C G)GC(G T)CTT(A G)TCGGAA(G
	T)(G T)A
	'oler_AFG26'TA(A G)GA(C G)(C T)(C G)(A C)(C T)(G T)TA(G T)ACGTA(A T)ACATTAC(C G)(A C)T(G T)TTGCGTGCGGCGCT(C T)G(A T)CGGA(A
	G)TGA
	'oler_GRC27'T(A C)(A G)GGG(C T)C(A C)T(G T)TA(G T)CCG(A T)AT(A T)TATTA(C G)C(A C)TT(C T)TG(C T)CNG(A C)(C G)AC(G T)CT(C T)GT(C
	T)AAAG(G T)(G T)A
	'oler_AFG28'T(A C)GCGCCCA(C T)(G T)(C T)(A G)G(A C)(C T)GTAATTAC(A T)(A T)(C G)G(A C)(C T)(G T)TT(A G)(C T)GTGA(C G)GCGTT(C
	T)G(A T)CGGAATGA
	'oler_CHN29'C(A C)G(C G)ACT(C G)CTTTGGC(C T)AT(A T)T(A T)TT(C T)TAGNCTGTAATCGG(A C)(C G)(A G)CTTC(C T)GAT(A G)(A G)CATGA
	'oler_SYR30'T(A C)GGAGT(C G)(A C)TT(C T)G(G T)CCGAAATC(A T)(C T)TACC(A C)TGT(A T)G(C T)CG(A G)(A C)GGCGC(C T)T(A G)TCGG(A
	C)(A G)TGA
	'oler_TUR31'TAAGGGC(C G)(A C)T(G T)(C T)G(G T)(A C)CGTA(A T)ACATTA(C G)C(A C)T(G T)TAGCGTGA(C G)ACG(C T)TTG(A T)(C T)GGA(A
	G)(G T)TA
	'oler_TUR32'TAG(C G)(A G)G(C T)C(A C)TT(C T)GG(A C)C(A G)(A T)(A T)A(A T)(C T)A(C T)(A T)(A T)(C G)C(A C)TTTAG(C T)CGGAGA(C T)(G
	T)T(C T)(C T)(A G)TC(A G)(A G)(A C)ATGA
	'oler_TUR33'TC(A G)G(A G)(C G)(C T)(C G)(A C)TT(C T)(A G)(G T)(A C)C(A G)T(A T)(A T)ATA(C T)(A T)(A T)(C G)C(A C)TGTTGC(C G)TGC(C
	G)(A G)(C T)T(C T)(C T)(C T)(A G)TC(A G)(A G)C(A G)(G T)(G T)A
	'oler_TUR34'(C T)CGG(A G)(C G)(C T)C(A C)TT(C T)(A G)G(A C)CG(A T)(A T)ATC(A T)CAT(C G)C(A C)(C T)GTT(A G)(C T)CGGACGCG(C T)(C
	T)(C T)(A G)TC(A G)(A G)AATGA
	'oler_TUR35'(C T)C(A G)(C G)AGTC(A C)TT(C T)(A G)G(A C)CGTTA(A T)(C T)A(C T)TACC(A C)TTT(A T)(A G)(C T)GTG(A C)(C G)G(C T)(G
	T)TCC(A G)TTGGA(A G)TGA
	'oler_IRN36'TC(A G)GGGT(C G)(A C)CTTGGCCGTA(A T)TCACTACC(A C)(C T)GT(A T)GC(C G)TAA(C G)GCTTTTAT(C T)AAAATGA
