DETERMINATION OF GENETIC DIVERSITY AND POPULATION STRUCTURE IN FABA BEAN (*Vicia faba* L)

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ABSTRACT

DETERMINATION OF GENETIC DIVERSITY AND POPULATION STRUCTURE IN FABA BEAN (*Vicia faba* L)

Faba bean (*Vicia faba* L.) is an important legume species because of the high protein and starch content of its seeds. Broad bean can be grown in different climatic conditions and is an ideal rotation crop because of the symbiotic relationship between the plant and nitrogen fixing bacteria in its roots. Broad bean seeds are consumed as fresh vegetables in many countries throughout the world. However, the genetic diversity found in this germplasm has not yet been characterized and has not been systematically used in broad bean breeding programs. In this project, faba bean individuals obtained from International Center for Agricultural Research in the Dry Areas (ICARDA), Centre for Genetic Resources (CGN), Aegean Agricultural Research Institute (AARI), Nordic Gene Bank (NGB) and Australia (The University of Adelaide, Jeffrey Paull) were examined for their genetic diversity and population structure. For this purpose, 259 faba bean germplasm accessions were characterized using 32 SSR primers. A total of 302 polymorphic SSR fragments were analyzed. According the results, faba bean individuals were divided into two main clusters based on Neighbor-joining algorithm (r = 0.9062) with some clustering based on geographical origin as well as seed size. STRUCTURE 2.2.3 program was used to determine population structure. K was determined as 2 subpopulations. Cluster 1 had 87 individuals; cluster 2 had 162 individuals and 10 individuals were intermixed with results generally agreeing with the dendrogram analysis. A total of 45 well-characterized faba bean individuals were selected for the core collection to be used in breeding studies.

ÖZET

BAKLA'DA (*Vicia faba* L) GENETİK ÇEŞİTLİLİK VE POPULASYON YAPISININ BELİRLENMESİ

Bakla (*Vicia faba* L.), tohumunun yüksek protein ve nişasta içeriğinden dolayı önemli bir baklagil türüdür. Bakla, değişik iklim koşullarında yetiştirilebilmektedir ve kök sisteminin azot bağlayan bakteriler ile oluşturduğu simbiyotik ilişki sayesinde ideal bir rotasyon bitki türüdür. Dünya genelinde pek çok ülkede taze sebze olarak tüketilir. Ancak, bakla germplazmlarının barındırdığı genetik çeşitliliğin karakterizasyonuna ilişkin çalışmalar yetersiz olup, bu baklagil türü için sistematik ıslah programları da oluşturulmuş değildir. Önerilen projenin esas amacı, ETAE, ICARDA, CGN, NGB ve Adelaide Üniversitesi'nden temin edilen bakla germplazmlarının genetik çeşitlilik ve populasyon yapısı bakımından kapsamlı bir şekilde incelemesinin yapılmasıdır. Bu amaçla, 259 bakla germplasmı 32 adet SSR primeri ile karakterize edilmiştir. Toplamda, 302 polimorfik SSR fragmenti analiz edilmiştir. Bakla örnekleri, Neighbor-joining algoritması (r = 0.9062) ile analizi sonucunda coğrafi orijin ve tohum büyüklüğüne göre iki ana kümeye ayrılmıştır. Popülasyon yapısının belirlenmesi için STRUCTURE 2.2.3 programı kullanılmıştır. K, 2 alt popülasyon olarak tespit edilmiştir. Küme 1'de 87, küme 2'de 162 ve hiçbir gruba bağlı olmayan 10 birey vardır. Genetik olarak iyi karakterize edilmiş 45 bakla bireyi çekirdek koleksiyon oluşturmak için seçilmiştir. Bu koleksiyon daha sonraki ıslah çalışmalarında kullanılabilecektir.

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CHAPTER 1

INTRODUCTION

1.1. Faba Bean and its Nutritional and Anti-nutritional Properties

Faba bean (*Vicia faba* L.), also named broad bean, field bean, tick bean, windsor bean, and horse bean was domesticated around 8000 BC in the Near East[1]. Faba bean belongs to the *Fabaceae*. Today this legume has a vital role in human consumption and animal feed worldwide because of the high protein and starch content in its seed. Moreover, immature faba bean has essential vitamins and numerous antioxidants. It also contains high amounts of vicine and convicine, components which are medicinally important [2]. These two anti-nutritional factors cause favism which is glucose-6 phosphate dehydrogenase deficiency. About 400 million people are affected by this disease and it is one of the most common human enzyme deficiencies worldwide [3]. Faba bean seed and seed coat accumulate another anti-nutritional component, tannin. This factor is an important problem for consumption both in food and feed. Tannins reduce protein digestibility and negatively affect taste and are usually relatively low in faba bean.

V. faba can also contain L-DOPA (L-3,4-dihydroxyphenylalanine**)** which is a precursor of the neurological factor dopamine. This biochemical compound is used to treat Parkinson's disease. Faba bean has relatively large amounts of L-DOPA which can account for up to 7% of the dry weight of pod tissue[4]. The biosynthetic pathway of L-DOPA is not well characterized in *V. faba*.

In addition to their nutritional qualities, legumes have nitrogen fixation bacteria in their roots. Due to this symbiotic relationship, these species are very important for agriculture [5].

1.2. Production of Faba Bean

Faba bean is the fourth most important cool season legume crop worldwide after chickpea, pea, and lentil[6]. China is the most important producer with an average of

1.779.800 tons production. Ethiopia (582.006 tons), France (344.014 tons), Egypt (285,172 tons) and Australia (217.577 tons) follow China (FAO 2012). In Turkey 18.410 tons faba bean were produced in 2012 and Turkey ranks sixteenth in faba bean production (FAO 2012). Worldwide faba bean production from 2007 to 2011 is shown in Figure 1.1.

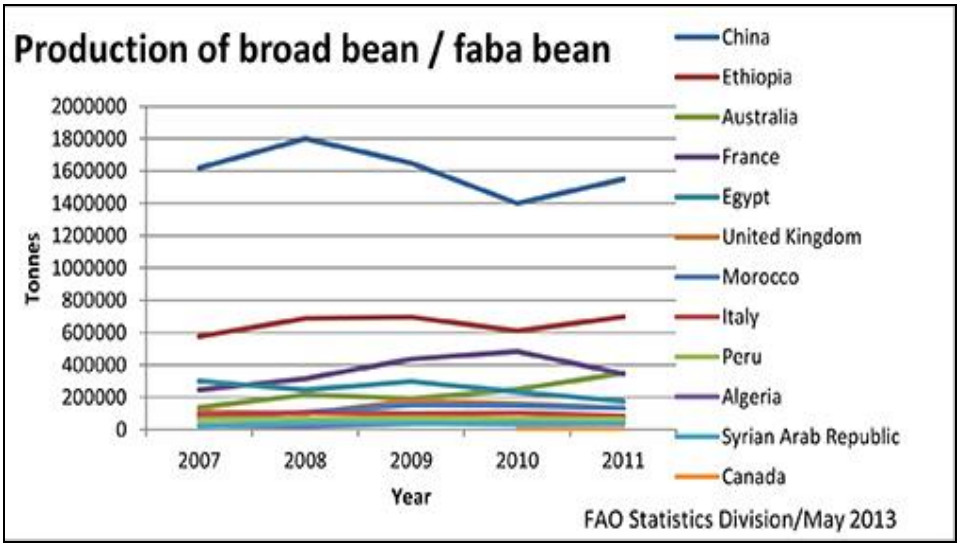

Figure1.1. Faba bean production around the world
(Source: FAO 2013)

Mediterranean types of faba bean are sown in late autumn. These types are also grown in parts of the UK and France that have relatively mild autumn climates. In the UK, more or less half of the faba beans are winter types. All UK types of faba bean can survive very mild frosts (to approximately -6°C). As a result, faba bean can be grown in different climate and soil conditions. Thus it is an important crop for sustainable agriculture on unfavorable land[7].

1.3. Types and Uses of Faba Bean

The agricultural importance of faba bean is increasing due to its high nutritional content. For example, protein content among faba bean genotypes ranges from 24% to 35% of seed dry matter[8]. Mediterranean countries, the Nile river valley, Ethiopia, Middle and East Asia, Latin America, North Europe, North America, Australia, the

Middle East, and North Africa use legumes as a main protein source in the daily diet[9]. Faba bean can be consumed as a fresh vegetable. In addition to fresh consumption, seeds can be dried, frozen, and canned.

From its origin in the Near East, faba bean spread around the world. During this spread, the crop gained specific adaptation factors and was selected for different agronomic traits related to plant architecture along with seed size, weight and shape. According to seed characters, four groups have been defined: *major*, *equina*, *minor*, and *paucijuga*[10]. These groups can still be recognized in the major areas of production. The largest seeds (*major*) emerged in South Mediterranean countries and China. Medium seeded types (*equina*) are grown throughout the Middle East, North Africa, and Australia while small seeds (*minor* and *paucijuga*) are found in Ethiopia and are the favored type in North European agriculture[11].

1.4. Faba Bean as a Genetic System

Faba bean is a facultative cross-pollinated legume crop and has $2n=2x=12$ chromosomes. The genome size is ~13.000 Mb[12] which is one of the largest described genomes among legumes. When compared to the model legume *Medicago truncatula*, faba bean has a genome that is 25 times larger[13]. The large genome of *V. faba* is due to high transposon activity[1]. Comparative genome analysis between *M. truncatula* and *V. faba* shows that faba bean contains more than 85% repetitive DNA[14].

A large genome size gives an advantage to the faba bean. This trait allowed faba bean to become one of the best characterized plant species in cytogenetics[15]. However, its large genome size hinders the development of saturated linkage maps and identification of relevant genes/QTLs in faba bean. In addition, it makes whole-genome shotgun assembly with next generation sequencing technologies more difficult. As a result, relatively little progress has been made in faba bean genomics and this has made it difficult to breed elite cultivars for adverse environmental conditions.

1.5. Breeding Traits

Faba bean breeders desire new cultivars with lower sensitivity to variable environmental conditions and stable yield[16]. There are remarkable morphological

differences between faba bean cultivars and breeding lines[17]. These differences are seen in traits such as plant height, pod number, seed size, seed color, susceptibility to drought, and resistance to diseases. The breeding objectives for this crop are grain yield, high protein content, resistance to drought, winter frost (in case of winter bean breeding), resistance to fungi and other pathogens and pests, and grain quality.

1.5.1. Environmental Influence

The environment enormously affects faba bean yield. Seed yield is influenced by the year and location[18]. The most influential environmental factor is rainfall from flowering to harvesting[19]. Faba bean crops are always sown in autumn and often on rainfed land in Mediterranean conditions. Variation in rainfall creates variation in yield. Faba bean is more sensitive to drought than other grain legumes and more affected by repeated drought and climate change. In the Mediterranean type climate, drought and heat waves have significant effects on the productivity of faba bean[20].

Drought stress is a very important problem for plants. Lack of water induces changes in anatomy and morphology because water plays an important role in many physiological and biochemical processes. Obviously, water deficiency reduces photosynthesis and induces oxidative stress. This situation is dangerous for lipids, proteins, and other biological components. The first result of water deficit is stomatal closure which prevents loss of water. This process is related with stomatal density, size, and opening. Stomatal size and density demonstrate differences in faba bean[21]. Thus, different faba bean genotypes have different responses to drought. Appropriate stomatal activity can be useful for improving drought response in faba bean[22].

Another factor of drought resistance is root growth. Total lateral root length, number, and mass are important factors. For example, the drought resistant cultivar Gobo has greater root length, mass, and number than the susceptible cultivar Victor[23].

Drought can also be avoided by minimizing the length of the critical growing period. This may be achieved by selection of varieties with an appropriate phenology and by adaption of appropriate crop management strategies. However, these strategies are not successful when intermittent drought occurs with unpredictable timing in the early growing season[24].

Another important environmental factor for faba bean is low temperature. Winter faba beans are generally sown in autumn in North-West Europe and can survive at -15°C, and even lower temperatures. Accumulation of polyunsaturated fatty acids (PUFA) could be an important adaptation mechanism for freezing tolerance[25]. In addition, accumulation of free proline or glycinebetaine can provide a cryoprotective mechanism to the plant[5].

1.5.2. Fungal Disease

V. faba is susceptible to several diseases caused by fungi, viruses, pests, and parasitic plants. Environmental conditions induce development of diseases. For example, ascochyta blight, which is a fungal disease, prefers cool, moist conditions but chocolate spot favours warmer humid environments[26].

The most important fungal foliar diseases are *Botrytis fabae*, *Ascochyta fabae*, and *Uromyces viciae-fabae*. *Rhizoctonia solani*, *Fusarium* species, and other fungi cause root rot[27]. *Botrytis* causes chocolate spot disease in faba bean and is effective in a wide range of growing conditions[28]. This fungus is often a serious problem and there is no effective prevention method. If environmental conditions are appropriate, the disease may become serious and cause significant yield loses. Different cultivars of faba bean show different quantitative resistance patterns and there are several less susceptible faba bean lines[29].

