Survey of Beta nana (Boiss. & Heldr.) in Greece

Lothar Frese¹, Richard Hannan², Barbara Hellier², Stelios Samaras³ and Lee Panella⁴ ¹Federal Centre for Breeding Research on Cultivated Plants (BAZ)¹¹, Braunschweig, Germany ²United States Department of Agriculture-Agricultural Research Service (USDA-ARS) National Plant Germplasm System (NPGS) Western Regional Plant Introduction Station (WRPIS), Pullman, USA

³Greek Gene Bank, Thessaloniki, Greece

⁴ United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Sugarbeet Research Unit, Crops Research Laboratory, Fort Collins, CO, USA

Introduction

The genus Beta is made up of the sections Beta, Corollinae, Nanae, and Procumbentes. Beta nana Boiss. & Heldr. constitutes a monotypic section within the genus, and is a relict species endemic to Greece that has a very limited distribution area (Buttler 1977; Letschert 1993). It occurs in the mountains at high altitudes, in limestone substrates and on short open turf at the edges of meadows with late snow. It is the only alpine species of the genus distributed in Greece (Strid 1995) but not the only alpine Beta species (Buttler 1977). Species of section Beta exist at lower elevations in Greece. The general habitat for B. nana is in closed or open depressions with relatively moist soil above 1800 m elevation. The climate at such an altitude is cool and moist because clouds often build up around the mountains. The plant populations are mainly found on ranges facing east or northeast, where temperatures are lower during the summer afternoons. Plants also grow in crevices between rocks and in disturbed areas, such as rough tracks or severely grazed open plant communities. The prostrate growth habit protects the head of the storage root from being damaged by grazing animals (Dale 1980, 1981). It is possible that a certain degree of grazing, however, may keep the associated flora short, thereby promoting the survival of the species. Strid (1995) described the species in detail. It is an inconspicuous, diploid (Franzen and Gustavsson 1983) plant species, with a small rosette of leaves approximately 10-20 cm in diameter, depending on the fertility of the soil. The plant is said to be self-fertile, producing few seed stalks with 10-25 flowers per spike between June and August. The monogerm seedballs dehisce to the ground in the vicinity of the seed plant while still green. Dale (1980) noted that germination of the seed has proved difficult, and assumed that the extremes of temperatures in the natural habitat, leaching of inhibitors, as well as enzymes in the gut of animals may all play a part in successful germination. He further argued that the species might not be easy to cultivate outside its very specific natural habitat. In contrast, Strid (1995) reported that the species is easy to cultivate from seed, and that plants have survived in the Copenhagen Botanic Garden for almost 15 years, where they have regenerated spontaneously.

¹¹ The German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) merged the former Federal Centre for Breeding Research on Cultivated Plants (BAZ), the Federal Biological Research Centre for Agriculture and Forestry (BBA), and parts of the Federal Agricultural Research Centre (FAL). Since January 2008 the new institution is called Federal Research Centre for Cultivated Plants – Julius Kühn-Institut.

Little is known about the patterns of genetic variation in *B. nana*. Phenotypic variation observed by Dale (1980) in the natural habitat was low within and between populations. Allozyme patterns of *B. nana* have been analysed by Nagamine and Ford-Lloyd (1989) who found five unique and invariant alleles compared to a range of other species investigated in the same study, indicating a unique phylogenetic position of *B. nana* within the genus. Shen and co-workers (1998a, 1998b) and earlier studies (Jung et al. 1993) showed that *B. nana* is more closely related to the section *Corollinae* than to any other section, although it was a clearly separated group when using random amplified polymorphic DNA (RAPD) banding patterns. Like *B. nana*, *Corollinae* species occur at high altitudes and are part of the secondary genepool in relation to cultivated beet (Buttler 1977). *Beta nana* is of interest to the beet breeding community because of its novel genetic variation, potential cold tolerance and monogermicity.

B. nana is considered a rare but not threatened species requiring no specific conservation measures. Some of the populations are growing in protected areas (Strid 1995). However, species living in alpine regions are particularly prone to extinction risk from climate change (Grabherr et al. 1994; McCarty 2001; Parmesan and Yohe 2003). B. nana is a highly specialized species of the alpine regions of the Greek mountains. Reproduction and migration mechanisms of the species have not been investigated, nor are demographic processes understood; thus the impact of climate change on the ecological niche of the species and its long-term population viability cannot be predicted. Moreover, it is not understood how the genetic variation is distributed within a population, among populations within a region, or among regions; and to what extent gene flow between populations within a region and among regions occurs. Short distance gene flow by seed dispersal (a few hundred metres) is facilitated, perhaps, by melt water flows, grazing animals, and birds. Because a few plants were found during the exploration in 2005 growing in cracks of steep cliffs, it is hypothesized that birds may play a role in long-distance dispersal by depositing undigested seeds.