	'oler_IRN37'(C T)(A C)AG(A G)GT(C G)(A C)TG(C T)G(G T)(A C)CA(A T)A(A T)TCTC(A T)(A T)CC(A C)TGT(A T)GC(C G)TN(A C)(C G)(A G)C(G
	T)TT(C T)(A G)TC(A G)(A G)AATGA
	'oler_AFG38'(C T)(A C)(A G)(C G)(A G)GTC(A C)T(G T)(C T)G(G T)(A C)C(A G)TAA(A T)C(A T)TTACN(A C)TGTT(A G)(C T)CG(A G)(A
	C)GGCGCTTGA(C T)AAAA(G T)(G T)A
	'oler_AFG39'CCGGA(C G)TC(A C)TTTGG(A C)CAT(A T)(A T)(A T)(C T)TCTA(C G)(C G)CT(G T)TTATCGGAGGTG(C T)(C T)TG(A T)TGGCATGA
	'oler_IND40'(C T)C(A G)GA(C G)TC(A C)TT(C T)(A G)GCC(A G)T(A T)(A T)ATT(C T)(A T)(A T)GC(A C)T(G T)TT(A G)(C T)(C G)TGAGG(C T)(G
	T)C(C T)T(A G)(A T)TGGAATGA
	'oler_AFG41'T(A C)GC(A G)GCC(A C)(C T)GTGGAC(A G)TTT(A T)T(A T)C(A T)(A T)CG(A C)CGTT(A G)(C T)CGG(A C)(C G)GC(G T)(C T)(C
	T)TGTCGG(A C)ATGA
	'oler_AFG42'TAGCGG(C T)C(A C)TGT(A G)(G T)CCGTA(A T)T(C T)(A T)(C T)(A T)(A T)GG(A C)TGTT(A G)(C T)CNGAGA(C T)G(C T)T(C T)(A
	G)(A T)(C T)GGAA(G T)(G T)A
	'oler_AFG43'TA(A G)CGG(C T)C(A C)(C T)(G T)(C T)(A G)GCCG(A T)(A T)(A T)ATTCTAGN(A C)TGTTGCGTGAG(A G)CTCT(C T)GT(C T)(A G)(A
	G)AATGA
	'oler_IRN44'T(A C)GGGG(C T)C(A C)(C T)(G T)C(A G)TCC(A G)T(A T)(A T)(A T)TATTA(C G)C(A C)(C T)(G T)TTGC(C G)T(A G)(A C)(C G)(A
	G)CTTTTAACGGA(A G)(G T)(G T)A
	'oler_NPL45'CCGGACTC(A C)TTTGG(A C)CGTTTATTCTAGNCTGTTATCGGAGGCG(C T)(C T)TGATAAAGTGA
	'oler_IRN46'T(A C)(A G)GG(C G)(C T)C(A C)TTT(A G)(G T)CCGTTA(A T)(C T)(A T)CTACC(A C)(C T)(G T)TTGC(C G)TGA(C G)(A G)(C T)(G
	T)(C T)T(C T)(A G)(A T)(C T)GG(A C)(A G)TGA
	'oler_TUR47'TA(A G)G(A G)GCC(A C)TTT(A G)T(A C)CGTTAA(C T)A(C T)TA(C G)N(A C)CGTTGC(C G)TG(A C)GGCTC(C T)(C T)(A G)TC(A G)(A
	G)A(A G)(G T)(G T)A
	'oler_MKD48'(C T)(A C)(A G)(C G)GGC(C G)(A C)T(G T)T(A G)TCCGATA(A T)(C T)ATTACC(A C)(C T)TT(A T)GTGT(A G)AGGCGCTTG(A T)(C
