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Exploring the needle's morphological, phytochemical, and genetic diversity of *Taxus baccata* L. in northern Iran

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Abstract

Objective: Golestan province, located in northern Iran, is recognized as one of the most important gene pools of the Yew tree (*Taxus baccata* L.) worldwide. Despite its significance, there is limited information on the diversity of yew populations in this region. This study aimed to investigate the diversity of these populations by examining morphological, phytochemical, and genetic traits.

Methods: To carry out the experiment, a completely randomized design with three replications was employed during the summer of 2020 in the Gorgan University of Agricultural Sciences and Natural Resources< Gorgan, Iran. Young shoots were collected from 30 individuals across 10 populations. Morphological traits of needles were measured using digital scales and calipers, while phytochemical properties, including phenolics, flavonoids, alkaloids, antioxidant activities, and taxol concentration, were analyzed using standard biochemical and HPLC methods. Genetic variation was evaluated through PCR using inter-simple sequence repeat (ISSR) markers. Data from morphological and phytochemical analyses were statistically processed with SPSS, whereas genetic data were evaluated using NTSYS and GenAlex software.

Results: Morphological analyses revealed considerable variability in traits among different yew populations, allowing for their classification into distinct groups. However, no significant variation was observed in taxol content among the sampled trees, although taxol presence indicates a promising potential for production. Differences in leaf antioxidant activity were observed among populations, suggesting varying levels of phytochemical activity. Using five ISSR primers highlighted genetic diversity among the populations. However, the majority of the genetic diversity (84%) was attributed to variations within populations, while only 16% was due to differences among populations. These findings enhance our understanding of the genetic diversity present within yew populations in the Golestan province and underscore the importance of conserving this diversity. Maintaining genetic variation is essential for the long-term adaptability, resilience, and survival of these populations. This study provides critical insights for developing sustainable management practices and highlights the potential medicinal and economic value inherent in the yew populations of this region.

Conclusion: Our investigation revealed no significant variation in taxol content among yew populations in the Golestan region, Iran. However,

morphological, phytochemical, and genetic analyses showed significant differences among populations, highlighting the potential for taxol production and the importance of conserving genetic diversity for sustainable management.

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Introduction

Yew (*Taxus baccata* L.) is a slow-growing evergreen coniferous tree belonging to the Taxaceae family (Siegle and Pietsch 2018). This tree is typically characterized by having two stems and an average annual longitudinal growth of only 10 cm, along with a transverse growth of 0.6 mm (Assareh *et al.* 2007). Yew has a wide distribution, spanning from North Africa to Scandinavia, and extending from the Iberian Peninsula to the Caspian Sea. It covers various regions in Europe, including the Alps, Carpathians, Caucasus, Turkey, and forests in North Africa and northern Iran (Olsson *et al*. 2018). In Iran, this species is found at altitudes ranging from 900 to 1800 meters, extending from Astara in the Guilan province to Aliabad Katoul in the Golestan province (Lesani 2000).

Yew serves as a prominent source of secondary and specialized metabolites with therapeutic properties. Afraz and Hosseini Hashemi (2018) identified 46 different compounds in the bark of the yew, such as phenols, flavonoids, alkaloids, fatty acids, alkanes, sugars, alcohols, and steroids. Moreover, it exhibits therapeutic potential for various ailments, such as rheumatism, urinary problems, and liver diseases, particularly in low doses (Chevallier 1996). The plant has also been associated with treatments for psoriasis, Alzheimer's, rheumatoid arthritis, and Parkinson's disease (Chen *et al*. 2024). Furthermore, yew demonstrates a wide array of pharmacological properties, including anti-cancer (Sarmadi *et al.* 2019), antioxidant (Karafakıoglu 2019), antifungal (Mahmutović-Dizdarević *et al.* 2019), antibacterial (Ghaedi *et al.* 2015), and promising anti-parasitic activity (Zarea *et al.* 2014).

Genetic diversity provides useful information about history and adaptive potential and is a basis for the phylogeny, classification of taxa, and preservation programs of endangered plant species (Han *et al*. 2007). It also provides useful information about the relationships in plants and promotes further insight for plant breeders (Abbasi Karin *et al.* 2023). Diversity has also emerged as a crucial

consideration in discussions about sustainability, as the resilience and stability of a forest ecosystem often hinge on the diversity of plant populations (Ruprecht *et al.* 2010). Species diversity can also have an indirect positive effect on recruitment because of an increase in regeneration niches in species-rich forests. Higher biodiversity can be maintained by interactions among tree species and their natural enemies that can help maintain or increase tree species diversity (Vencurik *et al.* 2019). Yew suffers from a lack of regeneration, high seedling mortality, and the inability of recruitment to develop beyond a sapling growth stage across a large part of the distribution area. The lack of light and low temperature, together with herbivory and seed predation by rodents, are the likely factors limiting the regeneration of yew in the northern and eastern parts of the geographic range of the species (Farris and Filigheddu 2008, Vencurik *et al.* 2019).

