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Review paper

A NOTE ON THE EARLIEST DISTRIBUTION, CULTIVATION AND GENETIC CHANGES IN BITTER VETCH (*Vicia ervilia*) IN ANCIENT EUROPE

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Bitter vetch (*Vicia ervilia* (L.) Willd.) was a part of the everyday diet of the Eurasian Neanderthal population and the modern human Palaeolithic hunter-gatherers at the end of the last Ice Age. The major criteria to determine the domestication in bitter vetch and other ancient grain legumes are non-dehiscent pods, larger seed size and smooth seed testa. Bitter vetch seeds were found among the earliest findings of cultivated crops at the site of Tell El-Kerkh, Syria, from 10th millennium BP. Along with cereals, pea and lentil, bitter vetch has become definitely associated with the start of the 'agricultural revolution' in the Old World. Bitter vetch entered Europe in its south-east regions and progressed into its interior via Danube. Its distribution was rapid, since the available evidence reveals its presence in remote places at similar periods. Recently the first success has been obtained in the extraction of ancient DNA from charred bitter vetch seeds. The linguistic evidence supports the fact that most of Eurasian peoples have their own words denoting bitter vetch, meaning that its cultivation preceded the diversification of their own proto-languages.

Key words: archaeobotany, bitter vetch, crop domestication, crop history, paleogenetics, *Vicia ervilia*

LEGUMES

Legumes (*Fabaceae* Lindl.) are one of the richest plant families in the world, extending over all continents with hundreds of genera and thousands of species (LEWIS *et al.* 2005).

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Numerous annual and perennial members of this family have been having a great economic importance throughout human history (RUBIALES *and* MIKIĆ 2015), such as chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris*), lucerne (*Medicago sativa* L.), pea (*Pisum sativum* L.) or clovers (*Trifolium* spp.).

Legume crops are used in diverse forms and for various purposes: as seedlings, immature pods, immature grains and mature grains for human consumption, as fresh forage, forage dry matter, forage meal, silage, haylage, straw, grazing and browsing in animal feeding and for various non-food uses, comprising pharmaceutical industry, human and veterinary medicine, biofuel, green manure and ornamental ones (MIKIĆ *et al.* 2011).

VETCHES

The genus vetch (*Vicia* L.) comprises at least 150 species, with faba bean (*V. faba* L.) and common vetch (*V. sativa* L.) as the economically most important (ORAK 2000). While faba bean is grown almost exclusively for grain, common and other vetches are common forage plant (MIKIĆ *et al.* 2014b), either as sole crops or in mixtures with cereals, brassicas or with each other (MIKIĆ *et al.* 2015) and only occasionally cultivated for grain (MIKIĆ *et al.* 2013b). Many vetch species are either heavily neglected and underutilised crops or wild species with a great economic potential, such as bitter (*V. ervilia* (L.) Willd.), large-flowered (*V. grandiflora* Scop.), Narbonne (*V. narbonensis* L.), Noë's (*V. noeana* Reut. ex Boiss.), Hungarian (*V. pannonica* Crantz) and French (*V. serratifolia* Jacq.) vetches (MIKIĆ *et al.* 2013a).

In South Europe and the Mediterranean, bitter vetch is cultivated for both forage dry matter and mature grain, with average yields of about 7 t ha^{-1} and between 300 kg ha^{-1} and 800 kg ha^{-1}, respectively (MIHAILOVIĆ *et al.* 2006). Today, bitter vetch is used solely in animal feeding and considered having a great nutritional value by the local farmers. The presence of canavanine, leading to a poorer palatability, has removed it from human diets a considerable time ago. Apart from improving grain yield, enhancing the potential of the bitter vetch crop for these regions includes increasing the resistance to black aphids (*Aphis fabae* Scopoli) and broomrape (*Orobanche* spp). In Serbia today, the only bitter vetch breeding programme is carried out at the Institute of Field and Vegetable Crops in Novi Sad, with the cultivar Perper registered in 2008 (Fig. 1).

