

CEPF FINAL PROJECT COMPLETION REPORT

Organization Legal Name:	Institute for Adriatic Crops and Karst Reclamation, Split, Croatia
Project Title:	Conservation of wild grapevine (<i>Vitis vinifera</i> L. subsp. <i>sylvestris</i>) in Bosnia and Herzegovina
Date of Report:	January 23, 2015
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CEPF Region: Mediterranean basin, Eastern Adriatic

Strategic Direction: 3. Improve the conservation and protection status of 44 priority key biodiversity areas, 3.3. Raise awareness of the importance of priority key biodiversity areas, including those that have irreplaceable plant and marine biodiversity

Grant Amount: \$16.970

Project Dates: June 28, 2013 – December 30, 2014

Implementation Partners for this Project (please explain the level of involvement for each partner):

The project was undertaken by the Institute for Adriatic Crops and Karst Reclamation, Split (IAC). IAC coordinated project tasks. Six IAC experts were involved in field expedition, on site evaluation, tissue sampling and laboratory work.

This project was done in collaboration with the Federal Agro Mediterranean Institute, Mostar, Bosnia and Hercegovina which assisted in locating wild grapevines. They also contributed in morphology evaluation, workshop organization and project results dissemination.

Nature Park "Hutovo blato" helped us finding wild grapevine populations within the protected area of park. Park "Hutovo blato" is situated on the south east Hercegovina on the left side of Neretva river. They also contributed in workshop organization and project result dissemination.

Conservation Impacts

Please explain/describe how your project has contributed to the implementation of the CEPF ecosystem profile.

The project goal was to locate the remaining populations of wild grapevines on the banks of Krka river with the aim to protect this very old plant that is on the edge of extinction. CEPF grants are just focused on the establishment of research and educational projects at the local level and support activities that protect endangered plants. This project provides an insight on the presence,

distribution and the basic characteristics of wild grapevines in the study area which is essential for biodiversity conservation, and management of wild grapevines within the study area (professional care, presentation to the wider range of visitors, monitoring etc.).

Please summarize the overall results/impact of your project.

In this project the presence of wild grapevines on seven separate locations along the Neretva river was determined (Distribution Map, annex 1). Dioecism within investigated grapevines (presence of male and female individuals), morphological characteristics of leaves, natural succession without human intervention and consociation with other woody plants suggest that investigated individuals fit in the subspecies of wild grapevine (*Vitis vinifera* subsp. *sylvestris*). Each of the seven locations was evaluated and grapevine individuals are characterized by a basic set of morphological descriptors (OIV, 1983; GENRES081 2001). The basic geographic information (latitude, longitude, elevation) of the study locations was collected through GPS device, and morphological characteristics of the young shoot, inflorescence and mature leaf was described (Morphology of wild grapevine samples, annex 2). The plant community within about 30 meters from investigated grapevine individuals was determined (Plant communities near wild grapevine, Annex 3). In total 61 plant species was identified on the basis of morphological characteristics using a standard identification keys for forest species (Pokorny, 1994; Pokorny, 1995; Kybalova, 1995; Šilić, 1998). The herbarium of identified forest species that grow close to the wild grapevines was made. Wild grapevine individuals was analysed using DNA markers. Set of nine SSR markers was applied. Root system of wild grapes was studied and we have found large biodiversity of mycorrhizal fungi, more diverse than in cultivated grapevines. The photo documentation of each of the seven locations and morphological characteristics of investigated grapevine samples was taken. In order to summarize results and give information to wider audience book about wild grape was published (annex 4). The book is published in Croatian language, contain 125 pages, entitled "*Divlja loza predak plemenite vinove loze, istraživanja uz Krku i Neretvu*" (ISBN number: 978-953-99819-5-0).

The results of this study fit into the activities that were planned in the Management Plan of Nature park "Hutovo blato". Only limited area along the Neretva river belongs to protected area within Nature park "Hutovo blato" which is administrated by Nature park while remaining area where we identified presence of wild grapes has open access to everyone. Protection and conservation of plant species is one of the fundamental tasks in this project and we have highlighted endangered areas which needing future monitoring. Actions we have taken in this project represent an inventory of the existing list of endemic and other plants. The data are stored in digital form and will be available to Federal Ministry of Environment and Tourism of Bosnia and Hercegovina, Nature park "Hutovo blato" Krka, and other stakeholders. Recently, Nature park "Hutovo blato" has been started with collecting information about presence for all plant and animal species that living in the protected area of Neretva river. Based on these results it will be possible to implement monitoring program for wild grapevines around the Neretva river, implement preventive procedures, improve the education system and visitors, and at the end, to continue further studies of wild grapevines. In addition, inventory is done for a large number of plants and forest vegetation which grow in the immediate vicinity of wild grapevines. That represents an added value of the project and will serve for monitoring and protection of these plants.

