

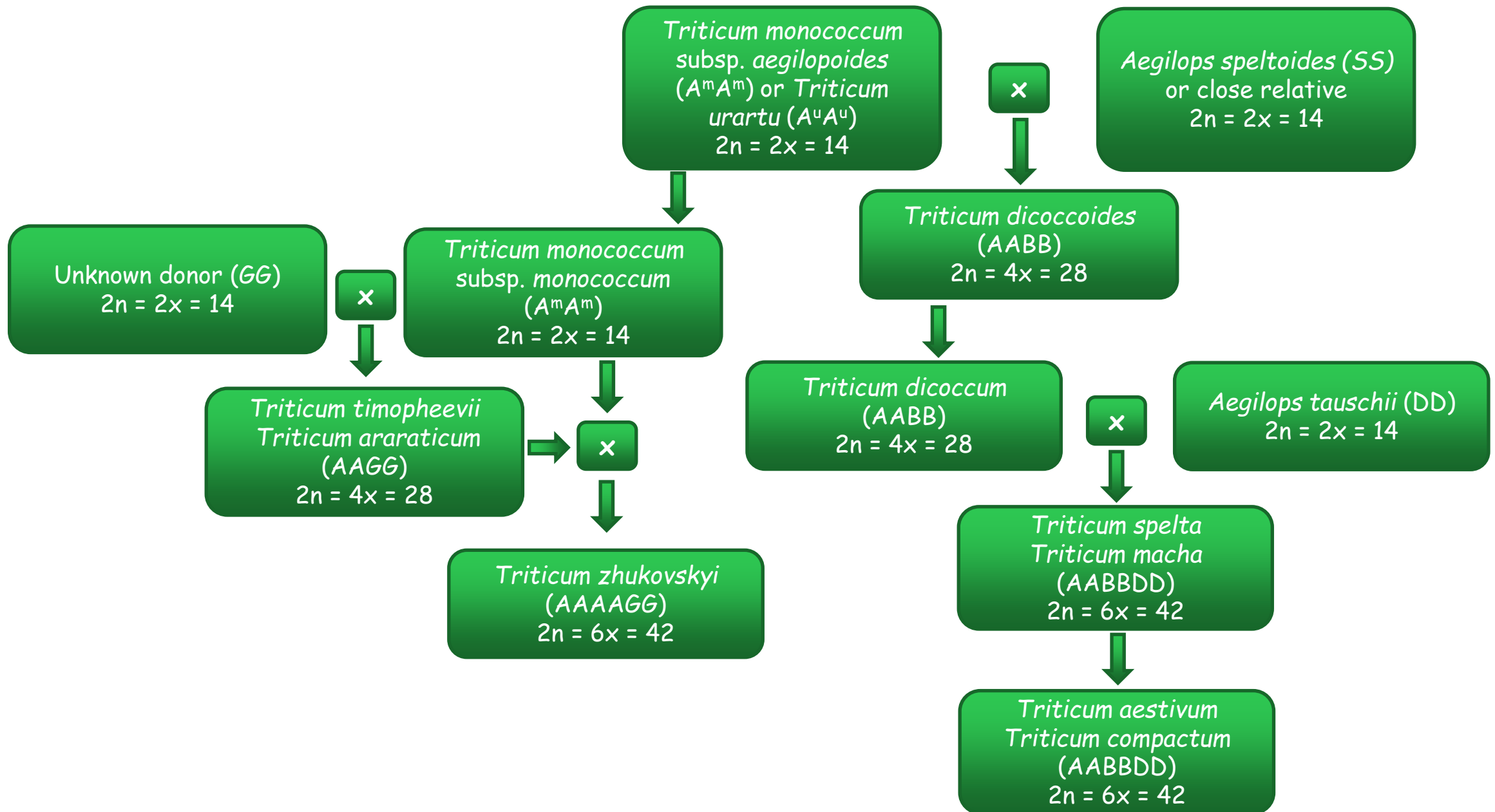
Pilot Study for the *In Situ* Conservation of Wild Wheat
Progenitors, *Triticum monococcum* subsp. *aegilopoides* and
Aegilops speltoides var. *ligustica* in Mesti Area, Greece

