Report of a *Spinacia turkestanica* collecting expedition to Uzbekistan

Daily itinerary, collected germplasm and data

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In 2023 heeft CGN, in nauwe samenwerking met lokale collega's, een expeditie ondernomen naar Oezbekistan. Dit rapport verslaat de resultaten van deze expeditie, waarin 27 monsters van zaden van *Spinacia turkestanica* verzameld werden. Deze nieuwe accessies zullen aan de spinazie collectie van het CGN toegevoegd worden. De meeste populaties van *S. turkestanica* in Oezbekistan worden mogelijk bedreigd omdat wilde spinazie alleen kan overleven in de marges van velden die extensief worden beheerd. Het was echter mogelijk om diverse populaties te vinden, dus waar de juiste condities te vinden zijn is nog voldoende *S. turkestanica* aanwezig. Dit betekent dat de landbouw in bepaalde gebieden niet erg is veranderd sinds de vorige expeditie in 2008. Maar hoe lang zal dit nog zo zijn?

In 2023 CGN carried out a collecting expedition in Uzbekistan, in close collaboration with its local counterparts. This report provides the results of this collecting expedition, during which 27 seed samples of *Spinacia turkestanica* were collected. These new accessions will be added to the spinach collection of the CGN. Potentially the majority of *S. turkestanica* populations in Uzbekistan are under threat as wild spinach can only survive in the margins of fields that are managed through low input farming. However it was possible to find a number of populations, so where the conditions are right there is still enough *S. turkestanica* present. This means that agriculture hasn't changed much in certain areas since the previous expedition in 2008. But for how long will this be the case?

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Preface

The mission of the Centre for Genetic Resources, the Netherlands (CGN) is to contribute to the conservation, development and sustainable use of plant, animal, forest and aquatic genetic resources, and hence to global food security, a more sustainable production, rural development, and the conservation of cultural heritage. To that end, CGN holds collections of over 20 crops and a total of number of accessions of more than 23.000 of interest to breeders, researchers and other users. Annually around 3700 seed samples are distributed.

To contribute to an effective global system of *ex situ* collections, for each of its collections CGN has analysed the coverage of the crop genepool by germplasm in its own collection and those of others. In a number of cases, CGN has been able to identify gaps in the total set of collections of a specific crop. Some genetic diversity that is known or can be assumed to exist, appeared poorly represented or even absent from the genebank collections. Such cases warrant new collecting missions, if we wish to conserve as wide a diversity for the genepool as possible.

The wild ancestors of the vegetable spinach (*Spinacia oleracea*) are such a case. CGN has made an effort to fill in gaps for *Spinacia turkestanica* and *S. tetranda*, that are considered to be the closest ancestors, and continues to do so. Therefore in 2023 CGN carried out a collecting expedition in Uzbekistan, in close collaboration with its local counterparts. The present report provides the results of this collecting expedition. During the expedition 27 seed samples were collected. Upon regeneration, the samples will be made available under the terms and conditions of the Standard Material Transfer Agreement of the International Treaty, with the agreement of the authorities in Uzbekistan involved.

This collecting expedition was an activity jointly undertaken by partners in Uzbekistan and the Netherlands. The support from the national authorities in Uzbekistan is duly recognized.

1 Introduction

The largest spinach collection in a genebank worldwide is maintained at CGN. This collection holds 541 accessions, of which 114 are wild relatives (Ribera et all. 2020), consisting of 39 *S. tetranda* and 75 *S. turkestanica* accessions. Per accession, the spinach collection is currently the most requested collection of CGN.

Resistance against downy mildew has been the main breeding target in spinach. The introgression of NBS-LRR resistance genes from wild relatives is the major strategy to develop downy mildew resistant cultivars. So far, abiotic resistance and quality traits have received minor attention in spinach research and breeding, but this is expected to change considering the potential effects of climate change on cultivated spinach. Accessions of the collection are used in breeding efforts and are also used for scientific studies on spinach (Ribera et all. 2021 and Gyawali et all. 2021).

