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BAZA PODATAKA MIKROSATELITNIH PROFILA HRVATSKIH SORTI MASLINA

Priručnik o rezultatima VIP projekta

DATABASE OF MICROSATELLITE PROFILES OF CROATIAN OLIVE CULTIVARS

VIP Project Handbook

CRO LIVE

MINISTARSTVO POLJOPRIVREDE

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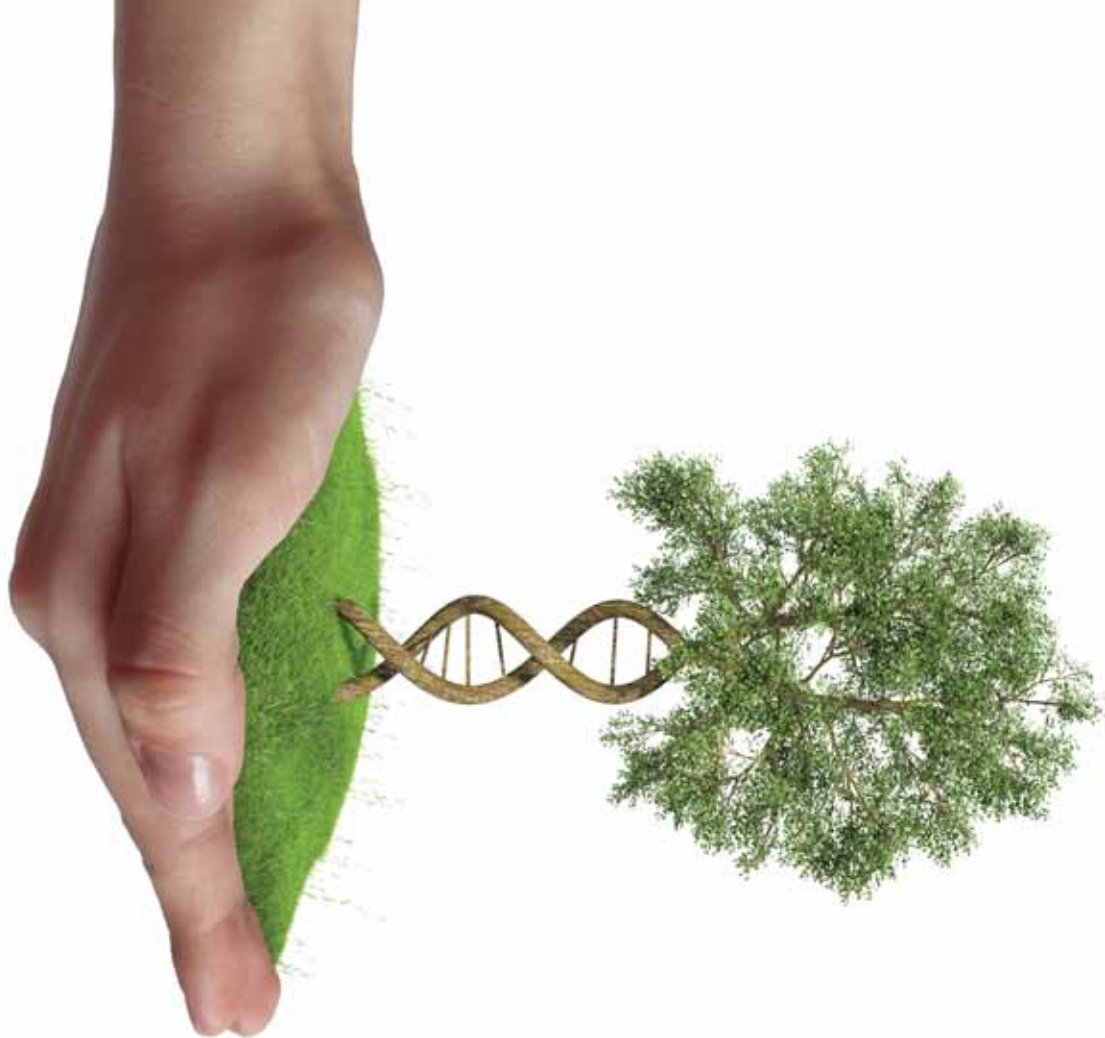

Uvodna riječ

Opojne arome maslinova ulja, koje je svaki put novo i drugačije, otkrivaju nam uvijek nanovo posebnost i dragocjenost toga žutoga zlata iznjedrenog iz stoljetnih stabala, ukorijenjenih u život i tradiciju. I dok ulje obogaćuje obilje mirisa i boja jeseni, moderni se i osviješteni uživalac (i potrošač) ponesen užitkom bogata okusa počinje pitati: Je li oduvijek ovdje, ta naša maslina, ili je odnekuda došla? Kada i otkuda? Kako se razvijala i mijenjala? Ima li sestre blizanke i bliske rođake negdje na Mediteranu ili je li baš ova naša posebna i jedinstvena? Je li joj aroma definirana genima ili je posljedica višestoljetne prilagodbe na okoliš?

Može li genetika ponuditi odgovore na ova pitanja potrošača i rješenja za sve strože zahtjeve koji se postavljaju pred proizvođače, koji radi konkurentnosti i ekonomske isplativosti proizvodnje, osim zadržavanja visoke kakvoće, moraju osigurati i specifična obilježja proizvoda garantirana oznakama izvornosti ili zemljopisnog podrijetla? Na dio njih, svakako može, a brzim napretkom modernih tehnologija,može se očekivati da će rješenja koja će nam genetika moći ponuditi u budućnosti biti sve preciznija i dostupnija. Molekularni alati već se neko vrijeme uspješno upotrebljavaju za identifikaciju sorata maslina. Iz vrlo male količine lisnog materijala, relativno jednostavno možemo izolirati DNA i analizirati je te izraditi genetski profil sorte. Profil se tada može usporediti s profilima u dostupnim bazama podataka koje trenutno više svjetskih timova znanstvenika razvija s ciljem uspostave standarda i sistema usporedbe.

Pred vama je rezultat projekta, prema mojim saznanjima prvog koji je okupio i ujedinio većinu renomiranih sudionika iz akademskog i stručnog sektora u području upravljanja i istraživanja genetskih resursa masline s cijelog područja Republike Hrvatske, u cilju objedinjavanja i usklađivanja svih postojećih znanja na temu genetskih profila naših maslina. Pred vama je, kao rezultat ove suradnje, i prva usklađena, jednoznačna i iskoristiva baza podataka genetskih profila hrvatskih sorata maslina. Ona je tek prvi, mali, ali vrijedan korak na zahtjevnom putu otkrivanja i zaštite biološke raznolikosti ove naše vrijedne kulture. U ime cijelog projektnog tima iskreno zahvaljujem svima koji su znanjem, radom, informacijama, financijskim sredstvima, ali i neizmjerno vrijednim i osvježavajućim entuzijazmom doprinijeli realizaciji ovog projekta. Nadam se da ćete vi koji ovaj priručnik čitate našu bazu smatrati korisnom i poticajnom za odgovore na nove izazove modernih, globalizacijom obilježenih, vremena. Kako započeta priča ne bi ostala svijetu neispričana, nastavimo je zajednički razvijati i novim poglavljima obogaćivati, za generacije koje dolaze...

Danijela Poljuha

Introductory note

While enjoying the smells and tastes of colourful autumn, rejuvenating him (her) self with the exciting aromas of constantly fresh and always different oils, perpetually rediscovering the uniqueness and preciousness of the yellow gold brought forth from centuries old and deeply entrenched olive trees, the modern and informed user (and consumer), inspired by the pleasure of rich taste, begins asking him (her) self: "Has our olive always been here, or was it brought over from some faraway land? Where from and when did it come? How has it developed and changed? Does it have a twin or possibly some cousins elsewhere in the Mediterranean or is it simply unique? Is its aroma defined by genes or is it merely the product of centuries of adaptation to its environment?"

Can genetics offer the answers to these consumer queries and possibly provide a solution for the ever-increasing demands placed upon producers, who, due to the need to maintain the competitiveness and economic cost effectiveness of production, while retaining a high level of quality, need to also ensure the specific characteristics demanded by the protected designation of origin or protected geographical indication? In part it certainly can, and, with the rapid advancement in modern technology, it is to be expected that the solutions which genetics will be able to provide us in the future will be increasingly accurate and even more accessible. Molecular tools have been successfully used to identify olive cultivars for some time. From a very small amount of leaf material, we can relatively easily isolate and analyse DNA and create a genetic profile of the given olive cultivar. The profile can then be compared with profiles present in various databases that are being developed by multiple teams of scientists from across the globe with the goal of establishing an international standard and a system for comparison.

Here before you is the result of our project which is, to the best of my knowledge, the first of its kind that has gathered and united most of the renowned experts from the academic and professional sector of management and research of olive genetic resources from the entire Croatian area, with the goal of uniting and synchronising all available knowledge concerning the genetic profiles of our olives. Before you, as the result of this cooperation, is also the first synchronised, unambiguous and usable database of genetic profiles of Croatian olive cultivars. It is only the first and is a small, but crucial, step on the long and arduous road to the discovery and protection of the biological diversity of this valuable plant culture. On behalf of the whole project team, I sincerely thank everyone who contributed to the realization of this project, be it with their knowledge, effort, information, financial means or their immeasurably valuable and refreshing enthusiasm. I hope that those who are reading this handbook find the database stimulating and useful in finding answers to new challenges presented in this time of globalisation. In order for this story to not remain untold, let us continue to develop it and enhance it with new chapters, to better serve future generations…

Danijela Poljuha

Sadržaj

Content

Determinacija, identifikacija, karakterizacija, genetski profili… U čemu je problem s našim maslinama?

Hrvatska se odlikuje vrijednim i raznovrsnim genofondom masline, koji je nedovoljno istražen. Ovo se podneblje, s posebno izraženom biološkom raznolikošću, kroz povijest pokazalo izuzetno važnim za razvoj i očuvanje sorata maslina. Hrvatska baza podataka o biljnim genetskim izvorima (CPGRD) trenutno sadrži 88 primki masline, dok je na popisu sorata voćnih vrsta trenutno njih 26. Međutim, sa sigurnošću možemo reći da ih je više. U Hrvatskoj se mogu naći i izolirane populacije divljih maslina, kao i feralne forme. Morfološke metode, bazirane na opisu agronomskih i morfoloških karakteristika koje se tradicionalno koriste za opis i razlikovanje sorata, imaju svoje nedostatke poput niske razine različitosti koju otkrivaju, ovisnosti o uvjetima okoliša, vremenskog ograničenja provedbe analiza te potrebe za iskusnim i educiranim ocjenjivačima, a ne smijemo zanemariti niti određenu subjektivnost u njihovoj procjeni. Biološka raznolikost maslina danas se u svijetu intenzivno istražuje molekularnim metodama od kojih je jedna od najčešćih genotipizacija analizom mikrosatelita (ponavljajućih sekvenci DNA), a koje su se pokazale objektivnima, točnim i ponovljivim. U Hrvatskoj, međutim, ne postoji potpuna i standardizirana baza podataka hrvatskih sorata maslina koja sadrži informacije o njihovim genetskim profilima. Mikrosatelitni profili mnogih sorata maslina nisu poznati, a postojeći profili nisu usklađeni, što ih čini međusobno neusporedivima te se na temelju postojećih podataka ne mogu dobiti relevantne informacije niti razriješiti izraženi problemi u nazivlju sorata (homonimi i sinonimi), kontroli sadnog materijala, dokazivanju autentičnosti i sljedivosti maslinovog ulja te održivom upravljanju nacionalnim genetskim resursima.

Determination, identification, characterisation, genetic profiles… Where is the problem with our olives?