A.L. Tsivelikas and Greek Genebank team



Photos courtesy: Greek Genebank

Wheat Evolutionary Pathway



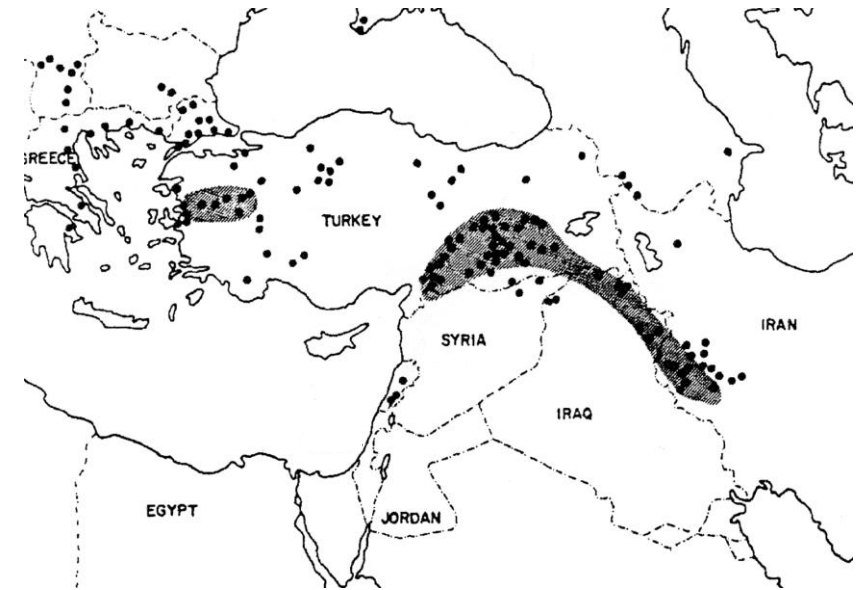
The Two Considered Species

A. *Triticum monococcum* subsp. *aegilopoides* (syn. *Triticum boeoticum*)

- Commonly named as wild einkorn wheat
- Donor of A genome at cultivated wheat ($2n = 2x = 14$)
- First record in Greece (in Argos region) from Link at 1833
- At 1844 Boissier finds it in Boeotia region (Greece) and records it as *Triticum boeoticum*
- Primary distribution in the fertile crescent (north Syria, southeast Turkey, north Iraq, west Iran)



Photo courtesy: Greek Genebank



Primary (shaded areas) and secondary distribution of wild einkorn wheat (*Triticum monococcum* subsp. *aegilopoides*) (modified from Harlan and Zohary, 1966)

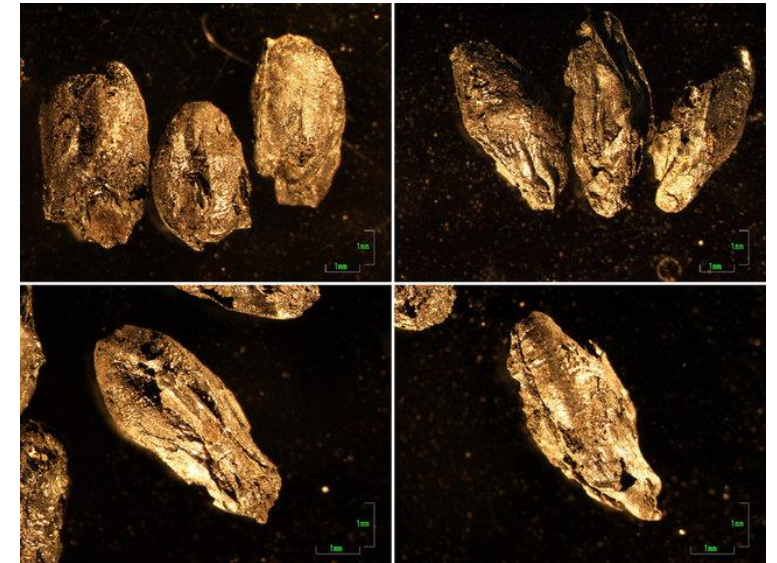
The Two Considered Species



From the wild to domesticated einkorn *Triticum monococcum* subsp. *monococcum*

- Domesticated einkorn (non-shattered) started to emerge ~10,400 years BCE in the Near East region
- Many neolithic settlements in Greece, where seeds of domesticated einkorn occurred
- Forms of *Triticum timopheevii* also found in neolithic settlements in Greece