Specific resistance mechanisms are known and molecular markers have been identified for *Ascochyta* and *Uromyces* resistance[30]. *Uromyces viciae-fabae* causes faba-bean rust which provokes partial defoliation and decreases photosynthesis and yield. Faba-bean rust resistance is seen in many cultivars. For example, the line 29H demonstrates the highest level of resistance and is used to improve *Ascochyta* resistance in the crop[30]. Interestingly, research revealed that zero-tannin materials are more susceptible to fungal diseases at germination than tannin-containing germplasm. There are several chemical and biological controls for fungal disease but none of them are effective. Therefore, there is a need to develop and breed new resistant cultivars.

1.5.3. Virus diseases

Although viruses are not as common a problem for *V. faba* production compared to fungal diseases or insect pests, virus diseases may occur as epidemics and become serious. For example, during the growing season of 1991/1992, a serious faba bean necrotic yellows virus outbreak happened in Middle Egypt and yield losses were over 90%[31]. Many viruses which affect faba bean are transmitted by aphids so aphid resistant cultivars should be developed to inhibit virus outbreak. For example, ILB132 is a natural resistant genotype for faba bean necrotic yellows virus. On the other hand, broad bean true mosaic virus and broad bean stain virus cannot be transmitted by aphids or seed. There are many other viruses which have local importance and, because there are no effective chemical controls, virus resistance is an important breeding goal.

1.5.4. Pests and Nematodes

The most important pest of faba bean is *Aphis fabae* which is a black aphid. This pest damages flowers and prevents the development of pods. Generally insecticides are used but *Aphis fabae* acts like as a vector of viruses and no useful resistance is known. Another important pest is *Sitona lineatus* which is a weevil. This pest feeds on the first, very young leaves. But more importantly, its larvae damage the plant by feeding on the root. This causes direct damage and also leads to root rot. *Bruchus* and *Callosobruchus* species also damage faba bean fields and stocks. They live in seeds generally in the Middle East. There is no information about the correlation between tannin content and resistance of pests. However, vicine and convicine content can influence colonization of faba bean by weevils[32].

In addition to aphids and beetles, nematodes can be a serious problem for faba bean. *Ditehlenchus dipsaci* and *Heterodera goettingiana* are stem and cyst nematodes, respectively. The cyst nematode is common in North Africa. Surprisingly, small seeded bean varieties are not preferred by nematodes and several resistant genotypes have been identified[33].

1.5.4. *Orobanche* Resistance

Numerous legume types are restricted by *Orobanche crenata* and this parasitic plant significantly restricts production of faba bean. Hand-weeding, herbicides like glyphosate, late sowing, and breeding are used to control *Orobanche*. There are also partially resistant types and several improved genotypes for example: Giza 402, Giza 429, Giza 674, Vf1071, Vf136 and Baraca. *Orobanche* resistance is a complex trait and QTLs for resistance have not yet been identified[1].

1.5.5. Biochemical Composition

Faba bean is used for human nutrition, animal and poultry feed because of its high protein and other essential nutrient content. *V. faba* is a source of cheap protein and energy in Africa, parts of Asia, and Latin America, where many people cannot buy meat. Like other legumes, faba bean is rich in lysine but is poor in sulfur amino acids and tryptophan[34].

There are some important metabolites which are not nutritional but affect taste or can stimulate disorders, like favism, in sensitive patients. Tannin can repress digestion system enzymes. Tannin content is controlled by the *zt-1* and *zt-2* genes. If a genotype is recessive for these genes, it does not accumulate tannin in its seed. There are some cultivars, such as Gloria, which have zero tannin[35].

Vicine and convicine accumulate in faba bean and they cause favism in people who have an inherited absence of the enzyme glucose-6-phosphate dehydrogenase in their red blood cells. *vc* mutation reduces the accumulation of vicine and convicine in *V. faba* by 20-fold[36]. Low vicine cultivars also exist, for example Melodie[35].

1.6. Importance of Genetic Diversity in Plant Breeding

Plant genetic resources are important for future food security. Crop germplasm diversity provides material to produce new improved crop cultivars. The total gene pool of a species includes landraces, advanced breeding lines, popular cultivars, and wild and weedy relatives. These are used as the raw material for any improvement program. Soil

and climatic variations, socioeconomic differences among regions and farmers induce evolution of landraces[37]. Variation and differentiation among landraces is contributed to the diversity of cropping systems. Nikolai Ivanovich Vavilov (1951)[38] recognized the importance of genetic diversity for improvement of crops and organized comprehensive germplasm collections from their origin. Since then, major crop germplasm collections have increased in the world[39].

Before modern plant breeding, farmers cultivated a large number of crop landraces. Due to modern plant breeding programs, within species diversity has been lost because uniform cultivars have taken the place of traditional varieties and landraces. Also, natural disaster, deforestation, road laying, environmental pollution, and change in dietary habits are other factors that negatively influence the situation. According to an estimate, approximately 75% of genetic diversity of crop plants was lost in the last century[40]. In addition, approximately 97% of U.S. Department of Agriculture listed germplasm has been lost in the last 80 years according to Rural Advancement Foundation International[40]. The diverse landraces, exotic and wild relatives not only have rich gene and allele content but also can help enhance some important agricultural traits in breeding programs.

Breeders prefer to use parental lines from their breeding study. Actually elite inbred lines are often thought to be the best genetic resources because each line contains a good combination of genetic traits[41]. Although plant breeders are aware of the lack of diversity in their working germplasm and the potential worth of wild and landrace, they do not tend to use such resources because of a lack of concrete information about the genetic value contained in large germplasm collections and because such material is known to contain many undesirable alleles.

There are two important expectations for genetic resources management: germplasm conservation and its utilization in crop development. There are several gene banks which store faba bean germplasm in the world including the collection at the Aegean Agricultural Research Institute in İzmir. These collections contain more than 38,000 faba bean accessions. Although there are 38,000 faba bean accessions, these individuals have not been characterized and have not been used to select core collections.

1.7. Ways of Studying Diversity

There are two major ways to study crop diversity: morphological and molecular analysis. These two techniques allow an understanding of the potential of species. During domestication some potentially important alleles can be lost, thereby reducing genetic diversity at the morphological, biochemical (protein), and molecular levels.

1.7.1. Morphological Markers

Faba bean morphological markers include plant height; fat, protein, and starch content; seed weight, color, shape, and size; number of pods per plant; number of seeds per pod; flowering time and days to maturity. These phenotypic traits give us valuable information about crop plant domestication. Morphological diversity is demonstrated by statistical comparisons of relationships among agronomical and morphological traits. Moreover, correlations can exist between these morphological traits. For example, there is a positive correlation between number of seeds per pod and the length of the pods or 100-seed weight whereas there is a negative correlation between days to flowering and days to maturity[17].

1.7.2. Molecular Markers

Molecular markers (DNA markers) show neutral sites of variation at the DNA level. 'Neutral' means that these variations do not reveal themselves in phenotype and each may be only a single nucleotide difference in a gene or repetitive DNA. This property and the fact that they are much more numerous than morphological markers are the primary advantages of molecular markers. In addition, such markers do not change with physiology of the organism. Table 1.1 shows the generally used marker systems and their advantages and disadvantages.

Simple sequence repeats (SSRs) are often used in plant studies because they are simple, PCR-based, highly polymorphic, and can be used to distinguish between closely related individuals. Plant genomes have large numbers of simple sequence repeats or microsatellites. Their size is 6 bp or smaller and they are tandemly repeated and distributed at numerous loci throughout the genome. Generally they are dinucleotides,

trinucleotides or tetranucleotides such as $(AC)_n$, $(AG)_n$, $(AT)_n$, $(TCT)_n$, $(TTG)_n$ or $(TATG)_n$ (n represents repeating units of microsatellite locus.). Plants are most abundant in $(AT)_n$ dinucleotides[42]. Different numbers of repeats between individuals produce polymorphisms which can be detected by electrophoresis after PCR amplification.

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Table 1.1.Summary of commonly used marker types [43].

Marker system	**Advantages**	**Disadvantages**
Restriction fragment length polymorphism (RFLP)	Co-dominant; highly reproducible.	High on time/labour.
Cleavage amplification polymorphism (CAP)	Insensitive to DNA methylation; no requirement for radioactivity.	Produces uninformative PCR products.
Random amplified polymorphic DNA (RAPD)	Low on time/labour.	Dominant; low reproducibility.
Amplified fragment length polymorphism (AFLP)	High reproducibility.	Dominant; moderate time/labour.
Simple sequence repeat (microsatellite) (SSR)	Co-dominant; highly reproducible; low on time and labour.	High cost of development.
Inter-simple sequence repeat (ISSR)	Technically simple; no prior genomic information needed to reveal both inter- and intraspecific variation.	Dominant markers; band staining can be weak.
Variable number tandem repeat (minisatellite) (VNTR)	Numerous multiallelic loci.	Low-resolution fingerprints in plants.
Sequence characterised amplification region (SCAR)	May be dominant or co-dominant; better reproducibility than RAPDs.	More difficult to reproduce than RAPDs.
Single nucleotide polymorphism (SNP)	Common; evenly distributed; detection easily automated; high throughput; low assay cost; useful for association studies.	Usually only two alleles present.
Expressed sequence tag (EST)	Easy to collect and sequence; reveals novel transcripts; good representation of transcripts.	Error-prone; isolation of mRNA may be difficult.
Sequence-related amplified polymorphism (SRAP)	Simplicity; high throughput; numerous co-dominant markers; high reproducibility; targets coding sequences; detects multiple loci without previous knowledge of sequence information; PCR products directly sequenced.	Detects co-dominant and dominant markers, which can lead to complexity; null alleles detected directly.
Target recognition amplification protocol (TRAP)	Simple to use; highly informative; produces numerous markers by using existing public EST databases; uses markers targeted to a specific gene.	Requires cDNA or EST sequence information for primer development.
Microarrays (arrangements of small spots of DNA fixed to glass slides)	Whole-genome scanning; high-throughput technology; genotype–phenotype relationship; expression analysis of large numbers of genes.	Expensive; needs gene sequence data; technically demanding.

Molecular markers are also used for studies of genetic diversity. Various genetic marker systems have been used in explaining the genetic diversity and relationships between accessions in faba bean *ex situ* germplasm collections such as restriction fragment length polymorphism (RFLP)[44], random amplified polymorphic DNA (RAPD)[44-45], amplified fragment length polymorphism (AFLP)[46], and inter-simple sequence repeat (ISSR)[47] markers. Molecular markers have been reported for faba bean (Table 1.2), but they are very limited when compared to other major crop species.

Table1.2. Summary of molecular markers for genetic diversity in faba bean.

Marker type	**Main output**	**Reference**
RFLP	Based on molecular markers, created a linkage map between genetically diverse faba bean parents	Van de Ven et al. (1990)[48]
RAPD	Classificationand identification of divergent heterotic groups	Link et al. (1995)[45]
SSAP	Determination geographical origins of *V. faba* genotypes	Sanz et al. (2007)[49]
AFLP	Validation of relationships	Zeid et al. (2003)[46a]
	Genetic diversity assessment of Chinese faba bean	Zong et al. (2009, 2010)[50]
ISSR	Determination of genetic variation among eight faba bean genotypes according to drought tolerance trait	Al-Ali et al. (2010)[51]
	Determination of Mediterranean faba bean genetic diversity	Terzopoulos and Bebeli (2008)[47]

Although molecular markers have been extensively used in crop breeding programs, there are really limited studies for faba beans. Faba bean has an enormous genome size so development of DNA markers is difficult. For example, Pozarkova et al. (2002)[52], Gong et al. (2010)[53], and Ma et al. (2011)[54], developed only 25, 11 and 21 SSR markers, respectively. Generally RAPD markers are used in faba bean genome mapping and QTL detection studies. But this marker system is dominant and has low reproducibility.

1.8. Aim of the Study

Faba bean is an important legume for human consumption and animal feed because of its high protein and starch content. Because faba bean has nitrogen fixation bacteria, it is an ideal rotation crop. More than 38000 faba bean accessions are housed in gene banks but there is not enough work studying its genetic diversity and breeding. In part this is due to the huge genome of faba bean (13 GB). Genetic diversity is important for maintaining variability in plant populations. When environmental conditions change, high variability of the gene pool provides a greater ability to adapt to such changes. In this study, a natural faba bean population was used because natural populations allow the possibility of sampling more different alleles than biparental populations. Moreover, the selection of parental lines for breeding studies from thousands of available germplasm accessions is very difficult. Therefore, it is useful to have a core set of accessions which represents all of the genetic diversity present in the population. In this study, genetic diversity and population structure of 259 faba bean accessions were determined. In addition, a core collection of genotypes for future breeding programs was constructed.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Materials

Plant materials (seeds) were provided by the International Center for Agricultural Research in the Dry Areas (ICARDA), Centre for Genetic Resources (CGN), Aegean Agricultural Research Institute (AARI), Nordic Gene Bank (NGB), and Australia (The University of Adelaide, Jeffrey Paull). A total of 259 different genotypes were tested genotypically. The individuals used in this study are listed in Table 2.1.