Croatia excels in a valuable and versatile olive gene pool, which is insufficiently researched. Throughout history, this environment, with particularly pronounced biological diversity, proved to be exceptionally important for the development and preservation of olive cultivars. There are currently 88 olive accessions in the Croatian Plant Genetic Resources Database as well as 26 olive cultivars on the list of fruit species. However, we can say with certainty that there are more than that. Isolated populations of wild olives, as well as feral forms, can also be found in Croatia. Morphological methods, based on the description of agronomic and morphological characteristics, traditionally used for cultivar description and differentiation, have their deficiencies such as the low level of differences which they reveal, dependence on environmental conditions, time limit set for conduct of analyses and the need for experienced and educated assessors, and we must not neglect also a certain degree of subjectivity in their evaluations. Today, the biological diversity of olives has been intensively investigated by means of molecular methods, of which one of the most frequent is genotyping, using microsatellite markers (repetitive DNA sequences) and which appears to be objective, accurate and reproducible. In Croatia, however, there is no complete and standardised database of the Croatian olive cultivars which contains information about their genetic profiles. Microsatellite profiles of many olive cultivars are not known and the existing profiles are not synchronised, which makes them mutually incomparable and, on the basis of the existing data, relevant information cannot be obtained, nor can pronounced problems be resolved in cultivar terminology (homonyms and synonyms), propagation

Danas još uvijek ne znamo što imamo u svojim maslinicima, kolekcijama i rasadnicima.

Znanje je moć! Ako znam što imam, znam i kako to iskoristiti!

Zašto je važno evidentirati i pravilno identificirati naše sorte?

Naše su sorte naše prirodno bogatstvo! Da bismo ga mogli što bolje zaštititi i sačuvati, moramo steći uvid u postojeću biološku raznolikost masline i genetske resurse područja. Ovo je znanje ključno za upravljanje kolekcijama, rasadničarsku proizvodnju i programe oplemenjivanja. Poznato je, naime, da se sorte razlikuju po agrobiološkim svojstvima, poput otpornosti prema bolestima, štetnicima, niskim temperaturama, uvjetima okoliša, itd. Upravo svojim različitostima pomažu u održavanju stabilnosti poljoprivrednih sustava te doprinose smanjenju opasnosti od genetske ranjivosti. Očuvanjem biološke raznolikosti, a posebno tradicijskih kultivara ili tzv. autohtonih/domaćih sorata, osigurava se široka genetska raznolikost biljnog materijala nužna za oplemenjivačke programe, sprječava genetska erozija i održava baza potencijalno vrijednih biljnih svojstava.

Samo dobrim poznavanjem resursa od kojih polazimo možemo kvalitetno doprinijeti planiranju njihova upravljanja i unaprjeđenja.

material control, proof of olive oil authenticity and traceability, as well as in the sustainable management of national genetic resources.

Today, we still do not know what we have in our olive groves, collections and nurseries.

Knowledge is power! If I know what I have, I then also know how to use it!

Why is it important to record and properly identify our varieties?

Our cultivars are our natural treasure! In order to protect and preserve them as best we can, we have to gain insight into the existing biological diversity of the olives and genetic resources of the area. This knowledge is crucial for the management of collections, nursery production and breeding programmes. Namely, it is well known that cultivars differ in their agro-biological properties, such as resistance to diseases, pests, low temperatures, environmental conditions, etc. It is with their diversities that they assist the maintenance of stability of agricultural systems and contribute to the reduction of risks to genetic vulnerability. By preservation of the biological diversity, and in particular of traditional cultivars or so-called autochthonous cultivars/old varieties, a wide genetic diversity is ensured of plant materials, essential for improvement programmes, genetic erosion is prevented and a base of potentially valuable plant properties is maintained.

Only with a sound knowledge of the resources from which we start can we contribute to the planning of their management and improvement in a quality manner.

A što je s uljem?

Kvaliteta maslinovog ulja, kao i njegova specifičnost, u velikoj mjeri ovise o odabiru sorte u proizvodnji, kao i teritoriju njenog uzgoja. U novije vrijeme stoga sve više raste interes za provjerom identiteta sorata korištenih u proizvodnji maslinovog ulja, prvenstveno radi pravilnog informiranja i zaštite potrošača, ali i proizvođača, odnosno ulja visoke kvalitete poput onih zaštićenih oznakom izvornosti. Suočena sa sve većom konkurencijom na tržištu maslinovog ulja, posebno izraženom nakon ulaska u EU, hrvatska maslinova ulja moraju se istaknuti svojim specifičnim svojstvima i visokom kvalitetom, što je prvenstveno uvjetovano sortom. Jedan od elemenata tržišne prepoznatljivosti i uspješne marketinške strategije jest i isticanje imena sorte na etiketi proizvoda. Iako važeća legislativa u Hrvatskoj ne obvezuje proizvođače na isticanje imena sorte, ono se pokazalo kao značajan element prepoznatljivosti u percepciji potrošača te stoga i kao faktor koji utječe na ostvarivanje više prodajne cijene proizvoda na tržištu. Isticanje sorte na etiketi proizvoda naročito je važno u brendiranju sortnih ulja kao prepoznatljivog proizvoda te će neosporno doprinijeti povećanju prepoznatljivosti i valorizacije hrvatskog poljoprivrednog potencijala.

> *Baza podataka genetskih profila hrvatskih sorata maslina osnova je za pouzdanu identifikaciju biljnog materijala te samim tim predstavlja vrijedan alat za održivo upravljanje genetskim resursima masline.*

And what with the oil?

The quality of olive oil, as well as its specificity, in great measure depend on the selection of cultivars in the production and the territories of their cultivation. In recent years, therefore, there is an increased interest in checking of the identity of the cultivars used in olive oil production, primarily due to adequate informing and protection of both consumers and producers, i.e. of high quality olive oils such as those protected by designations of origin. Facing ever-increasing competition in the olive oil market, especially pronounced following entry into the EU, Croatian olive oils must distinguish themselves by their specific properties and high quality, which primarily depends on the cultivar. One of the elements of market recognisability and a successful marketing strategy is also including the cultivar name on the product label. Although the legislation currently in force in Croatia does not oblige producers to show the cultivar name, it has proved to be a significant element of recognisability in the consumers' perception and therefore also a factor which affects realisation of a higher sales price of the product in the market. Displaying the cultivar name on the product label is particularly important in branding of varietal oils as a recognisable product and will, unquestionably, contribute to the increase in recognisability and valorisation of the Croatian agricultural potential.

> *The database of genetic profiles of Croatian olive cultivars forms a basis for reliable identification of plant materials, thus representing a valuable tool for sustainable management of olive genetic resources.*

DNA krije priču. Treba je samo znati ispričati…

Molekulu DNA volimo zvati molekulom života. Ona u sebi nosi informaciju o karakteristikama živih bića i nasljedni materijal za buduće generacije. Postavlja se pitanje **kako tu informaciju na pravi način pročitati i protumačiti?** Jedan od načina je **korištenjem molekularnih biljega**, u koje spadaju i mikrosateliti. **Mikrosateliti** (SSR; engl. Simple Sequence Repeats) su regije DNA sastavljene od kratkih, jednostavnih ponavljajućih sljedova (1-6 nukleotida; parova baza), koji se tandemski ponavljaju. Razlike u broju ponavljanja uzrok su DNA polimorfizma (različitosti) između jedinki.

DNA hides a story. We should just know how to tell it…

We like to call the DNA molecule a molecule of life. Within itself it carries information about the characteristics of living beings and hereditary material for future generations. The question is asked: ***how do we properly read and interpret this information?*** *One of the methods is* ***by using molecular markers****, which also include microsatellites.* ***Microsatellites*** *(SSR; Engl.* Simple Sequence Repeats*) are DNA regions made up of short, simple sequence repeats (1-6 nucleotides; bases pairs). Differences in the number of repeats are the cause of the DNA polymorphism (dissimilarities) between accessions.*

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Analizom dužine mikrosatelita na određenom mjestu (lokusu) u genomu dobiva se mikrosatelitni profil, određen veličinom alela, izraženom u parovima baza (bp). Mikrosateliti su danas najkorišteniji molekularni biljezi za identifikaciju sorte, odnosno genotipizaciju maslina (Bracci i sur. 2011). Brojna istraživanja pokazala su da se radi o pouzdanoj i reproducibilnoj metodi. U svjetskim istraživanjima ove tematike, mikrosatelitni biljezi se koriste za otkrivanje genetske raznolikosti i karakterizaciju kolekcija maslina, gdje pokazuju veliku genetsku varijabilnost među sortama. U istraživanjima je korišten veliki broj mikrosatelitnih biljega (Sefc i sur. 2000, Carriero i sur. 2002, Cipriani i sur. 2002, De la Rosa i sur. 2002, Baldoni i sur. 2009) i postoje pokušaji njihovog usklađivanja i stvaranja konsenzus-liste ovih biljega (Baldoni i sur., 2009).

Analiza mikrosatelita omogućila je utvrđivanje međusobne srodnosti različitih sorata, odnosa divljih i kultiviranih maslina, mogućih evolucijskih odnosa, ali i otkrivanje te karakterizaciju vrijednih izvora biljnog materijala kao potencijalnih izvora varijabilnosti korisnih za programe oplemenjivanja. Najnovija istraživanja koriste se mikrosatelitnim biljezima za utvrđivanje podrijetla biljnog materijala, dokazivanje autentičnosti i sljedivosti maslinovog ulja (Muzzalupo i sur. 2014, Pasqualone i sur. 2016), kao i proučavanje doprinosa genetskih faktora kvaliteti ulja, tj. povezanost prisutnosti određenih mikrosatelitnih biljega sa sastavom masnih kiselina u maslinovom ulju dobivenom iz tih sorata u cilju lakšeg odabira sorata za uzgoj (Ipek i sur. 2015).

Ukoliko je znamo pročitati, informacija skrivena u molekulama DNA naših maslina, može nam otvoriti nova područja istraživanja, ali i omogućiti prijenos postojećih znanja o njihovom podrijetlu i potencijalu, iz laboratorija u stvarni život.

By the analysis of the microsatellite length in a specific place (locus) in the genome, a microsatellite profile is obtained, determined by the size of the alleles expressed in base pairs (bp). Today, microsatellites are the most used molecular markers for identification of a variety, i.e. genotyping of olives (Bracci et al., 2011). Numerous researches showed that it concerns a reliable and reproducible method. Microsatellite markers are used for the discovery of genetic diversity and characterisation of olive collections, where they demonstrate great genetic variability among cultivars. A large number of microsatellite markers are used in different studies (Sefc et al. 2000, Carriero et al. 2002, Cipriani et al. 2002, De la Rosa et al. 2002, Baldoni et al. 2009) and there have been attempts to synchronise them and create consensus lists of these markers (Baldoni et al., 2009).

The microsatellite analysis facilitated the determination of mutual relatedness between different cultivars, of a relationship between wild and cultivated olives, of possible evolutionary relationships, but also the discovery and characterisation of valuable resources of plant materials as potential resources of variability, useful for breeding programmes. The latest researches use microsatellite markers for determination of the plant material origin, proving of olive oil authenticity and traceability (Muzzalupo et al. 2014, Pasqualone et al. 2016), as well as for studying of the contribution of genetic factors to the olive oil quality, i.e. a link between the presence of specific microsatellite markers and the composition of fatty acids in olive oil obtained from those varieties for the purposes of an easier selection of varieties for cultivation (Ipek et al. 2015).

Provided we can read it, the information concealed in the DNA molecules of our olives can open new research areas to us, but also facilitate the transfer of existing knowledge about their origin and potential, from the laboratory into real life.

DNA može reći sve! Fascinantna istina ili globalna zabluda?

Ni jedno, ni drugo. Iako su mikrosatelitni DNA biljezi zbog polimorfizma, reproducibilnosti i kodominantne prirode vrlo cijenjeni i često korišteni za razlikovanje, opisivanje i identifikaciju biljnog materijala, oni imaju i svoja ograničenja, vezana prvenstveno uz usporedivost rezultata dobivenih analizama u različitim laboratorijima i sadržanih u različitim bazama podataka.