Settlement region	Period (in years BC)	Domesticated einkorn (AA)	<i>Triticum timopheevii</i> (AAGG)
Gentiki	6000 - 5000	x	
Argissa	6000 - 5000	x	
Archontiko	3000	x	
Makri	5300 - 4500		x
Arkadikos	5300 - 4500		x
Makriyallos	5300 - 4500		x
Assiros	3000		x



Sprouted einkorn and barley grains from Archontiko (source: Valamoti, 2018)

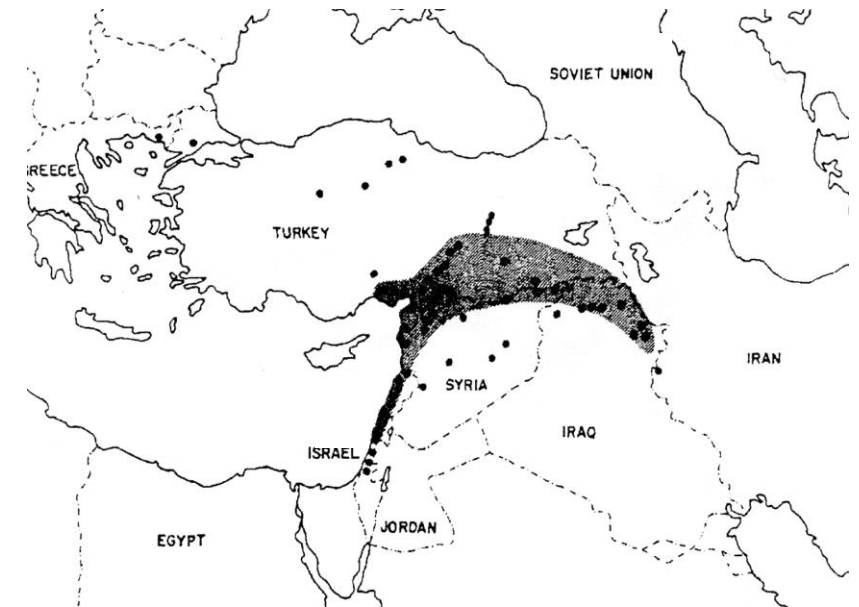
The Two Considered Species

B. *Aegilops speltoides* var. *ligustica*

- Donor of *B* genome at cultivated wheat ($2n = 2x = 14$)
- Primary distribution in the fertile crescent (Israel, Palestine, Syria, southeast Turkey, Iraq)
- Very narrow secondary distribution
- Open pollinated species
- First record in Greece by Zamanis et al. at 1987 in the area of Mesti, Evros prefecture in co-existence with *Triticum monococcum* subsp. *aegilopoides*



Photo courtesy: Greek Genebank



Primary (shaded areas) and secondary distribution of (*Aegilops speltoides* var. *ligustica*) (modified from Harlan and Zohary, 1966)

The Area of Mesti, Evros Prefecture

➤ Key characteristics of the area

- ✓ A low hilly pasture area along a provincial road network of approximately 20 km length
- ✓ Four nearby communities of mixed populations (Muslims and Christians) that tend to decline
- ✓ The forest of rare bush type black pine tree (approx. ~200 ha)
- ✓ Being part of the ancient Egnatia road constructed by Romans



Mesti
(*Triticum boeoticum* &
Aegilops speltoides)

Photo courtesy: Greek
Genebank



Preparatory Phase

- 
- 
- 2004: Desk survey and collection of related information
 - ✓ The climate of the area
 - ✓ The soil parameters
 - ✓ The agricultural production, the primary source of income and the economic development of the region
 - ✓ The population structure
 - ✓ The distribution of the species under study in other areas in Greek territory

However, the desk survey revealed...