CGN has conducted several expeditions in the past, and the 2023 expedition to Uzbekistan fits into this collecting program. Central Asia is not an unknown territory for CGN as Loek van Soest has carried out two multi-crop expeditions in Central Asia, in 1997 and 1999. More recently, Chris Kik organized single crop expeditions in 2008 (spinach), 2015 (carrot), 2017 (melon) and 2019 (lettuce). Visited countries were Kyrgyzstan, Tajikistan and Uzbekistan. The present and future collecting expeditions could therefore benefit from their knowledge and experience when travelling around in this part of the world.

Uzbekistan is located in Central Asia which is known to be the area of origin of *S. turkestanica*. The expedition was organized to expand the CGN spinach collection and to make more genetic resources of *S. turkestanica* available for research and breeding. After the successful collecting expeditions for *S. turkestanica* in 2008 and for *S. tetranda* in 2011 (van Treuren et all. 2019), breeding companies have used the collected material and introduced genes from it into modern Spinacia oleracea races.

In 2023 a Memorandum of Understanding adopting the Standard Material Transfer Agreement (SMTA) of the Internal Treaty for Plant Genetic Resources for Food and Agriculture (IT-PGRFA), as a basis for distribution, was signed between CGN and the national authorities on access and benefit sharing (ABS) in both countries. This formed the legal basis of the expedition. Good contacts with the Institute of Botany in Uzbekistan made it possible to collect *S. turkestanica* in Uzbekistan.

2 Set-up

2.1 Objectives

Major aims of this single crop collecting expedition in Uzbekistan:
- to broaden the spinach collection of CGN by collecting *S. turkestanica* for breeding and research purposes, and
- to contribute to the international need for the conservation of PGR.

2.2 Members of the collecting team

- Dr. Orzimat Turginov, Institute of Botany, Uzbek Academy of Sciences, Tashkent, Uzbekistan
- Mr. Doston Turdiyev, Institute of Botany, Uzbek Academy of Sciences, Tashkent, Uzbekistan
- Ir. Wouter Groenink, CGN, Wageningen
- Ir. Roel Hoekstra, CGN, Wageningen

2.3 Routes of exploration

In preparation of the expedition Orzimat Turginov made a map, based on the results of the 2008 spinach collecting expedition in Uzbekistan, indicating roughly five regions of interest for collecting *S. turkestanica* (Fig. 1). Prior to the expedition students and assistants of the Institute of Botany inventoried the presence of *S. turkestanica* when exploring these regions.
The expedition took place June 18th to the 2nd of July and the distance travelled was approximately 2500 km. The car that was used was a Chevrolet Cobalt (sedan model). The temperature in the field ranged from 34 till 40 degrees Celsius.