Zašto mikrosatelitni (SSR) profili najčešće nisu usporedivi? Kao prvo, koriste se različiti biljezi (SSR lokusi), jer u slučaju masline još uvijek ne postoji općeprihvaćeni konsenzus oko izbora optimalnih biljega. Također, rezultati mogu varirati između laboratorija zbog različitih sustava koji se koriste i različitih laboratorijskih uvjeta. Stoga je ključno standardizirati procjenu veličine alela, i to korištenjem referentnih profila, kako bi se različiti rezultati uskladili i učinili usporedivima. Osim toga, javljaju se i različiti tehnički problemi poput pomaka u dužini alela te ovisnosti očitanja o korištenim softverima pa je višegodišnje iskustvo u interpretaciji rezultata jedan od ključnih elemenata za uspješnu i pouzdanu analizu.

Uspostavom pouzdane baze podataka, nastale kao rezultat suradnje više stručnjaka i laboratorija, kao i njenom kontinuiranom nadogradnjom, informacija koju iščitavamo iz DNA može postati vrijedan alat u ostvarivanju zadanog cilja, a to je dobivanje jednoznačnih i usporedivih informacija o identitetu biljnog materijala.

DNA can tell everything! Fascinating truth or global delusion?

Neither of them! Although microsatellite DNA markers, due to their polymorphism, reproducibility and codominant nature, are very appreciated and often used for differentiation, description and identification of plant materials, they also have their limitations, related primarily to the comparability of the results obtained by means of analyses in various laboratories and contained in different databases.

***Why are microsatellite (SSR) profiles most frequently incomparable?** Firstly, different markers (SSR loci) are used, as, when it comes to olives, there is still no generally accepted consensus in choosing of optimal markers. Also, results may vary between the laboratories due to different systems which are being used and different laboratory conditions. This is why it is crucial to standardise allele scoring by using reference profiles, in order for different results to be aligned and made comparable. Apart from this, different technical problems are also emerging such as a shift in the length of the alleles and readings which depend on the software used and experience in result interpretation is one of the key elements for a successful and reliable analysis.*

By establishment of a reliable database, created as a result of cooperation of several experts and laboratories, as well as by its continuous upgrade, the information which we read from the DNA can become a precious tool in realisation of a set goal and that is obtaining unambiguous and comparable information about the plant material identity.

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Maslina u Hrvatskoj. Gdje smo? Kamo (i koliko brzo) idemo?

Genotipizacija hrvatskih maslina mikrosatelitnim biljezima izvršena je do sada na manjem broju sorata (Štambuk i sur. 2007, Poljuha i sur. 2008a, Poljuha i sur. 2008b, Miljković i sur. 2010, Klepo 2014), a dobiveni rezultati međusobno su teško usporedivi, što ukazuje na **potrebu sinkronizacije ove vrste analitike na području cijele Hrvatske te objedinjavanja i usklađivanja svih dosadašnjih rezultata u jednu cjelovitu, lako dostupnu bazu podataka** koja bi maslinarima, proizvođačima sadnog materijala te nacionalnoj i međunarodnoj znanstvenoj i stručnoj zajednici omogućila izravan pristup relevantnim podacima te pružila uvid u vrijednu biološku raznolikost sorata masline u Hrvatskoj. To bi doprinijelo i javnoj svijesti o nekim manje poznatim sortama te prepoznatljivosti i razvoju našeg poljoprivrednog potencijala kroz razvoj novih brendova sortnih ulja autohtonih hrvatskih sorata.

Upravo smo ove elemente ugradili u **ciljeve VIP projekta** „Baza podataka mikrosatelitnih profila hrvatskih sorti maslina", koji su bili:

- Formirati konsenzus listu mikrosatelitnih biljega za genotipizaciju hrvatskih sorata maslina;
- Razriješiti dio sinonima i homonima u nazivu sorata te na taj način doći do informacije o približnom broju uzgajanih autohtonih i introduciranih sorata u Hrvatskoj;
- Uspostaviti internetsku bazu podataka na hrvatskom i engleskom jeziku u kojoj će biti dostupne usklađene i jednoznačne informacije o mikrosatelitnim profilima autohtonih sorata masline s cijelog prostora Hrvatske;
- Omogućiti pregledan i jednostavan način uporabe internetske baze

Olives in Croatia. Where are we? Where (and how fast) are we going?

The genotyping of Croatian olives by microsatllite markers has been carried out so far on a smaller number of cultivars (Štambuk et al. 2007, Poljuha et al. 2008a, Poljuha et al. 2008b, Miljković et al. 2010, Klepo 2014) and it was hard to mutually compare the obtained results, ***which points to the need to synchronise this kind of analytics on the territory of the whole of Croatia and to consolidate and harmonise all the up to date results into a single, complete, easily accessible, database****, which would enable olive growers, producers of plant materials and the national and international scientific and professional community to directly access the relevant data and provide an insight into a valuable biological diversity of olive cultivars in Croatia. This would also contribute to public awareness about some lesser known cultivars and recognisability and development of our agricultural potential through the development of new brands of varietal olive oils of autochthonous Croatian cultivars.*

These are the very elements which we have integrated into the ***goals of the VIP project*** *„Database of Microsatellite Profiles of Croatian Olive Cultivars", which were the following:*

- *Establish a consensus list of microsatellite markers for genotyping of Croatian olive cultivars;*
- *Detect some synonyms and homonyms in the names of the cultivars and, in this way, obtain information about the approximate number of cultivated autochthonous and introduced cultivars in Croatia;*
- *Set up a web database in both Croatian and English languages, where harmonised and unambiguous information about microsatellite profiles of autochthonous olive*

podataka u kojoj će se točnost i usporedivost rezultata (profila) osiguravati uporabom referentnih svjetskih sorata;
- Povezati bazu podataka s postojećom i aktivnom hrvatskom bazom podataka o biljnim genetskim izvorima na način da se nova baza osmisli kao njena komplementarna dopuna;
- Osigurati dostupnost i vidljivost te mogućnost nadogradnje ove baze podataka tijekom narednog perioda od najmanje pet godina, u cilju povećanja prepoznatljivosti i valorizacije hrvatskog poljoprivrednog potencijala masline;
- Kroz realizaciju projekta usporednim analizama i međusobnim provjerama uskladiti rad dva nacionalna laboratorija (Poreč i Split), koji pokrivaju cijelu primorsku regiju, a koja je prirodni areal masline u Hrvatskoj;
- Podići razinu javne svijesti o problemu poznavanja, evidentiranja i pravilnog identificiranja sortimenta masline u Republici Hrvatskoj prezentiranjem rezultata putem predavanja, medija i publikacije.

Ciljeve projekta nastojali smo ostvariti uz jasne imperative: Ići naprijed, ići brže, bolje i svakako zajedno!

cultivars from the entire Croatian territory will be accessible;
- *Facilitate a clear and simple method of use of the web database, where the accuracy and comparability of the results (profiles) will be ensured using reference world cultivars;*
- *Link the database with the current and active Croatian Plant Genetic Resources Database (CPGRD) in such a way that the new base is conceived as its complementary addition;*
- *Ensure accessibility and visibility, as well as the possibility of upgrade of this database during the coming period of at least 5 years, for the purposes of an increased recognisability and valorisation of the Croatian agricultural potential of olives;*
- *Through project realisation, using parallel analyses and mutual checks, synchronise the work of the two national laboratories (Poreč and Split) which cover the entire coastal region, which is a natural olive areal in Croatia;*
- *Raise the level of public awareness about the problem of knowledge, recording and correct identification of olive assortment in the Republic of Croatia by means of result presentation through lectures, media and publications.*

We endeavoured to realise the project goals with clear imperatives: To go ahead, to go faster, better and, by all means, together!

Projekt u brojkama

Uvijek je dobro osvrnuti se na kraju projekta i kritički promotriti realizirano. Brojčana vizualizacija jedan je od atraktivnih i korisnih načina da to napravimo.

Project in Numbers

It is always good to look back at the end of the project and critically consider what has been achieved. Numerical visualisation is one of the attractive and useful methods of doing it.

VIP projekt „Baza mikrosatelitnih profila hrvatskih sorti maslina"

VIP project "Database of Microsatellite Profiles of Croatian Olive Cultivars"

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Kako je provedeno istraživanje?

Istraživanje je obuhvatilo **sorte masline** s područja cijele Hrvatske. Pretraživanjem literature i dostupnih baza podataka te međusobnom **komunikacijom hrvatskih istraživačkih grupa i nacionalnih tijela** prikupljeni su svi dosadašnji rezultati **genotipizacije** hrvatskih sorata provedene uporabom **mikrosatelitnih biljega.** U slučajevima gdje je to bilo moguće, izvršeno je usklađivanje genetskih profila. Na temelju prikupljenog izvršen je odabir reprezentativnih uzoraka i formiranje konsenzus liste mikrosatelitnih biljega usporedivih sa svjetskim bazama podataka. U slučajevima u kojima su nedostajali podaci za pojedine biljege ili se rezultati nisu mogu usporediti, **analiza mikrosatelita** je ponovljena. Analiziran je ukupno **51 uzorak** iz obje maslinarske regije (**Istra** i **Dalmacija**), čiji je odabir izvršen s naglaskom na provjerene i dostupne uzorke u registriranim kolekcijama. Analiza je provedena usporedno u dva laboratorija, kako bi se izvršila provjera i usklađivanje metodologije u cilju postizanja visokog stupnja reproducibilnosti i pouzdanosti. U istraživanje su uključene referentne sorte s provjerenim i usporedivim mikrosatelitnim profilima iz nacionalnih i svjetskih kolekcija. U cilju **usklađivanja rada dva nacionalna laboratorija** (Institut za poljoprivredu i turizam u Poreču (IPTPO) i Institut za jadranske kulture i melioraciju krša u Splitu (KRŠ)), nakon svih provedenih analiza izvršena je prezentacija provedenog za članove projektnog tima te razmjena optimiziranih protokola.

Svi rezultati usklađeni su i ugrađeni u **internetsku bazu podataka na dvama jezicima** (hrvatskom i engleskom) koncipiranu na principu „user-friendly" sučelja s mogućnošću jednostavnog pretraživanja na temelju imena sorte i veličine alela. Na stranici su dostupne i videoupute za korištenje baze na dvama

How was the research conducted?

*The research encompassed **olive varieties** from the entire Croatian territory. By examining the literature and available databases, as well as by mutual **communication between the Croatian research groups and national bodies**, all up to date results were collected of **genotyping** of Croatian cultivars, conducted by using **microsatellite markers**. In the cases where it was possible, synchronisation of genetic profiles was carried out. Based on the collected data, a selection was made of representative samples, followed by the establishment of a consensus list of microsatellite markers, comparable to the world databases. In the cases where data was missing for certain markers or where it was not possible to compare the results, the **microsatellite analysis** was repeated. A total of **51 samples** were analysed from both olive growing regions (**Istria** and **Dalmatia**), whose selection was carried out with an emphasis on the verified and available samples in the registered collections. An analysis was conducted in two laboratories in parallel, in order to carry out the check and synchronisation of the methodology for the purposes of obtaining a high degree of reproducibility and reliability. Reference cultivars with verified and comparable microsatellite profiles from national and world collections were included in the research. To **synchronise the work of the two national laboratories** (Institute of Agriculture and Tourism, Poreč (IPTPO) and Institute for Adriatic Culture and Karst Reclamation, Split (KRŠ)), following all the conducted analyses, a presentation was made of what has been done so far for the project team members, as well as the exchange of optimised protocols.*

*All the results were synchronised and built into the **web database in two languages** (Croatian and English), conceived on the principle of "user-friendly" interface, with a possibility of a simple search based on the cultivar name and the size of the alleles.*

jezicima. **Genotipovi su povezani s putovničkim i morfološkim podacima o primkama** te podacima o **sigurnosnoj kolekciji** u svim slučajevima u kojima je to moguće. Struktura internetske baze omogućava nadopunu baze novim genotipovima i nakon završetka projekta te njenu nadogradnju podacima o agronomski važnim karakteristikama te o kemijskim i senzorskim karakteristikama sortnih ulja.