Fig. 1. The only Serbian cultivar bitter vetch cultivar Perper, registered in 2008, in full bloom, Rimski Šančevi, Serbia, mid-may 2009

BEGINNINGS

Most of the traditional Eurasian grain legumes, such as chickpea, lentil, pea and common vetch originated primarily in the Near Eastern centre of diversity. On the other hand, some of their closest botanical relatives and economically important species, such as bitter vetch, red vetchling (*Lathyrus cicera* L.) and grass pea (*Lathyrus sativus* L.), evolved primarily in the Mediterranean centre of diversity and secondarily in Near East. All these species share a similar number of chromosomes of $2n = 12$ or $2n = 14$ (ZEVEN *and* ZHUKOVSKY 1975).

Bitter vetch and other grain legumes had been known to humans before they became cultivated crops. The earliest evidence of their use in human consumption are the fossilized microremains in calculus of the Neanderthal skeletons from Shanidar Cave in Iraq about 46,000 years old (HENRY *et al.* 2011), along with few other cereals. Together with pea and vetchlings (*Lathyrus* spp.), bitter and other vetches were present in the everyday diet of the hunter-gatherers at the end of the last Ice Age in Europe, as witnessed by the remains from the site of Santa Maira, Spain, from 10,000 to 7,000 BC (AURA *et al.* 2005).

DOMESTICATION

The remains of domesticated bitter vetch and other grain legumes often occur at high frequencies during the 10th and 9th millennia (WILLCOX *et al.* 2008) may contribute to the possibility that the domestication of grain legumes could predate cereals (KISLEV and BAR-YOSEF 1988). However, little is known about the early stages of grain legumes domestication. It is hard to determine there is very little evidence of how, when and where they were domesticated, mostly due to a fact that all those changes, being mostly morphological, do not survive to the present day. Among the earliest findings of cultivated grain legumes is the site of Tell El-Kerkh, Syria, from 10th millennium BP, with the seeds of bitter vetch, chickpea, grass pea, faba bean, lentil and pea (TANNO and WILLCOX 2006).

In all plant species, the process of domestication led to certain morphological changes that, in many aspects, strongly resemble the methods of selection used in contemporary plant breeding programmes. In grain legumes, the major criteria to determine the domestication are non-dehiscent pods, larger seed size and smooth seed coat. In the case of pea, the best studied close relative of bitter vetch, the gene *DPO*, controlling pod dehiscence (WEEDEN *et al.* 2002), underwent modifications during the domestication and thus became responsible for the development of non-dehiscent genotypes. Larger seed size in cultivated forms in comparison to that in wild ones is not typical only for grain legumes, but is often very hard to interpret. A smooth testa, due to the domestication, is the most reliable characteristic in telling wild from cultivated forms. Apart from these three major, there are several other indicators of the pea, vetchlings and other ancient Eurasian grain legumes domestication, such as absent seed dormancy, dwarf growing habit, less prominent basal branching, neutral photoperiodical reaction and improved grain quality (WEEDEN 2007).

DISTRIBUTION

It is quite certain that bitter vetch was one of the most ancient crops that entered Europe, after it had become more suitable place for living again, following the end of the last Ice Age. Thus bitter vetch, along with grass pea (MIKIĆ *et al.* 2009), lentil (LJUŠTINA and MIKIĆ 2010a) and pea (LJUŠTINA and MIKIĆ 2010b), and several cereals, has become definitely associated with the start of the 'agricultural revolution' in the Old World (ERSKINE 1998). Bitter vetch entered Europe in its southeast regions and roughly progressed into its interior via Danube. Its distribution was a rapid one, since the available evidence reveals its presence in mutually remote places at similar periods (Fig. 2).

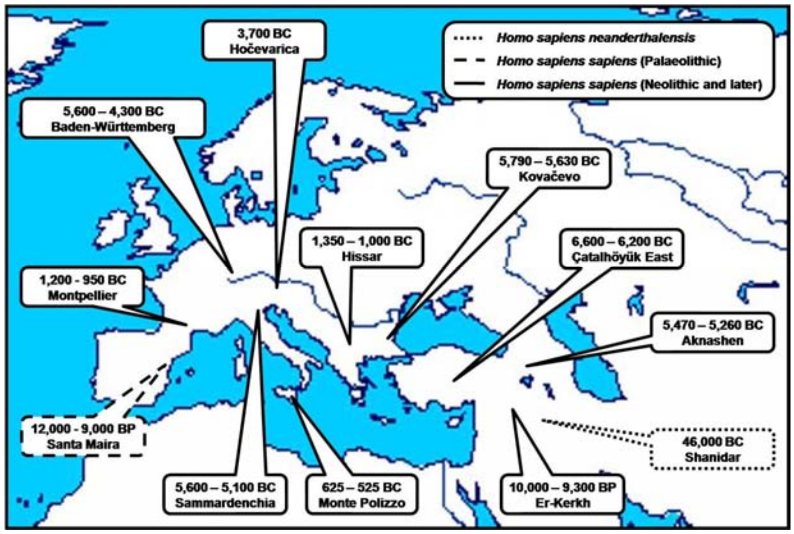

Fig. 2. Some of the archaeobotanical findings of bitter vetch in Europe and its neighbouring regions

