



Molecular approaches to origin, ancestry and domestication history of crop plants: Barley and clover as examples

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Abstract Knowledge of the origin and domestication history of crop plants is important for studies aiming at avoiding the erosion of genetic resources due to the loss of ecotypes and landraces and habitats and increased urbanization. Such knowledge also strengthens the capacity of modern farming system to develop and scale-up the domestication of high value potential crops that can be achieved by improving the knowledge that help to identify and select high value plant species within their locality, identify and apply the most appropriate propagation techniques for improving crops and integrate improved crop species into the farming systems. The study of domestication history and ancestry provide means for germplasm preservation through establishment of gene banks, largely as seed collections, and preservation of natural habitats. Information about crop evolution and specifically on patterns of genetic change generated by evolution prior, during, and after domestication, is important to develop sound genetic conservation programs of genetic resources of crop plants and also increases the efficiency of breeding programs. In recent years, molecular approaches have contributed to our understanding of the aspects of plant evolution and crops domestication. In this article, aspects of crops domestication are outlined and the role of molecular data in elucidating the ancestry and domestication of crop plants are outlined. Particular emphasis is given to the

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contribution of molecular approaches to the origin and domestication history of barley and the origin and ancestry of the Egyptian clover.

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1. Introduction

1.1. The process of domestication

Domestication is the most important development in the past 12,000 years of human history. It was prerequisite to the rise of civilization, and had transformed the global demography [17]. It may be simply regarded as the advent of agriculture to human evolution, which is perhaps one of the major cultural developments in human civilization. The agriculture could have independently originated multiple times in widely different areas [21]. The major event in the development of agriculture is the domestication of many plants and animals that provides most of our food today. Domestication is the outcome of a selection process that leads to increased adaptation to cultivation or rearing and utilization by humans. It is still being debated whether this selection took place consciously by humans or if it was an inadvertent phenomenon as a by-product of human cultivation of plants or rearing of animal [24]. What is certain is that such selection was associated with marked phenotypic changes that eventually arose during domestication. These changes have been so pronounced that plant taxonomists have often classified wild progenitors and domesticated descendants in different species or genera. The changes associated with domestication mark the transition from hunting-gathering to agriculture, in what is known as the Neolithic revolution [75]. Stiner [61] argued that this transition was preceded by the so-called broad-spectrum revolution that marked a switch in subsistence patterns during the Paleolithic. Evidence for this transition comes from an increase in the number of species in the diet, an abundance of milling tools and storage facilities, and a higher frequency of plant parts.

1.2. Changes accompanying domestication of crop plants

The domestication of crop plants had involved similar modifications in a set of traits including seed dispersal, seed dormancy, gigantism and increased harvest index; these features were for the first time called “domestication syndrome” by Hammer [23]. Traits in this Syndrome also include increasing adaptation and desirability of human consumption and use [25]. The characters associated with domestication arise, at least in part, from human selection and hence relate to ways in which the plants are cultivated and harvested. In addition to these features, Gepts [21] indicated that a shared feature among most domesticated plants is a marked genetic bottleneck. He also assumed that the genetic architecture of the domestication syndrome suggests that there was no genetic impediment to a fast domestication process and that the circumstantial evidence suggests that some species may be more amenable to domestication than others. Whatever, the characters selected for during domestication, crop domestication is a human-induced selection process for the adaptation of crops to human’s environment. The evolutionary features that occurred under cultivation was dismissed in the past as not typical of evolution at large, because the high level of selection pressure under cultivation, was thought to be unusual in natural environments. However, recent evidences indicate that strong selection also exists in natural environments [30].

The plant characteristics that were selected for during domestication may differ from a plant group to another. For cereals, the domestication syndrome was divided into seven components mostly concerned with loss of seed dispersal, increased grain size and nutrition quality [20]. In barley and wheat the main characters that have been selected for during



Figure 1 World map illustrating the centers of origins for crop plants as proposed by Vavilov (1926): 1. Mexico-Guatemala, 2. Peru-Ecuador-Bolivia, 2A. Southern Chile, 2B. Southern Brazil, 3. Mediterranean, 4. Middle East, 5. Ethiopia, 6. Central Asia, 7. Indo-Burma, 7A. Siam-Malaya-Java, 8. China.

domestication include brittle rachis, shattering of pods, reduction/loss of dormancy, rapid germination after sowing, shorter time to flowering and maturity, increased yield per plant, disease resistance, and stress tolerance [11]. The domestication syndrome of non-cereal crops might exclude some of these traits but include others such as reduction of defensive armor, and reduction in defensive toxins as in yams and various legumes [12]. In legumes, the crops grown for their seeds, including pea, lentil chickpea, common bean, cowpea, soybean, lupine and others, can be expected to exhibit the type of modifications typical of the domestication syndrome [25]. However, Weeden [69] concluded that like common bean [9], relatively few genes appear to have been modified during the domestication of pea contradicting the existence of common genetic basis to ‘domestication syndrome’ in the Fabaceae. On the other hand, an investigation of this question in the Poaceae [52] suggested that the convergent evolution observed during domestication in sorghum, rice and maize was produced by mutations in many of the same genes.