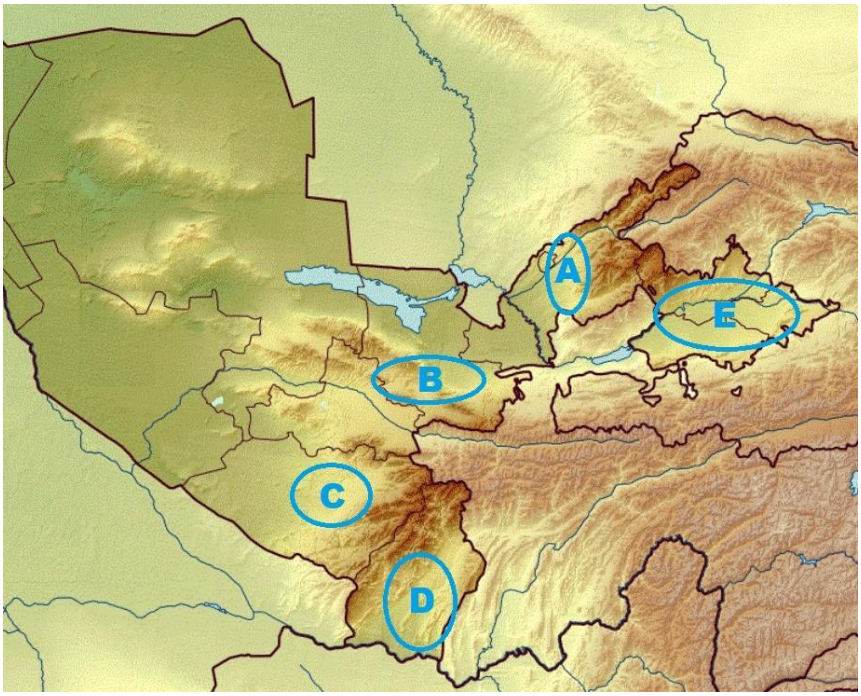

Figure 2.1 *Expedition collecting plan showing 5 regions to explore. The Southeast part of Uzbekistan is shown as only this part is of interest concerning* Spinacia turkestanica*.*

2.4 Field observations and sampling

Collecting of material during this single crop expedition was done according to the following protocol. Rough material, stems with seeds, of all plants collected at one site was put in a canvas seed bag. Immediately a collecting number was attached to the bag. On the collection site pictures were taken of the *S. turkestanica* plants and of the surroundings. On a digital map on a mobile phone the collection site was GPS-tagged. The GPS data of both map and pictures were used to determine the latitude, longitude and altitude of the location and spread of the population. Furthermore the type of collecting location, abundance of *S. turkestanica* plants and date and time of collecting were noted. Collecting numbers consist of four letters, namely TTGH, and two digits. The letters stand for the mission participants: Turginov, Turdiyev, Groenink, Hoekstra. Numbers were given according to the order of collecting. The rough material was cleaned in Tashkent, separating stems, debris and empty seeds from the good seeds. This resulted in clean seed samples ready for transport to the Netherlands. At Schiphol the seed bags were cleared by customs, using the Plant Health Certificate provided by Uzbek officials. After arrival at CGN the seed bags were put in the seed conditioning room at 15°C and 15% relative humidity.

3 Results

The daily itinerary is given in Annex 1, showing the route travelled and activities carried out each day of the collection expedition. During the expedition only natural locations of *S. turkestanica* were visited, where 27 samples were collected. No seed samples from vegetable gardens or from markets were collected. An overview of the collected germplasm can be found in Annex 2. During cleaning all samples that were handled were found to be free of insects, insect damage or any clear disease infestation. All seed samples contained only well dried seeds.

The distance between most of the collection sites was at least 8km. The first two days however the distance between sites was less, and sometimes even less than 2km apart. At the collecting sites GPS tagging was done, but only after we started plotting the locations on a map we found out that some collecting sites were quite close (e.g. on parallel roads) or close to spinach collecting sites in 2008. During the rest of the expedition we tagged locations on a digital map and kept better track of the distance in a straight line to the next collecting location.

Different areas were explored than those that were planned by Orzimat Turginov prior to the expedition (Figure 2.1). In area A the plan was to travel more northeast of Tashkent, but assistants from the Institute of Botany had determined that no *S. turkestanica* was present in that area. Area D was not explored at all because prior to the mission students from the institute had observed desert-like conditions in the area due to the drought in 2023, causing a low probability of success in finding suitable populations. In area C traveling southwards we noted that plants were becoming smaller and smaller (Figure 3.1), with the climate getting hotter. And in area E, the Fergana valley, we only explored the most north-eastern part where agriculture was expected to be the most primitive. After being unsuccessful in finding any *S. turkestanica* even along the Kasansay-Namangan road, where it was reported almost 50 years ago, we decided not to explore this valley any further.

Figure 3.1 *Habitat where* Spinacia turkestanica *is growing with loess soil. At this particular location where accession TTGH 27 was collected there was a very hot climate and plants were very short.*

The number of plants harvested per location varied from a few (5-10) to numerous (>100). Often plants from a location were not growing within a circular area but along a strip (e.g. field margins). Another observation was that male plants were not found. This might be due to a rapid degradation of these tiny plants in the dry environment, after they flowered. From the female plants few leaf material was left and mostly stems with seeds remained (Figure 3.2). Potentially the focus on seeds also attributed to not spotting male plants.