Video tutorials for using the base in two languages are also available on the web page. ***Genotypes are linked to passport and morphological data about accessions*** *and the data about* ***security collection*** *in all the cases where it was possible. The web base structure facilitates data updates with new genotypes even after the completion of the project and its upgrade with the data relating to agronomically important characteristics, as well as with the chemical and sensory characteristics of varietal oils.*

Metode kojima smo se koristili

Važno je bilo koristiti se metodama koje su univerzalne i primijenjene u literaturi. Još ih je važnije optimizirati i prilagoditi uvjetima u laboratoriju. U istraživanju su primijenjene sljedeće metode:

- Ekstrakcija DNA iz biljnog Ekstrakcija DNA iz biljnog materijala pomoću komercijalnog kita za pročišćavanje ukupne biljne DNA - PureLink® Plant Total DNA Purification Kit (Invitrogen), sukladno uputama proizvođača;

- Izrada konsenzus liste mikrosatelitnih biljega u suradnji s laboratorijima u Hrvatskoj i Italiji;

- Genotipizacija mikrosatelitnih regija genoma maslina prema optimiziranim standarnim postupcima iz literature (Sefc i sur. 2000, Cipriani i sur. 2002, De la Rosa i sur. 2002, Baldoni i sur. 2009.); PCR reakcije provedene su korištenjem komercijalnog kita DreamTaq PCR Master Mix (Thermo Scientific) pomoću termocycler-a MyCycler™ (Bio-Rad). Duljina fragmenata PCR produkata određena je kapilarnom gel-elektroforezom (Macrogen Inc., Korea) u dvije multipleks mješavine. Za kapilarnu gel-elektroforezu korišten

The methods we used

It was important to use methods which are universal and already applied in literature. Even more important was to optimise and adapt them to the conditions in the laboratory. The following methods were applied in this research:

- *Extraction of DNA from the plant material using the commercial kit for isolation of total plant DNA - PureLink® Plant Total DNA Purification Kit (Invitrogen), in accordance with the manufacturer's instructions;*

- *Preparation of a consensus list of microsatellite markers in cooperation with the laboratories in Croatia and Italy;*

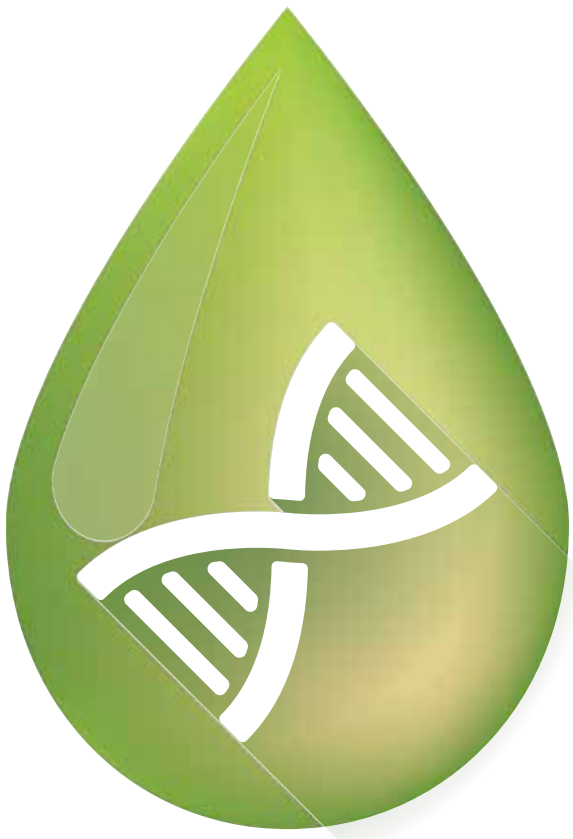
- *Genotyping of microsatellite regions in line with the optimised standard procedures from the literature (Sefc et al. 2000, Cipriani et al. 2002, De la Rosa et al. 2002, Baldoni et al. 2009.); PCR reactions were performed using the commercial kit DreamTaq PCR Master Mix (Thermo Scientific) and termocycler MyCycler™ (Bio-Rad). The length of the PCR products was determined by means of capillary gel-electrophoresis (Macrogen Inc., Korea), in two multiplex mixtures. An ABI3730XL DNA analyser (Applied Biosystems), with a marker of*

je ABI3730XL DNA analyzer (Applied Biosystems) uz biljeg standardnih veličina 400HD.

- Obrada podataka: Elektroferogrami koji su analizirani programima Peak Scanner™ Software v1.0 (Applied Biosystems) i GeneMarker® Software V2.7.0 (SoftGenetisc, USA) i obrađeni u programu Microsoft Excel 2010. Standardizacija svih mikrosatelitnih profila izvršena je na temelju mikrosatelitnih profila standardnih referentnih sorata 'Frantoio', 'Leccino', 'Arbequina' i 'Carolea'.

a 400HD size standard, was used for capillary gel-electrophoresis.

- *Data processing: Electropherograms were analysed using Peak Scanner™ Software v1.0 (Applied Biosystems) and GeneMarker® Software V2.7.0 (SoftGenetics, USA) programmes and processed in the Microsoft Excel 2010 programme. The standardisation of all microsatellite profiles was carried out on the basis of the microsatellite profiles of standard reference cultivars Frantoio, Leccino, Arbequina and Carolea.*



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Rezultati projekta

Odakle krenuti? Od prikupljanja svih dostupnih podataka!

Izvršeno je sakupljanje dostupnih literaturnih podataka o molekularnoj karakterizaciji hrvatskih kultivara masline (*Olea europaea* L.) mikrosatelitnim biljezima te podataka o svjetskim genotipovima, kao i pretraživanje svih dostupnih baza podataka. Pregledom dostupne literature u periodu od 1993. do 2016. godine utvrđeno je da je dostupno ukupno **225 znanstvenih radova** u kojima su u svrhu molekularne karakterizacije korišteni različiti molekularni biljezi (RAPD, RFLP, AFLP, SNP, SSR, ISSR, SCAR ili DArT). Od toga, u **75** publikacija korišteni su mikrosatelitni (SSR) biljezi, a u **18** publikacija SSR biljezi u kombinaciji s drugim biljezima. Pronađeno je ukupno **109** različitih **mikrosatelitnih biljega** čije su sekvence javno dostupne. Ukupno **465 mikrosatelitnih profila** (uključujući i međusobna preklapanja) s upotrijebljenim različitim biljezima dostupno je **u svjetskoj literaturi**. Od toga, analizirano je svega 50 uzoraka za koje se navodi hrvatsko podrijetlo. **U bazama podataka** sadržano je oko **900 profila**, od čega pronađenih **20** za koje se navodi hrvatsko podrijetlo (Istrian Olive Database http://old.iptpo.hr/iod/, 11 hrvatskih (HR) od ukupno (UK) 13 profila/ 14 biljega; OGDD - Olive Genetic Diversity Database https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4733328/, 0 HR/200UK; OLEAdb-OLEA databases http://www.oleadb.it/, 2HR/80UK, 15 biljega; OLVIVA http://www.iamb.it/news,186,186,152,progetto-olviva.htm, 0HR/197UK, 18 biljega; World Olive Germplasm Bank (WOGB) of Cordoba http://link.springer.com/article/10.1007%2Fs11295-013-0671-3, 7HR/411UK, 33 biljega).

Prikupljanje svih dostupnih podataka i njihova sinkronizacija nužni su za

Project Results

Where to start from? From collection of all the available data!

*Collection was conducted of all available literature data about the molecular characterisation of Croatian olive (*Olea europaea *L.) cultivars by microsatellite markers and the data about the world genotypes, as well as a search of all available databases. By a review of the available literature in the period between the years 1993 and 2016, it was determined that a total of **225 scientific papers** were available in which, for the purposes of the molecular characterisation, different molecular markers (RAPD, RFLP, AFLP, SNPs, SSR, ISSR, SCAR or DArT) were used. Out of this, microsatellite (SSR) markers were used in **75** publications and, in **18** publications, SSR markers in combination with other markers. A total of **109** different **microsatellite markers** were found, whose sequences are publicly available. A total of **465 microsatellite profiles** (including also their mutual overlapping), with use of different markers, are available in the **world literature**. Out of this, only 50 samples were analysed, for which the Croatian origin is stated. **In databases**, some **900 profiles** are contained, of which **20** were found for which the Croatian origin is mentioned (Istrian Olive Database http://old.iptpo.hr/iod/, 11 Croatian (CRO) out of the total (T) of 13 profiles/ 14 markers; OGDD - Olive Genetic Diversity Database https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4733328/, 0CRO/200T; OLEAdb-OLEA databases http://www.oleadb.it/, 2CRO/80T, 15 markers; OLVIVA http://www.iamb.it/news,186,186,152,progetto-olviva.htm, 0CRO/197T, 18 markers; World Olive Germplasm Bank (WOGB) of Cordoba http://link.springer.com/article/10.1007%2Fs11295-013-0671-3, 7CRO/411T, 33 markers).*

Collection of all available data and their synchronisation are necessary for solving the

razrješenje problema sinonima i homonima u nazivlju sorata i dobivanja informacije o približnom broju uzgajanih autohtonih i introduciranih sorata u Hrvatskoj. **Sinkronizacija podataka je ključan korak**. Ne postoji, naime, dogovor među laboratorijima vezan uz određivanje veličine alela te je stoga teško validirati usporedbu podataka/profila dobivenih u različitim laboratorijima.

> *Pronađeno je ukupno 465 profila u literaturi te oko 900 profila sadržanih u različitim bazama podataka. Od toga se za ukupno 50 profila u literaturi i 22 profila u bazama podataka navodi hrvatsko podrijetlo.*

*problem of synonyms and homonyms in the cultivar names and for obtaining information about the approximate number of autochthonous and introduced cultivars in Croatia. **Data synchronisation is a crucial step**. Namely, there is no arrangement between the laboratories with respect to the determination of the alleles size, which makes it difficult to validate the comparison of the data/profiles which are obtained in different laboratories.*

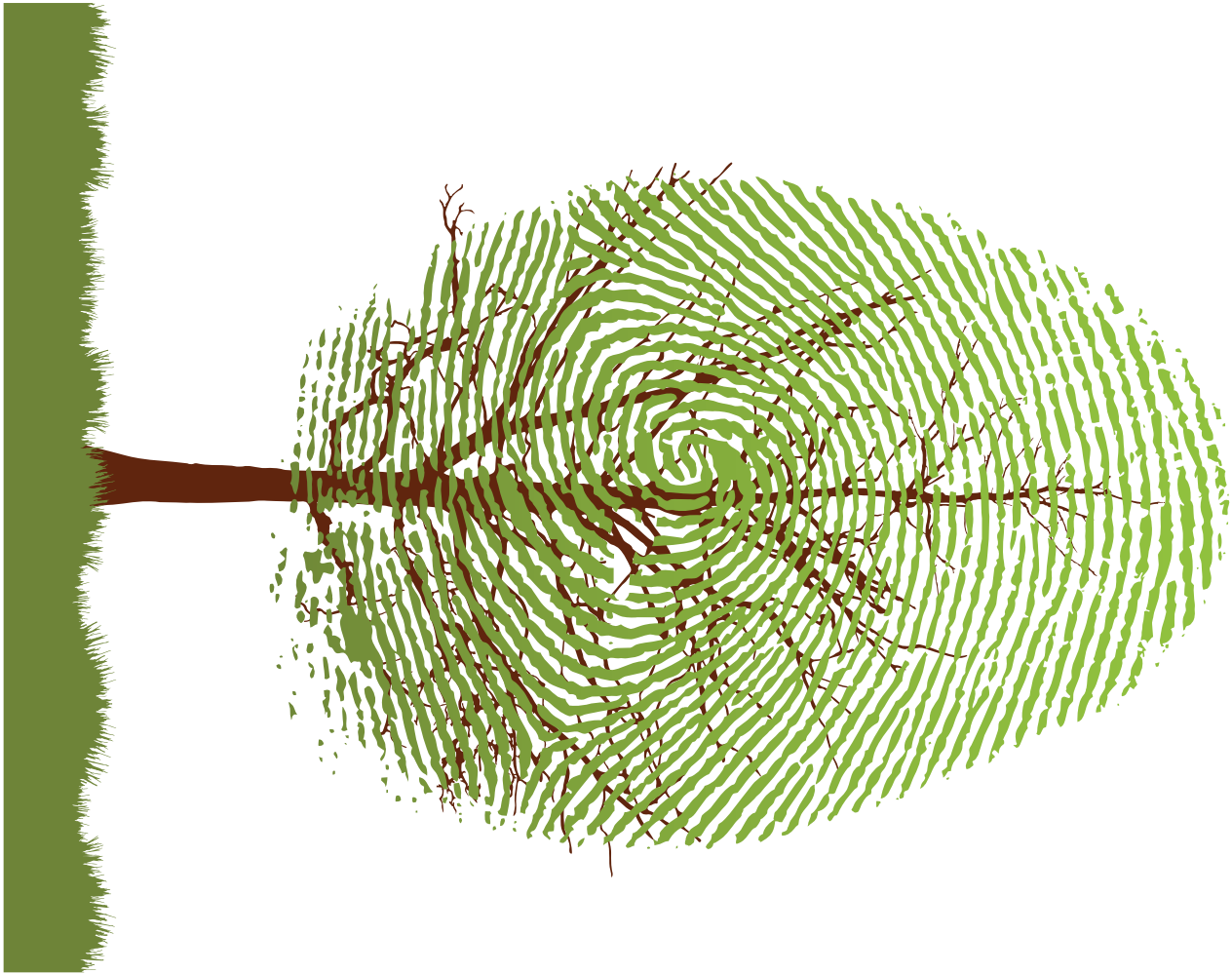
> *A total of 465 profiles were found in literature and approximately 900 profiles are contained in different databases. Out of this, Croatian origin is stated for a total of 50 profiles in literature and 22 profiles in databases.*