1.3. Centers of crop domestication

In 1882, Alphonse de Candolle proposed three centers of crop domestication in the World; Mesoamerica, the Fertile Crescent and South East Asia. He stated that historic events such as glaciations and domestication had paramount importance for crop plant distribution. Meanwhile [66] postulated that the region of greatest diversity of the wild relatives of a crop plant is also its center of origin and proposed eight centers of origin for cultivated crops (Fig. 1). He based his proposal on the presence of wild relatives of the major crops such as beans, wheat and rice and on archeological evidences. He realized the archaeological, botanical, historical and linguistic evidence could help to study the origin and history of plant domestication. However, domesticated crops are global travelers; a crop that was domesticated in a restricted area can spread from the

site of domestication to other sites in the region and then farther out, displacing other similar plants. Examples are illustrated by the domestication of corn, cotton and common bean in Latin America; rice and soybean in China; wheat, barley and lentil in the Middle East and sorghum, cowpea and groundnut in Africa.

Redman [54] proposed that the area of the Near East hosted wild progenitors of domesticated plants and animals that coexisted at the end of the last Ice Age. He further speculated that the hills and valleys of the Taurus Mountains in the present day South Turkey would have been an ideal location for the practice of agriculture - as the rainfall of this area is the optimum (250–500 mm per year). However, more recent views assume that domestication began in multiple places more or less simultaneously in different locations in the region of the Fertile Crescent some 8000 to 10000 years ago ([71,75]). This area has been the center of origin for over 30 crops; examples include einkorn wheat, durum wheat, common wheat, barley, rye, common oats, legumes e.g. lentil, beans and lupine as well as forage plants particularly the alfalfa, Persian clover, and Egyptian clover. The cereals and legumes that were domesticated in that area formed an important component of the human diet and animal feed at the early stages of civilization in the Middle East [75]. The most important founder crops in the Near East agriculture and their wild progenitors as compiled by Kilian et al. [34] include einkorn wheat, emmer wheat, rye, barley lentil, pea chickpea, vetch and flax.

1.4. Time frame of domestication

The process of domestication is an aspect of the transition from hunting-gathering to agriculture. It is generally thought that this transition has taken several millennia [60]. The point at which a crop or an animal can be considered to be domesticated is somewhat speculative. A domesticated crop usually displays several of characters encompassing the domestication

syndrome [23]. Yet, the archaeological record only consists of a few types of remains, usually those that have been able to withstand decomposition. Examples of these are seeds and inflorescence axes (rachis or cobs). Cereals generally offer more clues to the status of their domestication than other crops such as legumes. In addition to an increase in seed size, a tough rachis (in contrast to a brittle rachis) and free-threshing seeds (as opposed to hulled seeds) with their characteristic morphology are also useful in this respect. For legumes, seed size can generally be used; seed color and pod shape are may be additional possibilities [21]. Archaeological records within archaeological sites encompassing the transition from wild to domesticated forms in a number of domestication centers indicated early remains at about the same age – some 12,000 years ago [75].

Richerson et al. [55] argued that the period covering the last 12,000 years, known as the Holocene, has been characterized by a generally warmer and more stable climate than the preceding Pleistocene era. That climate change, which included a rise in CO₂ levels and increased rainfall, provided conditions for intensification of food production through cultivation of plants or rearing of animals, and eventually domestication, of highly productive plant and animal resources. Domestication can therefore be seen as an attempt some 12,000 years ago to further increase resource availability perhaps in response to ever increasing population levels or resource depletion or a combination of both. Richerson et al. [55] assumed that domestication was also made possible because humans had reached a higher cognitive and cultural level of advancement through successive bouts of intensification of land-efficient subsistence systems that requires and allows labor intensification correlated with population growth. The early adopters of these novel subsistence systems tended, through sheer increase of their population-to displace non-adopters by short- or long-range migration into territories occupied by non-adopters in a demic diffusion. The speed at which displacement was adopted was generally fast. Within the Fertile Crescent, it is difficult to identify gradients in age of the oldest remains of crops. Ammerman and Cavalli-Sforza [4] suggested that the introduction of agriculture into Europe from the Fertile Crescent had occurred in a demic diffusion fashion between 9000 and 5000 ybp at an average speed of about 1 km per year.

1.5. Rationale for tracing domestication of plant crops

The domestication involved the determination of wild relatives, related species, and new genes (especially dominant genes) as sources of useful traits. Knowledge of the origins of crop plants is also important in order to avoid genetic erosion, loss of germplasm due to the loss of ecotypes and land-races, loss of habitat (such as rainforests), and increased urbanization. It provides means for germplasm preservation through establishment of gene banks, largely as seed collections and preservation of natural habitats (especially in centers of origin). The rationale for conserving plant diversity as outlined by Given [22] is summarized in the following reasons: (i) the economic value of plants as resources for humanity, both now and in the future, (ii) role of plants in maintaining a stable environment, (iii) the scientific value of plants, (iv) maintaining future options, (v) cultural values and symbolic value, (vi) the role of plants in inspiring people and (vii) the right of species to

exist. Understanding the origin of crop plants domestication is also basic to breeding new varieties. Such understanding also helps to illuminate the evolutionary history of crop domestication that would explain further the origin and development of modern cultivation and agronomy. Furthermore, addressing issues of domestication strengthens the capacity of modern farming system to develop and scale-up the domestication of high value potential plants. This can be achieved by improving the knowledge that help to identify and select high value plant species within their locality, identify and apply the most appropriate propagation techniques for improving such trees crops and be able to integrate improved tree species into the farming systems.

1.6. Sources of information on crop domestication

Understanding of crop origin and evolution began in the mid 20th century with morphological studies and archeological finds of early domesticates as well as analysis of chromosome homology in crops and their wild relatives. Later, allelic variants of enzymes were used to explore the origin, ancestry of crop species. The greatest progress in studying crop domestication history came with the development of DNA-based molecular markers in the 1980s. Today evidences for addressing the origins and domestication of crop plants comes from three major sources (i) Archaeological excavations, (ii) Botanical observations, and (iii) Distribution of the relatives of domesticated crops.