Table 2.1. Plant individuals (*Vicia faba*) used in this study.

Sample Name	**Sample No**	**Origin**	**Sample Name**	**Sample No**	**Origin**
NGB8642	1	Finland	CGN7912	131	Ethiopia
NGB1540.1	2	Finland	CGN07875	132	India
NGB8643	3	Finland	CGN10387	133	Turkey
Melodie/2	4	France	CGN10385	134	Turkey
Mikko	5	Finland	CGN10383	135	Turkey
WitkiemManida	6	Germany	CGN10386	136	Turkey
Ukko	7	Germany	CGN10373	137	Syrian Arab Republic
Kontu	8	Germany	CGN18884	138	Ethiopia
NGB1550.1	9	Finland	CGN18880	139	Syrian Arab Republic
NGB1547.1	10	Finland	CGN18883	140	Ethiopia
NGB1552.1	11	Finland	CGN7735	141	Ethiopia
NGB1542.1	12	Finland	CGN19981	142	Italy
NGB1548.2	13	Finland	CGN10374	143	Syrian Arab Republic
NGB1546.2	14	Finland	CGN10325	144	Syrian Arab Republic
NGB20019.2	15	Finland	CGN18933	145	Israel
NGB8640	16	Finland	CGN18920	146	Finland
CGN10330	17	Ethiopia	CGN18923	147	France
CGN7874	18	Spain	CGN18856	148	Jordan
CGN13486	19	Pakistan	CGN15639	149	Australia
CGN7699	20	unknown	CGN7715	150	unknown
CGN10322	21	Turkey	CGN7710	151	Ethiopia

(Cont. on the next page)

Table 2.1. (cont.)

CGN7697	22	unknown	CGN15615	152	Ethiopia
CGN15563	23	Syrian Arab Republic	CGN10314	153	Egypt
CGN15619	24	Egypt	TR12123	154	Turkey
CGN10390	25	Lebanon	TR12271	155	Turkey
CGN19986	26	Netherlands	TR12540	156	Turkey
CGN19987	27	Turkey	TR12611	157	Turkey
CGN13487	28	Pakistan	TR12716	158	Turkey
CGN13485	29	Pakistan	TR12767	159	Turkey
CGN13464	30	United Kingdom	TR12773	160	Turkey
CGN13451	31	Syrian Arab Republic	TR22992	161	Turkey
CGN13450	32	Syrian Arab Republic	TR23018	162	Turkey
CGN13445	33	Germany	TR26194	163	Turkey
CGN12329	34	Ethiopia	TR26250	164	Turkey
CGN19993	35	Netherlands	TR26265	165	Turkey
CGN19995	36	unknown	TR26298	166	Turkey
CGN13518	37	Netherlands	TR26453	167	Turkey
CGN12321	38	Greece	TR26462	168	Turkey
CGN12309	39	Canada	TR26578	169	Turkey
CGN10391	40	Egypt	TR28096	170	Turkey
CGN7843	41	Italy	TR31590	171	Turkey
CGN10321	42	Turkey	TR31912	172	Turkey
CGN10344	43	Pakistan	TR31973	173	Turkey
CGN10347	44	Egypt	TR32925	174	Turkey
CGN10320	45	Turkey	TR33058	175	Turkey
CGN7826	46	Greece	TR33140	176	Turkey
CGN7827	47	Ethiopia	TR33421	177	Turkey
CGN19982	48	Netherlands	TR33517	178	Turkey
CGN07836	49	Ethiopia	TR33547	179	Turkey
CGN7839	50	Germany	TR33561	180	Turkey
CGN7842	51	unknown	TR35023	181	Turkey
CGN12320	52	Greece	TR35330	182	Turkey
CGN7716	53	Italy	TR37028	183	Turkey
CGN7844	54	Jordan	TR37041	184	Turkey
CGN10324	55	Spain	TR37200	185	Turkey
CGN7949	56	Spain	TR37255	186	Turkey
CGN07781	57	Netherlands	TR38142	187	Turkey
CGN07933	58	Afghanistan	TR39064	188	Turkey
CGN7848	59	Afghanistan	TR40217	189	Turkey
CGN7739	60	Egypt	TR40725	190	Turkey
CGN7728	61	unknown	TR42343	191	Turkey
CGN7872	62	Afghanistan	TR43564	192	Turkey
CGN15556	63	Ethiopia	TR44862	193	Turkey
CGN7725	64	unknown	TR44869	194	Turkey
CGN07734	65	Ethiopia	TR44876	195	Turkey
CGN18860	66	Netherlands	TR44914	196	Turkey
CGN18863	67	Netherlands	TR44928	197	Turkey
CGN18862	68	Netherlands	TR44931	198	Turkey
CGN15620	69	Ethiopia	TR44941	199	Turkey

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Table 2.1. (cont.)

CGN7950	70	China	TR46010	200	Turkey
CGN18893	71	Belgium	TR49377	201	Turkey
CGN18892	72	Netherlands	TR49380	202	Turkey
CGN7740	73	Egypt	TR49381	203	Turkey
CGN7757	74	Greece	TR49386	204	Turkey
CGN18906	75	United Kingdom	TR49387	205	Turkey
CGN7751	76	unknown	TR49388	206	Turkey
CGN15644	77	Spain	TR51377	207	Turkey
CGN18867	78	unknown	TR51378	208	Turkey
CGN15621	79	unknown	TR53648	209	Turkey
CGN7934	80	Ethiopia	TR53667	210	Turkey
CGN7938	81	Afghanistan	TR53683	211	Turkey
CGN15641	82	Netherlands	TR53724	212	Turkey
CGN07871	83	Afghanistan	TR53748	213	Turkey
CGN7766	84	unknown	TR53761	214	Turkey
CGN10315	85	United States of America	TR53770	215	Turkey
CGN18878	86	Germany	TR53781	216	Turkey
CGN7939	87	Afghanistan	TR53797	217	Turkey
CGN7940	88	Afghanistan	TR53947	218	Turkey
CGN10382	89	Turkey	TR53948	219	Turkey
CGN10313	90	Egypt	TR53949	220	Turkey
CGN18909	91	Germany	TR61267	221	Turkey
CGN7729	92	Germany	TR61300	222	Turkey
CGN7730	93	Czechoslovakia	TR71255	223	Turkey
CGN7738	94	Egypt	TR71521	224	Turkey
CGN7733	95	Iraq	TR71612	225	Turkey
CGN7948	96	Netherlands	TR74159	226	Turkey
CGN7727	97	United Kingdom	TR74184	227	Turkey
CGN7736	98	Italy	TR74214	228	Turkey
CGN7717	99	Ethiopia	TR74330	229	Turkey
CGN10371	100	Algeria	TR74337	230	Turkey
CGN10384	101	Turkey	TR75408	231	Turkey
CGN7947	102	Sri Lanka	TR75421	232	Turkey
CGN18927	103	Ethiopia	TR75431	233	Turkey
CGN7946	104	Lebanon	TR77139	234	Turkey
CGN7721	105	unknown	TR77143	235	Turkey
CGN19985	106	unknown	TR78048	236	Turkey
CGN10362	107	Turkey	TR79569	237	Turkey
CGN7752	108	Ethiopia	TR80277	238	Turkey
CGN7732	109	Iraq	ASCOT	239	Australia
CGN10329	110	Ethiopia	MANAFEST	240	Australia
CGN07873	111	Afghanistan	NURA	241	Australia
CGN7719	112	Italy	PBA RANA	242	Australia
CGN7931	113	Poland	FIORD	243	Australia
CGN18942	114	China	ICARUS	244	Australia
CGN18895	115	Netherlands	FIESTA	245	Australia
CGN7932	116	Ethiopia	FARAH	246	Australia
CGN18934	117	United Kingdom	AUADULCE	247	Australia
CGN18888	118	Turkey	Kıtık2003	248	Turkey

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Table 2.1. (cont.)

CGN19977	119	Spain	Eresen87	249	Turkey
CGN18856	120	Jordan	Filiz99	250	Turkey
CGN18855	121	Spain	Salkım	251	Turkey
CGN18941	122	Germany	BPL710	252	Colombia
CGN18892	123	Netherlands	Elizar	253	Lebonan
CGN18873	124	Pakistan	Lattaka2	254	unknown
CGN18905	125	Austria	Giza4	255	Egypt
CGN07723	126	Netherlands	ILB93812	256	unknown
CGN19979	127	Spain	Aurora/2	257	Sweden
CGN19987	128	Turkey	Melodie/2	258	France
CGN7895	129	Ethiopia	Disco/2	259	unknown
CGN7907	130	Afghanistan			

2.2. Methods

2.2.1. DNA Extraction

Genomic DNA extraction of faba bean plants was performed from the youngest and the lightest green leaves. DNA extraction was carried out using the Wizard Magnetic 96 Plant System (Promega Corp., Madison, WI, USA) with the Beckman Coulter Biomek NX Workstation (Beckman Coulter, Brea, CA, USA) according to the manufacturer's instructions. After extraction, all DNA samples were dissolved and homogenized in TE buffer. DNA quantity and quality were measured using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific™, Vantaa, Finland). All DNA samples were stored at -20°C.

2.2.3. Molecular Marker Analysis

2.2.3.1. SSR Analysis

According to DNA quantity and quality results, all samples were diluted to a final concentration of 10 ng/µl. For SSR analysis, 33 SSR primer pairs (GBSSR-VF and VfG) were selected to implement to the faba bean population. The faba bean SSR markers are listed in Table 2.2. GBSSR-VF (14) and VfG (19) primer information was obtained from Suresh et al. (2013)[55] and Zeid et al. (2009)[56], respectively.

Table 2.2. Simple sequence repeats (SSRs) markers which were used in this study [55, 56].

Primer name	Forward primer sequences (5'-3')	Reverse primer sequences (5'-3')	Annealing temperature
GBSSR-VF-8	TAAAGCAGCTCCGGATGA	TCGGTGGAGGAGTTGTTG	55 ^{o}C
GBSSR-VF-19	TCCATCAACCTCAAATCCA	CCGTACTTGTCCACGGAA	55 ^{o}C
GBSSR-VF-20	TCCACCAAGTCCACCTGA	AATAAGGGCGCAGGAGAG	55 ^{o}C
GBSSR-VF-22	CGAAGCCTCCTCCTCTTC	CAAGTGGCCGTTTTTCAA	57 ^{o}C
GBSSR-VF-52	GGTTTCTTGTCCAAATAAGACG	TGCGATTCTGGAAATTGG	56 ^{o}C
GBSSR-VF-113	TGGTGGTGCTTCTTTCCA	TGGTGAGCTTGGAACTGC	55 ^{o}C
GBSSR-VF-115	TGCTGCTTTTCCAACCAT	GTGCATGCCATAACAAAA	55 ^{o}C
GBSSR-VF-119	GTGGCCTGTACTGGTGGA	ACTCGTTGGGGCTAGGAA	57 ^{o}C
GBSSR-VF-131	CCGTACTAAATGAAGCCTTT	GGCAATCAAGTCCGGTAA	55 ^{o}C
GBSSR-VF-149	ACGACATGGTGATGAATCCT	ACGTGACCGAGTGACGAC	57 ^{o}C
GBSSR-VF-153	TCCCGACGCTACTTCTCA	CCGAGATCTGCAAACAGC	55 ^{o}C
GBSSR-VF-154	ACACCAATGTTTTTGCGG	TCCTGACTTTGCTGAGGC	55 ^{o}C
GBSSR-VF-159	GTGCCATCATCCTCGAAA	CAGCTGCTAGGTTGCCTG	57 ^{o}C
GBSSR-VF-164	ACCATTTGGCCTGTTCCT	CAAGGAGGGTTGTTTACGA	55 ^{o}C
VfG 1	TTTCAGCAAACTAGAACCAATC	GGCATTCAGTTTTTACCTTGTA	50 ^{o}C
VfG 3	TTCTTTGGTCCTCTCTCTATC	GCACTGTTGTTGCTGATACAA	51 ^{o}C
VfG 4	AAGGGGAGGGCATAACAGAA	AATCCGCAAGGGTCTTCTT	52 ^{o}C
VfG 9	GGTTTTGAATAGAAATGCAA	AAGATGTGTCAATATTGTTTT	50 ^{o}C
VfG 10	ACCAAAACGCGCACTTATCA	AAGAGAGAGAAGAGAGCTTC	50 ^{o}C
VfG 11	GCAAAAGGAGAGCAAGGGAA	CGAAAGAGGGGGACATTTTGT	52 ^{o}C
VfG 13	GGTTGGGATCTTTTAGGTTGAA	TGGCCTTATATCCGTCCAAT	51 ^{o}C
VfG 15	TCGATAGGGTTTCAGATTGA	GATGTTGACGGTGGTGTTT	50 ^{o}C
VfG 19	AGCGATGGTGCTCATGCTTA	TCTCTCACGGAATCACATCTTT	52 ^{o}C
VfG 27	CCCAAAAAGAGACGAACTGTAT	AGGGTTCATACGTTTGGCTT	51 ^{o}C
VfG 31	ATAAGAGAGAACGAGGGAGAA	TTATGGTGGGACGTCTTACAT	51 ^{o}C
VfG 34	GCACTCGAAGGAATTAATTTT	GAACAGTTGTTTCGTGTCGTA	51 ^{o}C
VfG 41	AGCCCATGGTTCAAATGCAA	GCAGTCATGCCACTGCTTA	51^{o}C
VfG 44	GATGTTGTTGGTGTTGTTTA	CAATTAGGAGCAAAATCAGA	50 ^{o}C
VfG 47	CGATTGTTTGCAGAGGAGATA	ACAGAGAGGGACAGAGAGAA	52 ^{o}C
VfG 53	GGTTCATGAAAAGAGGTTAG	CATTTTCCGTTCTCTCTCTA	50 ^{o}C
VfG 67	GTTCATCAAGCACCAATCTAAAC	TCAATTTGGTTTATCTCTCTCTCT	52 ^{o}C
VfG 69	ATTGGGGAGGATGAAGGTT	TTCCATTTTCCGTTCTCTCT	50 ^{o}C
VfG 87	AGGGCCAGCGTGATCCAATA	TGGGTTGGGATCTTTTGGTTG	53 ^{o}C