Figure 3.2 *Location of collecting number TTGH 16 where* Spinacia turkestanica *is growing among the cereal crop, in this case wheat. On the right a sample of the collected material.*

The habitat where *S. turkestanica* was found can be characterized as cultivated non-irrigated steppe having a loess soil, where the species grows along mostly extensively grown cereal fields (Figure 3.2). Wild spinach cannot be found in intensively grown, fertilized fields. A second habitat are areas that are located along a road, in between cereal fields (Figure 3.3) or on abandoned fields in which few other species grow. Individual plants can be small and have growth heights of 10-20 cm in exposed and hot conditions. Plants can be taller to ca. 50 cm, where they coexist with other taller species. As the populations we found were observed predominantly to be growing along field margins in rooted up soil, wild spinach can be characterized as a ruderal (so competition avoiding) species.

All the collected plants were already withered and dry and seeds detached from the stems easily. Sometimes only breaking a branch off a plant was sufficient to detach most of the seeds it carried. The accompanying cereal fields were close to harvesting in many cases. And often the outer most strip of a field is mowed even earlier, some time prior to the main cereal harvest. These margins is where *S. turkestanica* grows when it occurs along the field. In one field we collected material just minutes before a combine flattened that spot (Figure 3.4).

During the expedition we found plants with seeds from other crops that are of interest to the CGN collection. We noted the species and the locations where they were found for any further expeditions that CGN might undertake in Central Asia.

Figure 3.3 *An unused corner of land among cereal fields where* Spinacia turkestanica *is growing.*

Driving with a normal passenger car worked well, although occasionally a 4W-drive would have suited better to reach the fields further away from the main roads. We had more or less one designated driver for the trip. Driving with one driver is not ideal given the hot conditions in the Uzbekistan summer. With high temperatures and full sun, a regular change of driver is definitely advised for safety reasons.

Figure 3.4 *Harvesting of a cereal field right next to a collecting location. The ruderal margin was cut down minutes after the collecting finished.*

4 Discussion

The expedition resulted in 27 collected seed samples. A few of these may be disregarded for inclusion in the CGN collection as a separate accession due to the fact that sampling locations were rather close to one another and might be considered one population (e.g. TTGH 05 and 06). Two collecting numbers, TTGH 02 and TTGH 29, contain seeds from a limited number of plants, which may be a reason to disregard these samples as well.

The timing of the expedition turned out to be rather late. For future expeditions to the same area it is recommended to start two weeks earlier in order to avoid losing *S. turkestanica* populations due to cereal harvesting. Also the withered state of the collected plant material indicates that earlier collecting is to be preferred.

The visit to the Fergana valley (area E) did not yield any spinach samples. As this valley was not explored prior to the expedition we had hoped to collect some material in this area. Information about people traditionally collecting wild spinach indicates that *S. turkestanica* was present in this area. GBIF (the Global Biodiversity Information Facility) includes only one record for this valley, unfortunately without exact locality data. The Institute of Botany has a herbarium record from the 1970's. We revisited the collecting site of this specimen, but without success. In the Fergana valley farming is relatively intensive, which is not favorable for wild spinach. So, although we searched for only one day in the Northeast part of the valley, there was little hope of finding wild spinach elsewhere in this valley.

Potentially the majority of the *S. turkestanica* populations are under threat. Wild spinach can survive in the field margins when a field is managed through low input farming. When farming becomes more intensive with the application of (chemical) fertilizers, use of herbicides and irrigation, the crops will become denser and more competitive. As a result, ruderal species like wild spinach, experience a decreased probability to fulfil their life cycle in such habitats. At most areas that were visited during the expedition it was easy to find plants and mostly many plants could be found at a single location. So, where the conditions are favorable *S. turkestanica* is still abundant. Comparing the 2008 and 2023 expeditions showed that in the regions visited in both expeditions *S. turkestanica* is still present, indicating that agriculture hasn't changed much in these areas. The question is of course for how long.

The 2023 expedition was a single crop expedition. The advantage of this narrow crop focus was that all energy was centered on finding wild spinach. Under the given circumstances this was certainly justified as it was sometimes difficult to find wild spinach populations. Nevertheless, the collecting of other (wild relatives of) crop species as bycatch should be considered in future expeditions to increase the efficiency of the collecting program. This could have been the case for *Lactuca* and *Eruca* populations in the present expedition.