In 1980 the International Board for Plant Genetic Resources (IBPGR, now Bioversity International) charged M.F.G. Dale with a survey of *B. nana*. The major finding of his missions (summarized in Table 1) indicated genetic erosion within the species caused by overgrazing and road construction. The destruction of several populations of a species does not necessarily endanger the species as a whole. However, because plant breeders are especially interested in the maintenance of the within-species variation, the ECPGR Working Group on *Beta* recommended repeating the survey and assessing the need for *in situ* conservation actions (Maggioni et al. 2000).

Therefore, there are good reasons to monitor the demographic development of this wild crop relative and to acquire a better understanding of the biology, ecology and landscape genetics of the species. The *B. nana* exploration in 2005 was organized to provide a baseline to support future research to address these questions. Specific objectives of the joint plant exploration were:

- collection of fresh seeds from a few, larger populations for use in research;
- joint development of an in situ management programme with Greek collaborators;
- development of a database tool suited to document monitoring data; and
- determination of the environmental characteristics (soil, microclimate, associated vegetation) of the natural growing sites to design more efficient *ex situ* seed multiplication procedures.

Mountain surveyed	
Taigetos (1980, 1981)	1980: One population, extremely limited distribution, site intensively grazed by sheep and goats. 1981: Two quite large and other smaller populations were found.
Chelmos (1980, 1981)	No sign of <i>B. nana</i> . Vegetation heavily grazed by sheep and goats.
Parnassos (1980, 1981)	1980. <i>B. nana</i> widely distributed in this part of the mountain range. Intensively grazed by sheep and goats. Insect damage observed. Road construction may have caused drying out of previously suitable habitats. 1981: Disturbance caused by Athens Ski Club. Approximately 30-40% of the original suitable habitats/populations had been destroyed in 1981.
Giona Oros (1980, 1981)	1980: A number of populations observed. Man's activities are certain to have an effect on the populations of <i>B. nana.</i> 1981: Limited impact of bauxite mining on some populations.
Timfristos (1980, 1981)	No sign of <i>B. nana</i> . Natural habitat may not be suited for this species.
Lakmos (1980, 1981)	No sign of <i>B. nana</i> though potentially suitable sites were observed.
Smolikas (1980)	No sign of B. nana. Local shepherds recognized samples of the species.
Olympos (1980, 1981)	1980: Small populations were found throughout this area. The region was grazed by sheep and goats. 1981: Many populations were showing signs of drought stress.
Kato Vermiou (1981)	No sign of <i>B. nana</i> . Few suitable habitats.
Vitsi (1981)	No suitable habitats.
Tymphe (1981)	No sign of <i>B. nana.</i> Few areas retaining moisture.
Kaliakouda (1981)	No sign of B. nana. Little retention of surface water.
lti (1981)	No sign of <i>B. nana</i> . Suitable habitats were observed.
Vardousia (1981)	Populations were found in open depressions. Grazing by sheep and goats evident.

 Table 1. Results of a B. nana survey in Greece conducted by Dale (1980, 1981)

Materials and methods

The itinerary of the recent survey was based on Dale's reports and an extensive literature search. Unfortunately, Dale did not describe the exact collection sites nor did he estimate the geographical coordinates based on topographic maps. Populations were found using Dale's general site descriptions, current topographic maps, and local experts. Once found, the location of a population was determined using a Garmin 12XL global positioning system (GPS) (Garmin International, Inc., Olathe, Kansas, USA), and further site and plant characters were determined as shown in Table 2. Leaf tissue and plant sap samples were also collected from 12 to 24 plants from selected populations for future DNA extraction. The sap samples were collected using Clone Saver[™] cards (Whatman International, Middlesex, UK).

A data model for recording monitoring data was tested during the trip and is described by Frese and Germeier (see paper on "The International Database for *Beta* and *in situ* management – potential, role and functions", this volume, pp. 59-74). Using a model proposed by Guarino (1995), the threat of genetic erosion was assessed for each site by using proxy data. Proxy data are recorded to study a phenomenon for which a direct measurement in a given situation is not possible. The model uses scores for factors such as the extent of wild habitat of the target species within the study area, the ratio of present livestock density to estimated carrying capacity of a site, or the distance of the site to development projects such as tourism complexes or mining sites.

