Chapter 9

# Genetic Variability of US and Czech *Phalaris Arundinacea* L. Wild and Cultivated Populations

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Additional information is available at the end of the chapter

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**Abstract**

The spread of invasive plant species in natural habitats has become a worldwide problem with negative impacts. *Phalaris arundinacea,* an important forage and ornamental crop, is widespread worldwide. In recent years there has been a massive spread of *P. arundinacea* across North America and Canada. Production of *Phalaris* seed as a forage crop occurs in northern Minnesota; seeds are sold throughout the world, particularly in central Europe. We tested genetic similarities and differences between populations in the US (Minnesota) and the Czech Republic using ISSRs to determine potential gene flow for this forage crop. The cultivated forage and wild genotypes were dispersed into two groups that overlapped. At least four sets of wild US genotypes are dissimilar to European counterparts and potentially native to N. America. Future work to prove the ancestry of each accession will be necessary. Nonetheless, the sale of forage cultivars related to or derived from European types causes genetic mixing with N. American types. Part of this intercontinental gene flow is exacerbated by the production of *Phalaris* forage seed in Minnesota. The implications of these findings for management of invasive crops native to both continents are significant for forage producers, managers, and breeders.

**Keywords:** reed canarygrass, invasive species, forage cultivars, ornamental cultivars, ISSRs

## 1. Introduction

*Phalaris arundinacea* L., reed canarygrass, is widespread throughout the world, except Antarctica and Greenland [1]. The center of diversity for this genus is in the Mediterranean area; *Phalaris* occur in moist habitats from lower to alpine altitudes. About 22 *Phalaris* species are found mainly in temperate zones of Europe, N. America and South Africa. Among the

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most important species of *Phalaris* are: *P. arundinacea*, *P. aquatica*, *P. canariensis*, *P. amethystina*, *P. angusta*, *P. brachystachys*, and *P. minor* [1].

*Phalaris arundinacea* is a wind-pollinated, wetland grass cultivated as a forage and ornamental crop in temperate regions, widely used for soil stabilization, remediation and, more recently, for bioenergy [2–6]. Soil and water restoration projects have also used *P. arundinacea* for phytoremediation [7–10]. Wastewater treatment facilities employ *P. arundinacea* for removal of N [11–13].

*Phalaris* is widely cultivated both for forage and for ornamental (gardening) purposes. While long cultivated for forage in the US [14] and Sweden [15], its domestication has been relatively recent. Of greater significance is the breeding and cultivation of reed canarygrass in the US and Europe. Existing forage and ornamental cultivars resulted from as few as 1–2 selections and sexual recombination cycles removed from wild types [16], such as 'Chrastava', domesticated in the Czech Republic [17]. 'Chrastava' was genetically similar to wild Czech populations while all other ornamental cultivars differed [18]. High levels of seed dormancy, seed shattering, and low yield potential exist in most populations, e.g. Norwegian [19] but not French [20]. Thus, early forage production trials involved clonal transplants (rhizomes) in Connecticut (in 1834) and New Hampshire (in 1835) [21, 22]. Current seed production within the US occurs in Roseau, Minnesota, which is surrounded by wet meadows.

In Europe, the standard forage cultivar is 'Palaton' (from the US), while other important ones include: 'Luba son. Motycka' (Poland); 'Motterwizer' (Denmark); 'Peti', 'Szarvasi 50', 'Szarvasi 60', 'Keszthelyi 52' (Hungary); 'Lara' (Norway); 'Vantage', 'Venture' (US); 'Bellevue', 'Rival' (Canada); 'Chrastava' (Czech Republic) [23]. Current breeding is focused on improving of its yield potential as a fodder crop as well as for wastewater treatment plants and, more recently, biomass production. *Phalaris* is often used in gardening and ornamental horticulture [18]. It is cultivated mainly as decorative plants with longitudinal white or yellow variegated cultivars from the group *Phalaris arundinacea* var. *picta* and *luteopicta* [24, 25].

*Phalaris arundinacea* has high dry matter yield (8–12 t $ha^{-1}$) for forage as well as drought and flood tolerance when compared to timothy (*Phleum pratense*) and tall fescue (*Bromus inermis*) [26, 27]. Three forage cultivars ('Palaton', 'Vantage', 'Venture') responded to selection for establishment capacity with annual weeds [16]. Invasive genotypes possess wide genotype × environment (G×E) interactions across environments for emergence, tiller production, leaf number and biomass, indicating a lack of stability and wide genetic variation [28–30]. Recent molecular studies have shown that central European (Czech) wild populations were genetically similar to the forage 'Chrastava' while differing significantly from ornamental cultivars [18]. In contrast, within MN populations, forage/ornamentals were genetically similar to wild types [31].

Despite unverified assertions that "reed canarygrass is native to the northern half of the United States…" and "native to the temperate portions of Europe, Asia, and North America" [32], invasion biologists and ecologists have consistently postulated that *P. arundinacea* was native to Eurasia but introduced in N. America [33]. Untested hypotheses for *P. arundinacea* invasion in N. America [2, 34] encompass introduction of cultivated types from Eurasia [35], hybridization of Eurasian and N. American populations [28], and/or release of competitive hybrids from breeding programs [36]. However, native N. American *P. arundinacea* populations have

been discovered in Ontario, Canada [35] and remote areas elsewhere [37]; herbarium specimens collected in 1825 resembled diploid *P. arundinacea* subsp. *rotgesii* [36]. Recent molecular genetic analyses of herbarium specimens have confirmed the existence of native N. American populations across the continent [38–40]. Nelson et al. [30] determined that the population genetic structure of wild, forage, and ornamental exotic and N. American *Phalaris* harbored a high amount of genetic diversity within, as opposed to among, populations. Thus, range expansion of *P. arundinacea* in N. America is not a result of hybridization among exotic, forage, and native genotypes [38] despite previous theories [28].