Molecular markers have become very efficient and powerful tools in a wide range of applications, including fingerprinting the accessions, evaluation of phylogenetic relationships among accessions, and examining the level of genetic diversity (Nematollahi *et al.* 2013). Some research has been carried out in order to gain a deep understanding of the significance of diversity using molecular markers in different plants. Ghorbanzadeh *et al.* (2021) employed ISSR markers to investigate juniper populations (*Juniperus* spp.), revealing significant genetic diversity both within and among populations. They also explored morphological and phytochemical variation. This exploration led to the identification of myrtenil acetate for the first time, with a notable level in one species. Hematzadeh *et al.* (2023) examined genetic diversity in endangered yew populations using SSR markers in Iran, reporting relatively high genetic diversity for *T. baccata* in the Hyrcanian forest. They anticipated an increase in inbreeding depression over time, leading to the accumulation of detrimental alleles and intensification of the extinction process. Zarek (2009) detected significant polymorphism among four Iranian yew populations using RAPD markers. Interestingly, intra-species diversity was notably higher in plants grown at higher altitudes.

Ruprecht *et al.* (2010) conducted a structural diversity study on yew under diverse environmental conditions, linking low vitality to smaller mean distances between neighbors and greater tree height variation. Anthropogenic activities, habitat fragmentation, tree age, low regeneration, geographical barriers, and reduced gene flow among habitats were suggested as potential causes.

Although yew, as an endangered medicinal species in the world, has received more attention in conservation activities, the knowledge about conservation management is limited. Slow growth, delayed reproduction, and extraordinarily long lifespan suggest that yew requires long-lasting and stable ecological conditions (Kucbel *et al.* 2012). In this context, the genetic analyses within species and populations at global and local scales are the basis for developing and assessing strategies for the

sustainable preservation of precious tree resources (Aravanopoulos *et al.* 2016; Fady *et al.* 2016). Nonetheless, a comprehensive understanding of yew species diversity in northern Iran remains lacking. Therefore, this study aimed to foster conservation strategies based on genetic data and to explore potential differences or correlations in phenotypic, genotypic, and chemical diversity of yew populations in northern Iran, bridging this research gap.

Materials and Methods

To explore the diversity of yew populations in Golestan province, Iran, the current study was carried out during the summer of 2020. A completely randomized design with three repetitions was conducted. After collecting the samples, the measurements of interest were carried out in the laboratory of the Department of Horticultural Sciences, Gorgan University of Agriculture and Natural Resources, Iran.

Plant material and site identification

The collection involved obtaining young shoots from three distinct samples of female plants within each habitat, ensuring biological replication. The assessment encompassed a comprehensive analysis of 30 individuals across 10 populations, thereby encompassing a diverse spectrum of habitats located in close proximity within the region.

Table 1. Specifications of the soil of the studied habitats of yew in northern Iran.

No.	Location	pH	EC (µS/cm)	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)	Soil class
1	Abshar Ziarat	6.97	1372	2.53	92.54	3.76	3.7	Sand
2	Baran Koh	7.08	2086	3.70	55.14	31.4	13.46	Loam
3	Sefid Dareh Ziarat	7.28	1157	1.85	43.4	37.02	19.58	Loam
4	Dal Aram	7.28	851	2.82	38.78	53.56	7.66	Silt loam
5	Poneh Aram	7.23	1406	2.34	81.02	11.46	7.52	Loamy sand
6	Afra Takhteh	7.13	2111	3.70	76.12	10.12	13.76	Sandy loam
7	Tarkat Gahan Nama	7.28	1426	2.14	69.02	19.34	11.64	Sandy loam
8	Dareh Shor	7.37	1305	3.12	66.84	17.58	15.58	Sandy loam
9	Shast Kola	7.25	1247	3.70	68.9	21.54	9.52	Sandy loam
10	Mohavate Pardis	7.31	628	1.95	14.9	67.52	17.58	Silt loam

The geographic coordinates and elevation of each site were meticulously recorded using a GPS device (Garmin GPSMAP 176, USA). The soil-related data were outlined in Tables 1 and 2. Upon harvesting, aerial plant components were gathered and subsequently evaluated. The collected samples were stored in cool and dry conditions, facilitating optimal preservation until further analysis could be conducted.

Table 2. Geographical coordinates of the sampled areas.