Polymerase chain reaction (PCR) was performed with the following components in total of 20 µl volume for GBSSR-VF: 2 µl 10X PCR buffer (50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, pH: 8.3), 1 µl $MgCl_2$, 0.75 µl dNTP (0.2 mM), 0.75 µl

forward and 0.75 µl reverse primers (10 pmol), 0.75 µl *Taq* polymerase (0.25 U), 13 µl sterile double-distilled water, and 1 µl DNA (~10 ng/µl). To amplify DNA, PCR conditions were optimized as follows: one step of 10 min at 94°C for denaturation, 35 cycles with 30 sec at 95°C, 30 seconds at 55-57°C annealing temperature (Table 2.2) (depending on primer pair), 30 sec at 72°C for extension, and a final extension step of 10 min at 72°C in BIO-RAD Thermal Cycler™ (BIO-RAD, California, USA).

PCR was performed with the following components in total of 20 µl volume for VfG: 2 µl 10X PCR buffer (50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, pH: 8.3), 1.2 µl $MgCl_2$, 0.5 µl dNTP (0.2 mM), 1.5µl forward and 1.5 µl reverse primers (10 pmol), 0.5 µl *Taq* polymerase (0.25 U), 11.3µl sterile double-distilled water, and 1.5 µl DNA (~15 ng/µl). To amplify DNA, PCR conditions were optimized as follows: one step of 4 min at 94°C for denaturation, 30 cycles with 45 sec at 95°C, 1 min at 50-53°C annealing temperature (Table 2.2) (depending on primer pair), 1 min at 72°C for extension, and a final extension step of 7 min at 72°C in BIO-RAD Thermal Cycler™.

After PCR reactions, a Fragment Analyzer™ Automated CE System (Advanced Analytical Technologies, Inc., Ames, USA) was utilized to separate DNA fragments. This system has high resolution and can separate 3 bp differences between amplified fragments

2.2.3.2. Diversity Analysis

For determination of genetic diversity of the population, DARwin5 (Dissimilarity Analysis and Representation for Windows) was used. The allelic data were used for calculation of distance matrix using Dice's coefficient and clustering analysis with UnWeighted Neighbor-Joining algorithm.

2.2.3.3. Population Structure Analysis

Allelic data obtained from fragment analysis were scored according to band presence/absence. To detect the correct number of subpopulations explaining population structure, STRUCTURE 2.2.3 was used[57]. STRUCTURE is a clustering program. The analysis in STRUCTURE was run with parameters of burn-in period of 100,000 and 500,000 MCMC replications and a hoc statistic introduced by Evanno et al. (2005)[58]

was used to determine the correct estimated number of clusters with STRUCTURE HARVESTER online program.

Determination of population structure was done using the STRUCTURE program. The STRUCTURE HARVESTER program was used to find the correct number (K) of subpopulations. K was tested from 2 to 10 with 20 iterations for each group. ΔK was used to determine the most correct cluster number. If ΔK value is high, probability of population cluster number is the most correct

2.2.3.4. Core Collection

PowerCore (v.1.0) was used for construction of a core collection [59]. This program uses a heuristic search for establishing a core set based on the SSR marker data.

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1. DNA Extraction

DNA quantities and quality values were measured with Nanodrop ND-1000 spectrophotometer (Data not shown).

3.2. Molecular Marker Analysis

3.2.1. SSR Analysis

Allelic polymorphisms were determined using 32 SSR primer pairs[55-56] for the 259 individuals. All markers, except GBSSR-VF-113, VfG 31, and VfG 34, were polymorphic for the population. A total of 305 polymorphic loci were obtained for the 259 individuals (Table 3.1). Thus, each SSR primer combination gave an average of 8 polymorphic fragments.

Table 3.1.Number of polymorphic bands and amplified bands.

Primer Name	Polymorphic Bands	Amplified Bands
GBSSR-VF-8	10	10
GBSSR-VF-19	4	4
GBSSR-VF-20	5	5
GBSSR-VF-22	7	7
GBSSR-VF-52	7	7
GBSSR-VF-113	3	4
GBSSR-VF-115	5	5
GBSSR-VF-131	7	7
GBSSR-VF-149	7	7
GBSSR-VF-153	3	3
GBSSR-VF-154	4	4

(Cont. on the next page)

Table 3.1. (cont.)

GBSSR-VF-159	2	2
GBSSR-VF-164	2	2
VfG 1	18	18
VfG 3	9	9
VfG 4	3	3
VfG 9	13	13
VfG 10	13	13
VfG 11	11	11
VfG 13	10	10
VfG 15	12	12
VfG 19	12	12
VfG 27	15	15
VfG 31	15	16
VfG 34	10	11
VfG 41	16	16
VfG 44	13	13
VfG 47	15	15
VfG 53	16	16
VfG 67	12	12
VfG 69	12	12
VfG 87	11	11
Total bands	**302**	**305**

3.2.2. Diversity Analysis

A total of 255 individuals were analyzed with this program. Four individuals were omitted from the analysis because they had too much missing data. Thus, genotypes 183, 184, 245, and 258 were excluded. The maximum genetic dissimilarity between genotypes was 0.593 (59%) for genotypes 259 (Disco/2) and 23 (CGN15563). The minimum genetic dissimilarity (0.170 (17%)) was determined between genotypes 243 (FIORD) and 241 (NURA). The 254 units gave 32385 distance values with a mean value of 0.394 (Figure 3.1). A Mantel test gave a high correlation (r = 0.9062) between the dissimilarity matrix and dendrogram.

According to the UnWeighted Neighbor-Joining analysis, two clusters and one outgroup were obtained (Figure 3.2). Group A had 77 individuals, Group B had 177 individuals. The outgroup was genotype 89 (CGN10382)

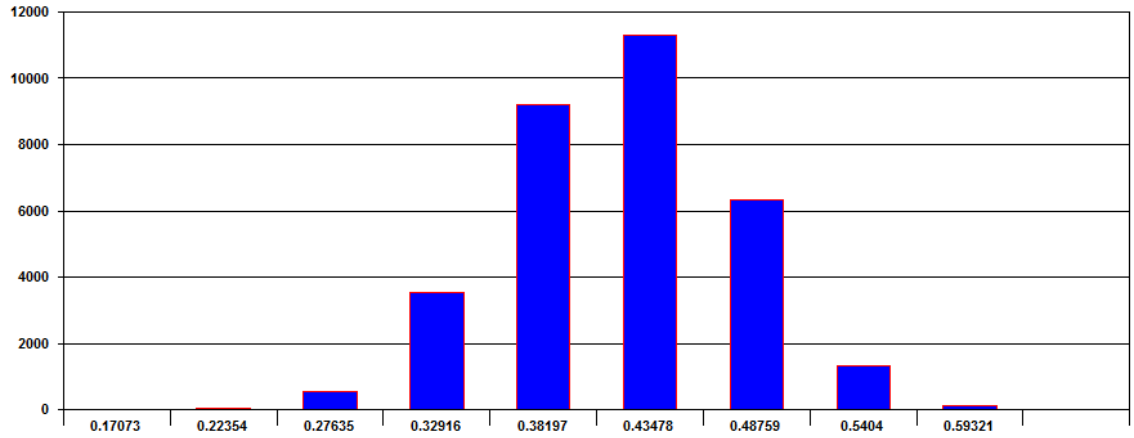

Figure3.1. Genetic dissimilarity between individuals of the faba bean population. X axis gives dissimilarity values of individuals and Y axis gives number of values.

According to the dendrogram (Figure 3.2), individuals demonstrated regional aggregation. Most of the Turkish varieties provided by AARI clustered together. Australian individuals also clustered together with Turkish individuals. Accessions from the Netherlands clustered together; all of these individuals were cultivars except for genotype 96. Accessions from Finland did not show tight clustering but they generally fell into the same main cluster. Individual from Afghanistan were found in both cluster A and cluster B. Ethiopian individuals also did not show tight clustering.

In addition, the dendrogram generally showed continental aggregation. Cluster A had individuals from European countries including the Netherlands, UK, Germany, Belgium, Greece, Spain, and Finland. Cluster B had individuals from Asian and African countries including Turkey, Afghanistan, Syria, Lebanon, Algeria, Ethiopia, Egypt, Jordan, and China. Australian individuals also grouped in cluster B indicating that these individuals could be originated from Asia.

The dendrogram was also analyzed to determine if there was any clustering based on seed size. This is important because there are four subspecies (*major*, *equina*, *minor*, and *paucijuga*) of faba bean based on origin and seed size, as can be seen from Figure 3.3, Cluster A tended to contain small and medium seeded types as is typical for genotypes from Northern Europe, Ethiopia, Afghanistan, and the Middle East. Similarly, Cluster B1 was composed of mostly small seeded types from Ethiopia and Afghanistan. Cluster B3 contained mostly medium and large seeded types and originated from Turkey and Australia. Large seeded types are typical of Mediterranean

countries like Turkey. It is also not surprising that the Australian genotypes fell into this cluster as they are all cultivars and, therefore, may have been bred for large seeds. In contrast Cluster B2 contained genotypes of all seed sizes and were from geographically diverse locations. Although there was a general correlation between seed size and origin, definite conclusions cannot be made as the subspecies identities of the faba bean genotypes studied in this work are not known.

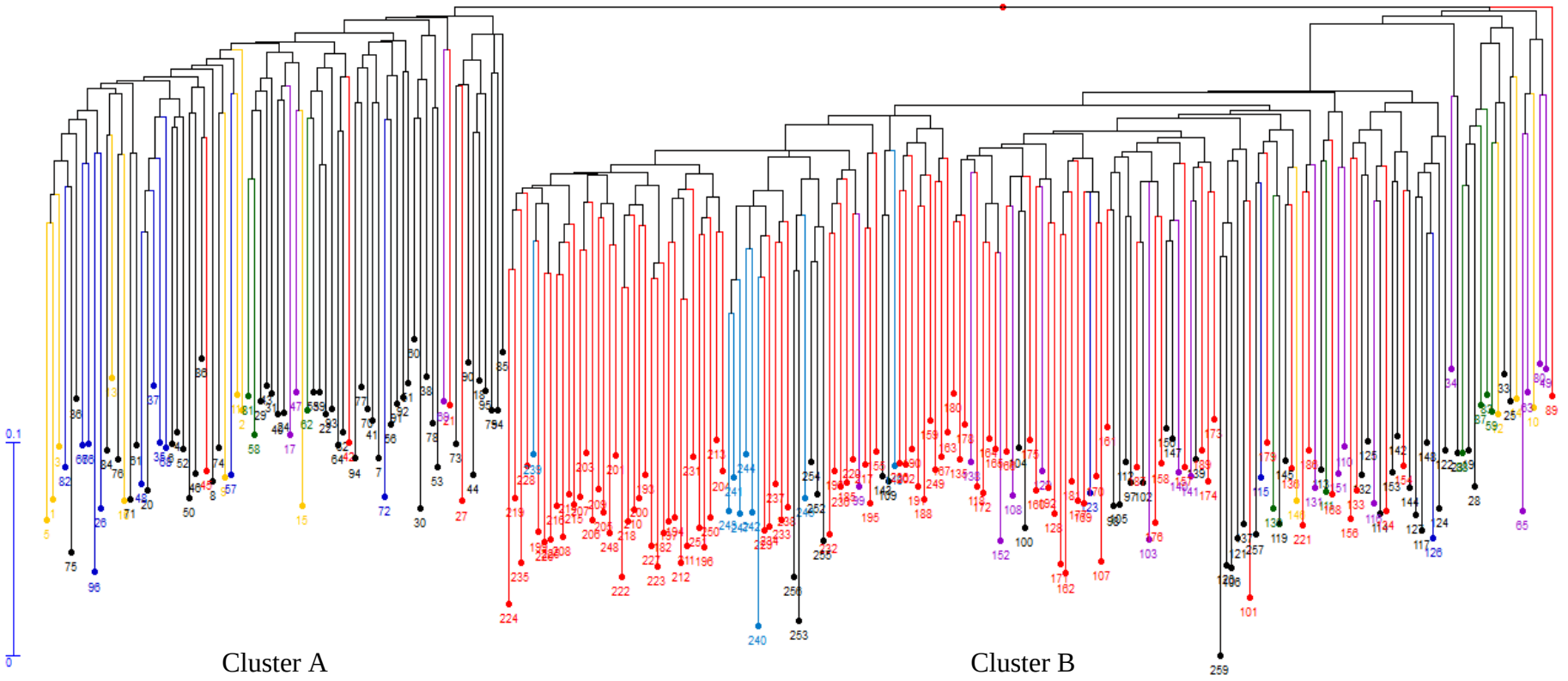

Figure 3.2.Dendrogram showing genetic diversity of the faba bean genotypes. Red, dark blue, light blue, yellow, green, and purple represent the regions with the most abundant accessions: Turkey, the Netherlands, Australia, Finland, Afghanistan, and Ethiopia, respectively. All other genotypes are in black.