2005
August
collected
data (
associated
and
nana accessions and associated
nana
Beta
Ň
ble 2.

collected		No. of	No. of	No. of	Site data								
	number	seed collected	plants collected	plants found	Site size (m²)		GPS Latitude		GPS Longitude	Elevation (m asl)	Aspect	Physical description	Locality
08/23/05	GR05-009	14	ъ	20	30	r z	36.95351	ш	22.35788	1873	East	Shallow gully on upper slope of a large ridge.	Mt. Taygetos
08/23/05	GR05-012	78 (*)	S	38	30	en Z	36.95905	ш	22.35826	1870	East	Medium sized rocky drainage on upper slope of major ridge.	Mt. Taygetos
08/24/05	GR05-023	109 (*)	6	>70	100	۳ z	37.97605	ш	22.19042	2077	West	Mid-slope on large ridge.	Mt. Chelmos
08/24/05	NO SEED Collected				-	en Z	37.97138	ш	22.19068	2174	West		Mt. Chelmos
08/26/05	GR05-024	80 (*)			-	en Z	38.68164	ш	22.13107	1938	West	Bench on mid-slope on large ridge. Site has large to small scattered rocks.	Mt. Vardousia
08/26/05	GR05-025	395 (*)	65	>300	1500 1	en Z	38.68099	ш	22.13164	1927	West	Bench on mid-slope on large ridge. Site has large to small scattered rocks.	Mt. Vardousia
08/26/05	NO SEED Collected		0	-	10	en Z	38.68272	ш	22.12892	1920	South	Eroded road cut.	Mt. Vardousia
08/26/05	GR05-026	51 (*)			÷	۳ z	38.68276	ш	22.13358	2080			Mt. Vardousia
08/26/05	NO SEED Collected				-	e Z	38.68432	ш	22.11334	2081			Mt. Vardousia
08/26/05	NO SEED Collected				-	en Z	38.68419	ш	22.13177	2000			Mt. Vardousia
08/26/05	NO SEED Collected				-	en Z	38.68486	ш	22.13065	1980			Mt. Vardousia
08/27/05	GR05-037	216 (*)	20	>300	400	e Z	38.64036	ш	22.28575	1917	North- northeast	Shallow vale (3000m ²) surrounded by rocky, limestone ridges. At or close to to to of range.	Mt. Giona
08/27/05	GR05-039	21	12	>200	100	en Z	38.63937	ш	22.28613	1912	West	Small shettered area surrounded by high rock walls.	Mt. Giona
08/28/05	GR05-040	276 (*)	25	>100	500	en Z	38.58549	ш	22.24103	1881	Northeast	Rocks over red sandy loam. Small (50x100m) bowl at top of high ridge.	Mt. Giona

48

2005
ted August
llec
l data co
associated
and
Beta nana accessions and associated
nana
Beta n
ole 2. (cont.) /
ň
ole

Date .		NO. OF	NO. OT	No. of	Site data								
collected	number	seed collected	plants collected	plants found	Site size (m²)		GPS Latitude		GPS Longitude	Elevation (m asl)	Aspect	Physical description	Locality
08/28/05	GR05-041	28	4	75	1200	z	38.59762	ш	22.23894	1937	Northwest	Rocks over red sandy loam. Small (50x100m) bowl at top of high ridge.	Mt. Giona
08/28/05	GR05-042	126 (*)	18	06<	200	z	38.59763	ш	22.23896	1950	West	Upper slope of high ridge.	Mt. Giona
08/28/05	GR05-043	338 (*)	25	>300	3000	z	38.61639	ш	22.22629	2095	South	Very rocky over red sandy loam. Not much soil. Large open bow//valley on top of ridge.	Mt. Giona
08/29/05	GR05-044	355 (*)	25	75	10000	z	38.54584	ш	22.59277	1928	North	Large bowl mid-slope on large ridge.	Mt. Parnassos
08/29/05	GR05-045	-		34	6000	z	38.53913	ш	22.61411	2052	AII	Flat area surrounded by steep rocky slopes	Mt. Parnassos
08/29/05	GR05-046	127 (*)	4	32	200	z	38.54222	ш	22.60944	2058	Southeast	Small gully. Very rocky.	Mt. Parnassos
08/29/05	GR05-047	290 (*)	69	143	3000	z	38.53795	ш	22.61266	2061	AII	Small (3000 m ²) flat area in a rocky bowl. Like a sink hole.	Mt. Parnassos
08/31/05	GR05-053	385 (*)	approx. 50	250	400	z	40.07534	ш	22.3592	2492	AII	Large, deep, open bowl on upper- slopes of mountain.	Mt. Olympus
08/31/05	GR05-054	222 (*)	>100	>1000	1200	z	40.07799	ш	22.35632	2639	AII	Small open gully off main ridge.	Mt. Olympus
08/31/05	GR05-055	103 (*)	100	>1000	75	z	40.07762	ш	22.35483	2675	East- northeast		Mt. Olympus
08/31/05	NO SEED Collected					z	40.07222	ш	22.35216	2626	East- northeast	Valley/saddle between two peaks. Plants found in lowest areas.	Mt. Olympus
08/31/05	GR05-056	82 (*)	100	006<	1600	z	40.07004	ш	22.35731	2558	East	Wide swale between rocky hill tops.	Mt. Olympus

