Genetic diversity in angelica (*Angelica archangelica* L.) populations assessed by ISSR molecular markers

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Introduction

Angelica is a tall herbaceous plant belonging to the Apiaceae family. It is a monocarpic cross-pollinated species, however not self-incompatible (Ojala, 1986). Long distance seed dispersal is mostly by water and the seeds can remain buoyant for more than 300 days (van den Broek *et al.,* 2005). The distribution of angelica ranges from Greenland in the west, Iceland, the Faroe Islands, Fennoscandia over Russia into the eastern parts of Siberia. It grows as far south as Central Germany, the Altai and Lake Baikal, with some occurrence also in the Himalayas (Ojala, 1984). *A. archangelica* is divided into two subspecies: *A. archangelica* ssp. *archangelica* and *A. archangelica* ssp. *littoralis* (Wahlenb.) Thell. There is inconsistency in the proposed distribution of the subspecies (Mossberg & Stenberg, 2003; Jonsell *et al.*, 2009). Jonsell & Karlsson (2010) propose that all angelica in Iceland is *A. archangelica* ssp. *archangelica*. Mossberg & Stenberg (2003) have a divergent distribution map, where all Icelandic angelica is stated to be *A. archangelica* ssp. *littoralis,* whereas Hultén (1971) reports both subspecies as distributed in Iceland. Tyler *et al.* (2007) reports that the two subspecies inter-cross where distribution overlaps.

Angelica is one of the oldest cultivated plants of origin in the Nordic region. Written records of angelica date back to the Norwegian *Gulathing's* law from the 11th century where theft of angelica was penalised (Fosså, 2004). The first vegetable gardens in Norway were called leek or angelica gardens; later they were followed by kale gardens (Fægri, 1951). In western Norway around the region Voss in Hordaland, a variety of angelica, ´Vosskvann´, with solid petioles is still scarcely cultivated. It reportedly has higher sugar content than *A. archangelica* ssp. *archangelica* (Fægri, 1951). Its history of origin is not known, but it is likely a result of long time of selection from wild angelica populations; Fægri (1951) proposed to call the ecotype *A. archangelica* ssp. *archangelica* var. *maiorum*. Collection of wild-growing angelica is mentioned in the Icelandic *Fóstbræðra Saga* (Fosså, 2004) indicating that its uses were well known when it was written in the 13th century. Even though angelica, given its abundance and wide distribution in Iceland, is likely to have grown on Iceland before settlement, it is not unlikely that settlers brought plant material with them on their journeys from Norway to Iceland, the Faroe Islands and Greenland (Fosså, 2004). If this was the case, angelica persisting on historically important sites may have a different genetic background as compared with isolated Angelica populations from the interior of Iceland.

The aims of the current genetic diversity study are (1) to assess diversity within and between angelica populations of different origin, (2) study genetic relationships between the subspecies *A. archangelica* ssp. *archangelica* and *A. archangelica* ssp. *littoralis*,

especially with regard to status on subspecies level of Icelandic angelica and (3) compare the cultivated *A. archangelica* ssp. *archangelica* var. *maiorum* with wild-growing angelica populations.

Material and methods

Plant material

95 DNA samples from six angelica populations from the Nordic region were included in the current study (see table 1 for origin and details on the samples). Two Icelandic populations were included two represent a historical site and an isolated inland population respectively. A population of origin by the seashore in the southernmost Sweden were chosen to represent *A. archangelica* ssp. *littoralis*; however by the time of seed collection the plants were not in the state to be assessed for their phenotypic characters. In addition, *A. archangelica* ssp. *archangelica* was included from a population growing in northern Finland where it is the only subspecies distributed (Hultén, 1971; Mossberg & Stenberg, 2003; Jonsell & Karlsson, 2010). A population of Vosskvann from Elje in Norway, as well as samples from a collection of angelica plants in Apelsvoll, Norway were included. Samples from *A. sylvestris* collected in Iceland were used as out-group in part of the analysis. Tissue from young leaves of the Icelandic and Norwegian populations was collected *in situ* and subsequently freeze-dried. The Swedish and Finnish populations were grown from seeds and young leaves were sampled and freeze dried prior to DNA extraction.

Table 1. Angelica samples included in the molecular marker study.