	T)(A G)(A G)CG(G T)TA
	'oler_PAK49'CCGGAGTC(A C)(C T)T(C T)GG(A C)C(A G)TATTTTCTAG(C G)CTTTTA(C T)CGGAGGTGCC(C T)GATGGCATGA
	'oler_TUR50'TC(A G)C(A G)(C G)C(C G)(A C)T(G T)(C T)(A G)T(A C)CG(A T)(A T)AA(C T)ACTA(C G)C(A C)TTTT(A G)(C T)CG(A G)A(C G)(A
	G)CG(C T)T(C T)(A G)T(C T)(A G)(A G)(A C)(A G)(G T)(G T)(A C)
	'oler_BGR51'TCAGGGCC(A C)TGT(A G)(G T)AC(A G)AAAA(C T)A(C T)(A T)(A T)(C G)N(A C)CTC(A T)GCGTG(A C)G(A G)CGCTTG(A T)(C
	T)GGAA(G T)(G T)A
	'oler_RUS52'TAGGGG(C T)C(A C)(C T)(G T)TG(G T)CC(A G)(A T)(A T)A(A T)CA(C T)(A T)(A T)(C G)N(A C)T(G T)TAGC(C G)T(A G)A(C
	G)GCGC(C T)TATC(A G)(A G)AATGA
	'oler_IRN53'(C T)AAGAGCC(A C)TT(C T)G(G T)ACGT(A T)TA(C T)TC(A T)(A T)CG(A C)TGT(A T)GCGTGAG(A G)(C T)(G T)(C T)(C T)(C T)G(A
	T)(C T)(A G)(A G)AG(G T)(G T)A
	'oler_PAK54'TA(A G)(C G)(A G)GTC(A C)T(G T)C(A G)(G T)(A C)C(A G)T(A T)(A T)(A T)(C T)TC(A T)(A T)(C G)G(A C)T(G
	T)TTATCGGAGGTGTTTGT(C T)GGCA(G T)(G T)A
	'oler_PAK55'CCGGA(C G)TC(A C)TT(C T)GGAC(A G)TATA(C T)TC(A T)(A T)(C G)GCTTTTATC(G T)G(A C)GGTT(C T)C(C T)GATGGAA(G T)(G
	T)A
	'oler_PAK56'CCGCAGTC(A C)(C T)(G T)(C T)GGCCATAT(A T)TTCTAGCCTTTTATC(G T)GAGGT(G T)TCTG(A T)TGG(A C)A(G T)(G T)A
	'oler_CHN57'CCG(C G)ACTC(A C)TTT(A G)GCC(A G)TAT(A T)(C T)TCTAGGCTTTTATCGG(A C)GGTG(C T)CTGA(C T)(A G)(A G)(A C)(A G)TGA
	'oler_GEO58'TC(A G)(C G)(A G)GCC(A C)TGTGG(A C)CG(A T)AA(A T)(C T)A(C T)(A T)(A T)CC(A C)C(G T)T(A T)ATGTAA(C G)GC(G T)CT(C
	T)(A G)T(C T)(A G)(A G)A(A G)(G T)(G T)A
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Appendix 8. NEXUS file

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'oler_SYR59'TAGGGG(C T)C(A C)TTTG(G T)CCG(A T)(A T)(A T)TCA(C T)TACN(A C)(C T)TTT(A G)(C T)CGGAG(A G)TGTT(C T)A(A T)(C T)(A G)(A G)AA(G T)TA