Suradnja nacionalnih laboratorija i grupa

Jedan od ciljeva projekta bio je i usklađivanje rada dva nacionalna laboratorija (IPTPO, Poreč i KRŠ, Split), što je i ostvareno kontinuiranom suradnjom u svakom dosadašnjem koraku realizacije projekta, od prikupljanja podataka preko odabira biljega i uzoraka, do provedbe analiza. Kako bi se došlo do relevantnih podataka te omogućila provjera analiza, uspostavljena je suradnja i s drugim relevantnim hrvatskim i svjetskim laboratorijima, stručnjacima, odnosno institucijama koje se bave istraživanjem biološke raznolikosti masline (Agronomski fakultet Sveučilišta u Zagrebu; Institute of Agricultural Research and Training (IFAPA), Cordoba, Španjolska; Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per l'Olivicoltura e l'Industria Olearia, Rende, Italija; Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro Di.S.S.P.A., Italija, i dr.) te nacionalnim tijelima (Ministarstvo poljoprivrede, Hrvatski centar za poljoprivredu, hranu i selo - radne grupe u sklopu Nacionalnog programa očuvanja i

Cooperation between national laboratories and groups

One of the project goals was also to harmonise the work of the two national laboratories (IPTPO, Poreč and KRŠ, Split), which has been achieved by means of continuous cooperation at every step of the project realisation so far, from data collection, through selection of the markers and samples, to the conduct of analyses. In order to obtain relevant data and to facilitate analysis checks, cooperation was established also with other important Croatian and world laboratories, experts, i.e. institutions dealing with research of the biological diversity of olives (Faculty of Agriculture, University of Zagreb; Institute of Agricultural Research and Training (IFAPA), Granada, Spain; Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per l'Olivicoltura e l'Industria Olearia, Rende, Italy; Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro Di.S.S.P.A., Italy and other) and national bodies (Ministry of Agriculture, Croatian Centre for Agriculture, Food and Rural Affairs – work groups within the National Programme for Conservation



Studiovin, Shutterstock

održive uporabe biljnih genetskih izvora za hranu i poljoprivredu).

Suradnja i koordinacija rada nacionalnih grupa i tijela ključna je za očuvanje nacionalnih genetskih resursa masline.

Konsenzus lista mikrosatelitnih biljega

Na temelju svega navedenog, u suradnji s Institutom za jadranske kulture i melioraciju krša iz Splita, izvršen je odabir optimalnog seta biljega tj. sastavljena konsenzus lista od devet mikrosatelitnih (SSR) biljega koji su preporučeni za ovu vrstu analiza, čiji se odabir temeljio na dosadašnjim analizama hrvatskih institucija, iskustvima svjetskih laboratorija te dobivenim podacima iz literature. **Odabrani su sljedeći biljezi: ssrOeUA-DCA3, ssrOeUA-DCA4, ssrOeUA-DCA8, ssrOeUA-DCA9, ssrOeUA-DCA14, ssrOeUA-DCA16, ssrOeUA-DCA18 (Sefc i sur. 2000), GAPU71B (Carriero i sur. 2002) i EMO90 (De la Rosa i sur. 2002)**.

Odabrani mikrosatelitni biljezi testirani su i primijenjeni na većem broju uzoraka. Odabir se pokazao optimalnim u sklopu provedenog projekta, no u budućnosti će ova lista biti proširena.

Jesmo li bliže odgovoru na pitanje koliko imamo sorata u Hrvatskoj?

Točan odgovor na ovo „pitanje za milijun dolara" zahtijevao bi uvjete koji znatno nadilaze trenutno raspoložive materijalne, ljudske i vremenske resurse. Međutim, u sklopu projekta napravili smo značajan, možemo reći i **ključan korak naprijed**, a to je **kreiranje sustava koji bi nas u budućnosti mogao dovesti do pravoga odgovora**.

and Sustainable Utilisation of Plant Genetic Resources for Food and Agriculture).

Cooperation and work coordination of national groups and bodies is crucial for the preservation of the national olive genetic resources.

Consensus list of microsatellite markers

*Based on what has been said so far, in cooperation with the Institute for Adriatic Culture and Karst Reclamation from Split, a selection was made of an optimal set of markers, i.e. a consensus list was established of 9 microsatellite (SSR) markers, which are recommended for this type of analysis and whose selection was based on analyses performed up to date by Croatian institutions, experiences of world laboratories, as well as data obtained from the literature. **The following markers were selected: ssrOeUA-DCA3, ssrOeUA-DCA4, ssrOeUA-DCA8, ssrOeUA-DCA9, ssrOeUA-DCA14, ssrOeUA-DCA16, ssrOeUA-DCA18 (Sefc et al. 2000), GAPU71B (Carriero et al. 2002) and EMO90 (De la Rosa et al. 2002)**.*

The selected microsatellite markers were tested and applied on a larger part of the samples. This selection proved to be optimal within the conducted project; however, in the future, this list will be extended.

Are we any closer to the answer to the question of how many varieties we have in Croatia?

The right answer to this "million dollar question" would require conditions which considerably surpass currently available material, human and time resources. However, within the project, we have made a significant, we could also say, a ***crucial, step forward***, and it is the ***creation of a system which, in the future, could lead us to the right answer***.

Na temelju svih prikupljenih profila te izvršene analize **51 uzorka** maslina s područja cijele Hrvatske, kao i usporedbom s rezultatima komplementarnih analiza provedenih u sklopu aktivnosti Nacionalnog programa očuvanja i održive uporabe biljnih genetskih izvora za hranu i poljoprivredu, kreirana je **internetska baza podataka CROLIVE**, dostupna na hrvatskom i engleskom jeziku, s usklađenim i sinkroniziranim profilima sorata s područja cijele Hrvatske, u kojoj je sadržano **ukupno 38 kompletnih genetskih profila sorata na devet mikrosatelitnih lokusa**. Omogućen je javni pristup bazi i ponuđene su **referentne svjetske sorte** na kojima se temelji pretraživanje baze podataka: 'Leccino', 'Frantoio', 'Arbequina' i 'Carolea'. Baza podataka CROLIVE nastala kao rezultat projekta **ugrađena** je u **Hrvatsku bazu podataka o biljnim genetskim izvorima (CPGRD)** i predstavlja njenu nadogradnju.

Baza CPGRD nudi sistematičan prikaz putovničkih podataka reprezentativnih uzoraka (primki) hrvatskih sorata te podataka o kolekcijama u kojima se oni(e) čuvaju. **Mikrosatelitni profili sorata** u bazi CROLIVE **povezani su s morfološkim i putovničkim podacima primki iz baze CPGRD**, čiji genetski profil odgovara profilu sorte dostupnom u literaturi ili postojećim bazama podataka. U nekim slučajevima, profili su **povezani i s morfološkim opisom sorata** dostupnim **u bazi podataka *Istrian Olive Database***. Ova je baza nastala kao rezultat bilateralnog hrvatsko-slovenskog projekta "Formiranje baze podataka autohtonih sorata maslina u Istri" i sadrži opise nekih važnijih sorata maslina zastupljenih u Istri.

*Based on all gathered profiles and performed analysis of **51 samples** of olives from the entire Croatian territory, as well as by comparison with the results of complementary analyses, carried out within the activities of the National Programme for Conservation and Sustainable Utilisation of Plant Genetic Resources for Food and Agriculture, a **web database CROLIVE** has been created, available in both Croatian and English languages, with harmonised and synchronised profiles of the cultivars from the entire Croatian territory, where **a total of 38 complete genetic profiles in 9 microsatellite loci** are contained. Public access is facilitated and world reference cultivars (Leccino, Frantoio, Arbequina and Carolea) provided, on which the search of the database is based. The CROLIVE database, created as the result of the project, is **built into the Croatian Plant Genetic Resources Database (CPGRD)** and represents its upgrade.*

*The CPGRD base provides a systematic illustration of passport data of the Croatian cultivars representative accessions and the data about the collections in which they are kept. In the CROLIVE base, **cultivars' microsatellite profiles are linked with the morphological and passport data of the accessions from the CPGRD base**, whose genetic profile corresponds to the cultivar's profile available in the literature or the existing databases. In some cases, profiles are **also linked to the morphological description of cultivars**, available **in the Istrian Olive Database**. This base was created as the result of a bilateral Croato-Slovene project "Formation of an Istrian Autochthonous Olive Variety Database" and contains descriptions of some of more important olive cultivars, represented in Istria.*

U našoj je bazi 38 sorata/profila sorata maslina uzgajanih u Hrvatskoj, čime smo se približili trenutku kada ćemo moći dati ne samo djelomičan nego i puno pouzdaniji odgovor na ovo pitanje. S postavljenim sustavom već se radi na sustavnoj identifikaciji dodatnih setova uzoraka, rješavanju kompleksnog problema sinonima i homonima u nazivlju te sinkronizaciji i internacionalizaciji podataka o našim genetskim resursima.

In our base, there are 38 cultivars/profiles of olives cultivated in Croatia, by which we have come closer to the moment when we will be able to give not only a partial, but a more trusted answer to this question. With the installed system, the work has already begun on the systematic identification of additional sets of samples, on resolving of the complex problem of synonyms and homonyms in the cultivar names, as well as on the synchronisation and internationalisation of the data about our genetic resources.

3D rendered DNA structure and barcode; Shutterstock

Baze podataka – alat 21. stoljeća

Internetske baze podataka genetskih resursa pokazale su se kao izvrstan alat u upravljanju genetskim resursima, u komunikaciji i umrežavanju znanstvenika i stručnjaka te drugih korisnika, rezultirajući povećanjem prepoznatljivosti i valorizacije nacionalnih poljoprivrednih potencijala.

U kreiranju baze težili smo što jednostavnijem pristupu, uporabi tzv. user-friendly sučelja i estetski i stilski čistom vizualnom identitetu, pogodnom za široki raspon potencijalnih korisnika. Iako poznavatelje tematike ona intuitivno vodi, ponudom videouputa za korištenje baze nastojali smo je učiniti što jednostavnijom za korištenje i onim potencijalnim korisnicima kojima ta tematika nije bliska. Svima koji žele znati više, ponuđeni su dodatni materijali poput letka i priručnika u elektroničkom obliku te poveznice sa značajnim komplementarnim projektima, programima i internetskim sadržajima.

Korištenjem dodatnih alata modernoga marketinga, poput društvenih mreža koje okupljaju različite profile korisnika, osigurana je vidljivost i diseminacija, ne samo rezultata i same baze, nego i svijesti o bogatoj biološkoj raznolikosti masline na našim prostorima.

