PLANT SCIENCE TODAY, 2020
Vol 7(3): 314–318
https://doi.org/10.14719/pst.2020.7.3.759
ISSN 2348-1900 (online)

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RESEARCH ARTICLE

Genetic diversity in threatened plant species *Alnus nitida* (Spach) Endl.

Muhammad Khalil Ullah Khan[1], Noor Muhammad[1*], Nisar Uddin[2], Niaz Ali[2], Muhammad Umer[3] & Shariat Ullah[4]

[1]Department of Pomology, College of Horticulture, Agricultural University of Hebei, Baoding 071000, Hebei China
[2]Department of Botany, Hazara University, Mansehra 21300, KP, Pakistan
[3]College of Plant protection, Hebei Agricultural University, Baoding 071000, Hebei China
[4]University of Malakand Chakdara 18800, KP, Pakistan
Email: noorpk_1990@yahoo.com

ARTICLE HISTORY
Received: 19 February 2020
Accepted: 19 March 2020
Published: 01 July 2020

KEYWORDS
Alnus nitida
conservation
genotypes
genetic variation
SDS-PAGE

ABSTRACT

Alnus nitida (Spach) Endl. is an ethnobotanically important threatened plant species. The genetic diversity among the 50 different genotypes of *Alnus nitida* was carried out using sodium dodecyl sulfate poly acrylamide gel electrophoresis (SDS-PAGE) characterization. A considerable amount of genetic diversity (90%) was observed among the genotypes of *A. nitida*. The protein characterization was carried out on 12% gel electrophoresis. A total of 10 protein bands were detected in *A. nitida* genotypes. SDS-PAGE procedure is a useful method for the investigation of both genetic diversity and phylogenetic relationship. Especially, B-5 was monomorphic in *A. nitida* genotypes and was considered as species specific. All other bands/loci were polymorphic. These polymorphic bands displayed 12, 16, 72, 88, 2, 44, 84, 54 and 12 percent variation respectively. In the present examination, the high intra-specific diversity was observed representing SDS-PAGE is a powerful tool for determining the genetically diverse germplasms in *A. nitida*. The results obtained by this study could be helpful in the identification and selection of desired genotypes of *Alnus nitida* for conservation programmes in future. Today, there is still a need to assess genetic variation and protect genetic resources, especially of wild species for prospective benefits in plant conservation programmes.

Introduction

Alnus nitida (Spach) Endl. belongs to the family Betulaceae, is a deciduous tree (1). The tree is tall up to 20-25 m or above. It is used for medicine, wood and fuel (1); and is mainly cultivated as an avenue tree in Pakistan. *Alnus nitida* is widely dispersed and is commonly found along river banks within its range (1, 3). Though possible threats have been recognized, there are no identified major threats presently for the existence of this species. The plant is classified as 'Least Concern' in the IUCN Red List of Threatened Species (1–4). In one of the studies the main threats identified were wood cut for fuel, unsustainable collection, over exploitation and overgrazing; these studies has elaborated the view of the local inhabitants that population has decreased up to 75% due to its medicinal potential and unsustainable collection (1, 5). *Alnus nitida* has been reported under the category A (a, c and d) of the endangered species in Swat valley (1).

Genetic diversity refers to the variation of genes in a species (6, 7). Genetic diversity is the main stake of biodiversity and diversity within species, inter species and of specie's surroundings (7). The objective of conservation genetics is to uphold genetic diversity at various stages and to offer tools for population monitoring and evaluation that can be used for conservation planning. All individuals are genetically unique by nature (6). Conservation efforts and related studies are rarely focused towards individual species but genetic variation is always measured in individuals/species (1) and this can only be assessed by the collection of individuals in a population/species (7). The conservation of the species is possible to detect the genetic variation from phenotypic variation either by quantitative traits or qualitative; furthermore, the quantitative traits are usually

To cite this article: Khan MKU, Muhammad N, Uddin N, Ali N, Umer M, Ullah S. Genetic diversity in threatened plant species *Alnus nitida* (Spach.) Endel. Plant Science Today. 2020;7(3):314-318. https://doi.org/10.14719/pst.2020.7.3.759

Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, etc. Full list at http://www.plantsciencetoday.online

governed by many genes whereas the qualitative traits that are governed by one or few major genes (7). Genetic diversity can also be recognized by investigating variation at the level of enzymes using the process of protein electrophoresis (1, 6, 7). Further, genetic variations can also be examined by the order of nucleotides in the DNA sequence and so on (1).