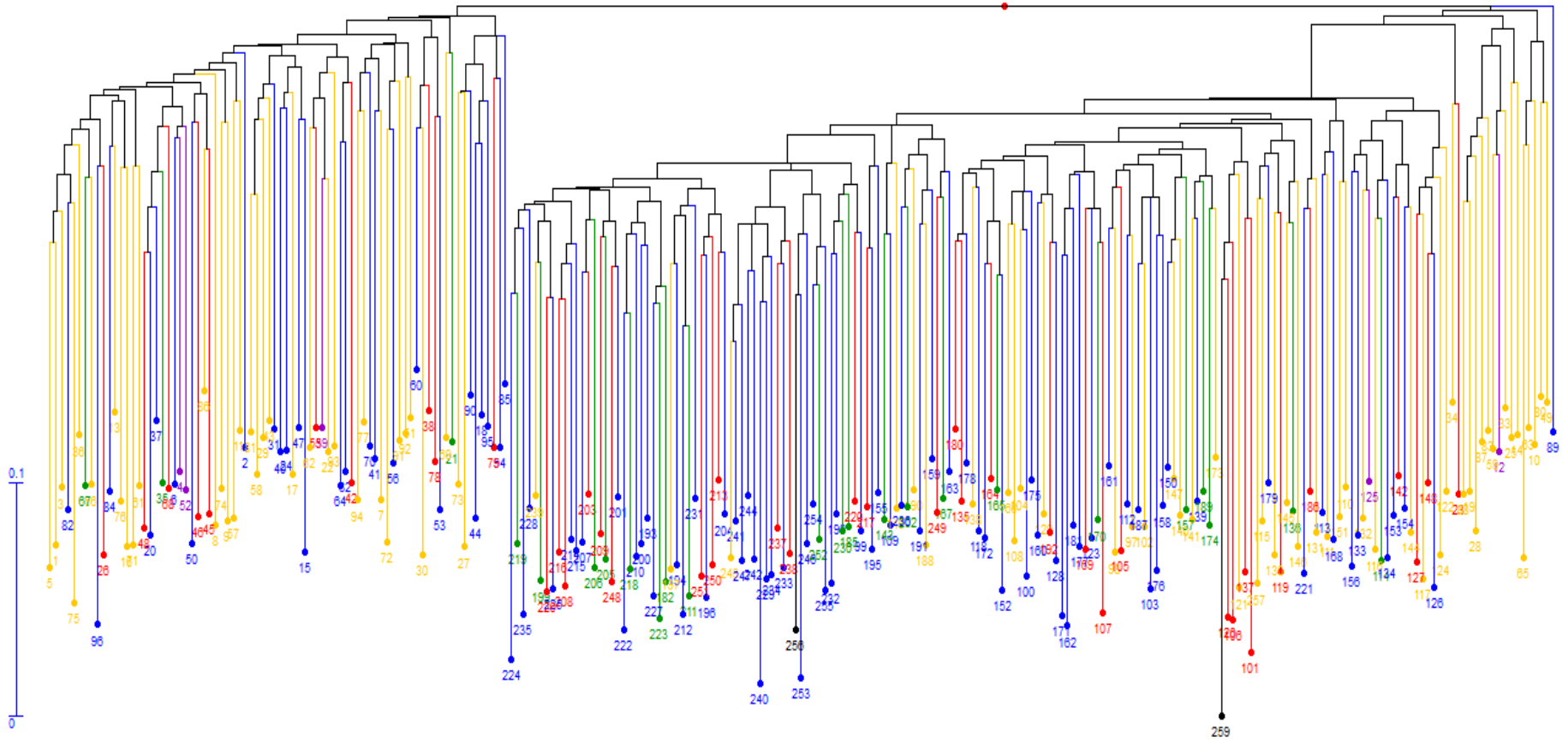

Figure3.3. Dendrogram showing seed size diversity of the faba bean genotypes. Yellow, blue, red, green, and purple represent small, medium, large, small/medium, and medium/large, respectively.

Principal coordinate analysis (PCoA) results are shown in Figure 3.4. Turkish and Australian genotypes were generally tightly clustered. But genotypes from the Netherlands, Finland, Afghanistan, and Ethiopia did not show similar tight grouping. According to this analysis, genotypes from Turkey and Australia showed low genetic diversity whereas those from the Netherlands, Finland, Afghanistan, and Ethiopia had higher genetic diversity. Some of the Turkish individuals were closely related to Ethiopian individuals. Genotypes from the Netherlands and Finland showed close grouping as also seen in the dendrogram (Figure 3.2).

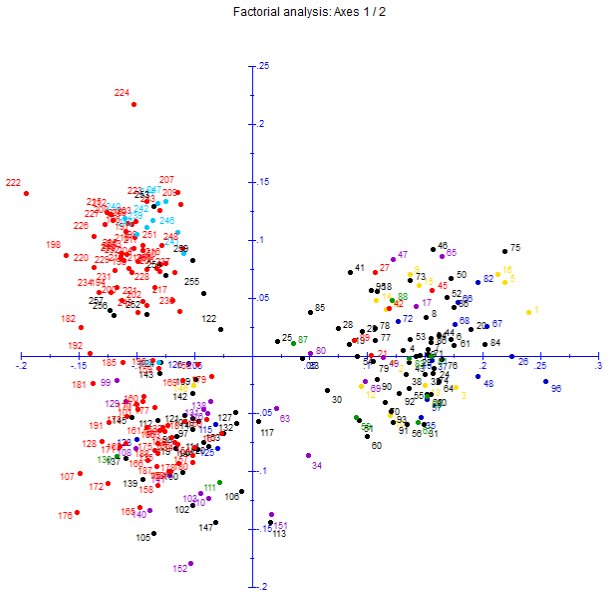

Figure 3.4. PCoA for all faba bean individuals. Red, dark blue, light blue, yellow, green, and purple represent genotypes from the most abundant regions: Turkey, the Netherlands, Australia, Finland, Afghanistan, and Ethiopia, respectively.

Landraces (154 individuals) and cultivars (105 individuals) were separately analyzed by UnWeighted Neighbor Joining analysis (Figures 3.5 and 3.7, respectively). One individual was omitted from each analysis because of missing data: genotypes 245 and 183 for cultivars and landraces, respectively. When we compared genetic dissimilarities between cultivars and landraces, cultivars (40%) were more diverse than landraces (38%) but this difference was not significant (2%). In general, it is believed that the genetic diversity present in cultivars is more limited than that in landraces;

however, this was not observed in our sample of faba bean genotypes. This may be because faba bean has a very large genome and has not undergone extensive breeding efforts. Therefore, the genetic diversity present in landraces has not yet been lost due to breeding.

When landraces were examined, minimum genetic dissimilarity between all landraces was determined between genotypes 178 (TR33517) and 180 (TR33561) and was 0.184 (18%). The maximum genetic dissimilarity was determined between 162 (TR23018) and 96 (CGN7948) and was 0.584 (58%). Mean value was determined as 0.380. The cophenetic r was 0.9102.

The most abundant landraces' genetic dissimilarities were separately analyzed by region using UnWeighted Neighbor Joining analysis. These landrace regions were Turkey (97 individuals), Ethiopia (10 individuals), Afghanistan (9 individuals), Syria (7 individuals), and Pakistan (5 individuals) (Table 3.2). Three main clusters were obtained. Minimum dissimilarity was observed for Turkish individuals. Maximum genetic dissimilarity was obtained for Turkish and Pakistani individuals. When mean dissimilarity values were compared, Pakistan and Syria had the highest value compared to other landraces. Faba bean is reported to have originated in the Near East[1] and then to have spread to Europe via Anatolia. Thus, it is not surprising that the highest levels of diversity were observed in Pakistan and Syria.

Table 3.2.Most abundant landrace regions and genetic dissimilarity values.

Region	Minimum dissimilarity	Maximum dissimilarity	Mean value	Fit criterion
Turkey	0.184 (18%)	0.536 (54%)	0.353	0.898
Ethiopia	0.255 (25%)	0.454 (45%)	0.372	0.919
Afghanistan	0.222 (22%)	0.458 (46%)	0.360	0.984
Syria	0.295 (29%)	0.476 (48%)	0.391	0.964
Pakistan	0.280 (28%)	0.534 (53%)	0.392	0.982

Most landraces were from Turkey (97 individuals) and they clustered together as observed when all genotypes were studied. The landraces of Afghanistan also

demonstrated tight clustering. According to the dendrogram (Figure 3.5), all other landraces did not show tight clustering and were found in both Clusters A and B.

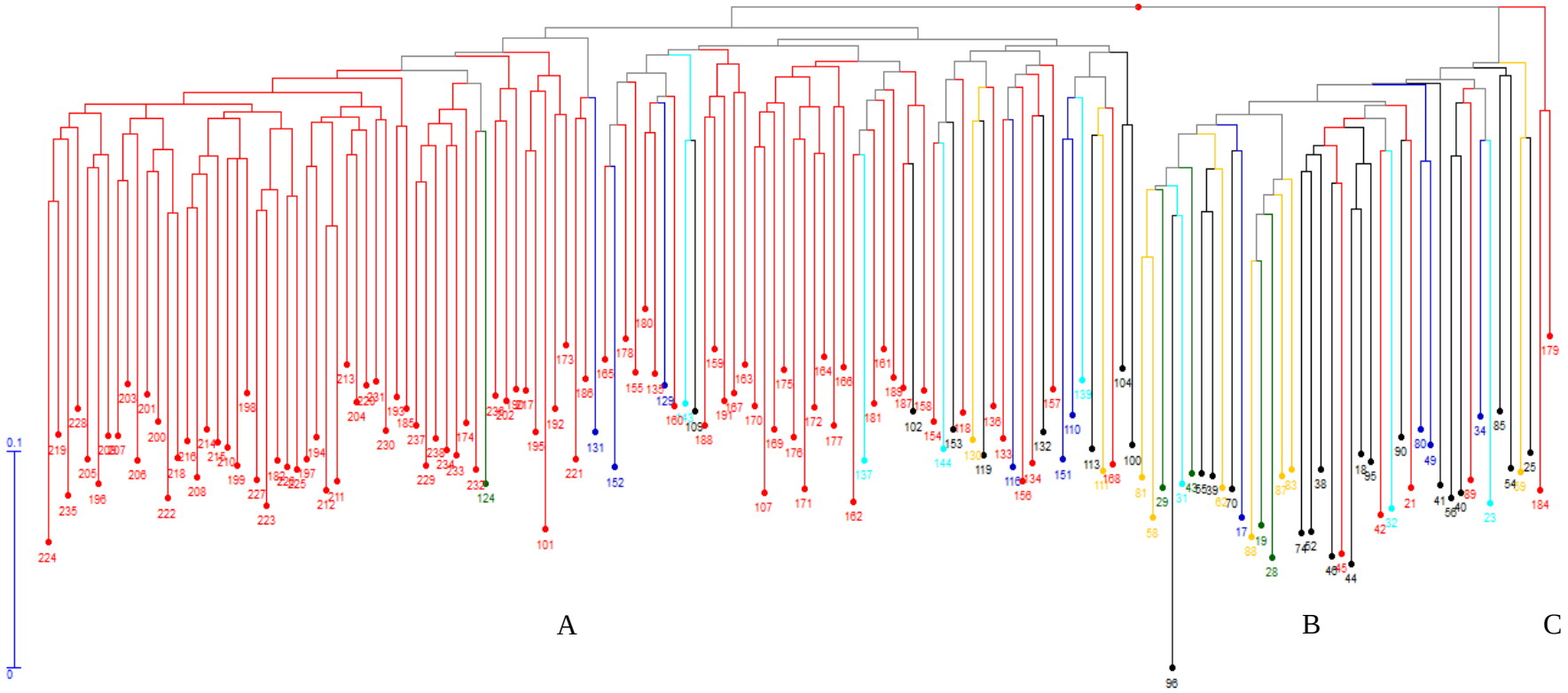

Figure 3.5.Dendrogram showing genetic diversity of the landraces. Red, dark blue, light blue, yellow, and green represent genotypes from the most abundant regions: Turkey, Ethiopia, Syria, Afghanistan, and Pakistan, respectively.