The latest records of the populations in the area were dated back in the year 1987

Action Phase

- On site visit during the period 2004 - 2008 (three to four visits per year)
 - ✓ Survey of the area and identification of target species populations
 - ✓ Setting semi-permanent quadrats and measuring demographic parameters of the populations under study (determine the dynamic, the threats and the direction of the populations)
 - ✓ Collecting representative seed samples for *ex situ* field trials and conservation.
 - ✓ Collecting soil samples at the defined populations sites
 - ✓ Collecting data on agricultural production, the primary sources of income and the economic development of the region
 - ✓ Investigation of the potential interest of local communities for *in situ* conservation actions
 - ✓ Meeting with local authorities for potential collaboration



Photos courtesy:
Greek Genebank

Action phase: Survey of the area for the target species

- A thriving population(s) of *Triticum monococcum* subsp. *aegilopoides* were found along the main road and up to 600 m wide inside the *Quercus* forest, occupying also parts of the ancient Egnatia road
- Individuals of *Aegilops speltoides* var. *ligustica* found in relatively disturbed environments (e.g., roadsides), with decreasing density towards the interior of the pasture, being absent inside the *Quercus* forest



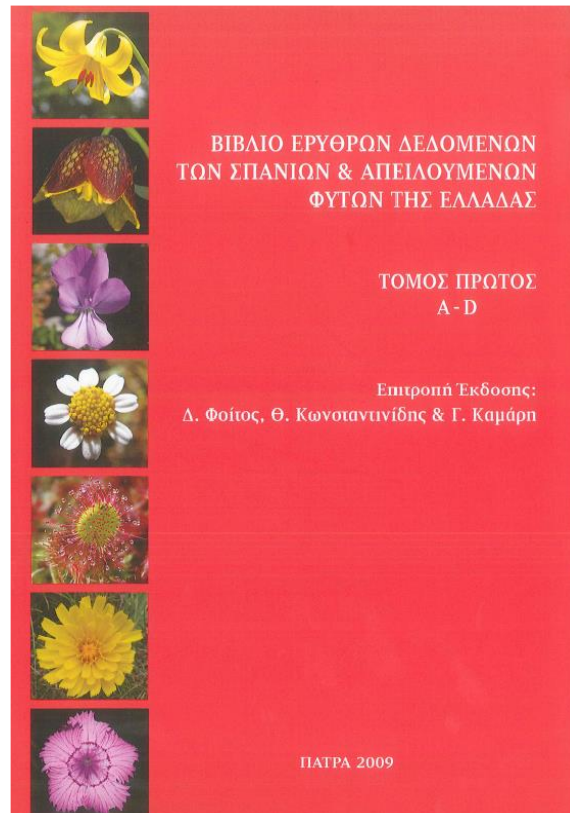
Part of ancient Egnatia road in the area of Mesti, where plants of *Triticum monococcum* subsp. *aegilopoides* occurred



Typical habitat for *Aegilops speltoides* var. *ligustica* in disturbed site with close proximity to the roadside

Action phase: Site description and demographic parameters

- Six semi-permanent sites for population monitoring were established along the road (two additional for *Triticum monococcum* subsp. *aegilopoides* inside the *Quercus* forest)
- Demographic parameters measured in each site visit to monitor the populations dynamic
- Data compilation and registration of *Aegilops speltoides* var. *ligustica* at the Red data book of rear and threatened species of Greece under the status "vulnerable"



Aegilops speltoides Tausch
var. *ligustica* (Savign.) Fiori

Gramineae

Aegilops speltoides Tausch var. *ligustica* (Savign.) Fiori, Fl. Italia 4: 33 (1907).

Τύπος: (υποείδη από τον van Slageren, 1994): [*Triloia*, *Aegilops*] "*tri*" (*tri*) *collis frearii* la porta Montaldo presso Genova", 1847, *Savignone* s.n. (Νότιος FI, Ισο-νέκος, LY-Gandoger).

Συνώνυμα: *Aegilops ligustica* (Savign.) Cass., App. II, jun. alt., Bull. Soc. Bot. France 11: 164 (1864).