Limited distribution of the *A. nitida* was observed in the study area (7). Unfair means of collection, cutting for traditional medication and habitat devastation by man-made activities might have led to extreme deterioration in natural populations of *Alnus nitida* (1). Instead of the urgent requirement of conservation plan for this species, the knowledge of population dynamics and diversity that may greatly contribute to limit the population decline. In this study, we investigated genetic diversity of *Alnus nitida*, a threatened species in Mansehra, KP, Pakistan. Today, various tools are in hand to judge the genetic diversity among the germplasms of plant species. At the beginning, morphological characterization was used but in majority cases morphological characterization is unstable due to the environmental instability (6). Germplasm evaluation and genetic variation through DNA based molecular marker is very prominent way, but is more expensive. Compared to DNA marker characterization, seed storage protein characterization is free of environmental fluxes and is easy to handle in the developing countries like Pakistan (5, 6).

Medicinal plants have a key role in folk medicinal systems like Unani, Traditional Chinese medicine, Ayurveda, Siddha, Yoga etc. and therefore the conservation of medicinal plants and traditional knowledge is important (8). *Alnus nitida* is well recognized for their folk ethnomedicinal values; since this species has been in use for the treatment of several ailments such as cancer, as a marginal astringent etc. (1). The bark is useful in mouth and throat inflammations and also applied to wash the lice and a variety of skin disorders like scabies and scabs (1).

Alnus nitida is one of the important medicinal plant species and have been found active against many life threatening disorders like hepatitis and cancer. This research was designed to examine the genetic diversity in threatened species *Alnus nitida*, using SDS-PAGE characterization among 50 genotypes of *A. nitida*. This species has an important local adaptation and widespread use by people for medicinal and fuel purposes. The aim of this study is to assess the intra-specific diversity in *A. nitida* to highlight the diversity among the compatible germlasms. The present study is the first ever report from Pakistan.

Material and Methods

Study area

In this study, the experimental tours were organized to diverse ecological zones of District Mansehra, a district in Hazara Division of KP province in Pakistan. A total of 50 samples were picked from the different zones given below for characterization of genetic diversity in seed storage protein profiling. The soil type of all zone almost same (Clay, silt loamy with uniform nutrients). The various zones are shown in Table 1.

Specimens for each genotypes have been collected and processed using standard herbarium techniques and were preserved in herbarium at Hazara University, Mansehra, KP, Pakistan as shown in Table 2.

Protein profiling

For the estimation of genetic diversity, sodium dodecyl sulfate poly acrylamide gel electrophoresis (12%) SDS-PAGE (for seed storage protein) was performed (7).

For seed storage protein profile, 3 to 5 seed of each genotypes was ground into a fine powder. About 400 µl of Protein Extraction Buffer (PEB) with a composition of 0.5M Tris-HCL, 0.2% SDS, 5M Urea, 1% β-mercaptoethanol under 8-pH was added to 0.01 gm of seed fine powder. The E-tube containing PEB and

Table 1. Fifty various localities of *Alnus nitida* in District Mansehra selected for current study