Original and introduced *P. arundinacea* populations coexisted in North America for more than a hundred years. We presume that there has been a myriad of migration and intraspecific crossing of this species. It is assumed that the European species and their hybrids are more aggressive [21, 41]. Casler et al. [42] investigated the genetic differences between European and North American genotypes. They found that, on the basis of nuclear DNA, genotypes can be divided into two distinct groups: group one consisted of three closely related genotypes from North America and a group two consisting from other assessed genotypes. Genotypes of first group from Oregon ('Superior'), Alabama ('Auburn') and Arkansas ('AR Upland') could be the sources of the original North American gene pool [42]. These genotypes significantly differed from all European genotypes and it supports the suggestion of their different origins. Casler et al. [42] found ample support for the action of the founder effect resulting from the migration of *Phalaris* from Europe or Asia in recent interglacial periods. These genotypes are, therefore, considered as originating in North America. The founding population in North America, therefore, probably has undergone many mutations that led to the creation genotypes different from Europe. These mutations had little effect on plant morphology and fitness-plant phenotypes remains completely unchanged. As a result, their lower genetic variability results in a bottleneck effect [42].

Previous work by our labs [18, 31] analyzed phenotypic and genotypic markers in genotypes obtained from wild populations growing along the six main rivers within the Czech Republic (Berounka, Dyje, Labe, Lužnice, Orlice, Vltava) and commercial cultivars (forage, ornamental types) grown in the Czech Republic to serve as a foundation for Central European reed canarygrass diversity. ISSRs or inter-simple sequence repeats, for the first time ever, showed distinct genetic differences between ornamental cultivars and wild *P. arundinacea* [18]. Interestingly, the Czech forage and biomass cultivar, 'Chrastava', could not be differentiated from the same wild populations. Most of the genetic diversity was within, rather than among, wild Czech populations [18].

The objective of the present study was to extend the focus on assessment of genetic structure to wild *Phalaris* populations collected in Minnesota (US) along the major rivers and wet meadows or fields with a larger sampling of comparative N. American forage cultivars. Since *Phalaris* seed for forage is commercially produced in Roseau, Minnesota for sale worldwide, sampling in and around production fields in Roseau is part of this study. First, the Minnesota genotypes along with forage comparisons from throughout North America were assessed for genotypic and population differences using ISSRs. Second we analyzed both the Minnesota and Czech [18] genotypic data together to compare differences among continents for genetic structural similarities and differences.

## 2. Materials and methods

### 2.1. Genotypes

A total of 16 wild *P. arundinacea* populations were collected in 2012 along the six major rivers in the State of Minnesota, U.S.A. (Des Moines, Minnesota, Mississippi, Red, Roseau, St. Croix) as well as wet meadows or cultivated fields (**Table 1**). The Des Moines, Minnesota, and St. Croix rivers empty southward into the Mississippi river, flowing to the Gulf of Mexico whereas the Roseau and Red rivers flow north into Manitoba, Canada, emptying into Lake Winnipeg. The headwaters for both the Mississippi and Red rivers watersheds originate in Minnesota. Collection protocols for wild *Phalaris* populations followed the same methodology used by Anderson et al. [18] for the Czech populations, with multiple collection sites along each river for a maximum of five genotypes/population (**Table 1**). Seeds of 13 forage cultivars bred, produced and/or grown across North America (**Table 1**) were obtained from the U.S. Department of Agriculture's Germplasm Resources Information Network or USDA-GRIN (http://www.ars-grin.gov/npgs/), germinated and grown to the juvenile stage for harvesting mature leaves. One to five genotypes were analyzed for each accession.

We included data from our previous paper [18] and that of Kávová's M.S. thesis [31] for comparative purposes, namely 110 European genotypes from Czech wild populations (1 site/river; 1–9 genotypes/collection site/river) collected in 2011 along the six main rivers of the

| Population or forage cultivar codes | River/wet meadow name and location or forage cultivar name and germplasm source | GPS[1] coordinates for site of collection (wild populations) or germplasm bank identifier number; [citations] |
|---|---|---|
| 2.1.2 | St. Croix River, South of Bayport, MN; by the Bayport Marina | Lat.[2] 45°0′32.8710″ N<br>Long.[2] −92°46′40.4286″ W |
| 6.1.3 | St. Croix River, St. Croix State Park; along the river by boat launch and swimming areas | Lat. 45°57.012′ N<br>Long. −92°34.044′ W |
| 8.I.A.1; 8.I.C.3; 8.I.G.3; 8.II.A.2; 8.II.F.3 | Wet Meadow, Chanhassen, MN; Horticulture Research Center's "Rice Paddy" wetlands | Lat. 44°51′43.3296″ N<br>Long. −93°35′59.4126″ W |
| 9.3.1 | Mississippi River, Reno, MN; along the dead arms, S from the dam of the "big" lake | Lat. 43°36.128′ N<br>Long. 91°16.151′ W |
| 14.2.1 | Mississippi River, Red Wing, MN; along the river banks in a wooded area | Lat. 44°35′03.9444″ N<br>Long. 92°38′39.6918″ W |
| 21.5.1 | Mississippi River, between Little Falls and Rice, MN; in open areas between wooded banks | Lat. 45°49.597′ N<br>Long. 94°21.262′ W |
| 34.3.1 | Mississippi River, near the headwaters; W of Bear Den Landing, Mississippi Headwaters State Forest | Lat. 47°26.012′ N<br>Long. 95°07.748′ W |
| 38.1.B.3 | Minnesota River, Blakeley, MN; W of Belle Plaine. MN; in open wet meadows | Lat. 44°36′47.1708″ N<br>Long. 93°51′32.8320″ W |
| 38.2.3 | Minnesota River, Blakeley, MN; W of Belle Plaine. MN; in open wet meadows | Lat. 44°36′43.7214″ N<br>Long. 93°51′35.2620″ W |