Archaeological evidences are concerned with and contribute to the determination of: (i) Time and place of earliest sign(s) of the crop, (ii) Time and means of crop spread, (iii) The form(s) of early cultigens, (iv) Changes to the crop during domestication and (v) Time and place of changes during domestication. Archaeological evidence is, however, not sufficient and contributions from related research fields, like archaeobotany and botany, have been a valuable contribution to our knowledge on the origin of agriculture [28,29,45,63,71].

Botanical evidences are concerned with the gene pool of the crop and contribute to the determination of: (i) Cultivated, weedy and wild elements in the gene pool of the crop, (ii) Botanical terms used for taxa of the crop complex, (iii) Fully divergent species placed in the same genus, (iv) Wild relative(s) and (progenitor(s) and (v) Geographic distribution and ecological adaptation. Botanical evidences are obtained from three main sources:

1. Classical taxonomy e.g. comparative morphology, anatomy etc.
2. Cytogenetic analyses e.g. chromosome affinities, crossing compatibility, stability of hybrids.
3. Molecular evidences e.g. DNA finger-printing, gene sequence, allele frequency.

1.7. Forms of cultivated species

The forms of cultivated crop vary in some traits and are of four types:

- (i) Commercial varieties developed by professional plant breeders and are characterized by high productivity and genetic uniformity.

- (ii) Breeding lines have a narrow genetic base and like commercial varieties are genetically vulnerable to mutation and recombination.
- (iii) Special genetic stocks include collections for use in developing commercial varieties.
- (iv) Land races or local varieties primitive cultivars evolved over centuries and thousands of years and have been influenced by both natural and artificial selection.
- (v) Land races and local varieties are adapted to survive in unfavorable conditions and are therefore regarded the reservoir of genes for sustainable agriculture.

1.8. Forms of wild relatives of crop plants

- (i) Species that man uses but does not cultivate. e.g. medicinal plants collected for extraction of pharmaceutical substances and forage species existing in natural pastures.
- (ii) Species for indirect use, the close relatives of cultivated species, that possess beneficial characters that can be transferred to cultivated relatives through sexual crossings.
- (iii) Potentially utilizable species, which are not used today but have probable use in the future. e.g. certain medicinal plants and agro-energy producers or those that have useful characters that may be transferred via gene transfer technologies.

1.9. Molecular markers as tools to study domestication

The development of increasingly informative molecular markers has allowed for detailed investigations of the evolution and domestication of a number of crops. Moreover, with the increasing ease and decreasing cost of molecular tools, the resources necessary for investigating the genetic underpinnings of phenotypic traits are now in place for most major crops. These advances not only allow for an investigation of the overall genetic architecture of the wild-crop transition, but also make possible the identification of genomic regions and genes that were subjected to selection during the evolution of various crops [6,59]. In some cases, researchers have been able to pinpoint the exact nucleotide changes responsible for the production of key crop-related trait. Kilian et al. [34] stated that the keys to obtain deeper insights to plant domestication using molecular biology are (i) a comprehensive germplasm collection covering the whole distribution area for each species; (ii) the comparison of many wild and domesticated accessions for each species; (iii) the identification of the wild progenitor in the wild gene pool and its comparison with domesticated descendants; (iv) the use of new molecular fingerprinting techniques at many loci and the access to new generation high throughput sequencing technologies [70]; and (v) improvement of analytical methods capable of treating domestication issues based on mathematical and statistical models.

Molecular information has for almost two decades provided new insights on genetic diversity of crop plants; in relation to wild relatives, identification of crop progenitors, centers of domestication, time frame of the domestication process and specific alleles supporting domesticated traits. This is due largely to the rapid accumulation of genomic resources that provided genome-wide markers for population and molecular

analyses of crops and their wild relatives [14,18,57,67]. One of the leading studies in this area of research was done by Brubaker and Wendel [13] who used the DNA markers derived from nuclear restriction fragment length polymorphisms (RFLPs) to reevaluate the origin of domesticated cotton. Matsuoka et al. [41] used multi-locus microsatellite genotyping to identify a single domestication for maize. Wills and Burke [72] used hyper-variable chloroplast simple-sequence repeat markers to search for evidence of a possible Mexican origin of domestication for sunflowers. The data provided further evidence that the extant domesticated sunflowers are the product of a single domestication event somewhere outside of Mexico. In the mean time Konishi et al. [38] used single nucleotide polymorphism (SNP) to elucidate loss of seed shattering during rice domestication. The localization and timing of domestication events and the demographics of domestication was addressed by Liu and Burke [39]. Further important contributions using molecular markers for other species include the works on barley [32,43]; einkorn wheat [33]; maize [73] and rice [40].

The most popular of the molecular approaches is the AFLP, a PCR-based procedure that resolves radioactively labeled electrophoretic bands (polymorphic loci) on sequencing gels or in gene sequencing machines. The AFLP approach became feasible in the 1990s when the development of high-throughput methods made it possible to type multiple markers in many individual plants [68]. For the AFLP fingerprinting, DNA is digested with *EcoRI* and *MseI* and specific double-stranded adapters with sequence complementary to newly formed ends of fragments, labeled with fluorescent dye moiety, are used to amplify AFLP fragments using PCR that are then separated in polyacrylamide gel or using automated DNA sequencing machine. The presence (1) or absence (0) of amplified fragments, in the range of 50–500 bp long is scored for data analyses [7].