Name	No. of samples	Sample	Origin	Species
Haukadalur[1]	20	Young leaf	Iceland	*A. archangelica*
Veiðivatn[2]	20	Young leaf	Iceland	*A. archangelica*
Elje	19	Young leaf	Norway	ssp. *archangelica* var. *maiorum*
Apelsvoll[3]	19	Young leaf	Norway	*A. archangelica*
Höllviken[4]	11	Seed	Sweden	ssp. *littoralis*
Pönttsö[5]	4	Seed	Finland	ssp. *archangelica*
A. Sylvestris[6]	2	Young leaf	Iceland	*A. sylvestris*

[1]From the historical settlement of Haukadalur; [2]A population from the highlands; [3]A collection of plants of varying Norwegian origin; [4]Seeds collected from a population of five plants; [5]Poor germination of seed resulted in four samples; [6]Used as outgroup in part of the analysis

Molecular analysis

Inter simple sequence repeat (ISSR) markers are unspecific dominant markers that do not need any previous genomic information from the studied taxon. The principle of ISSR markers is amplifying the DNA segment between two microsatellite (SSR) regions (Bornet & Branchard, 2001). The methods have been used with good results in a previous study of genetic diversity between populations of *Angelica lignescens* (Mendes *et* al., 2009).

DNA extraction and measurements of concentration was followed by dilution of the samples with to a concentration of 10 ng μl^{-1}. In all, 11 primers (Table 2) were tried on a

subset of samples in order to evaluate their efficiency to produce bands. Based on results from the trial, four primers were applied in the study. In the ISSR analysis 23 µl of master mix (2.5 µl PCR buffer, 0.5 µl dNTP, 0,75 µl primer 0.15 µl Taq and 19.1 µl H_2O) and 2 µl of DNA (20 ng) were added into each well of the PCR plate. The following PCR-program was run: 94˚ 3 min followed by touchdown 20 cycles (94˚ 30 sek, 55˚-53˚ 50 sec (-0.1˚ each cycle), 72˚ 50 sec) followed by 25 (primer 807, 836 and 841) or 18 (primer 880) cycles at 53˚C, ending with 72˚ 3 min. The amplified DNA fragments were separated by electrophoresis on CleanGel, using 10% polyacrylamide 52 well gels. The fragments were visualized by silver staining procedure.

Table 2. ISSR-primers tested in the study; the four selected primers are highlighted in bold.

Primers	Sequence 5' to 3'
807	**AGA GAG AGA GAG AGA GT**
812	GAG AGA GAG AGA GAG AA
836	**AGA GAG AGA GAG AGA GYA**
825	ACA CAC ACA CAC ACA CT
ISSR32	CCC GTG TGT GTG TGT GT
ISSR02	CAC ACA CAC ACA CAC
880	**GGA GAG GAG AGG AGA**
841	**GAG AGA GAG AGA GAG AYC**
834	AGA GAG AGA GAG AGA GYT
G11	GCT CTG GCG CAC CGA
G05	CAG AGG GGC ACC TGG

Y=C or T

Data analysis

All gels were scored manually for polymorphic bands. Where the bands had proved reproducible on multiple gels, they were scored as either present (1) or absent (0). The resulting binary matrix was analysed for molecular variance (AMOVA) and a principal coordinate analysis (PCoA) and pairwise population distance matrix (PhiPT) based on Eucledian distance were calculated with the software GenAlEx (Peakall & Smouse, 2006).

Results

Total number of polymorphic loci scored by the four ISSR markers was 51 and 46 of these were polymorphic among at least 5 % of the samples. AMOVA explained 53 % of the total diversity as variation between populations and 47 % among populations. Proportion of polymorphism among the scored loci (Table 3) which is a measure of the genetic variation within each population varied, with 73%, 61% and 55% in IS-Veidivatn, NO-Apelsvoll and IS-Haukadalur respectively. Voss-Elje had 31% of polymorphic loci.

Table 3. Proportion of polymorphic loci among six populations of angelica.

Population	Proportion of polymorphic loci
IS-Haukadalur	0.55
IS-Veiðivatn	0.73
Voss-Elje	0.31
NO-Apelsvoll	0.61
SE-Höllviken	0.29
FI-Pönttsö	0.37

The pairwise genetic differentiation (Table 4) was lowest among the two Icelandic and between NO-Apelsvoll and SE-Höllviken, respectively. Voss-Elje had the closest genetic distance to the two Icelandic populations.

Table 4. Pair wise population PhiPT (proportion of variance among populations relative to total variance) values (below the diagonal) with probability values based on 999 permutations (above the diagonal) based on Euclidean distance.