Naša je baza koncipirana tako da se osigura njena dostupnost i vidljivost te mogućnost nadogradnje i nakon završetka projekta, u cilju povećanja prepoznatljivosti i valorizacije hrvatskog poljoprivrednog potencijala masline.

Databases – the tool of the 21st century

The genetic resource web databases proved to be an excellent tool in genetic resource management and in communication and networking of scientists and experts, as well as other users, resulting in an increase in recognisability and valorisation of the national agricultural potentials.

In creation of the base, we strove towards as simple as possible access, use of the so-called user-friendly interface and aesthetically and stylistically clean visual identity, suited for a wide range of potential users. Although it intuitively guides those who are familiar with the topic area, by providing also a video tutorial with user's instructions, we tried to make the use as simple as possible for potential users who are not familiar with the topic area. All those who wish to find out more are provided with supplementary materials, such as leaflets and manuals in electronic form and links with significant complementary projects, programmes and web contents.

By using additional modern marketing tools, such as social networks which gather different profiles of users, visibility and dissemination are provided of not only the results and the base itself, but also of awareness about the rich biodiversity of Croatian olives.

Our base is conceived in such a way as to ensure its accessibility and visibility, as well as a possibility of upgrades even after the completion of the project, for the purposes of an increased recognisability and valorisation of the Croatian agricultural olive potential.

Internetske baze podataka genetskih resursa koristan su i suvremen alat u upravljanju genetskim resursima, koji olakšava komunikaciju i umrežavanju znanstvenika i stručnjaka te drugih korisnika, što doprinosi povećanju prepoznatljivosti i valorizacije nacionalnih poljoprivrednih potencijala.

The genetic resource web databases are a useful and modern tool in the management of genetic resources, which facilitates communication and networking between scientists and experts, as well as of other users, which, in turn, contributes to an increase in recognisability and valorisation of the national agricultural potentials.

Informacija je edukacija

Danas imamo brojne mogućnosti i alate za brz i učinkovit prijenos znanja i informacija. Jedan od ciljeva projekta je bio i **podići razinu javne svijesti o problemu poznavanja, evidentiranja i pravilnog identificiranja sortimenta masline u Republici Hrvatskoj**. Rezultati projekta prezentirani su stoga putem osam **predavanja**, **četiri** znanstveno-stručne **publikacije**, **četiri priloga na portalima i internetskim stranicama** te na **društvenim mrežama**.

Information is education

*Today, we have numerous possibilities and tools for a fast and efficient transfer of knowledge and information. One of the project goals was also to **raise the level of public awareness about the problem of knowledge, recording and correct identification of olive assortment in the Republic of Croatia**. The project results are, therefore, presented through **8 lectures** organised so far, **4** scientific and professional **publications**, **4 contributions at different portals and web pages**, as well as through **social networks**.*

KAKO PRETRAŽIVATI BAZU?

HOW TO SEARCH THE DATABASE?

CROLIVE

KRATKA ŠETNJA KROZ INTERNETSKU STRANICU
A SHORT WALK THROUGH THE WEB PAGE

POČETNA STRANICA – jednostavno sučelje s kojega direktno možete pretraživati bazu po veličini alela ili po imenu sorte. Ili možete potražiti više informacija o projektu, molekularnim biljezima upotrijebljenim u istraživanju te sortama koje baza sadrži.

HOME PAGE *– a simple interface, from which you can directly search the database by alleles size or by cultivars' names. Or, you can ask for more information about the project, molecular markers used in the research and cultivars which the database contains.*

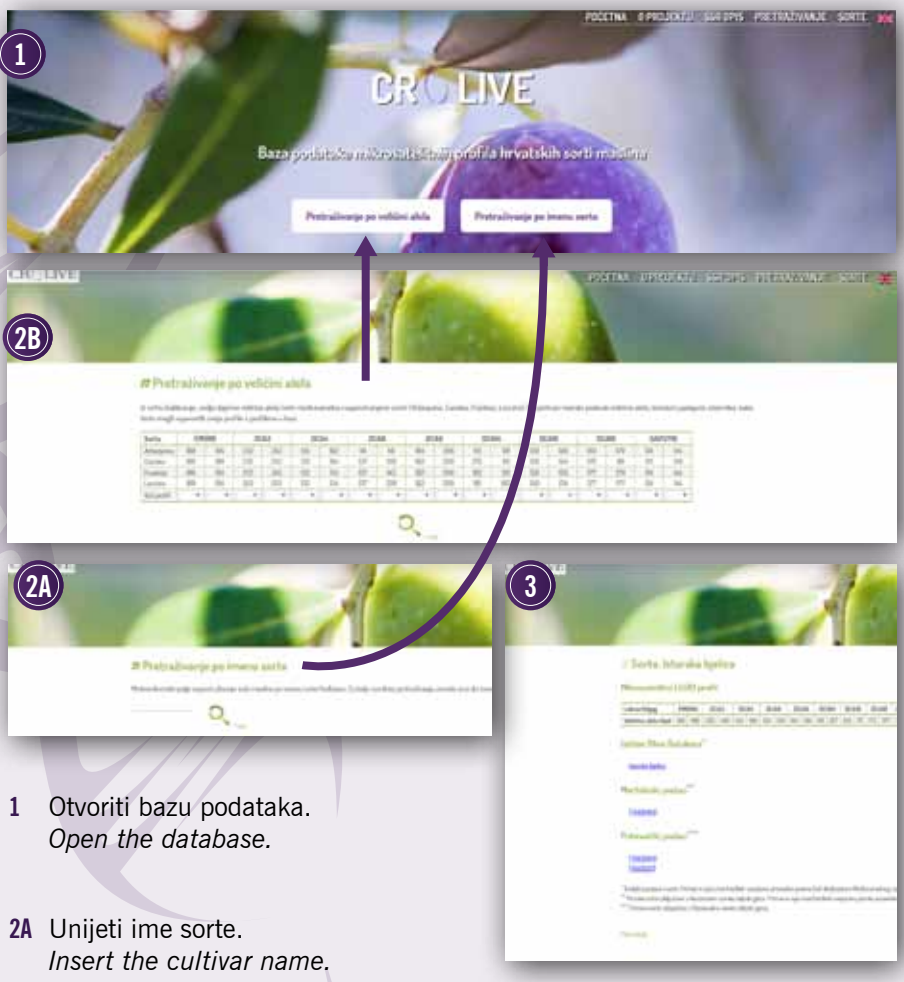
POČETNA
HOME

O PROJEKTU
ABOUT THE PROJECT

O MOLEKULARNIM BILJEZIMA
SSR DESCRIPTION

SORTE
CULTIVARS

KAKO PRETRAŽIVATI BAZU?
HOW TO SEARCH THE DATABASE?



1 Otvoriti bazu podataka.
Open the database.

2A Unijeti ime sorte.
Insert the cultivar name.

2B Unijeti profil sorte izražen veličinom alela, prethodno kalibriran prema referentnim sortama.
Insert the cultivar profile expressed in allele sizes, previously calibrated according to reference.

3 Ukoliko se tražena sorta nalazi u našoj bazi, sve će vam informacije biti dostupne na jednom mjestu.
If the requested cultivar is in our database, the complete information will be available for you.

KAKO KALIBRIRATI PROFIL TRAŽENE SORTE?

Da biste mogli pretraživati našu bazu, odnosno usporediti svoj profil s našim profilima, **morate napraviti kalibraciju, odnosno prilagoditi vaše vrijednosti (veličine alela) našima**. Kako ćete to napraviti? Vrlo jednostavno, ako imate profil barem referentne sorte. U tu svrhu, ponudili smo vam četiri svjetske, međunarodno rasprostranjene sorte, a to su 'Arbequina', 'Carolea', 'Frantoio' i 'Leccino'.

HOW TO CALIBRATE THE PROFILE OF THE CULTIVAR YOU ARE SEARCHING FOR

To be able to search our database, i.e. to compare your profile to our profiles, **you have to create a calibration profile, i.e. adapt your values (alleles size) to ours***. How will you do it? Very simply if you have a profile of at least a reference cultivar. For that purpose, we have offered you 4 world, internationally widespread cultivars, namely Arbequina, Carolea, Frantoio and Leccino.*

1. Dakle, imate profil vaše masline, za koji želite saznati nalazi li se u našoj bazi:

   So, you have a profile of your olive and you wish to find out whether it is in our database:

Lokus (Locus) / biljeg (marker)	EMO90		DCA3		DCA4		DCA8		DCA9		DCA14		DCA16		DCA18		GAPU71B	
Vaša sorta / Your cultivar	186	188	237	247	134	186	137	143	194	194	181	187	126	171	173	177	130	130

2. Najprije ga morate kalibrirati prema referentnim sortama. Što to znači? Znači da morate imati profil barem jedne referentne sorte koji je dobiven na isti način (u istom laboratoriju), s istim biljezima (na istim lokusima). Uzmimo primjer sorte 'Leccino':

First of all, you have to calibrate it according to the reference cultivars. What does it mean? It means that you have to have a profile of at least one of the reference cultivars which is obtained in the same way (at the same laboratory), with the same markers (at the same loci). Let us take the example of the Leccino cultivar:

Lokus (Locus) / biljeg (marker)	EMO90		DCA3		DCA4		DCA8		DCA9		DCA14		DCA16		DCA18		GAPU71B	
Vaš 'Leccino' /Your Leccino	188	194	241	251	132	134	141	143	162	206	181	183	150	174	177	177	124	144
Naš 'Leccino' / Our Leccino	188	194	243	253	132	134	137	139	162	206	181	183	150	174	177	177	124	144

3. Profil vašeg 'Leccina' razlikuje se nadva lokusa od našega profila te na tim lokusima morate prilagoditi profil tražene sorte. Drugim riječima, to je vaš **ključ za kalibriranje.**

The profile of your Leccino differs in two loci from our profile and, in these loci, you have to adapt the profile of the cultivar sought. In other words, this is your ***calibration key****.*

Lokus *(Locus)* / biljeg *(marker)*	EMO90		DCA3		DCA4		DCA8		DCA9		DCA14		DCA16		DCA18		GAPU71B	
Vaša sorta / *Your cultivar*	186	188	237	247	134	186	137	143	194	194	181	187	126	171	173	177	130	130

Lokus *(Locus)* / biljeg *(marker)*	EMO90		DCA3		DCA4		DCA8		DCA9		DCA14		DCA16		DCA18		GAPU71B	
Vaša kalibrirana sorta / Your calibrated cultivar	186	188	239	249	134	186	133	139	194	194	181	187	126	171	173	177	130	130

4. Sada, kada ste profil kalibrirali, možete krenuti s pretraživanjem. U za to predviđeno polje unosite (kalibrirani) profil vaše sorte, koristeći padajuće izbornike.
Možete unijeti kompletan profil (18 veličina alela), ali možete i djelomičan. Od 1 na dalje. Povećavajući broj unesenih vrijednosti, smanjujete izbor, odnosno dolazite do točnog profila svoje željene sorte.

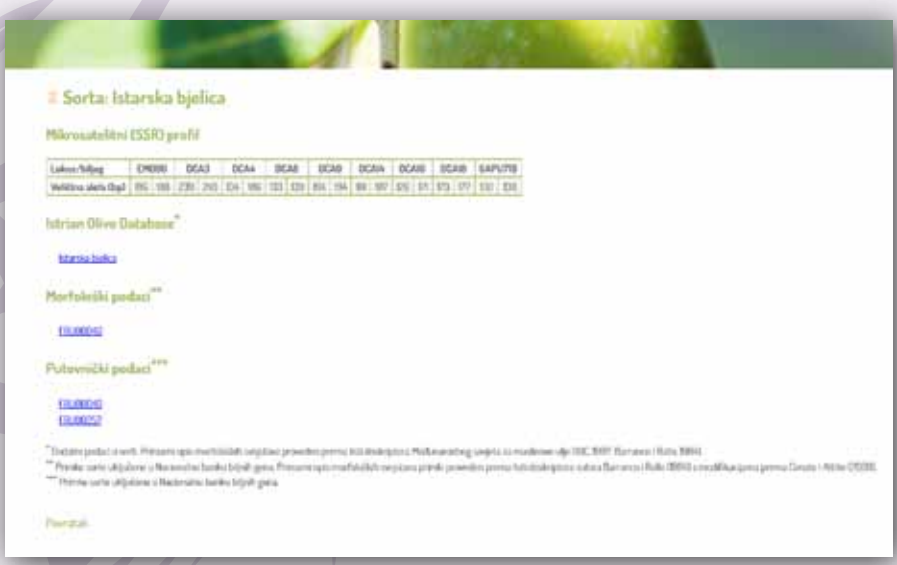
Now, when you have calibrated the profile, you can go ahead with the search. Youenter the (calibrated) profile of your cultivar in the designated box, using a drop-down menu.
You can enter a full profile (18 allele units) or a partial one. From 1 onwards. By increasing the number of entered units, you reduce the choice, i.e. you arrive atthe right profile of your desired cultivar.