This multi-locus analysis was first applied to einkorn wheat, through typing of 288 amplified fragment length polymorphisms (AFLP) in 338 wild and cultivated accessions [27]. Phylogenetic trees constructed from the AFLP data showed that domesticated einkorn is monophyletic, all modern crop plants rooting back to a single point, indicative of their common descent from a single progenitor population of early domesticate. The early domesticates were genetically most similar to wild plants from the Karacadag region of southeast Turkey. Similar AFLP analyses subsequently revealed an origin for barley in N Israel – NW Jordan area [7] and the tetraploid emmer wheat that was also found in Karacadag mountains region of southeast Turkey [50,51,2] questioned the use of AFLP markers in phylogenetic studies addressing crop domestication. Subsequently, Salamini et al. [58] cited several dozens of papers that correctly addressed domestication issues based on AFLP markers.

The monophyletic and localized event detected by AFLP typing in wheat and barley was thus interpreted as emergence of a ‘superior landrace’ possibly one possessing a major domestication phenotype such as the tough rachis (Salamini et al. [58]). This scenario is compatible with a lengthy period of plant utilization before domestication, but the difficulty remained that the tight affinity between each modern crop and a single wild population was consistent with a gradual transition only if during this transition the plants ancestral to the superior landrace either did not cross-hybridize with wild plants

or only cross-hybridized with their parent population. Brown et al. [12] argued that neither scenario is likely unless the early cultivators possessed the ability to isolate their crops from wild plants or if these pre-Neolithic communities were much less mobile than previously thought. They suggested that archaeo-botanical studies are showing that acquisition of the full set of traits observed in domesticated cereals was a protracted process, intermediate stages being seen at early farming sites throughout the Fertile Crescent. These authors favor a multi-regional nature of cereal domestication, in contrast to a previous view that each crop was domesticated by a rapid, unique and geographically localized process. Olsen and Gross [47] pointed out that a multi-locus cluster analyses can generate a monophyletic grouping for a hybrid species even if that species has evolved multiple times independently. This scenario potentially parallels that of independent domestication events followed by admixture of events. Thus, in assessing whether a crop's monophyletic grouping is real, it is critical to examine whether the alleles in the crop are truly a subset of those in the wild populations with which the crop is most closely clustered, as would be expected in a progenitor/derivative relationship [3].

1.10. Comments on barley origin and domestication history

The cultivated barley is an important element of the human diet, brewing malts, and the most important feed supplement for domestic animals. Taxonomists in the past recognized the two-rowed and the six-rowed cultivated barleys as two species; *Hordeum distichum* L. and *Hordeum hexastichum* L. but now the two forms are considered subspecies of one polymorphic species i.e. *Hordeum vulgare* L., which has been regarded to comprise the wild forms as *H. vulgare* L. subsp. *spontaneum* (C. Koch) Thell. and the cultivated forms as *H. vulgare* L. subsp. *vulgare*. A third and closely related form of barley that was first found in the Himalayan Tibet (*Hordeum agriocrithon* Åberg) is very close to the cultivated barley and is regarded as a form of the six-rowed cultivated subspecies [11]. The wild

spontaneum barley has been regarded as the living progenitor of cultivated barley [46,74]. *Hordeum spontaneum* is an annual spring growing, two-rowed, self-pollinated barley with long-haired and brittle rachis. Wild, brittle, two-rowed wild barley grow in central and southeast Asia and are also reported in Morocco. The Moroccan wild barley forms grow mostly as weeds and not as independent wild populations [11]. In barley the main characters that have been selected for during domestication include brittle rachis, shattering of pods, reduction/loss of dormancy, rapid germination after sowing, shorter time to flowering and maturity, increased yield per plant, disease resistance, and stress tolerance. Both the cultivated and wild forms of barley are diploid with $2n = 14$, interfertile and their hybrid is normal [11]. The oldest archaeological remains of domesticated barley are found in human Neolithic sites in the Fertile Crescent such as Abu Hureyra in Syrian and Jericho in Palestine and are dated to 10500 calibrated years (cal.) ybp [17,75].

1.11. Molecular approaches to barley domestication

Badr et al. [7] investigated the origin and the pathway of barley domestication, based on evidences derived from polymorphism in AFLP finger printing and also in the polymorphism of PCR amplification patterns of the *Bkn-3* gene alleles of the hooded mutation in the barley *Bkn-3* gene caused by a 305 bp duplication in intron IV of the gene. Three alleles of the gene were characterized: *wt*, *K* and *Ke* [44]. The wild type has three allele types; I, II and IIIa, the K allele is designated as allele type IIIc and the Ke as allele IIIb. It was also assumed that the area of barley domestication has been geographically stable for the last 10000 years and that the wild progenitor has not undergone significant genetic changes. Materials representing more 367 accessions of wild barley from the primary habitat in the Fertile Crescent and from secondary habitats from the Mediterranean area and Asia were used in addition to more than 100 cultivars and modern varieties of cultivated barley.