The PCoA (Figure 3.6) showed the same pattern as the dendrogram (Figure 3.5). Turkish and Afghan landraces had lower genetic diversity than those from Ethiopia, Syria, and Pakistan. Some Turkish and Syrian landraces were closely related to each other as may be expected because of the geographical proximity of the two countries. Afghan landraces were grouped with Syrian, Ethiopian, and Pakistani landraces.

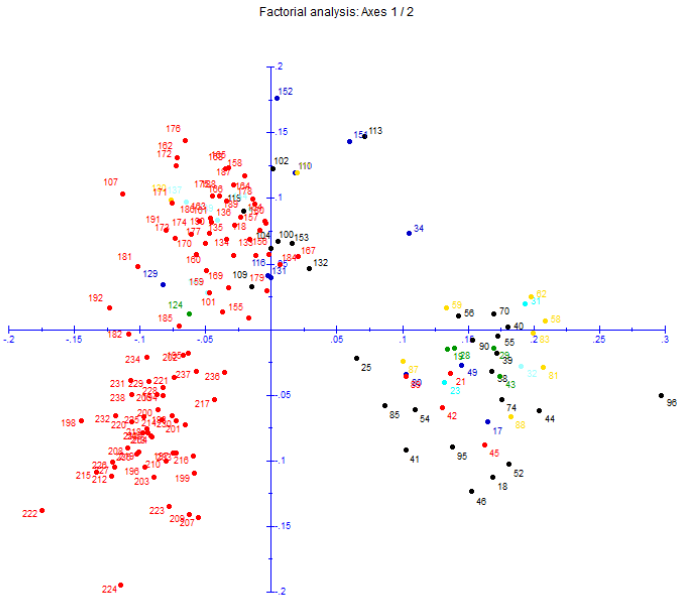

Figure 3.6. PCoA for all landraces. Red, dark blue, light blue, yellow, and green represent genotypes from the most abundant regions: Turkey, Ethiopia, Syria, Afghanistan, and Pakistan, respectively.

The minimum genetic dissimilarity between all cultivars was determined between genotype 243 (FIORD) and 241 (NURA) which were 0.170 (17%) dissimilar. The maximum genetic dissimilarity was between 26 (CGN19986) and 258 (Melodie/2) and was 0.593 (59%). Mean dissimilarity for the cultivars was 0.403. The cophenetic r was 0.9068. According to the dendrogram (Figure 3.7), cultivars generally did not show tight grouping. Only cultivars from Australia and the Netherlands grouped in the same main clusters which were cluster A and cluster B, respectively. The results demonstrated that, in general, origin was not an important factor for clustering of the cultivars except for those from Australia and the Netherlands. These results suggest that cultivars from these two countries have a narrower genetic base than those from other regions.

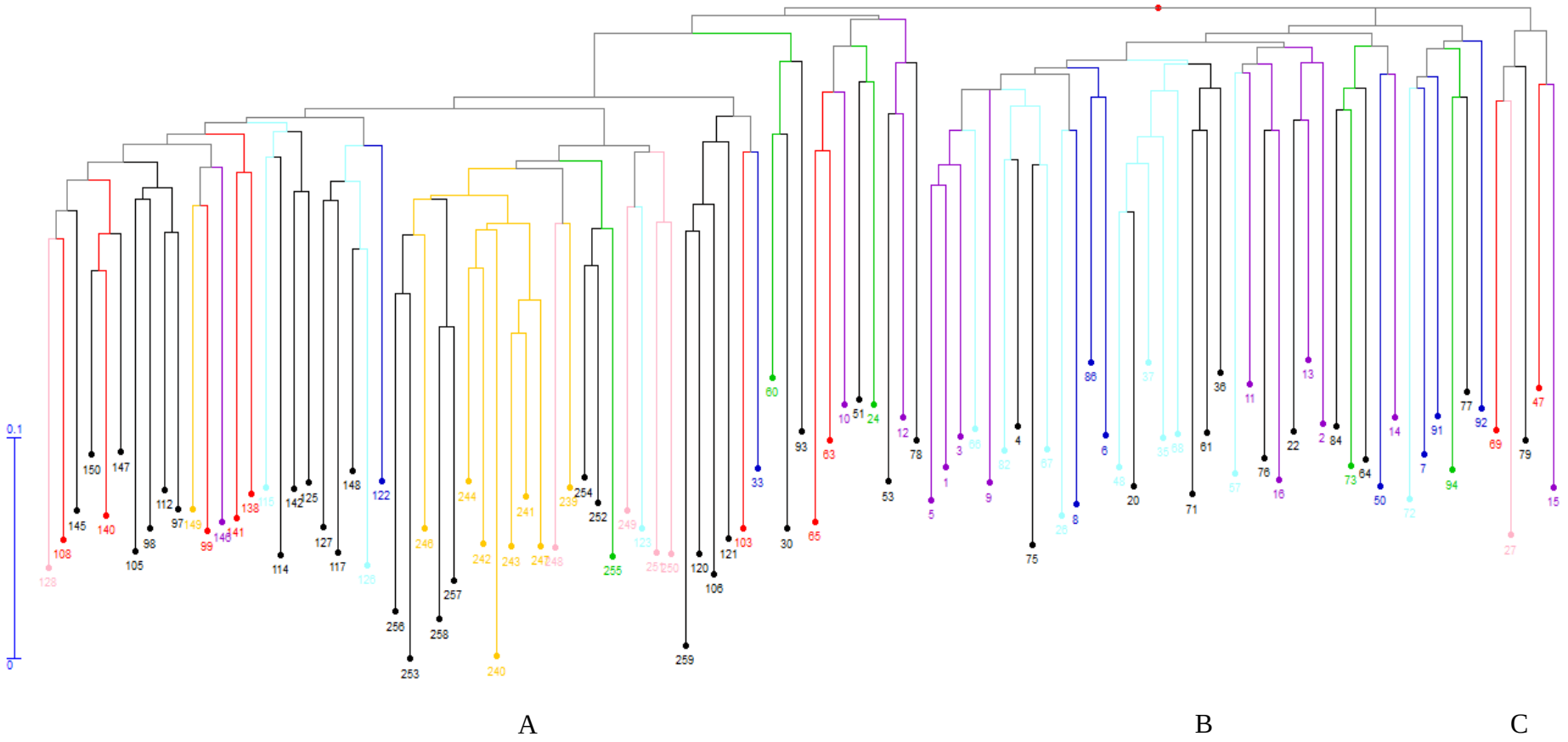

Figure 3.7. Dendrogram of genetic diversity of the most abundant cultivars. Red, dark blue, light blue, yellow, green, purple, and pink represent cultivars from the most abundant regions: Ethiopia, Germany, the Netherlands, Australia, Egypt, Finland, and Turkey, respectively.

According to the PCoA, cultivars from Australia, the Netherlands, Germany, and Ethiopia had low genetic diversity. Those from Finland, Egypt, and Turkey had relatively high genetic diversity (Figure 3.8). Australian individuals grouped together tightly and were distinct from other cultivars. These results indicate the narrow base of Australian cultivars and also suggest that the material is genetically distinct from other regions' cultivars. In the same way, Ethiopian individuals did not grouped together and were distinct from other cultivars. Cultivars from Netherlands and Germany grouped together and overlapped with cultivars from other regions.

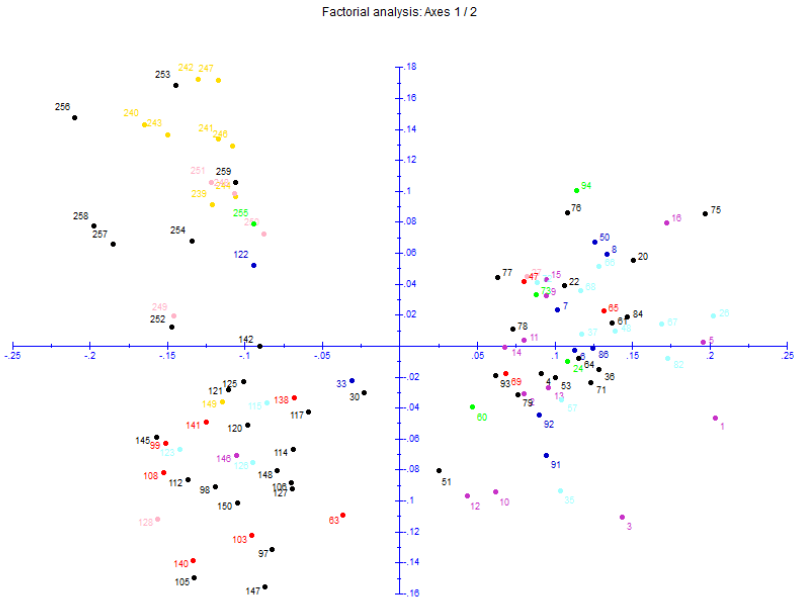

Figure 3.8. PCoA for cultivars. Red, dark blue, light blue, yellow, green, purple, and pink represent cultivars from the most abundant regions: Ethiopia, Germany, the Netherlands, Australia, Egypt, Finland, and Turkey, respectively.

These results partially coincide with the findings of Zeid et al. (2003)[46a] and Salem et al. (2012)[60]. They reported that regional origin was associated with clustering based on genetic relationships using AFLP [46a] and SRAP[60] markers. In our study, Asian faba beans grouped separately from those from Europe and Africa (Figure 3.3). Also, according to a previous study, Egyptian genotypes grouped together but in this study, there was no clustering for Egyptian faba bean[60]. SNP based studies as Kaur et al.

(2014)[61] reported that Australian cultivars clustered together as was observed in our study.

3.2.3. Population Structure Analysis

According to the results, K was determined as 2 subpopulations (Figure 3.9) because of the relatively high ΔK value at K = 2. The standard deviation (SD) for each value of K is also important when deciding the correct number of subpopulations. SD values are shown in Table 3.10 and support the choice of K=2.

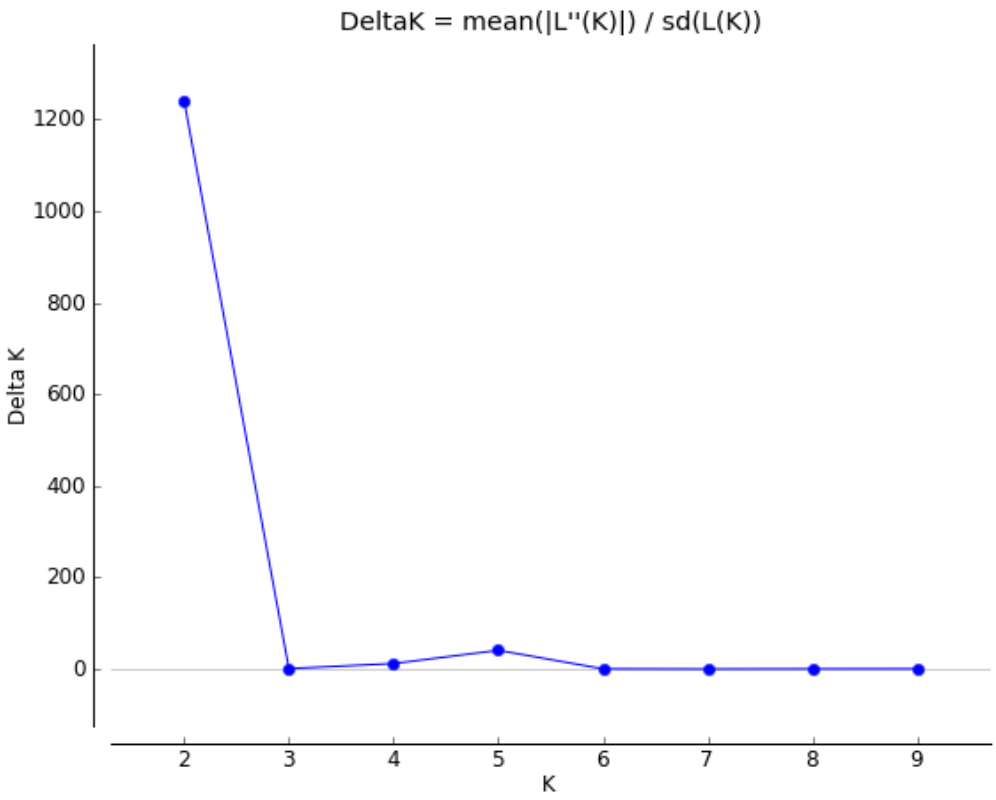

Figure 3.9. ΔK values for each number of subpopulations (K) for faba bean population.