'oler_UZB60'TAAGGGT(C G)(A C)T(G T)TG(G T)(A C)CGT(A T)AATA(C T)TA(C G)N(A C)CGTAGCGTG(A C)GGCGCTTATC(A G)(A G)A(A G)(G T)TA
'turk_TJK61'C(A C)GCACT(C G)(A C)TTTG(G T)(A C)C(A G)T(A T)(A T)(A T)(C T)TC(A T)(A T)(C G)N(A C)T(G T)(C T)T(A G)(C T)CGGN(C G)(A G)(C T)GT(C T)TGTTGGCATGA
'turk_TJK62'NC(A G)(C G)A(C G)TCCT(G T)(C T)G(G T)CCGT(A T)TATTCTA(C G)C(A C)TTT(A T)AT(C G)TGA(C G)GTTTCC(A G)(A T)TGGCATGA
'turk_TJK63'NCG(C G)A(C G)TCCT(G T)TGG(A C)CGTTA(A T)TTC(A T)(A T)GCCT(G T)(C T)T(A G)CGTGN(C G)GTGTC(C T)(A G)(A T)TGGCA(G T)(G T)A
'turk_TJK64'NC(A G)CACTC(A C)TTT(A G)(G T)(A C)CGTT(A T)A(C T)TC(A T)(A T)(C G)(C G)CTTTTATCG(A G)CGG(C T)(G T)TT(C T)G(A T)CGGCATGA
'turk_TJK65'C(A C)GCA(C G)T(C G)CTT(C T)G(G T)(A C)CGT(A T)T(A T)TTC(A T)(A T)GN(A C)TTTTACC(G T)GC(C G)GT(G T)TT(C T)(A G)(A T)TGGCATGA
'turk_TJK66'NCGGA(C G)T(C G)(A C)TGTGGC(C T)GT(A T)(A T)A(C T)TCATGNCTTTAA(C T)(C G)TGCGGT(G T)T(C T)(C T)(A G)(A T)TGGC(A G)TGA
'turk_UZB67'NC(A G)CACTC(A C)T(G T)TA(G T)ACGTTT(A T)CTCATCNCTTTTATCGGAG(A G)TGTTCGATGGCATGA
'turk_UZB68'TCGCA(C G)TC(A C)TTT(A G)(G T)CC(A G)T(A T)(A T)TC(A T)C(A T)(A T)GGCTTTTA(C T)(C G)TGCGGT(G T)TC(C T)GATGGCATGA
'turk_UZB69'CCG(C G)ACT(C G)CTT(C T)GG(A C)CGTT(A T)T(C T)TC(A T)(A T)(C G)NCTTTTA(C T)GTGC(C G)G(C T)GTT(C T)GA(C T)GGCATGA
'turk_UZB70'(C T)(A C)G(C G)ACT(C G)CTTTA(G T)(A C)CGT(A T)AA(C T)TCAT(C G)GCTTTTA(C T)(C G)TGCG(A G)TGT(C T)CGA(C T)GGCATGA
'turk_UZB71'(C T)(A C)(A G)CACT(C G)(A C)TTTGGCCAT(A T)(A T)(A T)CTC(A T)(A T)GGCTTTTA(C T)(C G)TG(A C)GG(C T)(G T)TTCGT(C T)GGCATGA
'turk_UZB72'(C T)(A C)GCAGTC(A C)TTTGG(A C)CGT(A T)A(A T)TTC(A T)(A T)GGCT(G T)TTATC(G T)GC(C G)G(C T)(G T)TTCGT(C T)GGCATGA