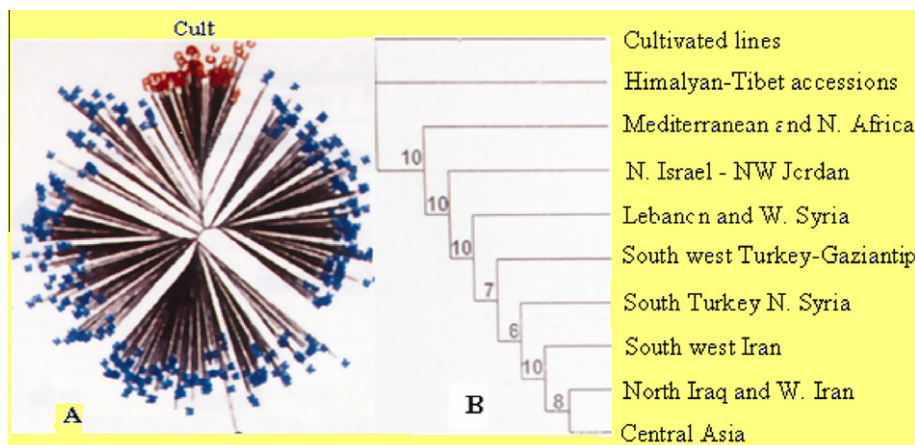


Figure 2 (A) Non-rooted phylogenetic tree illustrating monophyletic origin of cultivated barley in red compared to wild barley in blue. (B) Distance tree illustrating genetic distance between the cultivated barley and the groups of wild barley from the Middle East and the Mediterranean as well as Middle Asia and China. Genetic distance was computed according DICE, Roger-W and NEI72 algorithms using the SAS package based on the probability that a fragment in one genotype would be present in another. AFLP phylogenetic trees were constructed by the neighbor-joining (NJ), FITCH, UPGMA and CONTML methods in the PHYLIP package [19].



Figure 3 (A) Sampling sites of wild barley accessions collected in Israel and Jordan near the Syria border. Asterisks indicate lines with close genetic distances to the cultivated gene pool. Red dots indicate sites of collection of wild barley lines with *BKn-3* allele I. (B) Flow of alleles of the *BKn-3* gene from wild barley populations to cultivated germplasm. The borders of primary habitats of wild barley in the Fertile Crescent are represented by the dotted red line. Arrows indicate gene substitutions as barley domestication moved to the East and the development of western landraces [7].

The phylogenetic analysis of AFLP data (Fig. 2A) indicated that all cultivated barley lines are clustered as one group (Cult) and the wild accessions as another group. This result supports the view that the gene pool of the cultivated barley is a single monophyletic taxonomic entity and has a monophyletic origin. The analyses also showed that accessions from the Himalayan-Tibet area in Asia and from N. Africa and the Mediterranean are closer to the cultivated barley genotypes (Fig. 2B). However, the closest accessions of wild barley from its primary distribution area in the Fertile Crescent were accessions gathered from sites in the Jordan Valley region in N. Israel and N.W. Jordan (Fig. 2B).

Based on the AFLP fingerprinting analyses Badr et al. [7] indicated that the area of N Israel and NW Jordan host a stock of wild barley, which is genetically the closest, of the Fertile Crescent wild barley, to the present day cultivated barley. This area may be regarded as the primary area of barley domestication that could have taken place at multiple sites (Fig. 3A). The approaches applied by Badr et al. [7] pinpointed with loose precision two geographic areas within the Jordan Valley region in which the first domestication of barley may have taken place. Moreover, the diagnostic allele I of the *BKn-3* gene that is almost exclusively found in material from that area was pervasive in western landraces and modern cultivated varieties.

The analysis of the PCR amplification fingerprinting of the *Bkn-3* gene alleles showed that the *BKn-3* allele II that dominated in accessions from this area also dominated in all accessions in the west of the Fertile Crescent as well as, the Mediterranean and N African accessions of wild barley. The close genetic affinity of the N African and Mediterranean wild barley accessions to the cultivated barley may indicate that wild barley from these regions may have been introgressed

by cultivated two-rowed cultivated barley. Allele IIIa dominating in the Himalayan-Tibet wild barley accessions dominated in wild accessions from east of the Fertile Crescent, central Asia as well as in the Himalayan-Indian cultivated landraces indicating allelic substitution during the migration of barley from the Near East to South Asia (Fig 3B). The Himalayas may be considered a region of domesticated barley diversification.

Previous molecular evidence suggested that barley was domesticated, from populations in the Fertile Crescent in the western part of the range of its wild progenitor [7,58], subsequently expanding west into Europe and North Africa and east into Asia 8000 years ago [11]. More recent studies based on molecular markers comparing wild to domesticated barley, have shown that a large amount of nucleotide diversity has been lost in current domesticated varieties. Kilian et al. [32] determined the haplotypes at seven loci-*Adh2*, *Adh3*, *Amy1*, *Dhn9*, *GAPDH*, *PEPC* and *WAXY* for 20 cultivated barley lines and 25 wild barley lines. They calculated that the number of haplotypes, average nucleotide diversity, p and Watterson's theta at silent sites was reduced in domesticated lines; *Amy1* and *PEPC* were monomorphic in domesticated lines; *Amy1* and *GAPDH* produced significant values of Tajima's D when all domesticated and wild lines were considered. At *GAPDH*, p was slightly higher in domesticated than wild forms, due to divergent high-frequency haplotypes; for the remaining six loci, 87% of nucleotide diversity has been lost in the domesticated forms. Bottlenecks acting on neutrally evolving loci either during the domestication process, during subsequent breeding, or both, are sufficient to account for reduced diversity and the results of Tajima's test, without the need to evoke selection at these loci. Phylogenetic networks