S. No	Sites	Longitude	Latitude	Altitude
1	Muhandri	34˚12'03.23"N	73 ˚ 04'61.02"E	3314 ft
2	Neka Pani	34˚29'01.21"N	73 ˚ 08'50.01"E	2951 ft
3	Oghi	34˚ 29'09.22"N	73 ˚ 07'52.04"E	3351 ft
4	Pairaan	34 ˚ 22'68.18"N	73 ˚ 08'57.03"E	3350 ft
5	Perhenna	34 ˚ 22'03.12"N	73 ˚ 01'61.05"E	3311 ft
6	Phulrra	34 ˚ 04'09.11"N	73 ˚ 12'61.08"E	2829 ft
7	Sachaa Kaalan	34 ˚ 28'09.12"N	73 ˚ 12'06.01"E	2811 ft
8	Sandasaar	34 ˚ 18'12,19"N	73 ˚ 13'42,02"E	3110 ft
9	Satbaani	34 ˚ 29'13.12"N	73 ˚ 10'15.01"E	3310 ft
10	Shamdarra	34 ˚ 22'14.12"N	73 ˚ 07'61.03"E	3141 ft
11	Shohal Mazullah	34 ˚ 11'08.13"N	73 ˚ 08'01.05"E	3341 ft
12	Shoukatabad	34 ˚ 12'08.06"N	73 ˚ 07'11.05"E	3321 ft
13	Sum AlahiMong	34 ˚ 13'19.08"N	73 ˚ 11'02.01"E	3391 ft
14	Swaan Miara	34 ˚ 15'24.01"N	73 ˚ 02'11.08"E	3105 ft
15	Talhata	34 ˚ 34'34.91"N	73 ˚ 09'25.17"E	4557 ft
16	Tanda	34 ˚ 36'03.06"N	73 ˚ 06'14.26"E	3774 ft
17	Trangi	34 ˚ 31'01.25"N	73 ˚ 16'12.31"E	2611 ft
18	Sabir Shah	34 ˚ 11'23.28"N	73 ˚ 11'13.31"E	3121 ft
19	Battal	34 ˚ 13'20.11"N	73 ˚ 21'41.05"E	2938 ft
20	Attar Shisha	34 ˚ 22'37.11"N	73 ˚ 33'06.03"E	3011 ft

21	BadiShongli	34 ° 11'03.98"N	73 ° 71'01.08"E	3451ft
22	Baffa	34 ° 18'11.88"N	73 ° 36'02.28"E	3361ft
23	Balakot	34 ° 03'12.81"N	73 ° 26'01.36"E	3121ft
24	Behali	34 ° 11'05.33"N	73 ° 26'13.05"E	3412ft
25	Belian	34 ° 13'05.12"N	73 ° 15'12.06"E	3891ft
26	Bherkund	34 ° 16'05.11"N	73 ° 18'08.06"E	2881ft
27	Bhugerr Mung	34 ° 11'06.22"N	73 ° 11'01.04"E	2123ft
28	Chater Plain	34 ° 22'08.31"N	73 ° 02'04.05"E	2513ft
29	Darband	34 ° 21'05.42"N	73 ° 01'03.02"E	2541ft
30	Datta	34 ° 11'06.43"N	73 ° 02'04.02"E	2531ft
31	Daud Shah	34 ° 13'08.42"N	73 ° 01'05.11"E	2341ft
32	Deoli Jaberr	34 ° 22'01.34"N	73 ° 01'32.29"E	2411ft
33	Dhodial	34 ° 21'11.16"N	73 ° 01'31.29"E	2411ft
34	Delborri	34 ° 34'02.12"N	73 ° 02'11.41"E	2891ft
35	Gaarhi Habibullah	34 ° 12'01.41"N	73 ° 11'03.44"E	2893ft
36	Garlat	34 ° 11'03.33"N	73 ° 12'11.13"E	3041ft
37	Ghanool	34 ° 12'04.31"N	73 ° 13'02.41"E	3115ft
38	Hamsheerian	34 ° 81'05.41"N	73 ° 12'11.31"E	3211ft
39	Haengrai	34 ° 03'04.52"N	73 ° 10'21.34"E	4102ft
40	Helkot	34 ° 12'05.11"N	73 ° 01'31.21"E	2919ft
41	Icherro	34 ° 13'05.21"N	73 ° 11'30.12"E	3144ft
42	Inayat Abad	34 ° 34'15.32"N	73 ° 13'41.13"E	4121ft
43	Jaborri,	34 ° 34'31.11"N	73 ° 12'11.41"E	3941ft
44	Jaloo	34 ° 11'01.02"N	73 ° 45'01.08"E	3315ft
45	Kaghan	34 ° 11'54.08"N	73 ° 12'45.44"E	2923ft
46	Karnool	34 ° 02'11.3"1N	73 ° 16'02.31"E	3305ft
47	Kewal	34 ° 43'06.42"N	73 ° 42'09.24"E	3672ft
48	LaberKot	34 ° 44'16.43"N	73 ° 12'42.45"E	3456ft
49	LasanNawab	34 ° 06'12.44"N	73 ° 44'06.21"E	3243ft
50	Mansehra city	34 ° 17'13.99"N	73 ° 12'20.03"E	4201ft