Local residents in the area of the Hercegovina engaged in traditional viticulture and winemaking. The results of this project will be very interesting for the promotion of local wines and the story of a long tradition and rich history of wild grapevines in the area of the Neretva river. The landscape preservation in the contact zone between the protected areas and agriculture is priority objective for local decision makers, so the cooperation between decision makers and the local wine producers is essential for the protection and conservation of this area. The research of wild

grapevine fits into the broader context of grapevine history research for Hercegovina, and could explain the process of domestication of grapevine. Analysis of local cultivated and wild grapevines in this area may provide answers about the domestication of grapevine, and the results of this study provide a good starting point for future research.

Please provide the following information where relevant:

Hectares Protected: -

Species Conserved: -

Corridors Created: -

Describe the success or challenges of the project toward achieving its short-term and long-term impact objectives.

We have successfully achieved all objectives of the proposed project. We have located several sites where wild grapevines still persist and highlighted importance of their conservation. The best way for conservation of wild grapevine is at Nature parka "Hutovo blato" considering that area is under protection. One of the biggest challenges in the future would be establishing long term monitoring of wild grapevine. Local decision makers will have to work more closely with local NGOs, and other stakeholders to achieve long-term conservation.

From a scientific point of view wild grapes has great importance. Molecular identification and comparison of gained DNA profiles of tested samples with already known sequences from the world DNA database will enrich the data on wild grapevine phylogeny. This should be interesting to the academic community and from the practical point of view, hopefully open up the possibility of using wild grapevines in breeding programs.

Study of the mechanisms of wild vine adaptability in their natural habitats on the Neretva River where no intervention by humans is applied represents a scientific challenge and has the potential for improving conventional methods of growing cultivated grapevines. Therefore, the results of this study represent a good starting point for future projects on a wild grapevine in the terms of scientific as well as other project types.

Were there any unexpected impacts (positive or negative)?

The positive impact arose unexpectedly from the research. High diversity of plant species was noticed around wild grapevine in our previous CEPF project along Krka river in Croatia. The same we noticed here around Neretva River. So, we hypothesized that similar degree of biodiversity could be find in the soil and we decided to study the degree of association between mycorrhizal fungi found in the soil and root system of wild grapevines. Large diversity of root endophytic fungi was determined, which was significantly more than we expected. These results suggest that wild grape has great importance in ecosystem of Neretva River hosting diverse fungal colonisations on roots.

Also, surprisingly positively was the finding about grapevine distribution which was widely present along the Neretva river. With such appearance, larger than we expected it is more likely to stay preserved.

Project Components

Project Components: *Please report on results by project component. Reporting should reference specific products/deliverables from the approved project design and other relevant information.*

Component 1 Planned: Locating wild grapevine on sites

Component 1 Actual at Completion: Distribution map of wild grapevine

Information obtained during the project was entered into a digital database. We identified seven locations where the wild grapevine was present. The basic geographical information through the GPS (latitude, longitude, elevation) was recorded, and through the ArcGIS software the distribution map on which all information about the population of wild grapevine (the number of individuals, sex) for each location was made. In total, 61 different neighbouring plant species were determined and a list of plant communities for each location is made.

Component 2 Planned: Identifying wild grapevine

Component 2 Actual at Completion: Genetic profile of wild grapevine

Primary morphological identification was made at original locations. The characteristics of shoots, adult leaf, cluster and berries were described according to the system of OIV descriptors (OIV, 2001). Variables of adult leaves were measured using Superampelo software. The growth of wild grapevines without human impact at natural habitats affects a very small growth and therefore the leaves sizes were very small. Therefore, the surface of the leaf and other leaf variables were significantly lower than in other varieties of cultivated grapevines. Open petiole leaf sinus was noticed on all investigated individuals which could be one of morphological marker for wild grapevine. Another very specific morphological marker for wild grapevine is a flower type. The wild grapevine has separate male and female flowers which were observed on a good part of the studied individuals. Genetic analysis was conducted using DNA markers. Nine SSR loci were analysed, and genetic profiles were compared with SSR database of Croatian native varieties. All analyzed individuals have shown a unique genotype, and some unique alleles have been detected. This refers to the genetic divergence among wild and cultivated grapevines.