Ae. speltoides (Tausch) subsp. *ligustica* (Savign.) Zhuk., Bull. Appl. Bot., Gen. & Pl. Breeding 18(1): 530 (1928).

Ae. speltoides Tausch f. *ligustica* (Savign.) Hammer, Feddes Rept. 91: 231 (1980).

Triticum ligusticum (Savign.) Bertol., Fl. Ital. 6: 622 (1847).

T. speltoides (Tausch) Gren. ex K. Richt. subsp. *ligustica* (Savign.) Asch. & Graebn. in Aschers., Magyar Bot. Lapok 1 (6): 12 (1902).

T. speltoides (Tausch) Gren. ex K. Richt. f. *ligusticum* (Savign.) Bowden, Can. J. Bot. 37: 665 (1959).

T. speltoides (Tausch) Gren. ex K. Richt. subsp. var. *ligustica* (Savign.) Kimber & Feldman, Wild Wheat 26 (1987).

Περιγραφή: Μονοειδής πόα σχηματίζουσα σπείρες, με λίγα έως αρκετά λεπτομήκη στέλεχη, ύψους 30-75 cm εξαρτημένη του στέλεχους. Φύλλα αρσά, περισσότερο πυκνά στη βάση και βλαστά, λεπτός φύλλου γραμμωτός σπείρες, διαστάσεων 6-15 x 0.2-0.4 cm με περιθώριο κοκκώδους υαλίνης, ενίοτε βλαστηροειδές. Ταξιανθία λεπτός κυλινδρικός στέλεχος, διαστάσεων 7-10 x 0.3-0.6 cm χωρίς τα άνω. Στάχυς κατά την ωρίμανση σφαιροειδής αρθροειδής με το άνω στάχυδα της βάσης ή το χαμηλότερο γόνιμο στάχυδα να παραμένει προσκολλημένο στο στέλεχος. Στάχυς με 7-15 γόνιμα στάχυδα και κατώτερη ή ενίοτε ένα άγριο, ύψους 2 σε κάθε στάχυδα, δερματώδη, στενός ελλειψοειδής-ακρότατος, μήκους 5-7 mm, πρόσφατα έως πορφυροπράσινα, με τροχιά σφαιρική, κορυφή των λειψίων στα πλευρικά σταχυία ακέραιη, συνολικά με μικρή, τριές και στενό υαλίνες περιθώριο, κορυφή των λειψίων στα κορυφαία σταχυία αμβλύα, αυξανόμενου πάχους, με μικρές τριές, χωρίς αιχμή. Λεπίδες γόνιμων ανθιδίων στα πλευρικά και στα κορυφαία σταχυία με άνω στην κορυφή άνω με βραχέες τριές, χωρίς πλευρικούς σόνδους στη βάση, αυξανόμενου μήκους από 2.5 cm στη βάση του στέλεχους μέχρι 10 cm στην κορυφή. Χάλας στενό υαλίνης ελλειψοειδής, με δύο σφηνοειδείς, σπαστικές κορυφές, που καταλήγουν σε μία οξεία κορυφή. Καρπώση μήκους 6-7 mm, προσκολλημένη στις λεπίδες και στον κότυλο.

Χρονολογιστικός αριθμός: 2n = 2x = 14 (Chenopodaceae-ialia 1960, ως *A. aegypti* και *A. speltoides*, Waines & Barnhart 1992, Jones & Pašalkinskiene 2005).

Τρωάτο (VU)

Περίοδος ανθοφορίας: Στην Ελλάδα, από τα τέλη Μαΐου έως τα τέλη Ιουνίου.