IS-Haukadalur	IS-Veiðivatn	Voss-Elje	NO-Apelsvoll	SE-Höllviken	FI-Pönttsö	
-	0.001	0.001	0.001	0.001	0.001	IS-Haukadalı
0.119	-	0.001	0.001	0.001	0.001	IS-Veiðivatn
0.459	0.383	-	0.001	0.001	0.001	Voss-Elje
0.399	0.334	0.559	-	0.001	0.001	NO-Apelsvo
0.591	0.501	0.715	0.299	-	0.001	SE-Höllvike
0.274	0.246	0.597	0.312	0.557	-	FI-Pönttsö

A PCoA grouped IS-Haukadalur and IS-Veidivatn together, with FI-Pönttsö slightly overlapping the Icelandic populations. NO-Apelsvoll and SE-Höllviken and Swedish populations slightly overlapping, and Voss-Elje was distinct from the rest (Figure 5).

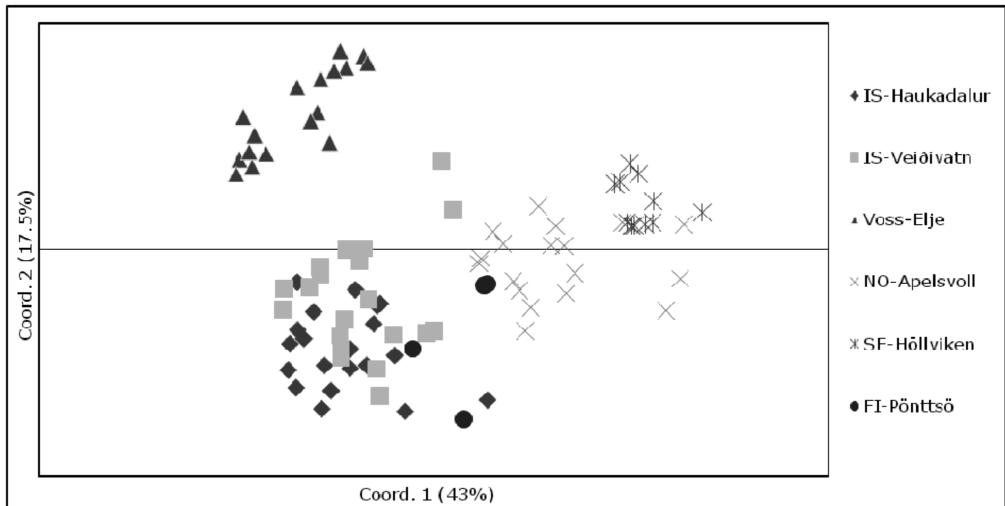

Figure 1. Principal coordinate analysis based on genetic distance among 93 samples of angelica from six populations.

Discussion

The variation in diversity within the populations could be explained by their different backgrounds. IS-Haukadalur and IS-Veidivatn were sampled from large natural populations and a relatively large intra-population variation was expected due to the out-crossing habit of the species. The NO-Apelsvoll population was a field collection of several different populations from a range of localities in Norway; hence the observed high proportion of polymorphic loci was expected. The NO-Apelsvoll was genetically closer to SE-Höllviken, which was assumed to be *A. archangelica* ssp. *littoralis*. This may indicate that hybridization on subspecies level occur where distribution area overlap, which has also been stated in the literature (Tyler *et al.*, 2007). Another explanation may be that the collected samples in Sweden were in fact *A. archangelica* ssp. *archangelica*. This needs verification by morphological characters.

Observed patterns of genetic diversity on a DNA level indicate that Icelandic angelica populations belong to *A. archangelica* ssp. *archangelica* as they were genetically most similar to the Finnish *A. archangelica* ssp. *archangelica*. This confirms the subdivision in Iceland as indicated by Jonsell & Karlsson (2010). However, both Icelandic populations were located off the coast, and the current study does not rule out that *A. archangelica* ssp. *littoralis* may coexist in Iceland along the shoreline, as proposed by Hultén (1971).

Observations in the study do not indicate genetic relatedness between IS-Haukadal from the Icelandic historical site with NO-Apelsvoll indicating that the settlers of Iceland likely utilised natural populations. Interestingly though, Voss-Elje had its closest genetic relatedness with the two Icelandic populations. Inappropriate samples of *A. archangelica* ssp. *archangelica* from Norway may explain this, where a sampling of a population growing nearby Voss might show a closer relatedness to Voss-Elje. Another theory may

be that plant material was in fact brought from Iceland to Norway to form the base for further selection resulting in ´Vosskvann´. The narrow genetic base and genetic distinctness of Voss-Elje implies that it is reproductively isolated from surrounding angelica populations.

All results in the current study are based solely on ISSR diversity. In order to validate the observations, careful observations of phenotypic diversity is needed. Plants from a number of the investigated populations are maintained and will be studied in detail during the coming year.

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