Component 3 Planned: Identification of current threats and impact and highlight of hotspot areas for this species

Component 3 Actual at Completion: Wild grapevine repository and current threats

Only one location was in the protected area of the Nature park, so it reduces the possibility of devastating the plants by direct injury or chemical pollution. However, remaining locations are exposed to the open access to everyone allowing the possibility of devastating the plants by visitors or local people who may use them for firewood. Fire also poses a serious threat to the wild grapevines and to other plants of forest vegetation community, therefore continuous monitoring is required.

Cuttings from wild grapevine individuals were taken and some of them propagate in pots. Seeds also taken from limited number of female individuals and germinated to collect wild grapevine in repository. Very tiny one year cuttings produced weak rooted plants in the pots; therefore we started with tissue culture method for propagation.

Close cultivated grapevines in the contact zone between the protected area and agriculture zone suggest the possibility of sexual hybridization between wild and cultivated grapevines. The degree of introgression of cultivated grapevines should be investigated by applying 20 SSR molecular markers.

Invasive plant species *Ailanthus altissima* Mill. (Croatian *pajasen*) was noticed at two locations which could be a serious threat for wild grapevine and other native plant species. *Ailanthus altissima* is originated in China. It threatens biodiversity, but in some cases completely destroy natural ecosystem striving to be the only plant species in particular area. *Ailanthus* grows faster than other plants in the vicinity, and through its root secretes allelopathic substances that inhibit growth of other species. It reproduces very quickly by seeds as well as by roots. *Ailanthus* has no special requirements for soil (grows on all soil types). Monitoring of this plant species should be high priority.

Were any components unrealized? If so, how has this affected the overall impact of the project?

No

Please describe and submit (electronically if possible) any tools, products, or methodologies that resulted from this project or contributed to the results.

Distribution Map, annex 1

Morphology of wild grapevine samples, annex 2

Plant communities near wild grapevine, annex 3

The book in Croatian language entitled "Divlja loza, predak plemenite vinove loze, istraživanja uz Krku i Neretvu", annex 4

Lessons Learned

Describe any lessons learned during the design and implementation of the project, as well as any related to organizational development and capacity building. Consider lessons that would inform projects designed or implemented by your organization or others, as well as lessons that might be considered by the global conservation community.

Wild grapevines grow in the natural habitat without human intervention and its growth is much smaller than in cultivated varieties affecting shoots, leaves etc. Therefore, wild grapevine has very thin one-year cuttings which hinders its vegetative reproduction. A very important lesson learned is the inclusion of tissue culture method (*in vitro*) for secure reproduction. The method of plant tissue culture requires sophisticated equipment, laboratory and expert knowledge which hamper the process of reproduction. This could be important for reproduction of wild grapes from natural habitats.

Analysis of wild grapevines using DNA markers at nine SSR loci fully meets the objective of identifying, however, for a more reliable analysis of relatedness between wild and cultivated grapevines will be necessary to expand the analysis to more loci (20 and over).

Finally, we did not spend all funding in this project. We have made this savings in large part because we did not spend all funds for travel, furniture and equipment. We have found wild grapevine very quickly but on the other side there was limited time in season when is optimal for sampling, evaluation and overall monitoring.

Project Design Process: (aspects of the project design that contributed to its success/shortcomings)

Several previous meetings of the researchers from the IAC were very effective for the preparation of a field expedition to Hercegovina. Given the experience of IAC workers in similar projects (especially previous CEPF project in Croatia) which included field expeditions we have been able

to previously observe the critical points in the project implementation. The project was designed and led by the project manager and open to the suggestions by the other members of the project team. Communication between the institutions relevant for the project realization (IAC as a project manager, Federal Agro Mediterranean Institute, Mostar, Bosnia and Herecegovina) was very effective and easy going.

This communication between Federal Agro Mediterranean Institute and local stakeholders was essential for smooth finding of wild grapevine locations. We would not be able to localize wild grapevine in such short period of time without help of local forest rangers, fisherman's, and wine producers.