| Population or forage cultivar codes | River/wet meadow name and location or forage cultivar name and germplasm source | GPS[1] coordinates for site of collection (wild populations) or germplasm bank identifier number; [citations] |
|---|---|---|
| 46.1.1 | Minnesota River, SE of Montevideo, MN at the confluence of Highways 212/15 | Lat. N 44°54′09.8″ N<br>Long. 95°41′07.9″ W |
| 50.1.1 | Des Moines River, S of Petersburg, MN at the border with the State of Iowa | Lat. 43°31′33.2″ N<br>Long. 94°55′07.4″ W |
| 54.3.2 | Des Moines River, SW of Dovray, MN; adjacent to Highway 8 | Lat. 44°00′09.1″ N<br>Long. 95°35′00.3″ W |
| 56.2.2 | Roseau River, in the Red Lake State Wildlife Mgt. Area, W of Mulligan Lake, adjacent to the Red Lake Indian Reservation; Co. Rd. 704, at headwaters (source) of the river | Lat. 48°32.774′ N<br>Long. 95°19.204′ W |
| 58.1.3 | Roseau River, N of Roseau, MN; wet meadows near Hwy. 3 | Lat. 48°54.504′ N<br>Long. 95°49.778′ W |
| 58.2.2 | Roseau River, N of Roseau, MN; wet meadows near Hwy. 3 | Lat. 48°54.546′ N<br>Long. 95°49.711′ W |
| 58.3.1 | Roseau River, N of Roseau, MN; wet meadows near Hwy. 3 | Lat. 48°54.562′ N<br>Long. 95°49.635′ W |
| 58.IV.A.1 | Roseau River, N of Roseau, MN; wet meadows near Hwy. 3; transect in cultivated field | Lat. 48°54.699′ N<br>Long. 95°52.130′ W |
| 58.IV.H.3 | Roseau River, N of Roseau, MN; wet meadows near Hwy. 3; transect in cultivated field | Lat. 48°54.753′ N<br>Long. 95°52.084′ W |
| 61.1.2 | Roseau River, Caribou, MN; Hwy. 4 near confluence with State Ditch; S of the Canadian Border | Lat. 48°59.006′ N<br>Long. 96°26.951′ W |
| 63.4.3 | Red River, S of McCauleville, MN and SW of Kent, MN | Lat. 46°26′43.0″ N<br>Long. 96°42′57.9″ W |
| 74.1.2 | Red River, Oslo, MN; S of Big Woods, County Ditch 38 | Lat. 48°18′40.3″ N<br>Long. 97°07′24.4″ W |
| VEN | 'Venture' (Minnesota); derived from crossing 'Vantage' × 'Flare'); low alkaloid variety; does not contain any tryptamine-carboline alkaloids; USDA-GRIN; https://npgsweb.ars-grin.gov | PI[3] 531089 [14, 42] |
| PAL | 'Palaton' (Minnesota); derived from 'Flare', 'Vantage' and 'Rise'); low alkaloid variety; does not contain any tryptamine-carboline alkaloids; USDA-GRIN; https://npgsweb.ars-grin.gov | PI 531088 [14] |
| AUB | 'Auburn' (Alabama); landrace, most likely derived from native N. American germplasm; USDA-GRIN; https://npgsweb.ars-grin.gov | PI 422031 [42] |
| IOR | 'Ioreed' (Iowa); high levels of alkaloids; USDA-GRIN; https://npgsweb.ars-grin.gov | PI 422030 [42] |
| 365 | 367 (British Columbia, Canada); USDA-GRIN; https://npgsweb.ars-grin.gov | PI 387929 |

| Population or forage cultivar codes | River/wet meadow name and location or forage cultivar name and germplasm source | GPS[1] coordinates for site of collection (wild populations) or germplasm bank identifier number; [citations] |
|---|---|---|
| PHA | *Phalaris arundinacea*; USDA-GRIN; https://npgsweb.ars-grin.gov | PI 241065 |
| PN-609 | Unknown origin; USDA-GRIN; https://npgsweb.ars-grin.gov | PI 371754 |
| GRO | 'Grove' (Ontario, Canada); USDA-GRIN; https://npgsweb.ars-grin.gov | PI 357645 [42] |
| MN-76 | MN-76 (Minnesota) 4-clone double cross hybrid; low alkaloid variety; does not contain any tryptamine-carboline alkaloids; USDA-GRIN; https://npgsweb.ars-grin.gov | PI 578797 [42] |
| CANA | 'Cana' (California); USDA-GRIN; https://npgsweb.ars-grin.gov | PI 578795 |
| VAN | 'Vantage' (Iowa); high alkaloid content; USDA-GRIN; https://npgsweb.ars-grin.gov | PI 578794 [14, 42] |
| MCRC1 | NCRC-1 (Minnesota); USDA-GRIN; https://npgsweb.ars-grin.gov | PI 578793 |
| SUP | 'Superior' (Oregon); most likely derived from native N. American germplasm; USDA-GRIN; https://npgsweb.ars-grin.gov | PI 578792 [14, 42] |

[1]GPS, global positioning system.

[2]Lat., latitude; Long., longitude.

[3]PI, plant introduction; USDA-GRIN, U.S. Dept. of Agriculture, Germplasm Resources Information Network.

**Table 1.** Minnesota (U.S.A.) population or North American forage cultivar codes, river/wet meadow name and location or forage cultivar name and germplasm source; GPS coordinates for site of collection (wild populations) or germplasm bank identifier number for *Phalaris arundinacea* wild populations collected in the State of Minnesota (MN; U.S.A.) along rivers and in wet meadows.

Czech Republic (Berounka, Dyje, Labe, Lužnice, Orlice, and Vltava). Similar to the Minnesota wild populations, five of the Czech rivers empty into the North Sea basin while the Dyje River flow into the Black Sea basin [18]. Additional wild population samples were made at the OSEVA PRO, Ltd., Grassland Research Station (Rožnov-Zubří, CZ); commercial forage, ornamental cultivars either bred and/or grown in the Czech Republic were also included. These all were grown and previously analyzed in our previous study [18] and ISSR molecular data from these were used herein to compare with the results found with the Minnesota and N. American types. Genotypic codes for all Czech germplasm consisted of the following: BE-1, 2, 3 (Berounka); DY1, 2, 3 (Dyje); LA1 (Labe); LU1, 2, 3 (Lužnice); OR1, 3 (Orlice); VL1, 3 (Vltava); CHR ('Chrastava'; forage cultivar); Z13, Z77, Z83, Z124, Z125 (OSEVA PRO, Ltd, Grassland Research Station, Rožnov-Zubří, CZ); ZP/COV1, 17 (Gardening Pelikán, Spálené Poříčí), AT/P6, 7 ('Picta'), AT/T2, 6 ('Tricolor'), F/L1, 4 ('Luteopicta'), F/Pa3, 4 (*Phalaris arundinacea*), F/P2, 3 ('Picta'), SF/P4, 5 ('Picta'). Any clonal ramets of genotypes were coded alphabetically (A, B, C, etc.) at the end of the genotypic code.