The following selection of archaeological findings offers nice examples that confirm the extreme importance pea had in the primeval agriculture in Europe and its adjacent regions.

- 6,600-6,200 BC, Çatalhöyük East, Turkey. In this Neolithic settlement near modern city of Konya in central Turkey, bitter vetch was one of the most important cultivated grain legumes along with cereals (FAIRBAIRN *et al.* 2007).
- 5,790-5,630 BC, Kovačevo, southeast Bulgaria. The final early Neolithic site in southwest Bulgaria, with red vetchling, grass pea, lentil and pea found along with bitter vetch and several cereal species (MARINOVA and POPOVA 2008).
- 5,600-4,300 BC, southwest Germany. The results of more than 100 archaeobotanical investigations from this region reveals that bitter vetch was one of the common grain legumes, with similar values of presence in early Neolithic and Iron Ages (RÖSCH 1997).
- 5,600-5,100 BC, Sammardenchia, northern Italy. In addition to cereals, this Neolithic site was abundant with a diversity of bitter vetch and other grain legumes, including red vetchling, grass pea, lentil, pea and other vetches (ROTTOLI and CASTIGLIONI 2009).
- 5,470-5,260 BC, Aknashen, Armenia. This evidence of bitter vetch along with lentil and pea is a nice example of the bitter vetch distribution to the easternmost regions of Europe (HOVSEPYAN *and* WILLCOX 2007; HOVSEPYAN 2014).

- 3,700 BC, Hočevarica, Slovenia. The first records of grass pea, together with common vetch, in the circumalpine Neolithic settlements of the fourth millennium BC. Other cultivated grain legumes included pea, although considerably less numerous in comparison to cereals (JERAJ *et al.* 2009).
- 2,500-2,000 BC, Titris¸ Höyük, Turkey. Along with cereals, grapes and vetchlings, bitter vetch was one of the major crops in this Early Bronze Age site in southeast Anatolia (HALD 2010).
- 1,350-1,000 BC, Hissar, Serbia. This fortified hill settlement near modern Leskovac in southeast Serbia is rather unique from archaeobotnaical viewpoint due to a lucky find of more than 3,000 bitter vetch seeds in a single storage (Fig. 3), making more than 90% of the total remains of cultivated plants (MEDOVIĆ *et al.* 2011). On the current territory of Serbia, the presence of bitter vetch was also attested at the sites dated to Eneolithic, Bronze Age, Early Iron Age, La Tène and Roman (barbaric) period (MEDOVIĆ and MIKIĆ 2011).
- 1,200-950 BC, southern France. Bitter vetch was present along with red vetchling, grass pea, lentil, faba bean and cereals in several late Bronze Age lagoon sites near modern Montpellier (BOUBY *et al.* 1999).
- 1,100-1,000 BC, Stagno Tuscany, Italy. Vetches, together chickpea, vetchlings, lentil and pea were included in the everyday use at this Final Bronze Age - Iron Age site near Livorno (BELLINI *et al.* 2008).
- 900-700 BC, Monte Trabocchetto, Italy. Bitter vetch, lentil and faba bean were found together with several cereal crops at this early Iron Age in Liguria (AROBBA *et al.* 2003).
- 625-525 BC, Monte Polizzo, Italy. Bitter vetch and other grain legume crops were subdominant to cereals in this early Iron Age site in Sicily (STIKA *et al.* 2008).
- 600-400 BC, four late Hallstat and early La Tène sites in Baden-Wurttemberg, Germany. Together with faba bean, lentil and pea, bitter vetch was an important crop in everyday nutrition, suggesting connections with the Mediterranean lands (STIKA 1999).