'turk_UZB73'C(A C)GCAGTC(A C)T(G T)T(A G)GA(C T)(A G)T(A T)A(A T)(C T)TC(A T)(A T)GGCTTTTATGTG(A C)(C G)G(C T)(G T)C(C T)CGA(C T)GGCATGA
'tetr_GEO74'CCGGAGTCCTTTAGCCNTNANTTCAANNCTNCTATCNGNNNCGCTNGTTGGCN(G T)GA
'tetr_GEO75'CCGGAGTCCTTTAGCCNTNAATTCAANNCTTCTATCGGNNNCGCTNGTTGGCN(G T)GA
'turk_TJK76'NCG(C G)ACTC(A C)TGTG(G T)A(C T)GTTT(A T)CTC(A T)(A T)(C G)NCT(G T)NT(A G)(C T)CGGCG(A G)C(G T)TTC(A G)ATGGCA(G T)(G T)A
'turk_UZB77'(C T)(A C)GCA(C G)T(C G)(A C)TTTGTA(C T)GT(A T)(A T)(A T)CTC(A T)(A T)CGCT(G T)(C T)TA(C T)CNGC(C G)G(C T)(G T)(C T)(C T)(C T)GATGGCATGA
'turk_UZB78'TCGCACTC(A C)TTTGG(A C)CGT(A T)AT(C T)AC(A T)(A T)(C G)N(A C)TT(C T)TA(C T)(C G)TGCGGTG(C T)(C T)TGATGGCATGA
'turk_UZB79'TC(A G)(C G)A(C G)T(C G)CTTT(A G)G(A C)CGT(A T)A(A T)(C T)TC(A T)(A T)GNCT(G T)TTATC(G T)G(A C)(C G)GTG(C T)(C T)(C T)GATGGCATGA
'oler_AZE80'T(A C)(A G)(C G)GG(C T)C(A C)TTT(A G)T(A C)CG(A T)TA(A T)CATTA(C G)C(A C)CGTTGCGTG(A C)(C G)GC(G T)CT(C T)(A G)(A T)TAAA(A G)(G T)TA
'oler_AZE81'T(A C)(A G)(C G)GG(C T)C(A C)T(G T)TAGACG(A T)A(A T)(A T)(C T)ACATCC(A C)CG(C T)AGC(C G)TAA(C G)GCGCTT(A G)TC(A G)(A G)(A C)(A G)TG(A C)
'oler_AZE82'TAG(C G)(A G)GTC(A C)T(G T)TAGACG(A T)A(A T)(A T)CATTACC(A C)C(G T)TAGC(C G)T(A G)AGGCGCT(C T)(A G)TC(A G)(A G)A(A G)(G T)(G T)A
'oler_ARM83'TCG(C G)(A G)(C G)CCATTTGTACGAAATCATTACN(A C)CGTTGCGTGAGGCGCTC(A G)TTAAAG(G T)(G T)A
'oler_ARM84'T(A C)GG(A G)GCC(A C)(C T)(G T)TAGACGAA(A T)(A T)C(A T)CATCN(A C)C(G T)(C T)AGCGTG(A C)(C G)(A G)CGC(C T)(C T)(A G)T(C T)AAAG(G T)TA
'turk_TJK85'NC(A G)CA(C G)T(C G)(A C)T(G T)T(A G)(G T)C(C T)G(A T)T(A T)(A T)CTC(A T)(A T)GGCT(G T)T(A T)(A G)(C T)(C G)TGCGGTTT(C T)CGA(C T)GGCGTGA