### 2.2. Genetic analyses

Genetic variability was assessed using ISSR markers. This molecular technique is often used in studies focused on genetic variation of plant populations and plant germplasm and we verified its suitability and stability in analyses of Phalaris genotypes. ISSR is also marker system with high detectable extent of genetic variation/diversity and also with the ability to detect the genetic diversity among individual accessions.

### 2.3. DNA extraction and ISSR analyses

DNA extraction from leaf samples and subsequent ISSR analyses of all Minnesota and N. American samples followed the protocols delineated by Kávová [31]. Four primers from the University of British Columbia were used to generate scorable ISSR markers: UBC 810—$[GA]_8T$, UBC 825—$[AC]_8T$, UBC 881—$G3[TGGGG]_2TG$, and UBC 890—$VHV[GT]_7$ [31]; these have been used in our subsequent studies for *Phalaris* [18, 30, 43]. Seventy-six markers (MW = 270–1200 base pairs [bp]) were scored and transformed into a binary character matrix (1 = present, 0 = absent).

### 2.4. Statistical analyses

Genetic distance matrices were created with Nei and Li's [44] metrics. PCoA (principal coordinate analysis) and UPGMA (unweighted pair group method with arithmetic mean) cluster analyses were calculated with MVSP, version 3.1 (Multi-Variate Statistical Package; Kovach Computing Services U.K.) and DARwin, version 5.0.158 (Dissimilarity Analysis and Representation for windows; CIRAD, F) software. Genetic structure was calculated using STRUCTURE version 2.3.4, a Bayesian clustering algorithm (Admixture Model; correlated allele frequencies; K = 2, K = 4, K = 6, and K = 10 groupings; 100,000 burnin repetitions) [43, 45]. STRUCTURE groupings refer to relationship patterns. After plotting, the K = 2 grouping had the necessary decrease in slope and increase in variance, diagnostic of the true K value, with the greatest number of genotypes/grouping; all other groupings were eliminated [30]. Only results from the K = 2 grouping will be shown.

## 3. Results

The four ISSR primers generated 76 scorable bands (56.6% were polymorphic). The UPGMA cluster analysis showed three distinct grouping of genotypes, all of which separated at a genetic distance of 0.0 (**Figure 1**). The first grouping consisted of strictly forage cultivars from Iowa (PAL, VEN), Minnesota (MN-76), California (CANA) and Missouri (AUB) (**Table 1**), all of which differed significantly ($p \leq 0.05$) from other forage cultivars and wild populations. The next grouping had 4 wild populations from the Mississippi (34.3.1, 38.2.3), Minnesota (46.1.1), Red (63.4.3) rivers in one small grouping, along with another grouping. This latter grouping was subdivided into (a) 7 wild populations from the wet meadow in Chanhassen (8.II.F.3), the Roseau (56.2.2, 58.IV.H.3; 61.1.2), St. Croix (2.1.2; 6.1.3), and Des Moines (50.1.1) rivers and (b)