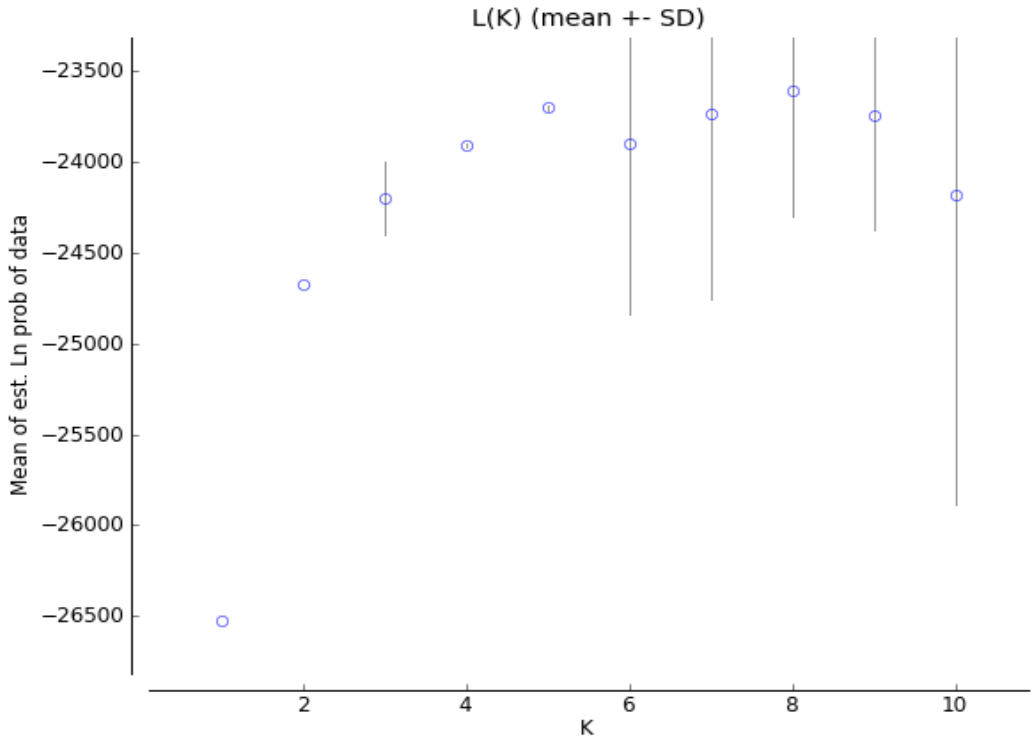

Figure 3.10. SD values for each number of subpopulations.

The bar plot demonstrating subpopulation composition of the faba bean genotypes is shown in Figure 3.11. Different colors represent different subpopulations. In the graph, the x-axis shows the individuals in the populations and the y-axis demonstrates the percentage identity to the group to which it belongs. Genotypes were grouped considering a cut-off of 70% probability of belonging to a given subpopulation. Genotypes with an identity value under this threshold were considered to be intermixed (Table 3.3). According to this evaluation, cluster 1 had 87 individuals; cluster 2 had 162 individuals and 10 individuals were intermixed.

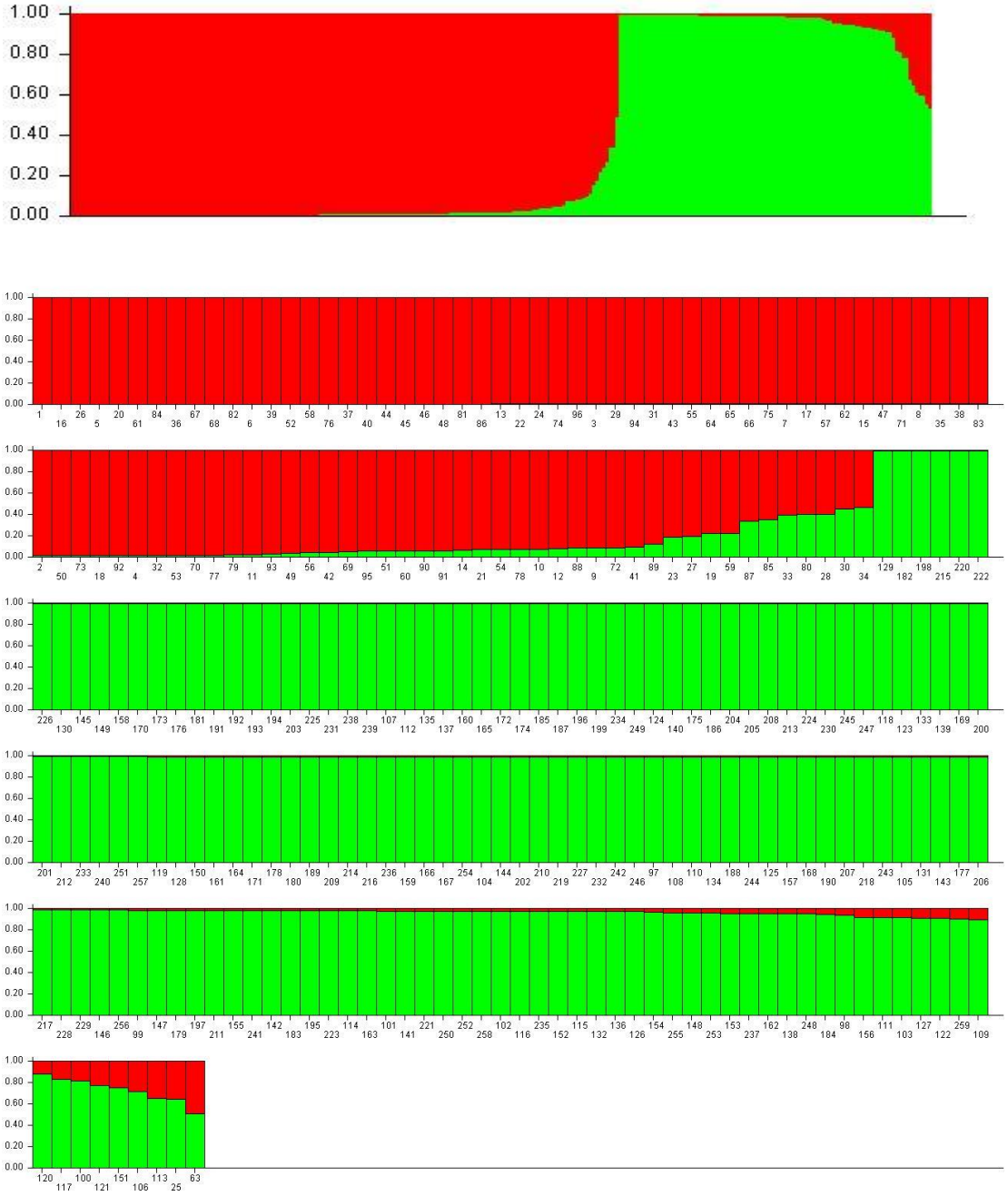

Figure 3.11. The bar plot for K=2. Red and green represent Cluster 1 and Cluster 2, respectively.

Table 3.3.Faba bean genotypes and their inferred subpopulation identities.

No	Genotype	Inferred Clusters		Cluster Number
1	NGB8642	0.997	0.003	1
2	NGB1540.1	0.983	0.017	1
3	NGB8643	0.991	0.009	1

(Cont. on the next page)

Table 3.3. (cont.)

4	Melodie/2	0.980	0.020	1
5	Mikko	0.996	0.004	1
6	WitkiemManida	0.994	0.006	1
7	Ukko	0.989	0.011	1
8	Kontu	0.986	0.014	1
9	NGB1550.1	0.913	0.087	1
10	NGB1547.1	0.924	0.076	1
11	NGB1552.1	0.976	0.024	1
12	NGB1542.1	0.921	0.079	1
13	NGB1548.2	0.992	0.008	1
14	NGB1546.2	0.931	0.069	1
15	NGB20019.2	0.988	0.012	1
16	NGB8640	0.997	0.003	1
17	CGN10330	0.989	0.011	1
18	CGN7874	0.982	0.018	1
19	CGN13486	0.778	0.222	1
20	CGN7699	0.996	0.004	1
21	CGN10322	0.928	0.072	1
22	CGN7697	0.992	0.008	1
23	CGN15563	0.811	0.189	1
24	CGN15619	0.992	0.008	1
25	CGN10390	0.354	0.646	intermixed
26	CGN19986	0.997	0.003	1
27	CGN19987	0.807	0.193	1
28	CGN13487	0.595	0.405	intermixed
29	CGN13485	0.991	0.009	1
30	CGN13464	0.549	0.451	intermixed
31	CGN13451	0.990	0.010	1
32	CGN13450	0.980	0.020	1
33	CGN13445	0.605	0.395	intermixed
34	CGN12329	0.533	0.467	intermixed
35	CGN19993	0.986	0.014	1
36	CGN19995	0.995	0.005	1
37	CGN13518	0.993	0.007	1
38	CGN12321	0.986	0.014	1
39	CGN12309	0.994	0.006	1
40	CGN10391	0.993	0.007	1
41	CGN7843	0.906	0.094	1
42	CGN10321	0.951	0.049	1
43	CGN10344	0.990	0.010	1
44	CGN10347	0.993	0.007	1
45	CGN10320	0.993	0.007	1
46	CGN7826	0.993	0.007	1
47	CGN7827	0.988	0.012	1
48	CGN19982	0.993	0.007	1
49	CGN07836	0.961	0.039	1
50	CGN7839	0.983	0.017	1
51	CGN7842	0.941	0.059	1
52	CGN12320	0.994	0.006	1
53	CGN7716	0.980	0.020	1

(Cont. on the next page)

Table 3.3. (cont.)

54	CGN7844	0.926	0.074	1
55	CGN10324	0.990	0.010	1
56	CGN7949	0.954	0.046	1
57	CGN07781	0.989	0.011	1
58	CGN07933	0.994	0.006	1
59	CGN7848	0.772	0.228	1
60	CGN7739	0.940	0.060	1
61	CGN7728	0.996	0.004	1
62	CGN7872	0.989	0.011	1
63	CGN15556	0.490	0.510	intermixed
64	CGN7725	0.990	0.010	1
65	CGN07734	0.990	0.010	1
66	CGN18860	0.990	0.010	1
67	CGN18863	0.995	0.005	1
68	CGN18862	0.995	0.005	1
69	CGN15620	0.950	0.050	1
70	CGN7950	0.979	0.021	1
71	CGN18893	0.987	0.013	1
72	CGN18892	0.909	0.091	1
73	CGN7740	0.983	0.017	1
74	CGN7757	0.992	0.008	1
75	CGN18906	0.990	0.010	1
76	CGN7751	0.994	0.006	1
77	CGN15644	0.979	0.021	1
78	CGN18867	0.926	0.074	1
79	CGN15621	0.978	0.022	1
80	CGN7934	0.597	0.403	intermixed
81	CGN7938	0.993	0.007	1
82	CGN15641	0.995	0.005	1
83	CGN07871	0.986	0.014	1
84	CGN7766	0.996	0.004	1
85	CGN10315	0.644	0.356	intermixed
86	CGN18878	0.993	0.007	1
87	CGN7939	0.660	0.340	intermixed
88	CGN7940	0.914	0.086	1
89	CGN10382	0.877	0.123	1
90	CGN10313	0.939	0.061	1
91	CGN18909	0.939	0.061	1
92	CGN7729	0.981	0.019	1
93	CGN7730	0.967	0.033	1
94	CGN7738	0.991	0.009	1
95	CGN7733	0.942	0.058	1
96	CGN7948	0.992	0.008	1
97	CGN7727	0.011	0.989	2
98	CGN7736	0.060	0.940	2
99	CGN7717	0.015	0.985	2
100	CGN10371	0.182	0.818	2
101	CGN10384	0.022	0.978	2
102	CGN7947	0.023	0.977	2
103	CGN18927	0.085	0.915	2

(Cont. on the next page)

Table 3.3 (cont.)