Table 2. Documentation of *Alnus nitida* with scientific name, local name and Voucher number

Botanical Name	Local Name	Family	Genotypes	Voucher No	Botanical Name	Local Name	Family	Genotypes	Voucher No
Alnus nitida (Spach) Endl	Giray /Sharoli	Betulaceae	An1	HUP-9503	*Alnus nitida* (Spach) Endl	Giray/ Sharoli	Betulaceae	An26	HUP-9528
		Betulaceae	An2	HUP-9504			Betulaceae	An27	HUP-9529
		Betulaceae	An3	HUP-9505			Betulaceae	An28	HUP-9530
		Betulaceae	An4	HUP-9506			Betulaceae	An29	HUP-9531
		Betulaceae	An5	HUP-9507			Betulaceae	An30	HUP-9532
		Betulaceae	An6	HUP-9508			Betulaceae	An31	HUP-9533
		Betulaceae	An7	HUP-9509			Betulaceae	An32	HUP-9534
		Betulaceae	An8	HUP-9510			Betulaceae	An33	HUP-9535
		Betulaceae	An9	HUP-9511			Betulaceae	An34	HUP-9536
		Betulaceae	An10	HUP-9512			Betulaceae	An35	HUP-9537
		Betulaceae	An11	HUP-9513			Betulaceae	An36	HUP-9538
		Betulaceae	An12	HUP-9514			Betulaceae	An37	HUP-9539
		Betulaceae	An13	HUP-9515			Betulaceae	An38	HUP-9540
		Betulaceae	An14	HUP-9516			Betulaceae	An39	HUP-9541
		Betulaceae	An15	HUP-9517			Betulaceae	An40	HUP-9542
		Betulaceae	An16	HUP-9518			Betulaceae	An41	HUP-9543
		Betulaceae	An17	HUP-9519			Betulaceae	An42	HUP-9544
		Betulaceae	An18	HUP-9520			Betulaceae	An43	HUP-9545
		Betulaceae	An19	HUP-9521			Betulaceae	An44	HUP-9546
		Betulaceae	An20	HUP-9522			Betulaceae	An45	HUP-9547
		Betulaceae	An21	HUP-9523			Betulaceae	An46	HUP-9548
		Betulaceae	An22	HUP-9524			Betulaceae	An47	HUP-9549
		Betulaceae	An23	HUP-9525			Betulaceae	An48	HUP-9550
		Betulaceae	An24	HUP-9526			Betulaceae	An49	HUP-9551
		Betulaceae	An25	HUP-9527			Betulaceae	An50	HUP-9552

seed fine powder (PEB-FP) was Vortexed thoroughly to homogenize the mixture. The Coomassie Brilliant Blue (CBB) was added to the E-tube as tracking dye to see the movement of PEB-FP on the separation PAG. The homogenated samples were centrifuged at 13000 rpm for 15 min under room temperature. The electrophoretic process was carried out using 12% polyacrylamide gel (composition of resolution gel: 3.0 M Tris-HCl pH9.0, 0.4% SDS and staking gel 0.4 M Tris-HCl pH 7.0, 0.4% SDS). The electrode buffer containing 0.025 M Tris, 129M Glycine and 0.125% SDS was poured in the Electrophoresis tank. Similarly, 15 µl PEB-FP was loaded in each well of

Table 3. Intra specific genetic diversity detected in *A. nitida* genotypes

Band/Locus	Present %	Absent %	status	variation %
B-1	44(88%)	6(12%)	Poly	12
B-2	42(84)	8(16%)	Poly	16
B-3	9(18%)	41(72%)	Poly	72
B-4	6(12%)	44(88%)	Poly	88
B-5	50(100%)	0.00	Mono	0.00
B-6	49(98%)	1(2%)	Poly	2
B-7	28(56%)	22(44%)	Poly	44
B-8	8(16%)	42(84%)	Poly	84
B-9	23(46%)	27(54%)	Poly	54
B-10	44(88%)	6(12%)	Poly	12
Genetic Diversity= (Poly/Total*100= 90%)				

12% PAG. The electrophoresis was run at 100V until the blue line passed through the bottom of gel plates. The PAG were then stained and destained for data scoring of seed storage protein profile.

Data analysis

The protein data was analyzed using MS Excel 2010 and PC-ord software.