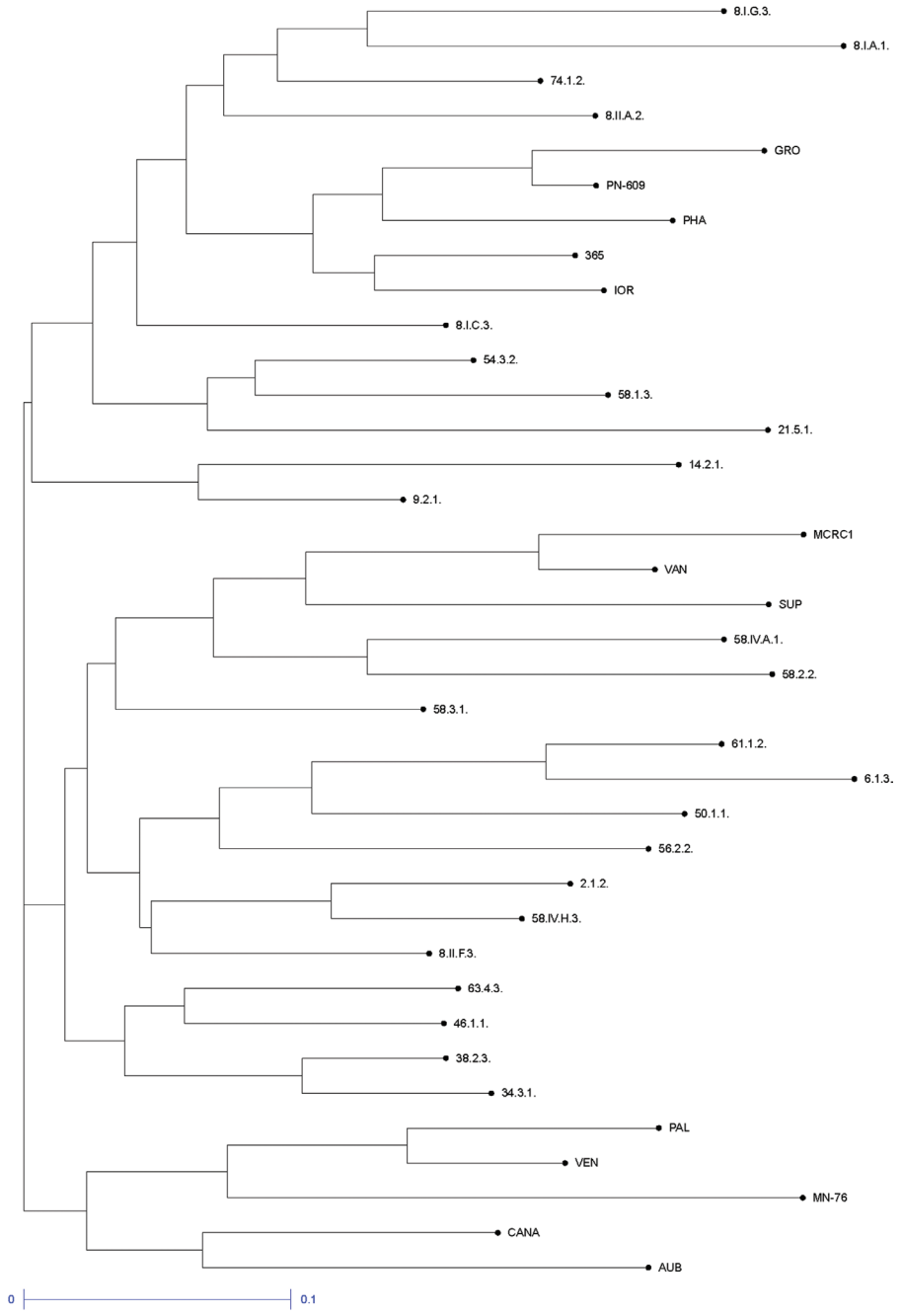



**Figure 1.** UPGMA, based on ISSR markers, for wild Minnesota populations and N. American comparative forage cultivars of *Phalaris arundinacea*. See **Table 1** for genotypic codes.

1 wild population from the Roseau river (58.3.1, 58.2.2, 58.IV.A.1) plus three forage cultivars from Oregon (SUP), Iowa (VAN) and Minnesota (MCRC1) (**Figure 1**). The final grouping consisted of two major subgroupings with (a) 2 wild populations from the Mississippi river (9.2.1; 14.2.1) and (b) a quadriplex set of (i) 3 wild populations from the Mississippi (21.5.1), Roseau (58.1.3), and Des Moines (54.3.2) rivers; (ii) the wet meadow in Chanhassen (8.I.C.3); (iii) 4 forage cultivars from Missouri (IOR), unnamed (PHA), unknown (PN-609), and Ontario, Canada (GRO); (iv) 2 wild populations from the wet meadow in Chanhassen (8.II.A.2; 8.I.A.1; 8.I.G.3) (**Figure 1**).

Principal coordinate analysis (PCoA) of inter-simple sequence repeat (ISSR) markers in reed canarygrass samples from Minnesota and N. America showed two overlapping groupings for the forage and wild genotypes (**Figure 2**). The forage cultivars AUB, VEN, PAL, and MN-76 were the farthest away from the wild populations collected along Minnesota rivers and in wet meadows or fields (**Figure 2**). Other forage cultivars (IOR, PHA, GRO, PN-609, VAN; **Figure 2**) were also categorically and genetically similar to these but more closely related to the wild genotypes.

When the wild and cultivated US genotypes were comparatively analyzed for PCoA together with the Czech/European genotypes [2] this resulted into forming two primary clusters (**Figure 3**). Cluster I (lower circle) included all samples from wild Czech (European) populations along rivers and the forage 'Chrastava' as established for European genotypes by Anderson et al. [18]; this cluster was enriched with all samples of US origin. All US genotypes

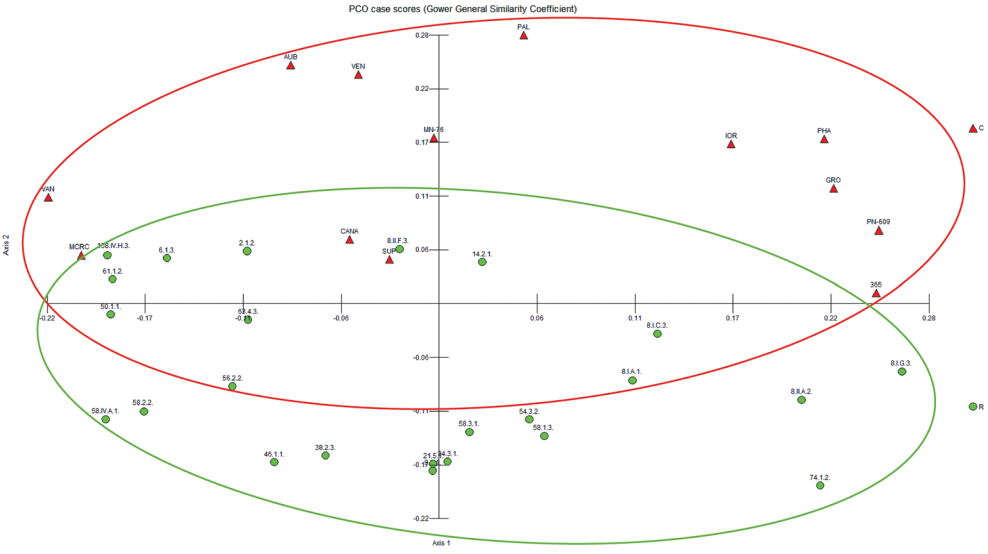



**Figure 2.** Principal coordinate analysis (PCoA) of inter-simple sequence repeat (ISSR) markers in reed canarygrass samples from US (Minnesota) and N. America. For genotype codes, refer to **Table 1**. Keys to symbols are: cultivated (triangles), wild (circles). The upper oval encompasses the majority of cultivated samples while the lower oval surrounds predominantly wild types.

were clustered into a small, oval sub cluster of Cluster I, on the border of the European wild genotypes and showing high similarity in ISSR marker pattern (**Figure 3**). Cluster II (upper oval) is represented by European horticultural and forage cultivars and genotypes from The Nursery of Genetic Resources, OSEVA PRO, Ltd., Grassland Research Station (Rožnov-Zubří, Czech Republic) with both variegated and nonvariegated leaf types [18].

Assessing the genetic structure of analyzed Czech cultivated and wild genotypes showed classification of genotypes according to Q1/Q2 values (membership probabilities in the C [rows or genotypes] × K [columns or clusters] matrix for a single cluster analysis); K = 2 had the best stratification in STRUCTURE (**Figure 4**). One group, 'PN-609', contains several forage cultivars and a few wild genotypes from Site 8. Whereas the larger group, '54.3.2' contains the remaining genotypes from all rivers, wet meadows and any remaining forage cultivars.