'turk_UZB86'C(A C)G(C G)AGT(C G)(A C)T(G T)T(A G)G(A C)(C T)GTATT(C T)TCTA(C G)GCT(G T)TTAT(C G)TGC(C G)GCGT(C T)CGATGG(A C)(A G)TGA
'turk_UZB87'C(A C)(A G)CA(C G)TC(A C)TGTAG(A C)(C T)(A G)T(A T)A(A T)CTCTAGGCT(G T)TTA(C T)(C G)TGC(C G)(A G)C(G T)(C T)(C T)TGACGGCATGA
'oler_UZB88'T(A C)AGGGCC(A C)(C T)(G T)T(A G)TAC(A G)TA(A T)TT(A T)(C T)(A T)(A T)CN(A C)T(G T)TAGCGTGC(C G)GCTCTT(A G)T(C T)(A G)(A G)AG(G T)TA
'oler_UZB89'TCGG(A G)G(C T)C(A C)(C T)TT(A G)TAC(A G)TAATC(A T)(C T)(A T)(A T)(C G)C(A C)TT(C T)(A T)GCCN(A G)A(C G)(A G)C(G T)TTTGT(C T)NAAGTGA
'turk_TKM90'TCG(C G)AGTC(A C)TTTAG(A C)CGT(A T)TTCTC(A T)(A T)(C G)GCTTTTATCGGC(C G)(A G)(C T)(G T)(C T)(C T)TGACGGCGTGA
'turk_TKM91'TCGCACT(C G)CT(G T)TGT(A C)CGA(A T)T(A T)(C T)T(C T)TACGCTTT(A T)ATCNGC(C G)G(C T)TCTCGATGGCGTGA
'turk_TKM92'TAA(C G)G(C G)T(C G)CTTTGTCCGT(A T)TA(C T)ACATCN(A C)TTTA(A G)(C T)(C G)TGCCGCTTTCGT(C T)GGAATGA
'turk_UZB93'(C T)(A C)GCAGT(C G)CTTTAGCCGTAA(A T)CTCTACNCTT(C T)T(A G)(C T)(C G)TGCG(A G)(C T)GT(C T)(C T)GATGGCATGA
'oler_AZE94'T(A C)GCGGCC(A C)TG(C T)(A G)(G T)ACGAAAACAC(A T)(A T)CN(A C)(C T)G(C T)AGCGTAC(C G)GCG(C T)TTAT(C T)AA(A C)(A G)(G T)(G T)A
'turk_TKM95'TCG(C G)AGCC(A C)TGTG(G T)ACG(A T)TAA(C T)(A T)CATCN(A C)(C T)(G T)C(A T)GC(C G)T(A G)AC(A G)(C T)TCCTG(A T)(C T)AACG(G T)(G T)(A C)

;
END;
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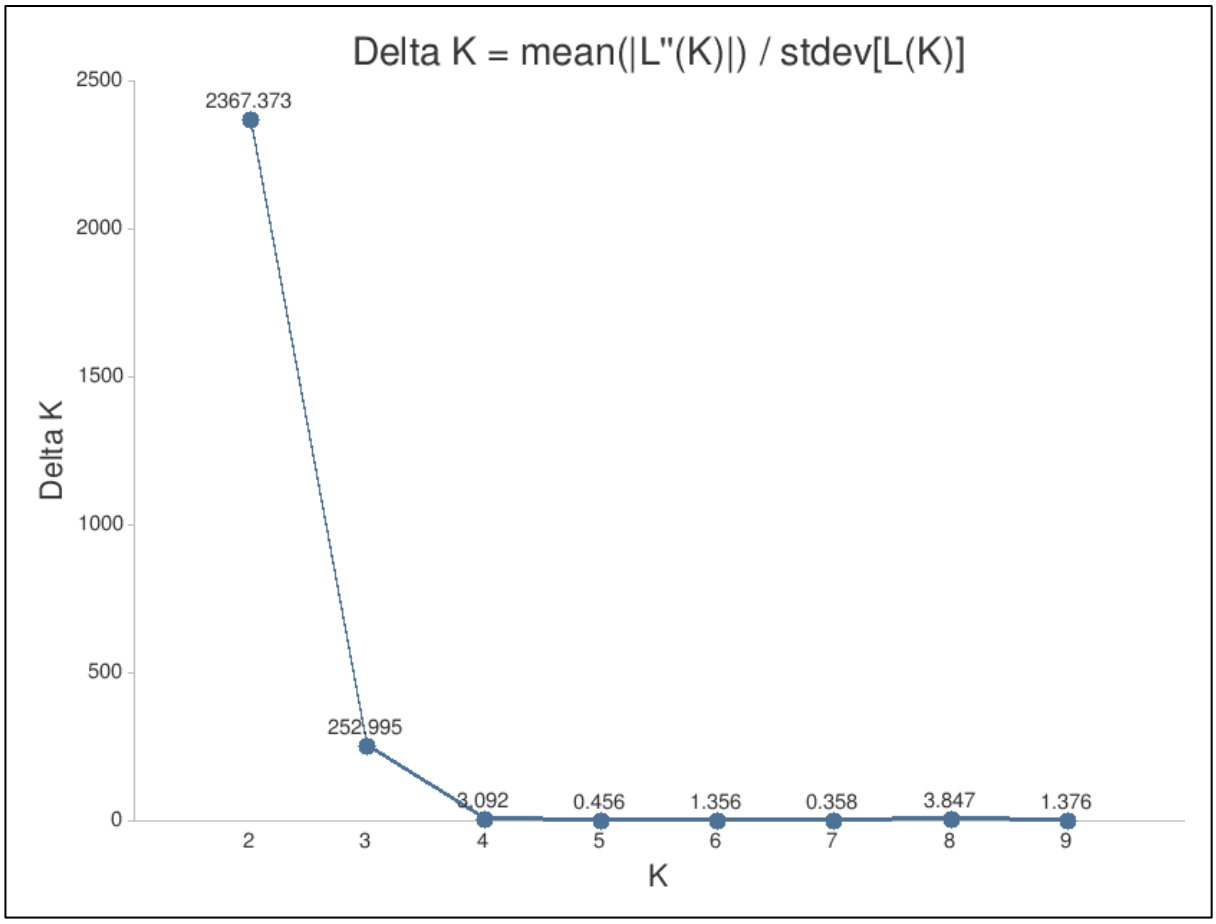
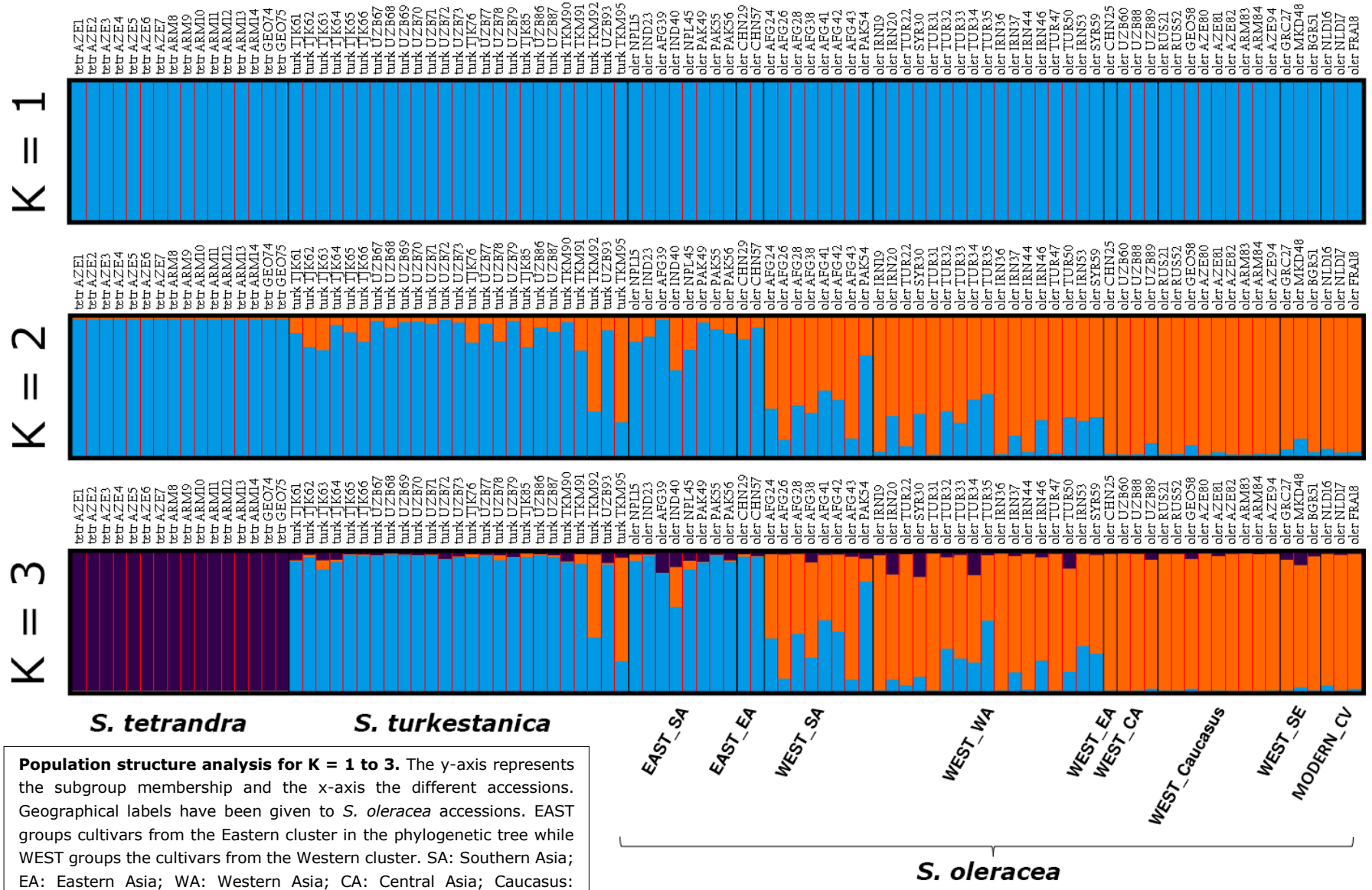
Appendix 9. ΔK analysis for estimating the population structure



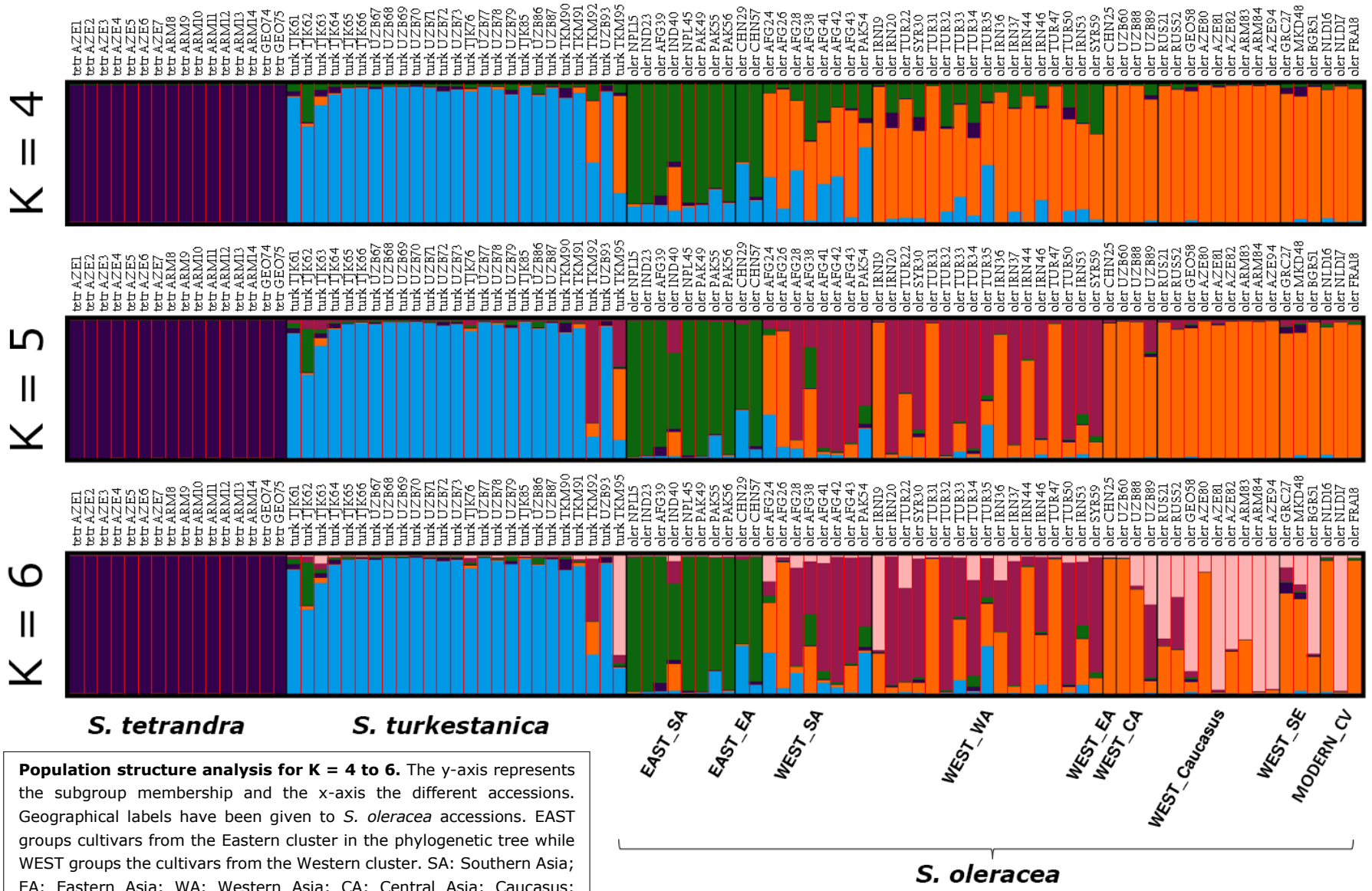
ΔK analysis to estimate the best number of subpopulations [K] for the population consisting of 95 *Spinacia* accessions.