Results

Ten bands were observed in the electrophoregram (Fig. 1; provided as supplementary file). The protein (0, 1) data of 50 genotypes of *A. nitida* based on SDS-PAGE was examined for the creation of a dendrogram (Fig. 2). This tree illustrates the diversity and similarity of various genotypes and the 50 genotypes of the *A. nitida* were studied and the tree was constructed (Fig. 1). The phylogenetic tree divided all the 50 genotypes of *A. nitida* into three regions (R-I – R-III). Region I is comprised of the genotypes; (An1 (Muhandri), An2 (Neka Pani), An3 (Oghi), An4 (Pairaan), An7 (Sachaa Kalan), An8 (Sandasar), An9 (Satbani), An10 (Shamdarra), An11 (Shohal Mazullah), An12 (Shoukatabad), An13 (Sum AlahiMong), An14 (Swan Miara), An15 (BadiShongli), An19 (DeoliJaberr) An18 (Talhata), An20 (Tanda), An33 (Trangi), An34 (Sabir Shah), An44 (Battal), An45 (Chattar plane). Region I (R-I) displayed 25% genetic similarity with R-II whereas The included genotypes in the Region II are An5 (Hamsherian), An6 (Hangrai), An16 (Helkot), An17 (Icherro), An21 (AtterShisha), An22 (BadiShungli), An26 (Baffa), An28 (Balakot), An29 (Behali), An23 (Beelian), An27 (Bharkund), An30 (BhugerrMung), An31 (Darbaand), An32 (Datta), An35 (Daud Shah), An41 (Deoli Jaberr), An42 (Dhodial), An43 (Dilburri), An46 (Garrhi Habibullah), An24 (Garrlat), An25 (Ghanol). The Region II & III are 43.75% similar with one another, The Region III composed of genotypes collected from An36 (Inayat Abad), An47 (Jaborri), An37 (Karnool), An38 (Karorr), An48 (Kaathai), An49 (Kewal), An39 (LaberKot), An50 (LasanNawab) and An40 (Mansehra) (Fig 2).

Intra- specific diversity in *A. nitida* genotypes

The overall genetic diversity among 50 genotypes of *Alnus nitida* is shown in Table 3 and remarkably, Locus 5 (B-5) was present all over in the total genotypes of *A. nitida* and was considered as monomorphic. This locus (B-5) was treated as species specific. B-1, B-2, B-3, B-4, B-6, B-7, B-8, B-9 and B-10 were polymorphic. These bands exhibited 12%, 16%, 72%, 88%, 2%, 44%, 84%, 54% and 12% respectively. The total genetic diversity in *A. nitida* genotypes was 90% (Table 3).

Discussion

The examination of genetic diversity in the germplasm of the plant species is very important for the conservation and their yield enhancement purposes (1, 6, 7, 9, 10). The seed storage protein characterization is the standard way for examining the genetic diversity among the threatened plant species (1). Study of genetic variability among