Results

Populations of *B. nana* were found in these mountains: Taygetos, Chelmos, Vardoussia, Giona, Parnassos and Olympos (Fig. 1). In contrast to Dale's reports (1980, 1981), the extent of the occurrence of the species was confirmed to be as reported in 1908 by Halacsy (cited by Akeroyd, 1986). Except for a very sheltered site at Mount Parnassos, all sites proved to be grazed to various degrees, primarily by goats. Estimates for the risk of genetic erosion were fluctuating around 100 on a scale of 0 (=no risk) to 200 (=very high risk). Especially on Mount Olympos, the species had reproduced well at several sites within the surveyed area. The overall population sizes ranged from more than 1000 individuals on Mount Olympos to a few individuals on Mount Taygetos. All sites had full exposure to the sun, and the slopes ranged from 0 to 25 degrees.

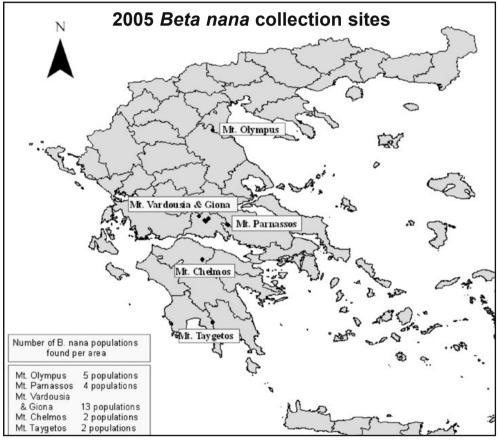


Fig. 1. 2005 Beta nana survey sites and population locations.

Maxted et al. (1997) suggested a new conservation technique called "genetic reserve conservation" to complement *ex situ* conservation efforts. Conservation of a wild species in its natural habitat requires the designation of an exact location for a genetic reserve, the development and implementation of a site management plan, the

engagement and funding of a reserve manager, and the monitoring of the location to assess the impact of the site management on the target species. The distribution area on Mount Olympos would be most suited for the establishment of a genetic reserve because the sites are already located within or close to the Nature Park. Two further sites, one on Mount Parnassos and a second on Mount Giona, would also be possible choices. Fig. 2 shows the Vathia Lakka site located on Mount Giona. In the background of the left part of the photo, rocks formed a U-like structure, which could easily be fenced by a shepherd and grazed by sheep or goats in a controlled manner favouring reproduction of the species.



Fig. 2. Vathia Lakka, Mount Giona. The site on the left part of the photo (arrow) would be suited to establish a genetic reserve.

Conclusions

Although populations of *B. nana* can be found on the mountains identified in this study, there still remain significant areas in Greece to survey to confirm the full extent of the range for this species. It is noted that sites which have extant populations of *B. nana* on Mount Olympos, Mount Parnassos and other mountains are located within or nearby protected areas. This is significant in that it may be that preservation of habitat is beneficial, but it is not known how effectively *B. nana* populations are protected by National or Provincial Nature Park statutes. This relationship needs further study. The extent of overlap between legally protected areas and natural sites of *B. nana* needs to be explored by integrating geographic information systems (GIS) and floristic studies to determine the scope of the relationship between conservation measures and plant species survival. One approach would be to match the distribution of the *B. nana* populations to existing protected areas, and then systematically determine the obvious overlaps. Researchers could then suggest locations for the development of genetic reserve conservation sites.