Project Implementation: (aspects of the project execution that contributed to its success/ shortcomings)

The project was performed very quickly and very effectively. Management of the project was done by a project manager. Six different expert profiles from the IAC were included in the project team (viticulture-enology, forestry, molecular biology, microbiology). Additionally, one expert in viticulture-enology was involved from Federal Agro Mediterranean Institute. Such an interdisciplinary approach was proved to be very effectively in the terms of locating and evaluating the locations and samples of wild grapevines. The financial management of the project was conducted by an accounting team within IAC. Efficient logistics was provided by Federal Agro Mediterranean Institute from Mostar and Nature park "Hutovo blato" as a result of good communication and great interests for project implementation.

Other lessons learned relevant to conservation community:

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Additional Funding

Provide details of any additional donors who supported this project and any funding secured for the project as a result of the CEPF grant or success of the project.

Donor	Type of Funding*	Amount	Notes

****Additional funding should be reported using the following categories:***

A) Project co-financing (Other donors contribute to the direct costs of this CEPF project)

B) Grantee and Partner leveraging (Other donors contribute to your organization or a partner organization as a direct result of successes with this CEPF project.)

C) Regional/Portfolio leveraging (Other donors make large investments in a region because of CEPF

investment or successes related to this project.)

Sustainability/Replicability

Summarize the success or challenge in achieving planned sustainability or replicability of project components or results.

Results of the project will be beneficial to Nature park "Hutovo blato" management plan and other stakeholders such as County of Čapljina and Federal Ministry of Environment and Tourism of Bosnia and Hercegovina. Inventory of large number of plant species along with wild grapevines was done. The exact geographical position found via GPS coordinates will facilitate further plant monitoring. The documentation clearly provides the methodology for evaluation and identification of wild grapevines and other plant species, which ensures repeatability of the project. This study could help to identify a wild grapevine on adjacent rivers in Bosnia and Hercegovina and could serve as a good example how to identify not only wild grapevines but other wild relatives of cultivated plants (e.g., figs, cherries, strawberries). Our intention is to apply for further funding to replicate our methods for investigation of wild grapevine along rivers in Croatia and Bosnia and Hercegovina. The equipment, skills learnt/bought with these funds will be used for other projects in the same field for further study.

In addition, we are planning to apply new project to one of suitable call for biodiversity conservation, and continue monitoring and research of wild grapevine.

Summarize any unplanned sustainability or replicability achieved.

Safeguard Policy Assessment

Provide a summary of the implementation of any required action toward the environmental and social safeguard policies within the project.

Project activities were conducted in accordance with the Occupational Safety and Health whereby all involved employees respected regulations concerning accident prevention based on the Croatian legislation. Also, during the project we followed the Law on the nature protection and behave nature-friendly.

Additional Comments/Recommendations

Information Sharing and CEPF Policy

CEPF is committed to transparent operations and to helping civil society groups share experiences, lessons learned, and results. Final project completion reports are made available on our Web site, www.cepf.net, and publicized in our newsletter and other communications.

Please include your full contact details below:

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Performance Tracking Report Addendum

CEPF Global Targets

(Enter Grant Term)

Provide a numerical amount and brief description of the results achieved by your grant.
Please respond to only those questions that are relevant to your project.

Project Results	Is this question relevant?	If yes, provide your numerical response for results achieved during the annual period.	Provide your numerical response for project from inception of CEPF support to date.	Describe the principal results achieved from July 1, 2013 to June 30, 2014. (Attach annexes if necessary)
1. Did your project strengthen management of a protected area guided by a sustainable management plan? Please indicate number of hectares improved.	NO			Please also include name of the protected area(s). If more than one, please include the number of hectares strengthened for each one.
2. How many hectares of new and/or expanded protected areas did your project help establish through a legal declaration or community agreement?	NO			Please also include name of the protected area. If more than one, please include the number of hectares strengthened for each one.
3. Did your project strengthen biodiversity conservation and/or natural resources management inside a key biodiversity area identified in the CEPF ecosystem profile? If so, please indicate how many hectares.		YES	500 ha	
4. Did your project effectively introduce or strengthen biodiversity conservation in management practices outside protected areas? If so, please indicate how many hectares.		NO		
5. If your project promotes the sustainable use of natural resources, how many local communities accrued tangible socioeconomic benefits? Please complete Table 1 below.		NO		

If you answered yes to question 5, please complete the following table