Fig. 3. A charred bitter vetch seed from the site of Hissar in southeast Serbia, 1,250-1000 BC

PALEOGENETICS

Probably the first successful extraction of ancient DNA (aDNA) from the charred legume seeds was done from the bitter vetch and pea samples from the site of Hissar in southeast Serbia (JOVANOVIĆ *et al.* 2011). In comparison to more recent sources, the amounts of aDNA extracted from charred seeds are much lower, mostly due to largely anoxic or low-oxygen events occurring with different archaeological remains. In the case of the charred bitter vetch seeds from Hissar, about 2 ng μl^{-1} aDNA using both the standard and a modified cetyltrimethylammonium bromide (CTAB) method and 7.2 ng μl^{-1} aDNA by the commercial QIAGEN DNAesy kit were obtained, respectively. The modification of the CTAB method included increasing the concentration to 3%, in order to improve the osmolarity and obtain better seed disruption, as well as using an insoluble polyvinylpolipyrrolidone (PVPP), remarkably efficient in absorbing polyphenols during purifying DNA and thus preventing them from deactivating proteins and inhibiting the downstream reactions like polymerase chain reaction (PCR).

Since the yield of the extracted aDNA from the charred legume seeds was considerably low in comparison to those of some modern DNA, the whole genome amplification was applied, together with a fragment of nuclear ribosomal DNA gene, 26S rDNA, eventually resulting to the detection of the aDNA among the PCR products (Fig. 4).

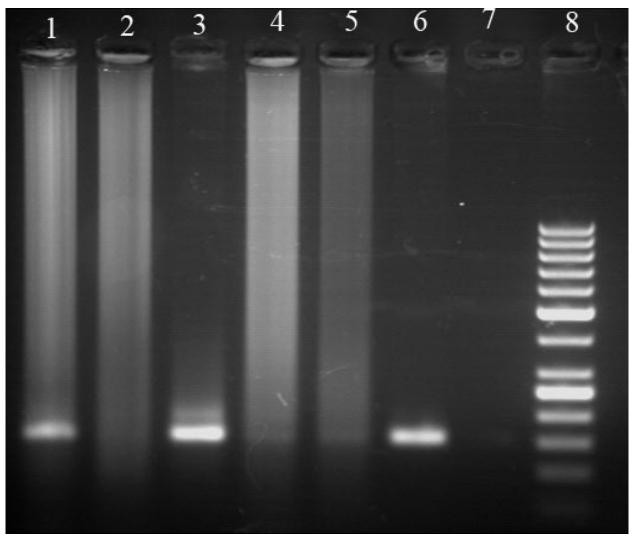

Fig. 4. Polymerase Chain Reaction (PCR) products obtained after amplification of ancient and modern DNA from pea and bitter vetch with 26S rDNA primers. Lanes: 1 - ancient pea DNA extracted using kit, 2 - ancient pea DNA extracted using CTAB method, 3 - modern pea DNA, 4 - ancient bitter vetch DNA extracted using kit, 5 - ancient bitter vetch DNA extracted using CTAB method, 6 - modern bitter vetch DNA, 7 - negative control, 8 - DNA size marker (50bp Fermentas) (JOVANOVIĆ *et al.* 2011)

This was an evidence that the aDNA from charred grain legume seeds, such as those from Hissar, may be relatively easily extracted using a commercial kit, as well as that the DNA fragments, such as 26S rDNA, can be amplified by PCR and found useful for further archaeobotanical and paleogenetic analyses.

The whole genome amplification (WGA) and the sequencing of this chloroplast aDNA should bring more data on the traits of the ancient bitter vetch population from Hissar, encouraging further attempts in extracting also aDNA from nucleus and mitochondria.

HISTORICAL LINGUISTICS

The linguistic evidence supports the fact that bitter vetch and other ancient Eurasian grain legumes had been present in nearly all regions of Europe before the modern European language families were developed. Peoples like Indo-Europeans, Turkic, Caucasians or Basques, each have their own words denoting bitter vetch, meaning that it preceded the diversification of their own proto-languages into their contemporary descendants (MIKIĆ 2015).

Among the most ancient words denoting bitter vetch in Eurasian proto-languages are the Proto-Indo-European **erəg^{w}[h]-* (Fig. 5), the Proto-Turkic **burčak* and the Proto-Basque **iłhař* (MIKIĆ 2012). Similar is with other most ancient legume crops such as lentil (MIKIĆ 2010), pea (MIKIĆ 2009) or faba bean (MIKIĆ 2011).