5 Conclusions

- A Memorandum of Understanding, based upon the SMTA of the IT-PGRFA, was signed between CGN and the Institute of Botany in Uzbekistan. A Cooperation Agreement was also signed stating the intention to organize more collection expeditions together in case this expedition was a success.
- The collecting expedition took place in good cooperation between all team members. Although there were some misunderstandings because of language barriers. No major problems were encountered with local or national authorities.
- The choice to make it a single crop expedition meant that a good number of *Spinacia turkestanica* populations could be found and sampled. Only a few other species were seen that could be of interest to the CGN.
- The *Spinacia* collection of CGN was expanded with 27 *S. turkestanica* accessions. Unfortunately the most isolated and unique location did not deliver any material.
- Plotting collecting sites directly on a map and checking distance in a straight line to the next site, prevents from unintentionally collecting populations that are close together.
- For collecting wild spinach the timing of the expedition was a little bit late; the beginning of June would have suited better to collect seed.

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Annex 1 Daily itinerary

- **June 19**
  After arriving by plane in the morning, the Institute of Botany in Tashkent was visited. There we visited the herbarium and were introduced to the deputy director of science Farhod Karimov Isomiddinovich and the institute director Komiljon Tojibaev Sharobitdinovich. In the afternoon the first collecting trip was caried out in Region A, the road from Taskent to Ohangaron and 4 *S. turkestanica* accessions were collected.
- **June 20**
  The second day of collecting was also in Region A, easterly from the road from Taskent to Ohangaron we collected 5 accessions.
- **June 21**
  A transfer from Tashkent to Jizzax was made. During an evening collecting trip 2 accessions in Region B, south of the M39 between G'allaorol and Qorayantoq, were collected.
- **June 22**
  During a day of collecting, we collected 4 accessions in Region B, east of G'allaorol and 1 accession just South of the Jizzax water reservoir. This was next to a road between Qorayantoq and Nonisangil.
- **June 23**
  This day a transfer from Jizzax to Samarkand was made. On the way we went further along the road in Region B, east of G'allaorol, and collected 3 more accessions, before turning southwards towards Samarkand.
- **June 24**
  We moved from Samarkand to Shahrisabz. In Region C, along the road from Samarkand to Qarshi we collected 5 accessions. Along the connecting road between the afore mentioned road and Yakkabog 1 accession was collected.
- **June 25**
  Travelled from Shahrisabz back to Tashkent. In the area around Pskent we looked for *S. turkestanica*, but our searches were unsuccessful.
- **June 26**
  This day we spent cleaning the seeds of 16 of the collected accessions.
- **June 27**
  We made another trip in Region A, further eastwards of the road we explored the first few days. Along the fields south of the road between Dostlik and Kursoy we found only 2 solitary plants. They were in nearby locations, and they were merged into 1 accession. Further east our search was unsuccessful. After returning to Tashkent the seeds of another 5 accessions were cleaned.
- **June 28**
  This day was a Muslim religious holiday, and therefore a day off for Orzimat and Doston. We cleaned the seeds of the last 6 accessions that were collected so far. We started writing the report.
- **June 29**
  Travel to Chust in the Fergana valley.
- **June 30**
  Searching north of the road from Chust to Namangan in region E. First north of Baymoq in fields near the border with Kirghizstan. And then further along the road to Varashilov, which would be the most promising area. The search also included the road from Kosonsoy to Namangan, along which the only Fergana valley herbarium specimen from The Institute of Botany was collected. The search was unsuccessful.
- **July 1**
  Return trip from Chust to Tashkent. Before the flight back to the Netherlands we spend some time saying goodbye to our local contacts from The Institute of Botany.