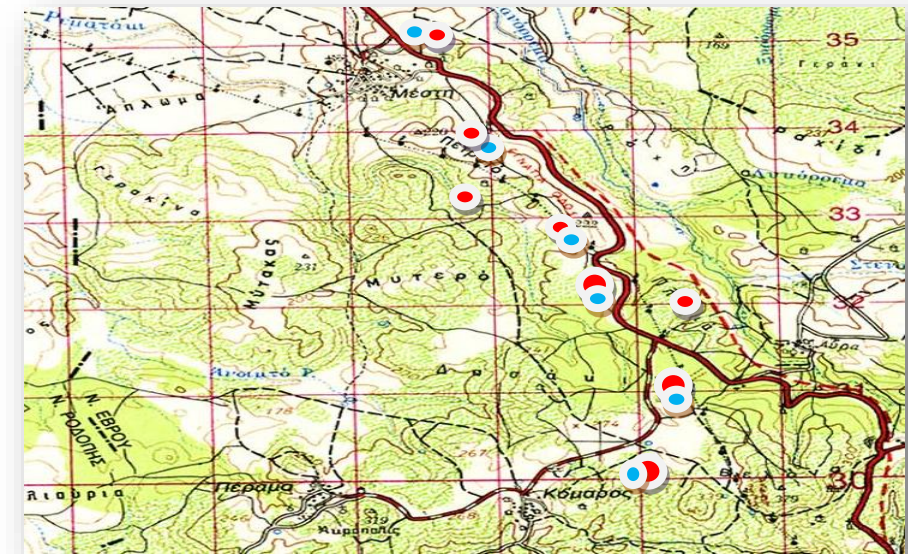
Γεωγραφική εξάπλωση: Ο *Aegilops speltoides* καταγράφεται από την περιοχή του τόπου της "έλασης ημεσλήνου", δηλαδή την περιοχή που εκτείνεται από το Ισραήλ, τη Συρία, την Α. Τουρκία, το Ιράν, το Ιράκ μέχρι την Αρμενία και τον Ν. Κακάσο. Σε συνδυασμό με την τυπική μορφή του είδους (var. *speltoides*), που περιορίζεται στον ασιατικό στο τόπο αυτό, ο *Ae. speltoides* var. *ligustica* επικρατεί μέχρι το νοτιοανατολικό όριο της Ευρωπαϊκής Ηπείρου, καθώς αναφέρεται στα όρια της Α. Πολωνίας, στην Βουλγαρία (Stojanov & Stefanoff 1948, Stojanov & al. 1966, Delipavlov 1992), όπου βρίσκεται να αναπτύσσεται στην ίδια περιοχή με το αυτοφυές διπλοειδές σπείρι.

Στην Ελλάδα αναφέρεται για πρώτη φορά από μέλη των ερευνητικών αποστολών που πραγματοποιήθηκαν από το 1987 και κατόπιν (Zamanis & al. 1988, Σταυρούλας κ.α. 2008) στην ευρύτερη περιοχή της Μέσης, στα σύνορα των νομών Έβρου και Ροδόπης. Εκεί συναντάται με το αυτοφυές διπλοειδές σπείρι *Triticum monococcum* subsp. *boeoticum* και αποτελεί το μοναδικό μέχρι σήμερα γνωστό σημείο καταγραφής στο ελληνικό κράτος και ένα από τα ελάχιστα σημεία στον ευρωπαϊκό χώρο.

Διαδόση, αξίζει να σημειωθεί ότι σύμφωνα με στοιχεία



Γεωγραφική εξάπλωση του είδους *Aegilops speltoides* var. *ligustica* στην Ελλάδα.



Capture of marking sites of the two species under study in the area of Mesti

- *Aegilops speltoides* var. *ligustica*
- *Triticum monococcum* subsp. *aegilopoides*

Action phase: Site description and demographic parameters

Site 3:	
Geographical coordinates:	40°56'018''N; 25°39'980''E
Altitude:	190 m
Related flora:	<i>Paliurus</i> sp., <i>Aegilops</i> spp., <i>Onobrychis</i> sp., <i>Trifolium campestre</i> , <i>Haynaldia villosa</i> , <i>Vicia</i> spp., <i>Medicago minima</i> , <i>Cardus</i> sp.
Habitat and soil elements:	Site along the provincial road, in the kennels, without high vegetation presence, where plant communities of <i>Aegilops speltoides</i> subsp. <i>ligustica</i> and <i>Triticum monococcum</i> subsp. <i>boeoticum</i> coexist. Silty-sandy soil with neutral to alkaline pH and poor in organic matter. It is characterized by particularly low concentrations of potassium and trace elements, which makes it almost borderline even for the development of any form of plant vegetation.