UPGMA analyses of both the US and Czech populations, based on ISSRs, showed distinct groupings of reed canarygrass genotypes (**Figure 5**). The first group was a small set of 6 genotypes, ZPCOV, collected at The Nursery of Genetic Resources, OSEVA PRO, Ltd., Grassland Research Station (Rožnov-Zubří, Czech Republic). The second grouping was a large series of sub clusters divided as follows. The most distant genotypes from the ZPCOV cluster were primarily horticultural cultivars from the Czech Republic along with one sole US genotype from the wet meadow in Chanhassen, MN (8.I.A.1; **Figure 5** and **Table 1**). The next cluster

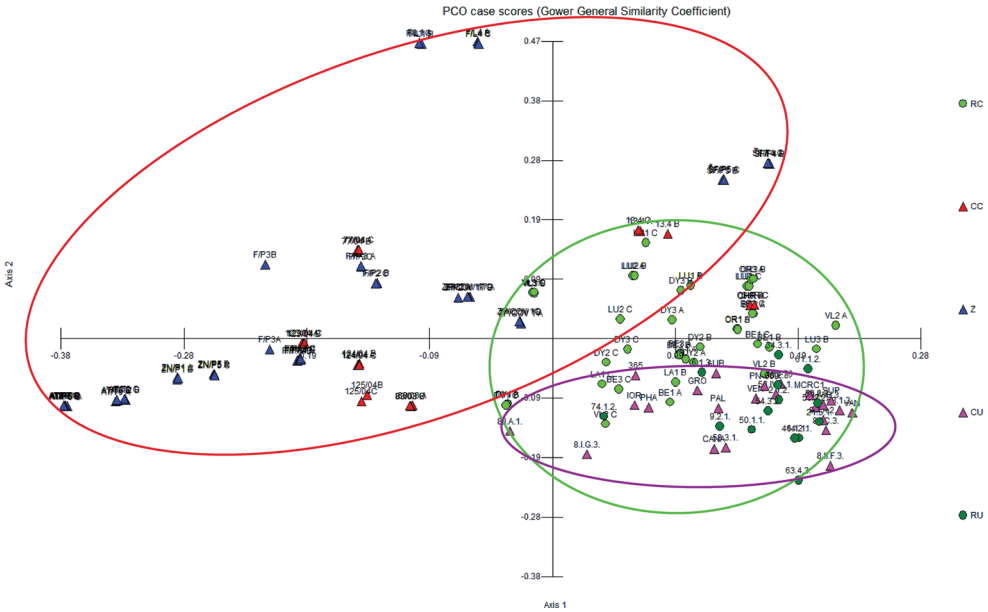



**Figure 3.** Principal coordinate analysis (PCoA) of inter-simple sequence repeat (ISSR) markers in reed canarygrass samples from Minnesota and N. America compared with Czech wild populations and cultivars [18]. For Minnesota and N. American genotype codes, refer to **Table 1**; for the Czech genotypes, refer to [18] (*cf.* **Table 1**). Keys to symbols are: cultivated (triangles), wild (circles). The large oval (Cluster II) encompasses the majority of cultivated samples while the circle (Cluster I) surrounds predominantly wild types. Key: RC—rivers CZ, RU—rivers MN, CC—CZ forage cultivars, CU—MN forage cultivars, Z—horticultural genotypes.

A

| Sample | Type | Q1 | Q2 |
|---|---|---|---|
| PN-609 | C | 0.022 | 0.978 |
| 365 | C | 0.024 | 0.976 |
| PHA | C | 0.024 | 0.976 |
| IOR | C | 0.029 | 0.971 |
| GRO | C | 0.030 | 0.970 |
| 8.I.G.3. | W | 0.032 | 0.968 |
| 8.II.A.2. | W | 0.096 | 0.904 |
| PAL | C | 0.126 | 0.874 |
| 74.1.2. | W | 0.159 | 0.841 |
| 8.I.C.3. | W | 0.260 | 0.740 |
| 8.I.A.1. | W | 0.347 | 0.653 |
| MN-76 | C | 0.389 | 0.611 |
| VEN | C | 0.428 | 0.572 |
| 54.3.2. | W | 0.563 | 0.437 |
| 14.2.1. | W | 0.572 | 0.428 |
| 58.1.3. | W | 0.573 | 0.427 |
| 8.II.F.3. | W | 0.648 | 0.352 |
| AUB | C | 0.650 | 0.350 |
| CANA | C | 0.675 | 0.325 |
| 21.5.1. | W | 0.692 | 0.308 |
| 58.3.1. | W | 0.723 | 0.277 |
| SUP | C | 0.759 | 0.241 |
| 34.3.1. | W | 0.808 | 0.192 |
| 9.2.1. | W | 0.828 | 0.172 |
| 2.1.2. | W | 0.886 | 0.114 |
| 6.1.3. | W | 0.936 | 0.064 |
| 56.2.2. | W | 0.936 | 0.064 |
| 63.4.3. | W | 0.947 | 0.053 |
| 38.2.3. | W | 0.949 | 0.051 |
| 46.1.1. | W | 0.951 | 0.049 |
| 50.1.1. | W | 0.970 | 0.030 |
| 58.IV.A.1. | W | 0.971 | 0.029 |
| 61.1.2. | W | 0.972 | 0.028 |
| MCRC1 | C | 0.973 | 0.027 |
| 58.IV.H.3. | W | 0.974 | 0.026 |
| VAN | C | 0.975 | 0.025 |
| 58.2.2. | W | 0.976 | 0.024 |