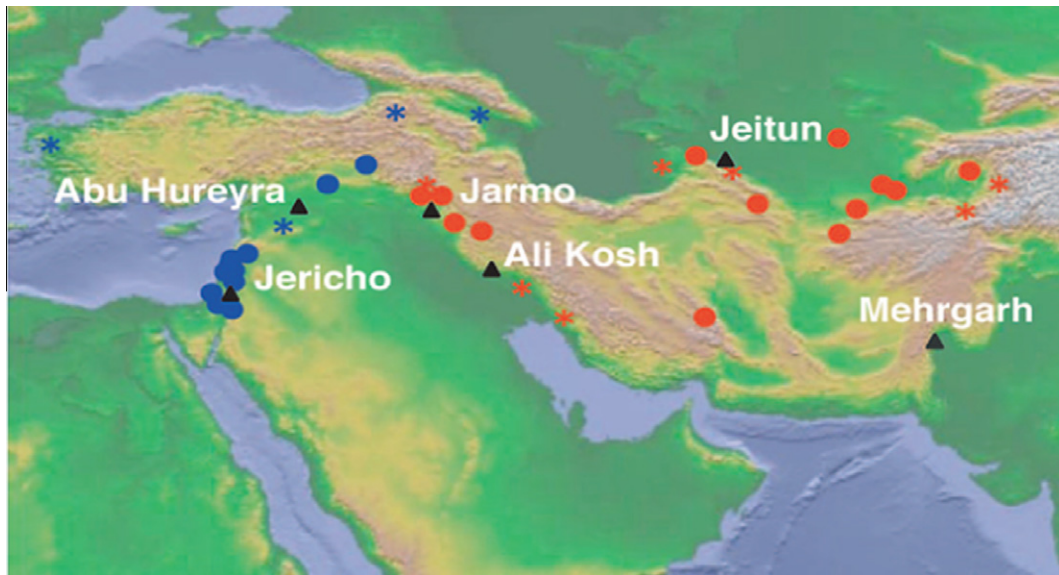


Figure 4 The neolithic sites indicated in Morrell and Clegg [43] illustrating probable domestications sites of barley: Jericho (Palestine), Abu Hureyra (Syria), Jarmo (Iraq), Ali Kosh (Iran), Jeitun (Turkmenistan), and Mehrgarh (Pakistan).

data uncover distinct wild and domesticated barley genotypes and indicated that barley may have been domesticated in the Jordan Valley. These new findings are in agreement with the previously inferred area of barley domestication in the Jordan Valley as indicated by the analysis of AFLP data [7].

Other recent data have agreed with the conclusion that two-rowed and six-rowed genotypes may have different, independent origins [32,36,75]. These new data however open the possibility that barley domestication might have been diphyletic. However, the diphyletic origin that was not inferred from the AFLP data presented by Badr et al. [7] is favored by some authors of works that have addressed the origin of barley material growing in secondary habitats of the wild barley distribution. Examples include [5,15,37,42,49]. Meanwhile Taketa et al. [62] concluded a monophyletic origin of naked barley inferred from molecular analyses of a marker closely linked to the naked caryopsis gene (*nud*). The particular matter concerning single versus multiple origins of barley is however complicated by the following two views (i) Multiple independent introgressions of genes from wild relatives to cultivated varieties can mimic multiple domestication events [31,1]; and (ii) Splitting of domesticated genotypes in two alternative groups based on two-six-rowed ears, hulled-naked caryopsis, western-eastern varieties, and brittleness of the rachis may have followed, and not be coeval with, the domestication process.

Recent evidence reported by Morrell and Clegg [43] indicates that a second domestication event may have occurred in this cereal crop species, possibly in Central Asia at the eastern edge of the Iranian Plateau, and that this separate origin may have been the progenitor of present day barleys found in East and South Asia. Morrell and Clegg [43] used differences in haplotype frequency among geographic regions at multiple loci to infer at least two domestications of barley; one within the Fertile Crescent and a second 1500–3000 km farther east. They proposed that the Fertile Crescent domestication contributed the majority of diversity in European and American cultivars, whereas the second domestication contributed most of the diversity in barley from Central Asia to the

Far East. This view is in line with the assumption of Badr et al. [7] that allele substitution has occurred during the migration of barley cultivation from the Near East to Middle and South Asia. Morrell and Clegg [43] concluded that the earliest remains of barley have been recovered alongside einkorn and emmer wheat at Neolithic sites in the Fertile Crescent (Fig. 4) including Jericho (Palestine) and Abu Hureyra (Syria) about 10500 years BC. In the Zagros Mountains, at sites such as Ali Kosh (Iran) and Jarmo (Iraq), domestic barley has been found at sites dated between 9000 and 10,000 ybp. Further east, domesticated barley has been found at Mehrgarh (Pakistan) about 9000 BC, and at Jeitun (Turkmenistan) as early as about 8000 ybp.

1.12. Molecular approaches to clover ancestry and domestication

The Egyptian clover (*Trifolium alexandrinum* L.) belongs to section *Trifolium* of the genus *Trifolium* L., which comprises 242 species, of World-wide distribution, and includes 16 cultivated species all as forage crops [76]. The Egyptian clover (Berseem) has been widely cultivated, as a forage crop, in western Asia and North Africa for a long time. Its cultivation was extended to Middle Asia, particularly Pakistan and India and also the United States of America since the beginning of the 20th century [35]. In their comprehensive monograph on the whole genus, Zohary and Heller [76] recognized two varieties of *T. alexandrinum*; Var. *alexandrinum* with the local name Fahli and Var. *serotinum* with the local name Muscavi. The Fahli variety gives basal branching and one crop per cultivation, whereas the Muscavi variety gives apical branching and 4–6 crops per cultivation. A third variety, the Saidi, produces both basal and apical branching and gives 2–3 crops per cultivation.