The choice to be made, of which populations and sites should be protected in genetic reserve conservation sites, needs to be based on genetic distance measures and/or the amount of genetic variation detected within and among populations. For this work, which is still to be done, it is proposed to use microsatellite markers. The question of how effectively agro-environmental measures subsidized by the EU Commission can be deployed for the controlled management of selected sites also needs to be investigated.

Acknowledgements

The plant exploration was permitted by Mr A. Chatziathanssiou, Ministry of Agriculture Development and Food, General Directorate of Agricultural Extension and Research, Directorate of Natural Planning and Environmental Protection, Athens, Greece. This exploration was funded by the USDA-ARS National Plant Germplasm System and supported by the German Ministry of Consumer Protection, Food and Agriculture (BMVEL).

References

- Akeroyd JR. 1986. 1. Beta L. In: Strid A, editor. Mountain Flora of Greece, Volume 1. Cambridge University Press, Cambridge, UK. pp. 78-79.
- Buttler KP. 1977. Revision von Beta Sektion Corollinae (Chenopodiaceae). I. Selbststerile Basisarten. [Revision of Beta Section Corollinae (Chenopodiaceae). I. Self-sterile base species]. Mitteilungen aus der Botanischer Staatssamlung München 13:255-336. (in German).
- Dale MFG. 1980. Report on 1980. Survey on *B. nana* in Greece. Copy available at the Greek Gene Bank, Thessaloniki.
- Dale MFG. 1981. Report on 1981. Collection of *B. nana* in Greece (Supplement to 1980 report). Copy available at the Greek Gene Bank, Thessaloniki.
- Franzén R, Gustavsson L.-Å. 1983. Chromosome numbers in flowering plants from the high mountains of Sterea Ellas, Greece. Willdenowia 13:101-106.
- Grabherr G, Gottfried M, Pauli H. 1994. Climate effects on mountain plants. Nature 369:448.
- Guarino L. 1995. Assessing the threat of genetic erosion. In: Guarino L, Ramanatha Rao V, Reid R, editors. Collecting Plant Genetic Diversity. Technical Guidelines. CAB International, Wallingford, UK. pp. 67-74.
- Jung C, Pillen K, Frese L, F\u00e4hr S, Melchinger AE. 1993. Phylogenetic relationships between cultivated and wild species of the genus *Beta* revealed by DNA "fingerprinting". Theoretical and Applied Genetics 86:449-457.
- Letschert JPW. 1993. *Beta* section *Beta*: biogeographical patterns of variation and taxonomy. PhD Thesis. Wageningen Agricultural University Papers 93-1.
- Maggioni L, Frese L, Germeier C, Lipman E, compilers. 2000. Report of a Working Group on *Beta*. First meeting, 9-10 September 1999, Broom's Barn, Higham, Bury St. Edmunds, UK. International Plant Genetic Resources Institute, Rome, Italy.
- Maxted N, Ford-Lloyd BV, Hawkes JG. 1997. Complementary conservation strategies. In: Maxted N, Ford-Lloyd BV, Hawkes JG, editors. Plant Genetic Conservation. The *in situ* Approach. Chapman and Hall, London, UK. pp. 15-39.
- McCarty JP. 2001. Ecological consequences of recent climate change. Conservation Biology 15(2):320-331.
- Nagamine T, Ford-Lloyd BV. 1989. New genetic markers in a wild species of beet (*Beta nana* Boiss. et Heldr.): prospects for utilisation. Plant Breeding 102(4):23-26.
- Parmesan C, Yohe G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37-42.
- Shen Y, Ford-Lloyd BV, Newbury HJ. 1998a. Genetic relationships within the genus *Beta* determined using both PCR-based marker and DNA sequencing techniques. Heredity 80:624-632.
- Shen Y, Newbury HJ, Ford-Lloyd BV. 1998b. Identification of taxa in the genus *Beta* using ITS1 sequence information. Plant Molecular Biology Reporter 16:147-155.
- Strid A. 1995. Beta nana Boiss. & Heldr. Chenopodiaceae. In: Phitos D, Strid A, Snogerup S, Greuter W, editors. The Red Data Book of Rare and Threatened Plants of Greece. World Wildlife Fund, Athens, Greece. p. 86.