5. Nakon unosa profila, kliknete na pretragu. Ukoliko profil vaše sorte odgovara profilu iz naše baze, ime sorte pojavljuje se u dijelu „Odgovarajući profili".

After having entered the profile, click on search. If the profile of your cultivar matches the profile from our base, the cultivar name appears in the part entitled „Corresponding profiles".

6. Klikom na ime sorte otvara vam se stranica sa svim podacima o toj sorti sadržanima u našoj bazi, ali i s njom povezanim bazama podataka.

By clicking on the cultivar name, a page opens featuring all the data about thatparticular cultivar contained in our database, but also in databases related to ours.



Free vector: Freepik

Od maslinika do laboratorija i natrag: Znanost i tehnologija u službi (pre)poznavanja naših sorata maslina

Kako znanost može izaći iz laboratorija?

Molekularni biljezi, analiza DNA , mikrosatelitni profili… Sve su to stručni izrazi vezani uz laboratorij i znanost. „Imam mikrosatelitni profil, što ću s tim?", „Kakve koristi mogu imati od ove informacije?", „Kakva je veza između mojega maslinika i laboratorija?" najčešća su pitanja koja si postavlja prosječan potencijalni korisnik ovakve baze i rezultata molekularno-genetičkih i općenito, znanstvenih analiza.

Problem koji se postavlja pred znanstvenike koji se bave primijenjenom znanošću jest kako na jednostavan i širem spektru korisnika prihvatljiv način prezentirati što znanost i tehnologija mogu učiniti za opću dobrobit. To, međutim, nije lak zadatak. Što zbog složene metodologije, a što zbog njenih nedostataka i ograničenja koja dovode do vrlo čestih situacija u kojima znanost i analitika ne mogu striktno i jasno iznijeti nedvosmislene i trajno realne i objektivne rezultate.

Primjerice, analiza DNA pokazala je da je genetski profil vaše masline jedinstven, odnosno da se razlikuje od svih do sada analiziranih genotipova masline u Hrvatskoj i šire. lako je analiza relativno pouzdana, samo na bazi ovih dobivenih rezultata ne možemo sa sigurnošću tvrditi radi li se u ovom slučaju zaista o jedinstvenom genotipu vrijednom čuvanja, umnažanja i zaštite. Prvenstveno zato što to zahtijeva kompleksan pristup koji uključuje opis jedinke osim na molekularnoj, također i na agronomskoj te morfološkoj i fiziološkoj razini. Promatramo li samo molekularni aspekt, nažalost još uvijek ne postoji

From olive grove to laboratory and back: Science and technology to know and recognise our olive cultivars

How can science get out of the laboratory?

Molecular markers, DNA analysis, microsatellite profiles…these are all professional terms associated with the laboratory and the science. "I have a microsatellite profile, what shall I do with it?", "How can I benefit from this information?", "What is the relationship between my olive grove and the laboratory?" are the most frequent questions asked by an average potential user of such a database and the results of molecular and genetic and, generally, scientific analyses.

The problem which the scientists who work in applied science face is how to, in a simple way and in a way acceptable to a wider circle of users, present what science and technology can do for the general well-being. This, however, is not an easy task. On one hand, due to a complex methodology, on the other, due to the imperfections and limitations which lead to very frequently emerging situations in which the science and the analytics cannot strictly and clearly put forward unambiguous and permanently realistic and objective results.

For example, the DNA analysis showed that your olive's genetic profile is unique, i.e. that it differs from all so far analysed olive genotypes in Croatia and wider. Although the analysis is relatively reliable, on the basis of the obtained results alone we cannot claim with certainty whether, in this case, it really concerns a unique genotype worth keeping, propagating and protecting. This is primarily due to the fact that this would require a complex approach, which includes the description of the accession not only on the molecular, but also on the agronomic, morp-

univerzalni protokol za molekularnu karakterizaciju i razlikovanje genotipova, niti opće prihvaćen analitički pristup koji bi identificirao regije genoma koji utječu na agronomske karakteristike masline. Još nije razvijena zajednička baza podataka koja bi sadržavala molekularne i agrobiološke informacije o najvažnijim svjetskim genetskim resursima. Ne postoji ni organizirana mreža proizvođača, rasadničara, znanstvenika, stručnjaka, voditelja nacionalnih kolekcija i upravitelja nacionalnim genetskim resursima te drugih dionika uključenih u sektor, koja bi omogućila usporedbu i razmjenu informacija.

Dobra je vijest, međutim, da poznati svjetski istraživački timovi intenzivno rade na rješavanju ovih pitanja i da nam u budućnosti sa znanstvenog aspekta predstoji uzbudljivo razdoblje u kojemu ćemo vjerojatno doći do vrijednih i općenito primjenjivih spoznaja koje će nam omogućiti poduzimanje značajnih mjera u rješavanju problema zaštite i unaprjeđenja genetskih resursa masline.

> *Da bi znanstveno-istraživački rezultati i inovacije našli svoju primjenu na terenu, ključno je krenuti sa sistematičnom i globalno metodološki usklađenom karakterizacijom svih nacionalnih kolekcija maslina koja uključuje molekularni, morfološki, agrobiološki te gospodarsko-tehnološki aspekt, kao i procjenu kvalitete ulja povezanu s najvažnijim sortama. Suradnja znanstveno-istraživačkog i proizvodnog sektora nužna je u ostvarivanju ovoga cilja.*

hological and physiological levels. Should we observe only the molecular aspect, a universal protocol for molecular characterisation and differentiation of genotypes unfortunately still does not exist, nor is there a generally accepted analytical approach which would identify the genome regions which affect olive agronomic characteristics. A common database has not been developed yet, which would contain molecular and agro-biological information about the most important world genetic resources. An organised network, which would facilitate information comparison and exchange and consisting of producers, nursery workers, scientists, experts, national collection managers and directors of national genetic resources, as well as of other stakeholders involved in the sector, does not exist either.

The good news is, however, that renowned world research teams work intensively on resolution of these issues and that, in the future, from a scientific aspect, an exciting period is before us in the course of which we will most probably arrive at valuable and generally applicable knowledge, which will enable us to undertake more considerable measures in the resolution of the problems of protection and improvement of the olive genetic resources.

> *In order for scientific and research results and innovations to be used on-the-spot, it is crucial to begin with a systematic and globally methodologically synchronised characterisation of all the national olive collections, which includes molecular, morphological and agro-biological aspects, as well as assessments of oil quality, associated with the most important cultivars. Cooperation between the scientific and research and production sectors is essential for the realisation of this goal.*

Korisni linkovi
http://www.beforeproject.eu/
http://www.oleumproject.eu/

Useful links
http://www.beforeproject.eu/
http://www.oleumproject.eu/

Učinci projektnih rezultata

Imajući u vidu sve ranije navedeno, možemo rezimirati **sve ekonomske i društvene aspekte** potencijalne primjene projektnih rezultata te koristi od njegove realizacije:

- Bolje poznavanje i učinkovitija zaštita biološke raznolikosti masline;
- Bolja eksploatacija prirodnih dobara kroz unaprjeđenje ponude maslinovog ulja i održivog razvoja poljoprivrede, turizma i poduzetništva;
- Učinkovitije planiranje i održivo upravljanje nacionalnim genetskim resursima;
- Kvalitetnije upravljanje nacionalnim ex situ kolekcijama;
- Kreiranje kvalitetnih programa oplemenjivanja;
- Podizanje svijesti o važnosti pravilnoga prepoznavanja, evidentiranja i identificiranja hrvatskog sortimenta masline;
- Podizanje novih nasada;
- Kvalitetnija i intenzivnija kontrolirana proizvodnja i prodaja sadnog materijala autohtonih sorata;
- Razvoj novih proizvoda;
- Povećanje prepoznatljivosti i valorizacije hrvatskog poljoprivrednog potencijala kroz brendiranje sortnih ulja;
- Pouzdanije dokazivanje autentičnosti i sljedivosti maslinovog ulja,
- Bolji tržišni plasman autohtonih maslinovih ulja kroz pouzdaniju determinaciju sorte u postupku izrade specifikacije u svrhu stjecanja oznaka izvornosti i zemljopisnog podrijetla;
- Porast ugleda i konkurentnosti autohtonih poljoprivrednih proizvoda, a samim time i poboljšanje uvjeta života proizvođača maslina i

Project result effects

*Bearing in mind all the aforesaid, we can make a summary of **all the economic and social aspects** of potential implementation of the project results and benefits from its realisation:*

- *A better knowledge and more efficient protection of the biological diversity of olives;*
- *A better exploitation of natural resources through improvement of olive oil offer and of sustainable development of agriculture, tourism and entrepreneurship;*
- *A more efficient planning and sustainable national genetic resource management;*
- *A higher quality management of the national ex situ collections;*
- *Creation of quality breeding programmes;*
- *Raising awareness about the importance of proper recognition, recording and identification of the Croatian olive assortment;*
- *Establishing of new plantations;*
- *A higher quality and a more intensive controlled production and sale of autochthonous cultivars planting materials;*
- *New product development;*
- *Increasing recognisability and valorisation of the Croatian agricultural potential through olive oil branding;*
- *A more reliable proving of olive oil authenticity and traceability;*
- *A better market placement of autochthonous olive oils through a more trusted determination of a cultivar in the process of preparation of a specification for the purposes of acquisition of designations of origin and geo-*

maslinovog ulja u regiji te stanovništva općenito.

Potencijalne koristi od ovog i sličnih projekata, s posebnim naglaskom na inovacije proistekle iz suradnje svih dionika sektora, od znanosti do gospodarstva, moramo imati na umu u svakodnevnom djelovanju, komunikaciji i suradnji.

graphical indications;

- *Increasing the image and competitiveness of autochthonous agricultural products, thus also improving the living conditions of olive growers and olive oil producers in the region and of the population in general.*

In everyday work, communication and cooperation, we must bear in mind the potential benefits from this and similar projects, with a special emphasis on the innovations derived from the cooperation between all sector stakeholders, from science to economy.

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Quo vadis homine modernicus?

Pitanja i izazovi budućnosti

Maslinu kao jednu od najvažnijih i najstarijih voćnih vrsta na Mediteranu, karakterizira bogato genetsko naslijeđe, zastupljeno kultiviranim i divljim oblicima, drevnim stablima i srodnim formama. Bogatstvo ove prirodne baštine ističe se među hortikulturnim vrstama, zbog dugovječnosti masline, nedostatka novih genotipova s boljim svojstvima, kao i milenijske tradicije uzgoja. Radi se o izuzetno bogatom izvoru genetske raznolikosti koji se može izravno koristiti ili održavati za potrebe budućih programa oplemenjivanja.
Zamislimo maslinu budućnosti: otporna na epidemije bolesti i štetnika, prilagodljiva na klimatske promjene, odolijeva suši i poplavama, visokoga prinosa i kakvoće, prilagođena specifičnim lokalnim agroekološkim uvjetima te specifičnih svojstava što se odražavaju na kvalitetu i posebnost ulja. Sva su ta svojstva sadržana u bogatstvu genetske baštine sadržane u kultiviranim i divljim maslinama koji predstavljaju izniman potencijal u oplemenjivanju.