Appendix 10. Population structure analysis for K = 1 to 10



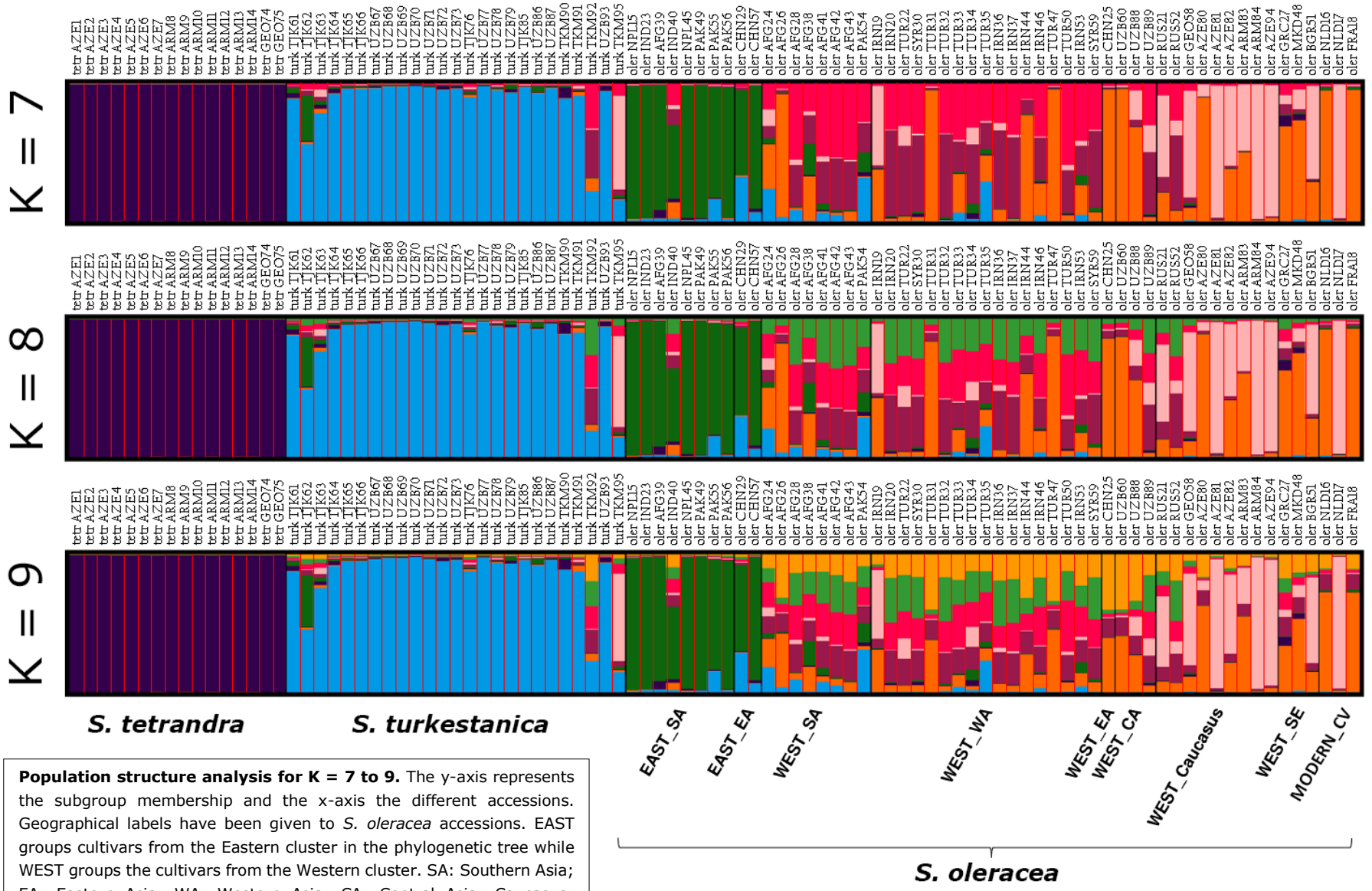
Population structure analysis for K = 1 to 3. The y-axis represents the subgroup membership and the x-axis the different accessions. Geographical labels have been given to *S. oleracea* accessions. EAST groups cultivars from the Eastern cluster in the phylogenetic tree while WEST groups the cultivars from the Western cluster. SA: Southern Asia; EA: Eastern Asia; WA: Western Asia; CA: Central Asia; Caucasus: Western Asia (Caucasus region); SE: Southern Europe (Balkans); MODERN_CV: modern cultivars from Western Europe.