1.13. Views on the possible ancestors of the Egyptian clover

Trabut [65] assumed that *T. berytheum* Bioss, from the coasts of Lebanon, might be a wild form of *T. alexandrinum* and its

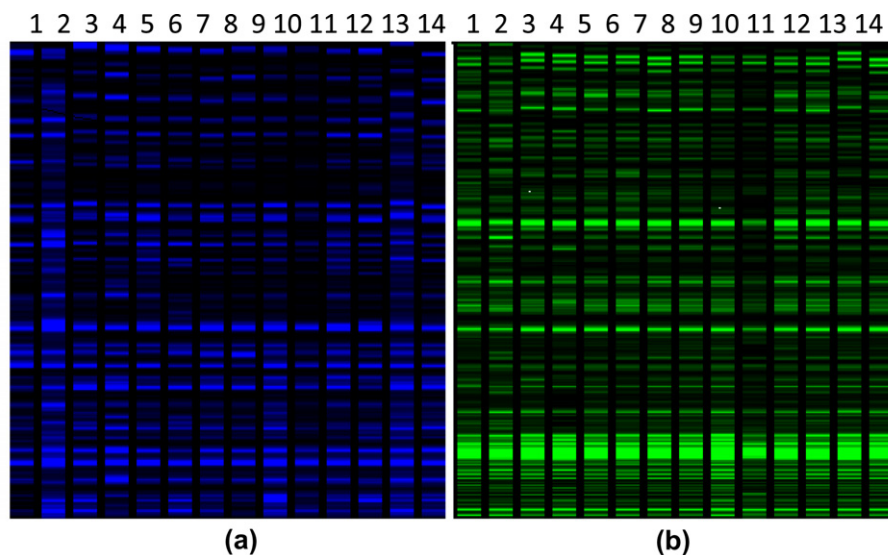


Figure 5 AFLP banding profile for nine accessions of *Trifolium alexandrinum* (1–9), *T. salmoneum* (10), *T. apertum* (11–12) and *T. berytheum* (13–14). DNA was cut with *EcoRI* and *MseI* and fragments were amplified using PCR in the presence of the *MseI* adapter CAC, and two *EcoRI* adapters; ACA (a) left and AAG (b).

immediate progenitor. This idea was supported by Oppenheimer [48] who invalidated claims for other possible ancestors such as: *T. echinatum*, *T. carmeli*, *T. vavilovii*, *T. constantinopolitanum*, *T. leucanthum*, *T. phleoides*, and *T. salmoneum*. On the other hand, Bobrov [10] assumed that *T. apertum* is the true progenitor of *T. alexandrinum*. Comprehensive cytogenetic studies by Putiyevsky et al. [53] indicated that *T. berytheum*, and *T. salmoneum* seem to be the true ancestors of the Egyptian clover. Delile [16] mentioned that seeds of clover, in his time, were frequently imported, into Egypt, from Syria where this clover was cultivated and grows wild Hegi [26] claimed that the Mamluks (rulers of Egypt from 12th to 15th century AD) introduced clover into Egypt, from Caucasus. Becker-Dellinger [8] proposed that clover was introduced into Egypt in the 6th century AD. However, Putiyevsky et al. [53] suggested that clover was probably the earliest forage crop to be sown as from the first Egyptian dynasty (5500 – 5800 ybp). This view is supported by Taylor [64] who stated that Egyptian clover (*T. alexandrinum*) was probably native to the Nile Valley in the ancient Lower Egypt.

1.14. Clover material and AFLP approach

To locate the origin of the cultivated Egyptian clover and trace its ancestry using AFLP, 30 accessions cultivated accessions including landraces and varieties of the Egyptian clover and 26 accessions from other eleven related wild species have been obtained from different sources and areas of the World. AFLP fragments labeled with fluorescent dye were amplified and separated using automated DNA sequence machine. Examples of the AFLP fingerprinting produced by two adapter combination are given in Fig. 5. Distance trees demonstrating the genetic diversity among the accessions were constructed based on Dice and Jaccard similarity coefficients using UPGMA and Neighbor-joining tree building methods with the software NTSYS-pc 2.1 [56]. In addition, the PAUP software (Swofford 2002) was also used to conduct a parsimony analysis, using

heuristic search with MULTREES in effect TBR branch swapping and 100 replicate random additions. Bootstrap values were calculated for 1000 replicates and plotted onto the strict consensus tree of 2149 most parsimonious trees.

1.15. Results and comments inferred from AFLP trees

The distance trees illustrating the genetic relationships of 30 cultivated clover and 26 accessions of eleven of its closely related wild species grouped the 30 accessions of cultivated clover as one major group indicating a monophyletic origin for the Egyptian clover. In this major group, 21 accessions were distinguished from a smaller group of nine mostly Syrian and Egyptian varieties that also comprise one accession representing *T. salmoneum* (Fig. 6A). The accessions representing *T. apertum* and *T. berytheum* formed a small cluster assigned to the latter group whereas accessions representing other species were delimited as another separate group. However, close genetic similarity between the cultivated accessions from Syria and Egypt and accessions representing the three wild species *T. salmoneum*, *T. apertum* and *T. berytheum* was demonstrated by the strict consensus tree illustrating the genetic relationships of the examined accessions and (Fig. 6B). In this tree, two cultivated accessions from Syria (alex 94 and 99) and two Egyptian accessions (alex 22 and 57) are clearly grouped with one sample representing *T. salmoneum* (salm 15), two samples of *T. apertum* (aper 84 and 10) and two samples of *T. berytheum* (bery 59 and 12).