Stoga budućnost pred nas kao prioritet postavlja nekoliko **zadataka i izazova**:

1. Bolje poznavanje, upravljanje i korištenje genetskih resursa sadržanih u kultiviranim, divljim i drevnim stablima maslina;
2. Uspostava univerzalnih protokola za molekularnu identifikaciju i imenovanje sorti;
3. Pouzdana morfološka i agrobiološka procjena biljaka u kolekcijama i bankama gena te uspostava komparativnih pokusa za procjenu agronomskih svojstava;
4. *In situ* ali i *ex situ* očuvanje divljih maslina, koje je u svrhu detaljnije

Quo vadis homine modernicus?

Questions and challenges of the future

Olive, as one of the most important and oldest fruit species in the Mediterranean, is characterised by a rich genetic heritage, represented by cultivated and wild forms, ancient trees and related forms. The wealth of this natural heritage stands out among horticultural species due to its longevity, lack of new genotypes with better properties, as well as to its millennial cultivation tradition. It concerns an exceptionally rich source of genetic diversity, which can either be directly used, or maintained for the needs of future breeding programmes.
Let us imagine an olive of the future: resistant to disease and pest epidemics, adaptable to climatic changes, resists droughts and floods, high yield and quality, adapted to the specific local agro ecological conditions and of specific properties, reflected in the quality and specificity of its oil. *All these properties are contained in the richness of the genetic heritage, contained in cultivated and wild olives, which represent an exceptional potential in improvement.*

*Therefore, as a priority, the future sets before us several **tasks and challenges**:*

1. *A better knowledge, management and use of the genetic resources contained in cultivated, wild and ancient olive trees;*
2. *Establishment of universal protocols for molecular identification and naming of the cultivars;*
3. *A reliable phenotypic and morpho-agronomic evaluation of plants in collections and gene banks and establishment of comparative experiments for assessment of agronomic properties;*
4. In situ *but also* ex situ *preserva-*

evaluacije i procjene moguće upotrebe u oplemenjivačkim programima, potrebno selekcionirati, kolekcionirati i evaluirati u istim proizvodnim uvjetima u kojima se uzgajaju kultivirane masline;

5. Razvoj novih metoda i kombinirana primjena tehnologija nove generacije u istraživanju genoma i metoda fenotipske procjene u svrhu učinkovitije identifikacije regija genoma povezanih s agronomskim karakteristikama masline, što bi trebalo omogućiti i ubrzati genetsko unaprjeđenje ove drevne kulture.
6. Planiranje oplemenjivačkih programa baziranih na novim tehnologijama (biljezima potpomognuta selekcija, „spašavanje embrija" u kulturi tkiva, stvaranje dihaploida, somatska hibridizacija, genetska transformacija, itd.) kao nadopuni i potpori klasičnim programima oplemenjivanja. Primjenom ovih tehnika ubrzat će se proces dobivanja novih sorti maslina s vrijednim osobinama i sposobnošću da se odupru izazovima novog vremena poput brzog sušenja masline izazvanog bakterijom Xylella fastidiosa, verticilioznoga venuća masline izazvanog fitopatogenom gljivom Verticillium dahliae ili klimatskim promjenama.
7. Uspostava međunarodnih prava intelektualnog vlasništva za očuvanje i razmjenu genetskih resursa masline.

tion of wild olives, which, for the purposes of a more detailed estimate and assessment as to possible use in breeding programmes, need to be selected, collected and evaluated in the identical production conditions in which cultivated olives are raised;

5. *Development of new methods and a combined application of new generation technologies in the genome research and the methods of phenotype assessment for the purposes of a more efficient identification of the genome regions, associated with the olive agronomic characteristics, which should facilitate and accelerate genetic improvement of this ancient culture;*
6. *Planning of improvement programmes based on new technologies (marker-assisted selection rescue of embryos, the generation of dihaploids, somatic hybridisation genetic transformation, etc.) as a supplement and support to classical improvement programmes. By application of these techniques, the process will be accelerated of getting new olive cultivars with valuable characteristics and ability to resist the new times challenges, such as fast olive drying caused by the Xylella fastidiosa bacteria, olive tree verticillium wilt, caused by phytopathogenic fungi Verticillium dahliae or by climatic changes.*
7. *Establishment of international laws on intellectual property for preservation and exchange of olive genetic resources.*

Priča se nastavlja i nakon projektnog ciklusa

Ovaj je projekt samo dio zajedničkih nastojanja svih relevantnih dionika maslinarskog sektora za unaprjeđenjem maslinarske proizvodnje te upravljanja genetskim resursima u cilju povećanja prepoznatljivosti i valorizacije hrvatskog poljoprivrednog potencijala.

Rezultati projekta iskoristit će se u aktivnostima koje se provode u sklopu Nacionalnog programa očuvanja i održive uporabe biljnih genetskih izvora za hranu i poljoprivredu u Republici Hrvatskoj. Ove su višegodišnje aktivnosti vezane uz ex situ i *in situ* očuvanje i on farm upravljanje, održivo korištenje te izgradnju ljudskih i institucionalnih kapaciteta za očuvanje biljnih genetskih izvora za hranu i poljoprivredu, što uključuje i maslinu. Učinkovita provedba ovog programa omogućit će da svi važni biljni genetski izvori u Republici Hrvatskoj budu identificirani, prikupljeni, opisani i očuvani u kolekcijama Nacionalne banke biljnih gena te dostupni za korištenje.

The story goes on even after the project life-cycle

This project is only a part of mutual attempts of all relevant olive sector stakeholders to improve olive production and manage genetic resources with the aim to increase the recognisability and to valorise the Croatian agricultural potential.

The project results will be used in the activities conducted within the National Programme of Conservation and Sustainable Utilisation of Plant Genetic Resources for Food and Agriculture in the Republic of Croatia. These multiannual activities are related to ex situ and in situ *conservation and on farm management, sustainable use and building of human and institutional capacities for preservation of plant genetic resources for food and agriculture, which also include olives. An efficient implementation of this programme will facilitate identification, collection, description and storage in the National Plant Gene Banks of all important plant genetic resources in the Republic of Croatia, which will be available for use.*

Literatura / *Literature*

Baldoni L, Cultrera NG, Mariotti R, Ricciolini C, Arcioni S, Vendramin GG, Buonamici A, Porceddu A, Sarri V, Ojeda MA, Trujillo I, Rallo L, Belaj A, Perri E, Salimonti A, Muzzalupo I, Casagrande A, Lain O, Messina R, Testolin R (2009) A consensus list of microsatellite markers for olive genotyping. Molecular Breeding 24(3), 213–231.

Bracci T, Busconi M, Fogher C, Sebastiani L (2011) Molecular studies in olive (*Olea europaea* L.): overview on DNA markers applications and recent advances in genome analysis. Plant Cell Reports, 30(4), 449–462.

Carriero F, Fontanazza G, Cellini F, Giorio G (2002) Identification of simple sequence repeats (SSRs) in olive (*Olea europaea* L.). Theoretical and Applied Genetics 104(2-3), 301–307.

Cipriani G, Marrazzo MT, Marconi R, Cimato A, Testolin R (2002) Microsatellite markers isolated in olive (*Olea europaea* L.) are suitable for individual fingerprinting and reveal polymorphism within ancient cultivars. Theoretical and Applied Genetics 104(2-3), 223–228.

De la Rosa R, James CM, Tobutt KR (2002) Isolation and characterization of polymorphic microsatellites in olive (*Olea europaea* L.) and their transferability to other genera in the Oleaceae. Molecular Ecology Notes 2, 265–267.

Ipek M, Ipek A, Seker M, Gul MK (2015) Association of SSR markers with contents of fatty acids in olive oil and genetic diversity analysis of an olive core collection. Genetics and Molecular Research, 14(1), 2241–2252.

Klepo T (2014) Genetska raznolikost kultiviranih i divljih maslina (*Olea europaea* L.) u Hrvatskoj. Doktorska disertacija. Zagreb: Agronomski fakultet, 18. 6. 2014.

Miljković I, Žužić I, Pucci C, Baldoni L, Mariotti M, Cultrera NGM (2010) Molecular characterization of an ancient *Olea europaea* tree located on the Brijuni islands of (Croatia) by SSR markers analysis. Pomologia Croatica 16(1-2), 3-11.

Muzzalupo I, Vendramin GG, Chiappetta A (2014) Genetic biodiversity of Italian olives (*Olea europaea*) germplasm analyzed by SSR markers. The Scientific World Journal 2014, 1-12.

Pasqualone A, Montemurro C, diRienzo V, Summo C,Paradiso V, Caponio F (2016) Evolution and perspectives of cultivar identification and traceability from tree to oil and table olives by means of DNA markers. Journal of the Science of Food and Agriculture 96(11), 3642–3657.

Poljuha D, Sladonja B, Brkić Bubola K, Radulović M, Brščić K, Šetić E, Krapac M, Milotić A (2008) A multidisciplinary approach to the characterisation of autochthonous Istrian olive (*Olea europaea* L.) varieties. Food Technology and Biotechnology 46(4), 347-354.

Poljuha D, Sladonja B, Šetić E, Milotić A, Bandelj D, Jakše J, Javornik B (2008) DNA fingerprinting of olive varieties in Istria (Croatia) by microsatellite markers. Scientia Horticulturae 115(3), 223-230.

Sefc KM, Lopes MS, Mendonça D, Rodrigues Dos Santos M, Laimer Da Câmara Machado M, Da Câmara Machado A (2000) Identification of microsatellite loci in olive (*Olea europaea*) and their characaterization in Italian and Iberian olive trees. Molecular Ecology 9(8), 1171–1173.

Štambuk S, Sutlović D, Bakarić P, Petričević S, Anđelinović Š (2007) Forensic botany: potential usefulness of microsatellite-based genotyping of Croatian olive (*Olea europaea* L.) in forensic casework. Croatian Medical Journal 48, 556–562.

O autorici

Danijela Poljuha završila je studij biologije, smjer molekularna biologija na Prirodoslovno–matematičkom fakultetu Sveučilišta u Zagrebu, gdje je i magistrirala i doktorirala u području Prirodnih znanosti, polju biologije. Izabrana je u znanstvena zvanja znanstveni savjetnik u području biotehničkih znanosti te znanstveni suradnik u području prirodnih znanosti. Radila je kao istraživač na Prirodoslovno-matematičkom fakultetu Sveučilišta u Zagrebu, u Institutu za poljoprivredu i turizam Poreč i Centru za istraživanje materijala Istarske županije METRIS (Istarska razvojna agencija) te u više navrata boravila na usavršavanju u Hrvatskoj i inozemstvu. Sudjelovala je na brojnim nacionalnim i međunarodnim projektima i objavila više od 50 znanstvenih radova iz područja biologije i biotehnologije. Osnivač je dva laboratorija i Biotehničkog odjela istraživačkog centra. Istraživački interes usmjeren joj je na biljnu genetiku i primjenu molekularnih biljega u zaštiti i očuvanju genetskih resursa u hortikulturi. Osim znanstvenim istraživanjem, bavi se i popularizacijom znanost, nalazeći neiscrpan izvor inspiracije u spoju znanosti, tehnologije i umjetnosti.

About the author

Danijela Poljuha completed the Faculty of Biology, the course in Molecular Biology at the University of Zagreb, Faculty of Science, where she also did her Master's and Doctoral degrees in the field of Natural Sciences, Biology. She obtained the scientific title of a Scientific Advisor in the field of Biotechnical Sciences and a Research Associate in the field of Natural Sciences. She worked as a researcher at the University of Zagreb, Faculty of Science, at the Institute of Agriculture and Tourism in Poreč and at the County of Istria Material Research Centre METRIS (The Istrian Development Agency) and, on several occasions, attended residential trainings both in Croatia and abroad. She took part in numerous national and international projects and published over 50 scientific papers from the field of Biology and Biotechnology. She is a founder of two laboratories and the Research Centre Biotechnical Department. Her research interest is focused on plant genetics and implementation of molecular markers in the protection and preservation of genetic resources in horticulture. Apart from scientific research, she also engages in the popularisation of the science, finding an inexhaustible source of inspiration in the blend of science, technology and art.

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