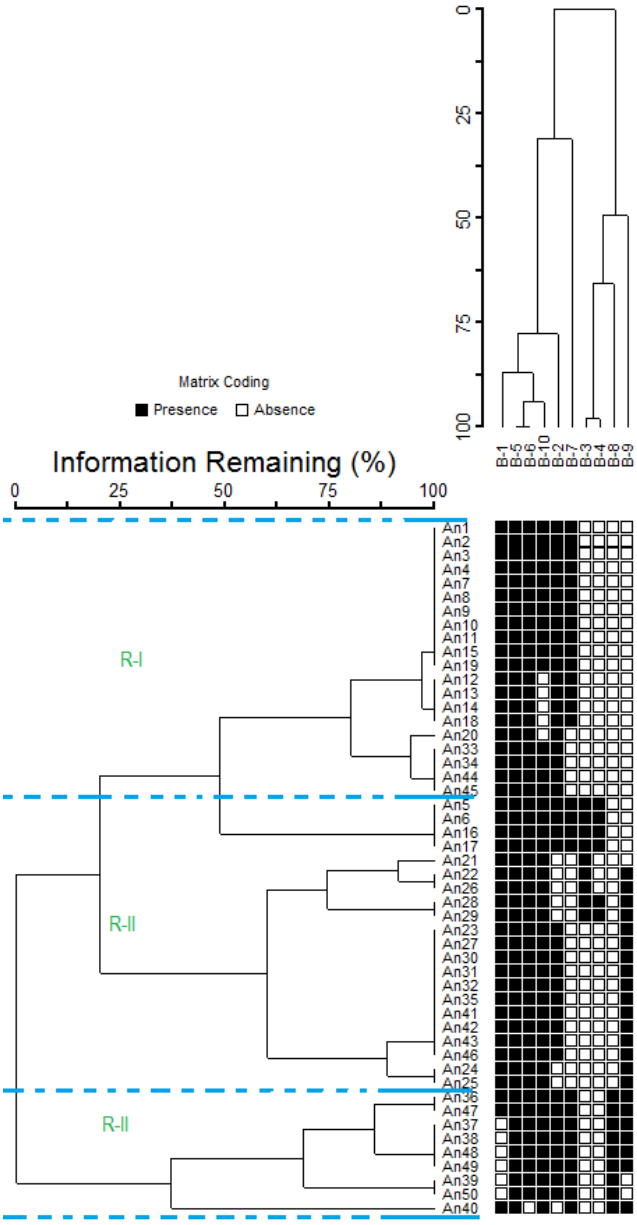

Fig. 2. Genetic diversity recognized through SDS-PAGE analysis among 50 various samples of *A. nitida* picked from Mansehra, KP, Pakistan: An: indicates genotypes of *Alnus nitida*

genotypes is of supreme economic importance to crop enhancement studies (6, 7, 10, 11). Among various plant species of agronomic and medicinal importance, the genetic diversity has been carried out using electrophoretic arrays of total seed storage proteins (6, 10, 12, 13). Numerous studies of genetic diversity have been carried out in the cultivated plant species using SDS-PAGE; however this attempt is negligible for wild threatened plant species (6, 10, 12-16). This is the first attempt to find out the genetic diversity in term of conservation of the ethnomedicinally important threatened plant, *Alnus nitida* using SDS-PAGE (1, 16).

Medicinal plants are very important in the traditional healthcare system (8, 17-19). *Alnus nitida* is medicinally very important plant species, locally used for the treatment of various ailments (1). The main threats are wood cut for fuel, overgrazing, unsustainable collection and over exploitation (6, 10, 12-16).

In this investigation, 50 genotypes *A. nitida* unveiled a significant intra genetic diversity examined through SDS-PAGE characterization. The greatest genetic diversity among the *A. nitida* has been observed. The phylogenetic tree based on SDS-PAGE distinguished all the genotypes into three regions. R-1 and R-II has 25% similarity whereas the genotypes of R-II and R-III are 43.75% genetically similar. Due to high intra-species locus contribution toward genetic diversity, SDS-PAGE is a reliable method for identification of the genotypes of this species (5, 6) and intra-specific genetic diversity in genotypes of *A. nitida* was 90%. Mainly, B-5 was monomorphic in *A. nitida* and was considered as species specific.

Conclusion

SDS-PAGE of (Seed storage protein) electrophoresis is an authoritative practice for assessment of genetic diversity and this machinery is especially thought to be a reliable method, as the seed storage proteins are mainly independent of environmental fluctuations. The genetic diversity using the seed storage protein is mainly important for conservation strategies of threatened and endangered plant species. A considerable amount of genetic diversity (90%) was observed in the genotypes of *A. nitida*, this diversity suggests that the genotypes of diverse genetic makeup could better be helpful for the existence and survival of *A. nitida*. In this investigation, struggles have been made to define the genetic diversity and phylogenetic relationship among the genotypes of *A. nitida*, and the B-5 in the studied genotypes is considered as species specific locus representing that all the genotypes belong to the same species (*A. nitida*). Considerable genetic variance will be helpful for the identification of elite germplasm of *Alnus nitida* that could ensure their survival in adverse climatic conditions. This study could be helpful in conservation of this important genetic resource.

Authors' contributions

MKK did the experiment, NM wrote the manuscript, NU, NA, MU and SU read the manuscript and made suitable changes.

Acknowledgements

The authors are thankful to the Hebei Agricultural University for providing some chemicals for this research work.

Competing interests

Authors have no competing interests to declare.

Supplementary file

Supplementary Figure 1

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