	<i>Triticum monococcum</i> subsp. <i>aegilopoides</i>		<i>Aegilops speltoides</i> var. <i>ligustica</i>	
Date:	23/05/2008	29/06/2008	23/05/2008	29/06/2008
Plant density (at heading)	20 plants/m ²	28 plants/m ²	6 plants/m ²	53 plants/m ²
Mean plant height	60 cm	65 cm	30 cm	58 cm
Spike length	9.5 cm	9.0 cm	5.5 cm	7.0 cm
Awn length	8.0 cm	8.0 cm	6.0 cm	7.0 cm

Action phase: Collecting soil samples for analysis

	Site 1	Site 2	Site 3	Site 4
Mechanical texture	Organic	CL	SL	C
pH	7,04	6,90	7,68	6,99
CaCO ₃ (%)	1,3	0,9	4,4	1,3
Ec (mS/cm)	0,513	0,340	0,299	0,440
Organic matter (%)	4,56	1,96	0,55	1,46
P (ppm)	65,29	57,5	60,9	57,7
K (ppm)	260	70	40	160
Exchange Ca ²⁺ (ppm)	1213	1000	1310	1050
Exchange Mg ²⁺ (ppm)	134	153	150	104
B (ppm)	0,58	0,24	0,04	0,17
Mn (ppm)	8,95	12,79	2,54	19,62
Zn (ppm)	1,90	0,43	0,17	0,39
Fe (ppm)	11,59	5,87	3,68	9,50
Cu (ppm)	0,54	0,55	0,20	0,48

- ✓ Wide variation on soil types between the different sites in terms of soil texture and chemical properties
- ✓ Site 3: Marginal soil for plant growth and development



Action phase: Assessing the major threats

- Three major threats were identified:
 - ✓ Overgrazing
 - ✓ Fire
 - ✓ Competitive development risks (e.g. widening or construction of new road network)



Photos courtesy: Greek Genebank

Action phase: Collecting seed samples for *ex situ* field trials

- Spikes from 10-15 plants within each quadrat for each of the two species were collected (~200 seeds/quadrat/species)
- Field trials at the Greek Genebank (*ex situ*) were established for each growing season
- Phenotypic description and assessment of morphological diversity
- Seed collection from *ex situ* trials for *ex situ* conservation

Preliminary assumptions...

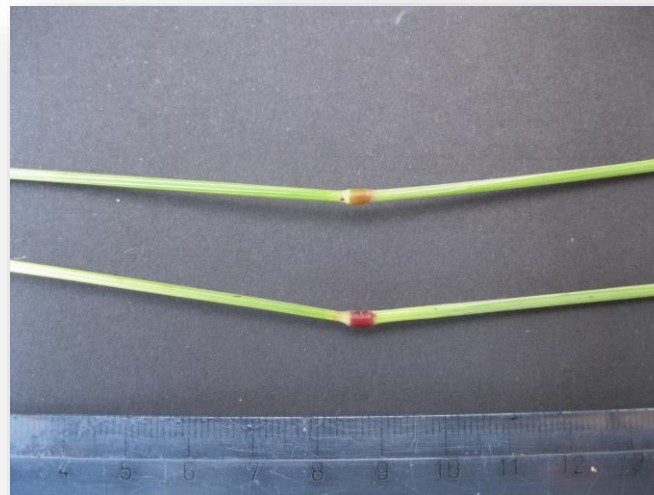
Each of the species constitutes a distinct population in the region, with the detectible genetic diversity as expressed for many morphological traits.