Appendix 10. Population structure analysis for K = 1 to 10



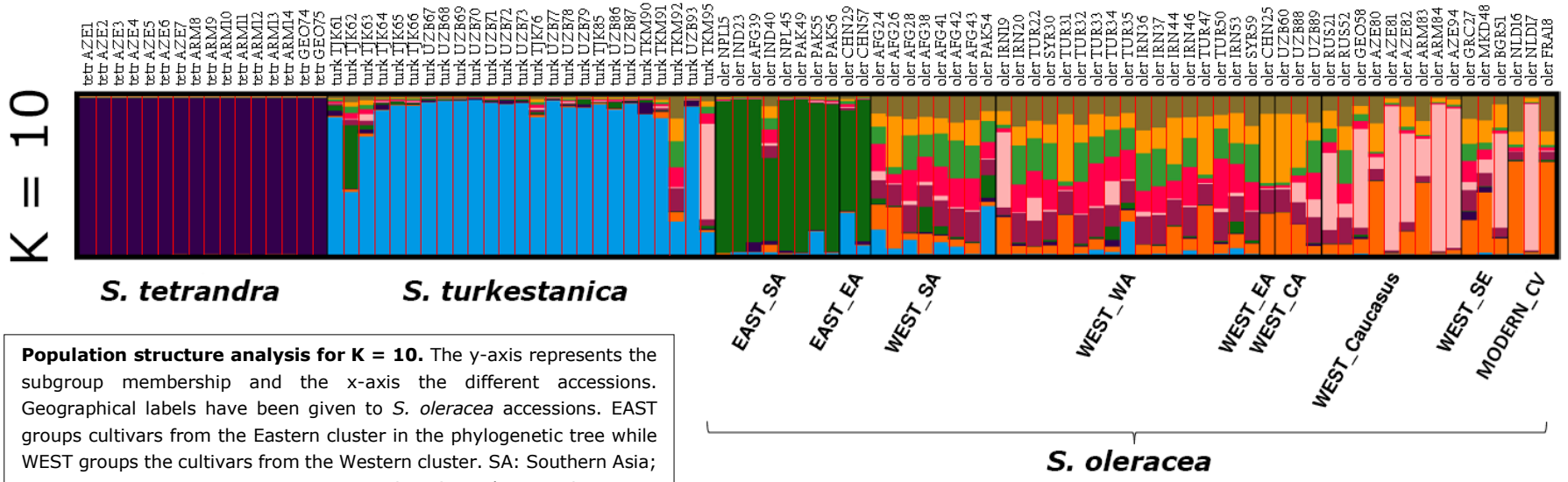
Population structure analysis for K = 4 to 6. The y-axis represents the subgroup membership and the x-axis the different accessions. Geographical labels have been given to *S. oleracea* accessions. EAST groups cultivars from the Eastern cluster in the phylogenetic tree while WEST groups the cultivars from the Western cluster. SA: Southern Asia; EA: Eastern Asia; WA: Western Asia; CA: Central Asia; Caucasus: Western Asia (Caucasus region); SE: Southern Europe (Balkans); MODERN_CV: modern cultivars from Western Europe.

Appendix 10. Population structure analysis for K = 1 to 10



Population structure analysis for K = 7 to 9. The y-axis represents the subgroup membership and the x-axis the different accessions. Geographical labels have been given to *S. oleracea* accessions. EAST groups cultivars from the Eastern cluster in the phylogenetic tree while WEST groups the cultivars from the Western cluster. SA: Southern Asia; EA: Eastern Asia; WA: Western Asia; CA: Central Asia; Caucasus: Western Asia (Caucasus region); SE: Southern Europe (Balkans); MODERN_CV: modern cultivars from Western Europe.

Appendix 10. Population structure analysis for K = 1 to 10



Population structure analysis for K = 10. The y-axis represents the subgroup membership and the x-axis the different accessions. Geographical labels have been given to *S. oleracea* accessions. EAST groups cultivars from the Eastern cluster in the phylogenetic tree while WEST groups the cultivars from the Western cluster. SA: Southern Asia; EA: Eastern Asia; WA: Western Asia; CA: Central Asia; Caucasus: Western Asia (Caucasus region); SE: Southern Europe (Balkans); MODERN_CV: modern cultivars from Western Europe.