104	CGN7946	0.010	0.990	2
105	CGN7721	0.013	0.987	2
106	CGN19985	0.282	0.718	2
107	CGN10362	0.005	0.995	2
108	CGN7752	0.011	0.989	2
109	CGN7732	0.103	0.897	2
110	CGN10329	0.011	0.989	2
111	CGN07873	0.082	0.918	2
112	CGN7719	0.005	0.995	2
113	CGN7931	0.346	0.654	intermixed
114	CGN18942	0.021	0.979	2
115	CGN18895	0.026	0.974	2
116	CGN7932	0.023	0.977	2
117	CGN18934	0.165	0.835	2
118	CGN18888	0.007	0.993	2
119	CGN19977	0.008	0.992	2
120	CGN18856	0.116	0.884	2
121	CGN18855	0.222	0.778	2
122	CGN18941	0.091	0.909	2
123	CGN18892	0.007	0.993	2
124	CGN18873	0.006	0.994	2
125	CGN18905	0.012	0.988	2
126	CGN07723	0.027	0.973	2
127	CGN19979	0.088	0.912	2
128	CGN19987	0.008	0.992	2
129	CGN7895	0.003	0.997	2
130	CGN7907	0.004	0.996	2
131	CGN7912	0.013	0.987	2
132	CGN07875	0.026	0.974	2
133	CGN10387	0.007	0.993	2
134	CGN10385	0.011	0.989	2
135	CGN10383	0.005	0.995	2
136	CGN10386	0.026	0.974	2
137	CGN10373	0.005	0.995	2
138	CGN18884	0.047	0.953	2
139	CGN18880	0.007	0.993	2
140	CGN18883	0.006	0.994	2
141	CGN7735	0.022	0.978	2
142	CGN19981	0.019	0.981	2
143	CGN10374	0.013	0.987	2
144	CGN10325	0.010	0.990	2
145	CGN18933	0.004	0.996	2
146	CGN18920	0.014	0.986	2
147	CGN18923	0.015	0.985	2
148	CGN18856	0.039	0.961	2
149	CGN15639	0.004	0.996	2
150	CGN7715	0.008	0.992	2
151	CGN7710	0.246	0.754	2
152	CGN15615	0.025	0.975	2
153	CGN10314	0.043	0.957	2

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Table 3.3. (cont.)

154	TR12123	0.030	0.970	2
155	TR12271	0.017	0.983	2
156	TR12540	0.081	0.919	2
157	TR12611	0.012	0.988	2
158	TR12716	0.004	0.996	2
159	TR12767	0.009	0.991	2
160	TR12773	0.005	0.995	2
161	TR22992	0.008	0.992	2
162	TR23018	0.045	0.955	2
163	TR26194	0.021	0.979	2
164	TR26250	0.008	0.992	2
165	TR26265	0.005	0.995	2
166	TR26298	0.009	0.991	2
167	TR26453	0.009	0.991	2
168	TR26462	0.012	0.988	2
169	TR26578	0.007	0.993	2
170	TR28096	0.004	0.996	2
171	TR31590	0.008	0.992	2
172	TR31912	0.005	0.995	2
173	TR31973	0.004	0.996	2
174	TR32925	0.005	0.995	2
175	TR33058	0.006	0.994	2
176	TR33140	0.004	0.996	2
177	TR33421	0.013	0.987	2
178	TR33517	0.008	0.992	2
179	TR33547	0.016	0.984	2
180	TR33561	0.008	0.992	2
181	TR35023	0.004	0.996	2
182	TR35330	0.003	0.997	2
183	TR37028	0.019	0.981	2
184	TR37041	0.055	0.945	2
185	TR37200	0.005	0.995	2
186	TR37255	0.006	0.994	2
187	TR38142	0.005	0.995	2
188	TR39064	0.011	0.989	2
189	TR40217	0.008	0.992	2
190	TR40725	0.012	0.988	2
191	TR42343	0.004	0.996	2
192	TR43564	0.004	0.996	2
193	TR44862	0.004	0.996	2
194	TR44869	0.004	0.996	2
195	TR44876	0.019	0.981	2
196	TR44914	0.005	0.995	2
197	TR44928	0.016	0.984	2
198	TR44931	0.003	0.997	2
199	TR44941	0.005	0.995	2
200	TR46010	0.007	0.993	2
201	TR49377	0.007	0.993	2
202	TR49380	0.010	0.990	2
203	TR49381	0.004	0.996	2

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Table 3.3. (cont.)

204	TR49386	0.006	0.994	2
205	TR49387	0.006	0.994	2
206	TR49388	0.013	0.987	2
207	TR51377	0.012	0.988	2
208	TR51378	0.006	0.994	2
209	TR53648	0.008	0.992	2
210	TR53667	0.010	0.990	2
211	TR53683	0.016	0.984	2
212	TR53724	0.007	0.993	2
213	TR53748	0.006	0.994	2
214	TR53761	0.008	0.992	2
215	TR53770	0.003	0.997	2
216	TR53781	0.008	0.992	2
217	TR53797	0.013	0.987	2
218	TR53947	0.012	0.988	2
219	TR53948	0.010	0.990	2
220	TR53949	0.003	0.997	2
221	TR61267	0.022	0.978	2
222	TR61300	0.003	0.997	2
223	TR71255	0.019	0.981	2
224	TR71521	0.006	0.994	2
225	TR71612	0.004	0.996	2
226	TR74159	0.003	0.997	2
227	TR74184	0.010	0.990	2
228	TR74214	0.013	0.987	2
229	TR74330	0.013	0.987	2
230	TR74337	0.006	0.994	2
231	TR75408	0.004	0.996	2
232	TR75421	0.010	0.990	2
233	TR75431	0.007	0.993	2
234	TR77139	0.005	0.995	2
235	TR77143	0.024	0.976	2
236	TR78048	0.008	0.992	2
237	TR79569	0.043	0.957	2
238	TR80277	0.004	0.996	2
239	ASCOT	0.004	0.996	2
240	MANAFEST	0.007	0.993	2
241	NURA	0.017	0.983	2
242	PBA RANA	0.010	0.990	2
243	FIORD	0.012	0.988	2
244	ICARUS	0.011	0.989	2
245	FIESTA	0.006	0.994	2
246	FARAH	0.010	0.990	2
247	AUADULCE	0.006	0.994	2
248	kıtık2003	0.050	0.950	2
249	eresen87	0.005	0.995	2
250	filiz99	0.022	0.978	2
251	Salkım	0.007	0.993	2
252	BPL710	0.022	0.978	2
253	Elizar	0.041	0.959	2

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Table 3.3. (cont.)

254	Lattaka2	0.009	0.991	2
255	Giza4	0.036	0.964	2
256	ILB93812	0.014	0.986	2
257	Aurora/2	0.007	0.993	2
258	Melodie/2	0.022	0.978	2
259	Disco/2	0.097	0.903	2

The STRUCTURE analysis agreed with the dendrogram analysis in that two clusters were found in the population. Figure 3.12 shows the DARwin5 dendrogram which was re-labeled based on STRUCTURE results. Group A and Cluster 1 are approximately 98% identical whereas Group B and Cluster 2 are approximately 90% identical. Thus, both analyses gave similar grouping in the faba bean population, although they based on different methods which are distance based and model based.

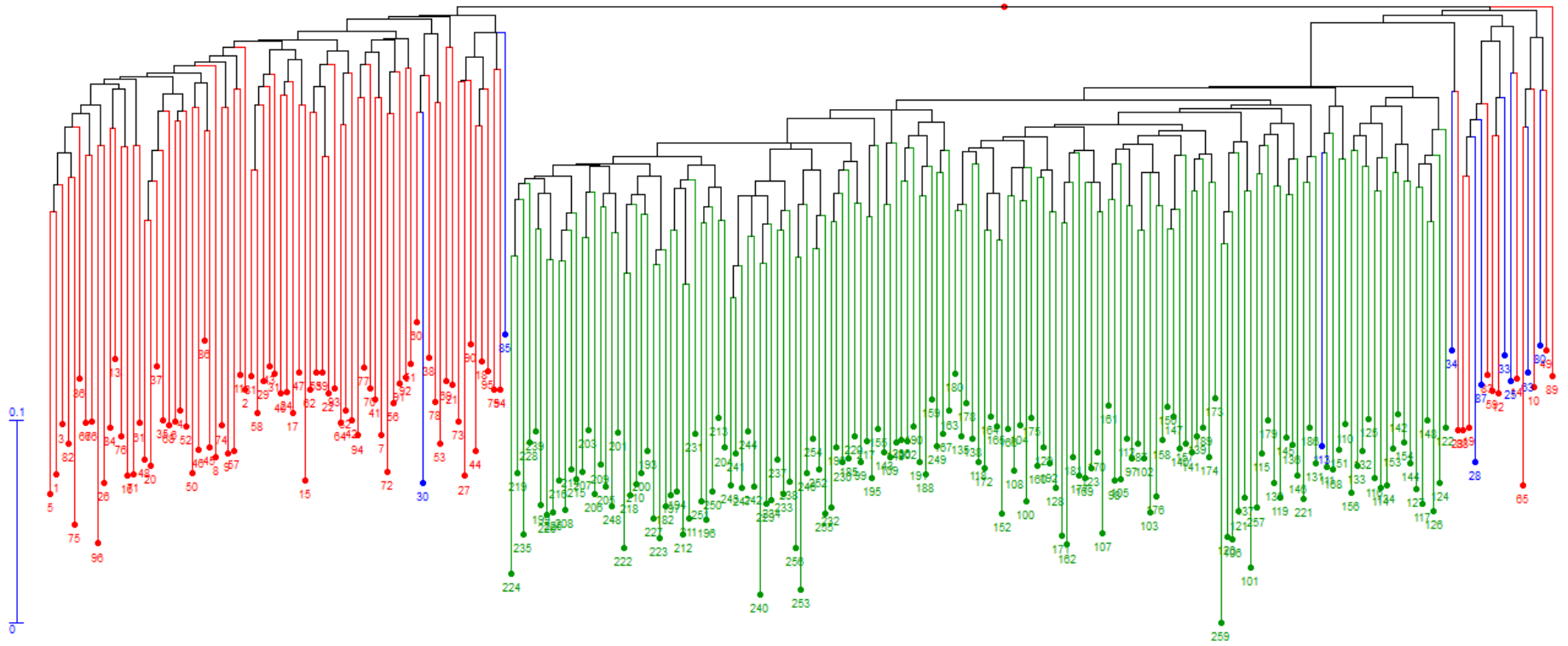

Figure 3.12. Comparison of DARwin5 and STRUCTURE analysis. Red, green, and blue represent Cluster 1, Cluster 2, and intermixed individuals from STRUCTURE, respectively.

3.2.4. Construction of Core Collection

A total of 45 individuals among 259 individuals were selected to construct a core collection (Table 3.4). These individuals represent the maximum genetic diversity present in all of the accessions used in this study in the minimum number of genotypes. Individuals which belong to the core collection should be morphologically characterized and can then be used in breeding studies to develop new varieties.

Table 3.4.Individuals selected from PowerCore program to construct core collection.

Genotype Number	**Genotype Name**	**Genotype Number**	**Genotype Name**
5	Mikko	134	CGN10385
6	Witkiem Manita	143	CGN10374
8	Kontu	144	CGN10325
10	NGB1547.1	154	TR12123
18	CGN07874	162	TR23018
20	CGN07699	171	TR31590
23	CGN15563	176	TR33140
24	CGN15619	183	TR37028
27	CGN19987	186	TR37255
29	CGN13485	195	TR44876
30	CGN13464	197	TR44928
40	CGN10391	202	TR49380
44	CGN10347	213	TR53748
46	CGN07826	221	TR61267
53	CGN07716	239	ASCOT
54	CGN07844	248	Kıtık2003
57	CGN07781	249	Eresen87
76	CGN07751	252	BPL710
82	CGN15641	255	Giza4
89	CGN10382	256	ILB938/2
100	CGN10371	257	Aurora/2
119	CGN19977	259	Disco/2
132	CGN07875		

CHAPTER 4

CONCLUSION

Faba bean has high protein and starch contents in their seed so it can be used in both the human diet and for animal feed. It can also be a rotation crop due to nitrogen fixation bacteria on its roots. Although there are 37 gene banks[62] in the world which contain faba bean germplasm, there are not enough studies about the genetic diversity of these natural populations. Genetic diversity studies are important for understanding of domestication and evaluation of species. For maintain population continuity during breeding, core collections should be created. Individuals belonging to the core collection should represent all of the genetic diversity present in the population.

In this study, 259 faba bean accessions were analyzed to assess their genetic diversity. A total of 32 SSR primer pairs were utilized and 302 polymorphic bands were obtained. For detection of genetic diversity and population structure, DARwin5 and STRUCTURE programs were used, respectively. According to the neighbor joining results, the population was divided into 2 main groups: Group A had 77 individuals, Group B had 177 individuals and 1 individual did not belong to any group. We detected 59% maximum genetic dissimilarity and 17% minimum genetic dissimilarity. We found nearly identical levels of dissimilarity for both cultivars and landraces. According to these results, faba bean cultivars have not been yet lost genetic variability due to breeding. Population structure analysis agreed with the dendrogram: 2 subpopulations were determined. Cluster 1 had 87 individuals; Cluster 2 had 162 individuals and 10 individuals were intermixed. Overall, clustering of the accessions based on molecular marker analysis showed some correlation with geographic origin and seed size. A genetically well characterized core collection was selected containing 45 faba bean individuals. This core collection can be used as a starting point for future faba bean breeding.

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