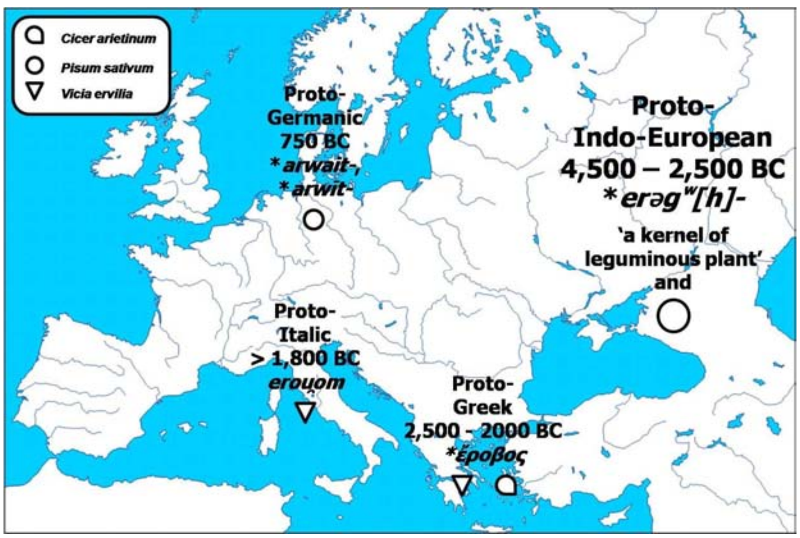

Fig. 5. Initial evolution of the Proto-Indo-European root **erəg^{w}[h]-* (MIKIĆ 2012)

INTEGRATION

Integrating plant aDNA studies with the research on ancient human populations, archaeobotany of cultivated plants and historical linguistics may assess the origin and derivation of the 'agricultural' vocabulary. Such joint efforts could produce verily seminal discoveries, such as casting much more light onto the language spoken by the first farmers in the world, confirming that the bearers of the ‘agricultural revolution’ in Europe were immigrants from Near East or making possible to concurrently follow the human migrations and language development and assessing the connections among the well-established ethnolinguistic families at a significantly earlier time than conventionally considered (MIKIĆ *et al.* 2014a).

PROSPECTS

As one of the most ancient crops in the world, pea played an important role in the introduction of agriculture in post-glacial Europe, often representing the main pulse in the diets of local communities across the continent. The future research on this subject certainly must make a more detailed map of its paths over Europe and, especially, its long-term and essentially important ties with the pea domestication and distribution in Asia Minor, Near East and North Africa. This will hopefully be a useful reminder how widespread and important bitter vetch used to be, as well as a tool for its re-introduction as a presently neglected and underutilised crop.

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BELEŠKA O NAJRANIJOJ RASPROSTRANJENOSTI, GAJENJU I GENETIČKIM PROMENAMA UROVA (*Vicia ervilia*) U DREVNOJ EVROPI

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Izvod

Urov (*Vicia ervilia* (L.) Willd.) bio je deo svakodnevne ishrane neandertalskog čoveka u Evroaziji, kao i savremenog čoveka u zajednicama lovaca i sakupljača krajem poslednjeg ledenog doba. Glavni kriterijumi za određivanje odomaćivanja urova i ostalih drevnih zrnenih mahunarki su nepucajuće mahune, krupnije seme i glatka semenjača. Semena urova pronađena su među najstarijim ostacima gajenih biljaka na lokalitetu Tel El-Kerh, u Siriji, iz 10 milenijuma pre današnjice. Zajedno sa žitaricama, graškom i sočivom, urov je neraskidivo povezan s početkom tzv. poljoprivredne revolucije Starog sveta. uriv je u Evropu u njenim jugoistočnim oblastima i napredovao u unutrašnjost uz Dunav. Njegovo rasprostiranje bilo je brzo, pošto postojeći dokazi ukazuju na prisustvo na međusobno udaljenim mestima u slično doba. Nedavno, ostvaren je prvi uspeh u izdvajanju drevne DNK iz ugljenisanih semena urova. Jezička svedočanstva potvrđuju činjenicu da je većina evroazijskih naroda posedovala svoje sopstvene reči za urov, što podrazumeva da se gajio pre raslojavanja njihovih prajezika.

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