Annex 2 Collected germplasm

Col. number	date	plants	Pop. size	environment	Latti-tude	Longi-tude	Alti-tude	district
TTGH 01	19-6-23	7	7	Herbaceous border of an oat field, between grasses and thistles	41,0649	69,4808	468	Ohangaron
TTGH 02	19-6-23	15	15	Herbaceous border of an unused cereal field, between grasses, thistles and *Lactuca orientalis*	40,9988	69,5269	458	Ohangaron
TTGH 03	19-6-23	4	4	Herbaceous border of a wheat field, between grasses and thistles	40,9685	69,5486	514	Ohangaron
TTGH 05	19-6-23	15	15	Herbaceous border of a wheat field, between grasses and thistles	40,9456	69,5781	531	Ohangaron
TTGH 06	20-6-23	50	200	Herbaceous strip within a wheat field with grasses, thistles and caper and grazing cows	40,9512	69,5887	548	Ohangaron
TTGH 07	20-6-23	50	50	Herbaceous border next to a barley field with grasses, thistles and caper	40,9545	69,6113	573	Ohangaron
TTGH 08	20-6-23	30	30	Herbaceous strip next to and within a barley field	40,9431	69,6191	576	Ohangaron
TTGH 09	20-6-23	50	50	Herbaceous border next to a cereal field with grasses, thistles and caper	40,9772	69,559	510	Ohangaron
TTGH 11	20-6-23	60	80	Herbaceous border next to a cereal field with grasses (a.o. *Aegilops*), thistles and caper.	41,0010	69,5689	519	Ohangaron
TTGH 12	21-6-23	88	150	Herbaceous strip next to and within a barley field	40,0464	67,7386	633	Jizzax
TTGH 13	21-6-23	50	50	Herbaceous strips within a field with an unknown crop with regrowth of barley from last season.	40,0199	67,8936	449	Jizzax
TTGH 14	22-6-23	60	60	Herbaceous border next to a barley field with grasses, thistles and caper	39,9355	67,5194	719	Forish
TTGH 15	22-6-23	40	40	Herbaceous strip bordering barley fields with grasses, flowers, thistles and caper	39,9273	67,4236	800	Forish
TTGH 16	22-6-23	120	2000	Outer 2m of a wheat field that was thinly sown	39,9854	67,3412	846	Forish
TTGH 17	22-6-23	70	70	Herbaceous strip around an electricity pole within a barley field, with grasses, flowers, thistles (and a few *Eruca* plants)	40,0569	67,2653	766	Gallaorol
TTGH 18	22-6-23	120	2000	Within a thinly sown barley field	40,0884	67,1693	726	Gallaorol
TTGH 19	22-6-23	50	70	Herbaceous border next to a cereal field with grasses and thistles	40,0357	67,9285	398	Jizzax
TTGH 20	23-6-23	70	70	Herbaceous strip bordering barley fields with grasses, flowers and thistles	40,1333	67,0675	752	Gallaorol
TTGH 21	23-6-23	120	2000	Within a thinly sown part of a barley field	40,1784	66,9645	841	Gallaorol
TTGH 22	23-6-23	100	400	Unused field with grasses, flowers and thistles and grazing cows	40,2302	66,8607	730	Pastdargom
TTGH 23	24-6-23	30	30	Unused field with herbaceous plants including *Cichorium* and grasses. Wild spinach is harvested in spring by the owner	39,5421	66,7666	806	Payariq
TTGH 24	24-6-23	50	50	Within a poorly grown barley field	39,4172	66,4012	644	Nurobod
TTGH 25	24-6-23	70	100	Outer 4m of a wheat field that was poorly sown	39,2895	66,2948	503	Chiroqchi
TTGH 26	24-6-23	120	1000	Herbaceous border between road and wheat field, tiny completely parched plants	39,1690	66,2038	475	Chiroqchi
TTGH 27	24-6-23	70	100	verge of road with grasses and shrubs, next to an unused field	39,1019	66,1130	459	Chiroqchi
TTGH 28	24-6-23	100	400	Herbaceous border next to a cereal field with grasses and thistles	39,1873	66,4764	582	Chiroqchi
TTGH 29	27-6-23	2	2	Herbaceous border next to a cereal field with grasses, thistles and caper	41,0808	69,5667	577	Ohangaron

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The mission of Wageningen University & Research is "To explore the potential of nature to improve the quality of life". Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 7,600 employees (6,700 fte) and 13,100 students and over 150,000 participants to WUR's Life Long Learning, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.

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