Action phase: Assessing morphological diversity of populations

Differences in spike density within the population of *Aegilops speltoides* var. *ligustica*



Differences in spike morphology for *Triticum monococcum* subsp. *aegilopoides* population

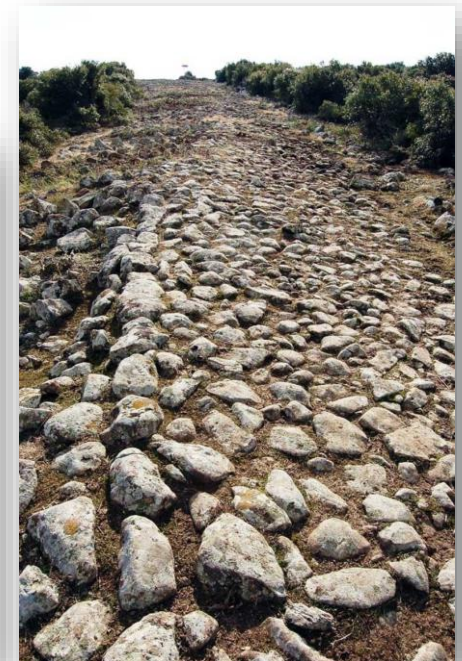


Differences in stem and auricle coloration within the population of *Aegilops speltoides* var. *ligustica*



Action phase: Investigation of the potential interest of local communities and authorities

- Meeting with local communities to ensure the consent and participation of the local community or individual site owners → incentives needed (such as subsidies)
- Meeting with governing body of Evros prefecture to set a plan for the *in situ* conservation of *Triticum monococcum* subsp. *aegilopoides* and *Aegilops speltoides* var. *ligustica* in the area of Mesti that will include also the forest of bush type black pine tree
- Meeting with the archeological services for setting a management plan within the sites in ancient Egnatia road



Recommended Management Plan

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- 
- Needed approach should take into consideration the limited size of the area and spread of populations and the distinct elements of the site.
 - ✓ No core area, buffer zone and transition zone to be defined

Basic elements of action plan

- 
- ✓ Detailed mapping of the populations distribution of the two protected species in the region and in the country (if elsewhere occurred)
 - ✓ Periodic monitoring and recording of the demographic parameters of their populations (number and size of populations, spread, direction, etc.)
 - ✓ Periodic observation and recording of ecogeographical parameters (flora, soil, climate, hydrological basin and water reserves, threats, etc.) (employment of appropriate software, e.g. ArcGIS)
 - ✓ Study of phenotypic and genetic diversity of the populations, genetic constitution of populations, determining N_e , (crossability within and between populations and subpopulations, etc.)
 - ✓ Necessary protection interventions (e.g., management of grazing, weed removal, minimization of other risks) for the targeted populations and their associated habitats

Recommended Management Plan



Basic elements of action plan

- ✓ Complementary *ex situ* conservation as a security back up for long term conservation at the premises of the Greek Genebank'
 - ❖ Use for restoring populations in case of destruction by fires, diseases and enemies or unforeseen human interventions
 - ❖ Use for reinforcement of the populations in the protected area, when necessary
 - ❖ Use for relocation of the populations to safer sites at the same habitat (e.g., at sites that are out of private land, where population management and conservation presents less legal and bureaucratic difficulties)
- ✓ Establishment of *ex situ* field trials for phenotypic, agronomic and genetic studies for the populations under consideration
- ✓ Maintenance of appropriate documentation system that can host all the data collected for the populations and the habitats and interventions according to management plan (e.g., weed removal, grazing management, etc.)

Recommended Management Plan

Basic elements of action plan

- ✓ Generating governmental financial support for the local community and through the development of a wider agro-environmental program in the region
- ✓ Promoting a new type of touristic development for the region - scientific tourism (schools, students, researchers from different disciplines, such as archeology, history, botanist, agronomist)
 - ❖ Diligent interventions in ancient Egnatia road to highlight its importance and scientific value (weed cleaning, selection of appropriate lighting along the ancient pathway, information kiosks nearby the protected populations to aware the public, etc.)

Estimated budget

- ✓ For 10 defined observation sites for each of the two species and 6 observation sites for the mixed populations → 130.000 Euros/year
 - ❖ Includes only equipment needs, transportation, cost for defining and marking the populations, collection, *ex situ* conservation, field trials, genetic and reproductive biology studies (based on Iriondo, 2008), as well as subsidies for local community.



***THANK YOU FOR YOUR KIND
ATTENTION!***