1.16. General comments and conclusions on Egyptian clover domestication

The analysis of AFLP data by distance and parsimony methods showed that accessions of *T. apertum*, *T. berytheum*, and *T. salmoneum* are most similar to *T. alexandrinum* accessions from Syria and Egypt. Based on the cross-ability of these species [53] and their geographic distribution [76], *T. apertum* may

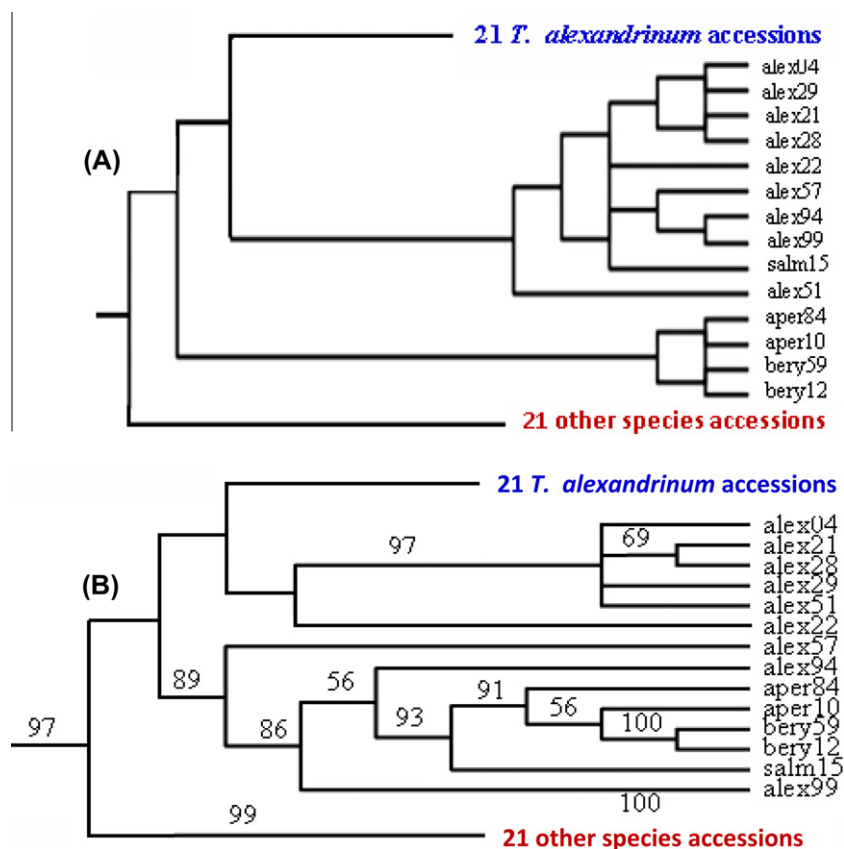


Figure 6 Distance tree (A) and a consensus tree (B) illustrating close genetic relationships of cultivated *T. alexandrinum* and to *T. salmoneum* in a cluster of *T. alexandrinum*.

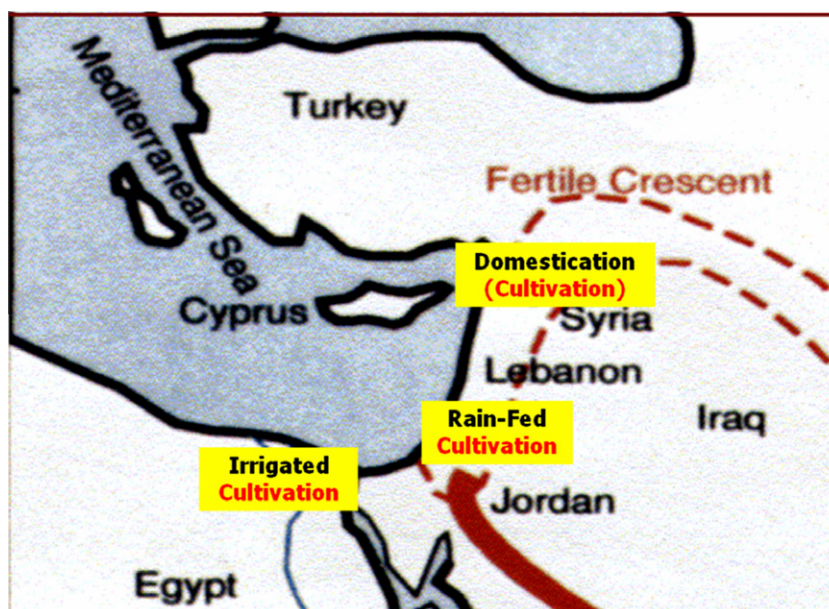


Figure 7 Diagrammatic representation of domestication sites of the Egyptian clover by rain-fed cultivation in Syria and Palestine and irrigated cultivation in Egypt.

be regarded unlikely ancestor of the Egyptian clover. In the meantime, *T. salmoneum* has been found the most probable ancestor for Syrian material of the Egyptian clover. Close relationship has been revealed between *T. salmoneum* and *T.*

berytheum and the same accessions of *T. alexandrinum*. The ability of these species to cross freely as reported by Putiyevski et al. [53] and others may indicate that *T. salmoneum* and *T. berytheum* may be regarded as the main ancestors from that

had given rise to the Egyptian clover by selection in Syria under rain fed irrigation (Fig. 7). After domestication, the early forms of the domesticated crop could have been taken into rain-fed cultivation in Palestine and irrigated cultivation in Egypt. In this regard, the domestication of the Egyptian clover may be analogous to other crops, such as barley and wheat that were also domesticated in the Fertile Crescent and taken into cultivation in the Nile Valley as one of the early domesticated legume. It seems that genetic improvement of the crop had occurred in Egypt after cultivation and that varieties developed in Egypt were later widely distributed.

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