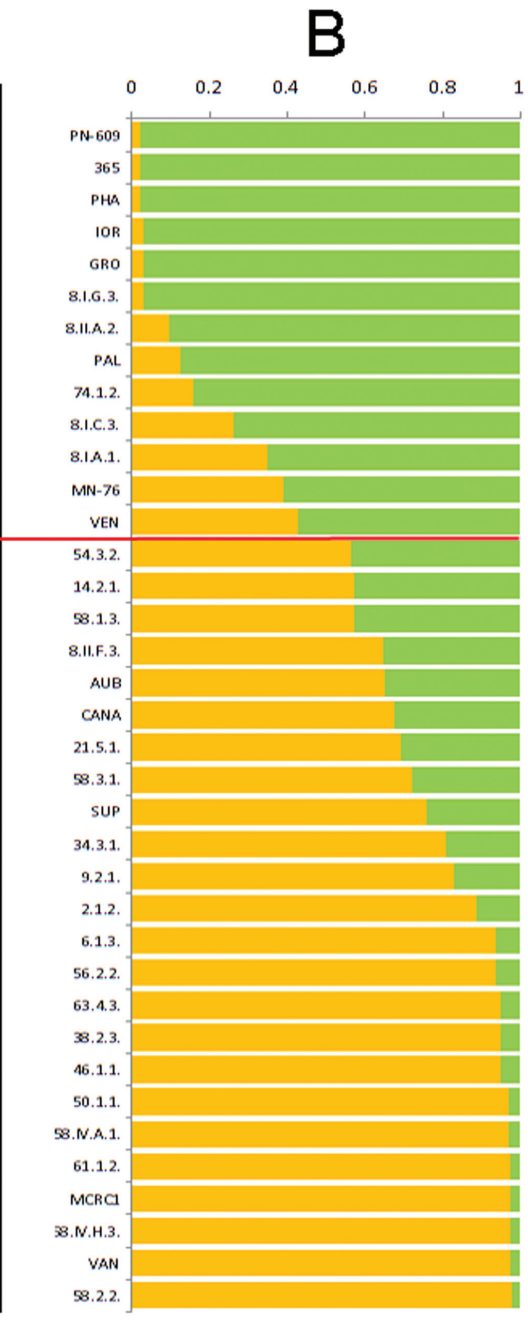



**Figure 4.** Genetic structure analysis of the US and N. American reed canarygrass collection using STRUCTURE software package (Admixture Model, allele frequencies correlated, K = 6, length of burnin period: 100,000). Key: the population code is located left from the corresponding color bars with two groups of accessions: black— 'PN-609'; grey—'54.3.2'.

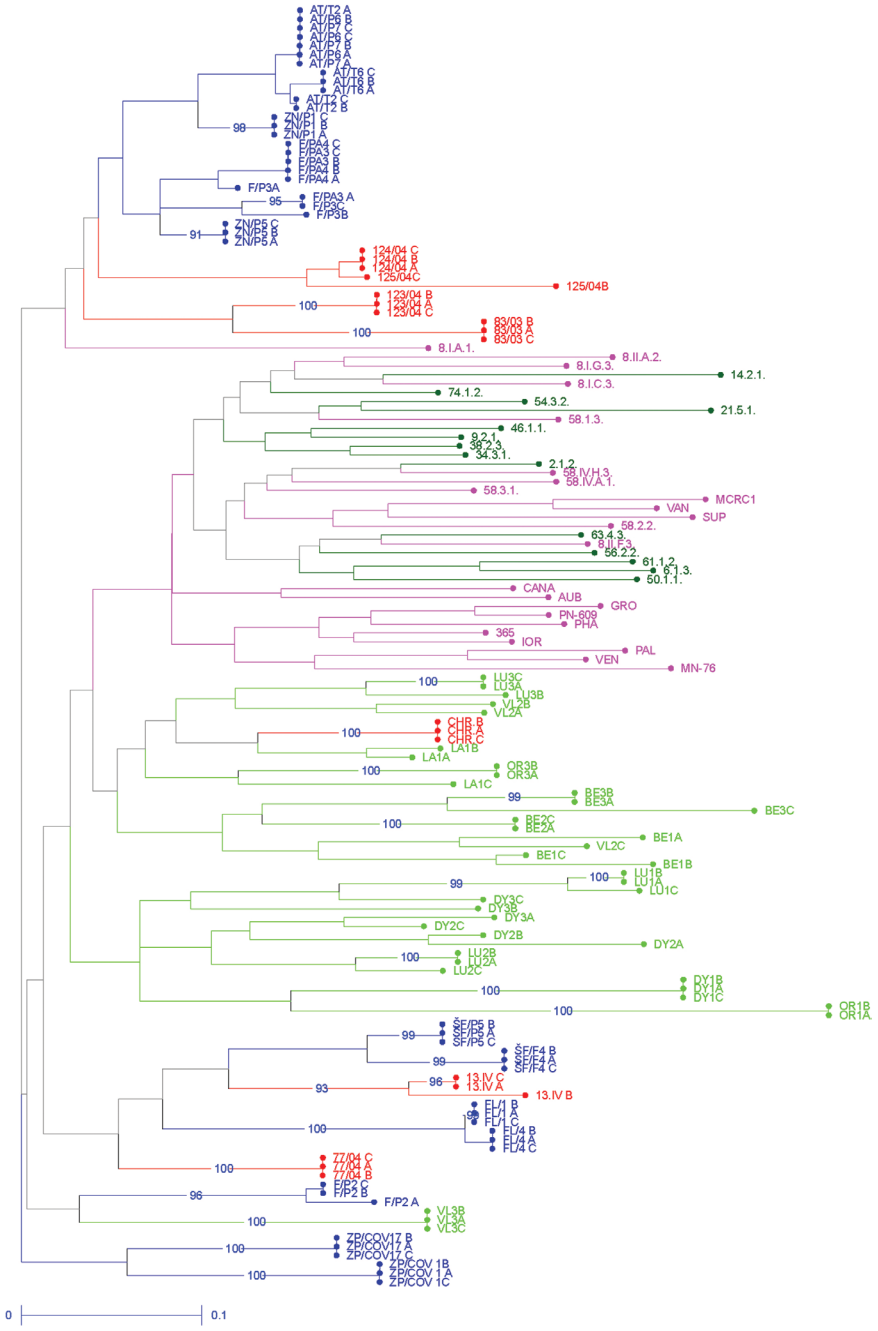



**Figure 5.** UPGMA analysis, based on ISSR data, of combined Czech, Minnesota and N. American reed canarygrass samples analyzed (*cf.* **Table 1** [18] for genotypic codes of US genotypes for CZ genotypes).

was divided into two groups of: (a) 6 Czech genotypes (3 wild from the Vltava River and 3 GFP/GNP or 'Picta'). Next were two sub clusters which bifurcated at a genetic distance of ~ 0.2 (**Figure 5**). One formed a small grouping of 18 genotypes, namely Czech accessions and MN genotypes while the other was a large grouping of all remaining wild and cultivated US and CZ genotypes.

## 4. Discussion

There were two overlapping groupings for the forage cultivars and wild reed canarygrass samples from Minnesota and N. America (**Figure 2**). The forage cultivars AUB, VEN, PAL, and MN-76 were the farthest away from the wild populations collected along Minnesota rivers and in wet meadows or fields (**Figure 2**). This included at least one forage cultivar, AUB ('Auburn'), which is most likely derived from native N. American strains (**Table 1**) [7]. SUP ('Superior'), also derived from native N. American strains, was more closely aligned with some wild genotypes, particularly one from the wet meadow in Chanhassen, MN (8.II.F.3; **Table 1**). Other forage cultivars (IOR, PHA, GRO, PN-609, VAN; **Figure 2**) were also categorically and genetically similar to these but more closely related to the wild genotypes.

Based on the UPGMA analysis of US cultivated and wild types of reed canarygrass (**Figure 1**), potentially the 4 wild populations from the Mississippi (34.3.1, 38.2.3), Minnesota (46.1.1), and Red (63.4.3) rivers are the least related to the N. American forage cultivars SUP ('Superior'), VAN ('Vantage') and MCRC-1 and may be native American genotypes. These MN wild populations also differed from the Czech wild populations (**Figure 4**). Casler et al. [42] and Jakubowski et al. [38, 39] used 15 SSR molecular markers to distinguish among N. American native and exotic (European) *P. arundinacea* herbaria specimens. They found that the forage cultivars AUB ('Auburn') and SUP ('Superior'), used in the present study, were native American in origin. However, in our study, these two forage cultivars were even further away from the 4 wild populations identified above. Thus, it may be possible that additional native N. American strains included herein exist. Future work will be devoted to identifying this possibility using the 15 SSR markers specific to N. American *Phalaris* already identified [38, 39, 42].

In the STRUCTURE analysis of the US reed canarygrass collected along Minnesota rivers and in wet meadows, along with the North American cultivars, the cultivars were distributed throughout both groups (**Figure 4A** and **B**). This was unexpected and surprising since, for instance, the Red and Roseau Rivers running through northern Minnesota do not flow to the Gulf of Mexico and the Atlantic Ocean via the Mississippi River, but instead flow to Manitoba, Canada into Lake Winnipeg and would have limited opportunities for gene exchange. Additionally, since reed canarygrass is native in Minnesota, there could have been divergent evolution within isolated rivers creating distinct populations but this was not found to be the case. This could be due to wind pollination, which may allow for gene flow (pollen) between rivers. Also likely could be the small sample sizes collected along all rivers and/or the choice of genetic markers that, even though they are polymorphic among the populations and cultivars, may not be able to discriminate among *Phalaris* along all rivers.

In the PCoA and STRUCTURE analyses of both the N. American and European sample sets from river habitats and forage cultivars, no clear differentiation among groupings was evident (**Figure 5**). The pattern of genetic markers in the European (Czech) genotypes from alluvial habitats was inclusive of all US wild and cultivated forage genotypes. Both groups of genotypes (wild/cultivated) overlapped and, in contrast with our previous analysis of the European genotypes [18], it was not possible to distinguish between wild and cultivated genotypes with precision. One reason for this may be the low genetic variation and differentiation among genotypes and their high genetic similarity. What is surprising is the very low extent of genetic variability among US genotypes, which formed one "dense" group in this pooled analysis, with low levels of genetic dissimilarity. This fact may also explain poor differentiation between US wild genotypes and US cultivated forage, because of their low genetic dissimilarity. Another reason may be the small sample sizes tested herein. Future work will be devoted to conducting a more thorough sampling of wild and commercial *P. arundinacea* throughout Minnesota and analyzing the SSR genetic alignment into the distinct European vs. native N. American haplotypes.

Since all grasses, including reed canarygrass, are anemophilous (wind-pollinated), it would be easy for genetic mixing to occur in adjacent plantings of cultivated and wild types. Likewise, as most forage cultivars bred and/or produced in Minnesota and N. America are closely related to or derived from European types [42], this also could be a reason why the wild and forage types overlapped in their genetic similarity (**Figures 1** and **2**). The numerous influxes of exotic, European types and cross-pollination effects (either occurring naturally or by hand pollination by plant breeders), combined with migration have mixed the gene pools [42]. For instance, while 'Rival' has both European and Scandinavian ancestors, 'Ioreed' is a hybrid mixture with the European nuclear haplotype but N. American cytoplasmic haplotype [42]. However, maintaining the integrity of N. American *Phalaris* germplasm, distinct from the exotic or European forage types commonly distributed on the continent [33], is of paramount importance given its historical and cultural significance in weavings by native Americans [46–49]. Destruction of native *Phalaris* genotypes would violate Treaty Rights.

## 5. Conclusion

In Minnesota populations of *Phalaris*, the cultivated and wild genotypes formed separate groups, which did overlap significantly. At least four sets of wild U.S. genotypes are the most dissimilar to European counterparts and, as such, could be native to N. America. Future work to prove the ancestry of each accession will be necessary. Nonetheless, the sale of forage cultivars related to or derived from European types continues to cause genetic mixing with N. American types. Part of this intercontinental gene flow and exchange is exacerbated by the production of *Phalaris* forage seed in Minnesota, which is sold both in N. America and Europe. While the expectation that forage/ornamental reed canarygrass cultivars should have a similar genetic makeup with the wild populations across continents (due to limited breeding and genetic selection pressures in this forage and ornamental crop) this is clearly not the case despite *Phalaris* being an invasive, wind-pollinated grass. The implications of these findings for management of invasive crops native to both continents have significant implications for forage producers, managers, and breeders.

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