No	Location	Abbreviation	Geographical coordinates	Annual temperature (ºC)	Annual rainfall (mm)	Relative humidity (%)	Height (m)
1	Abshar Ziarat	AZ	54º 27′ 51″ E 36º 40′ 29″ N	17	600	71	1285
2	Baran Koh	BK	54º 27′ 06″ E 36º 45′ 55″ N	17	600	71	600
3	Sefid Dareh Ziarat	SDZ	54º 24′ 16″ E 36º 40′ 14″ N	17.7	649	71	1925
4	Dal Aram	DA	55º 05′ 13″ E 36º 47′ 14″ N	21	693	80	1180
5	Pooneh Aram	PA	55º 03′ 52″ E 36º 45′ 17″ N	21	693	80	1350
6	Afra Takhteh	AT	54º 58′ 22″ E 36º 47′ 58″ N	15	700	80	1600
7	Tarkat Gahan Nama	TGN	54º 23′ 39″ E 36º 51′ 58″ N	12	570	75	1600
8	Dareh Shor	DSH	55º 07′ 30″ E 36º 47′ 50″ N	21	693	80	1325
9	Shast Kola	SHK	54º 22′ 30″ E 36º 46′ 12″ N	17.7	700	80	250
10	Mohavate Pardis	MP	54º 23′ 44″ E 36º 50′ 20″ N	17.7	600	71	90

Measurement of needle morphological traits

Upon the delivery of the plant material to the laboratory, assessment of eight distinct traits about the needles of the plants was conducted across all 10 populations. The quantification of sample weight traits was accomplished through the utilization of digital scales, whereas measurements for plant size traits were undertaken using digital calipers. To increase the accuracy of each replication, a systematic approach was employed whereby the average value obtained from 10 technical replications was deemed representative of a single authentic replication.

Measurement of phytochemical factors

Preparation of methanolic extract: For the extraction of phytochemicals, a cold maceration technique was applied to the desiccated and powdered plant samples. In a concise overview, a gram of the dried powder was combined with 10 ml of an 80% methanol solvent, followed by agitation on

a shaker for 24 h. Subsequently, the resultant solution underwent filtration using filter paper and was subsequently preserved at a temperature of 4 °C until the analysis phase. This method mirrors the approach outlined by Jondoaghleboob *et al.* (2019).

Phenolic content: The determination of the phenolic content of the plant material was carried out utilizing the Folin method, as outlined by Wojdyło *et al.* (2007). In brief, 0.5 mL of the plant extract was combined with 2 mL of a 10% Folin-Ciocalteu reagent ($C_{10}H_5NaO_5S$), and the resulting mixture was incubated for a period of 5 minutes. Subsequently, 2 mL of a 5% sodium carbonate solution (Na_2CO_3) was introduced, and the amalgamation was subjected to an additional 2-hour incubation period. To quantify the phenolic content, the absorbance of the samples was measured at a wavelength of 760 nm and then compared to a control sample comprising all compounds except for the plant extract. For the establishment of a calibration curve, gallic acid ($C_7H_6O_5$) was employed as the standard ($y = 0.003x-0.0254$). The quantification of phenolic acid was expressed in mg gallic acid/ gr DM.

Flavonoids: The quantification of the flavonoid content was carried out following the methodology outlined by Kim *et al.* (2002). To provide a succinct overview, 500 µl of the methanolic extract obtained from the plant material was combined with 1.5 ml of an 80% methanol solution, 100 µl of 10% aluminum chloride ($AlCl_3$), 100 µl of potassium acetate (CH_3CO_2K) at 1 M concentration, and 2.2 ml of deionized water. The resulting amalgamation was vigorously vortexed and allowed to stand at room temperature for 40 min. Following this, the absorbance of the resultant samples was meticulously recorded at a wavelength of 415 nm and subsequently juxtaposed with a control sample lacking the plant extract. To establish the calibration curve, quercetin ($C_{15}H_{10}O_7$) was employed as the standard ($y = 0.0046x + 0.112$). The reported flavonoid content was expressed in mg quercetin/ gr DM.

Alkaloid content: The extraction and quantification of alkaloids followed the methodology detailed in the study by Jondoaghleboob (2019). Initially, a sample weighing 3 g was combined with 15 ml of ammonia in an Erlenmeyer flask and allowed to incubate for 20 min. Following this, 30 ml of chloroform was introduced, and the mixture was subjected to agitation for a span of 2.5 h on a shaker. The resultant solution underwent filtration through filter paper, and subsequently, the solution was incubated at a temperature range of 50-60 °C for a period of 20 min in order to reduce the volume of chloroform to 15 ml. The solution was then carefully poured into a decanter containing 15 ml of a

2% tartaric acid solution to facilitate the separation of the aqueous and organic solutions. The aqueous solution was isolated and then re-alkalized through the addition of 25% ammonia until reaching a pH value of 9. This alkalized solution was mixed with 20 ml of chloroform, with this process being repeated twice. The samples were subsequently incubated with 10 ml of glacial acetic acid, and after 10 min, three drops of violet crystal reagent were introduced to all samples. This addition was continued until complete dissolution of the alkaloids was achieved. The samples were subjected to titration with normal perchloric acid, and the observable transformation of the violet crystal reagent's color was noted as it transitioned from purple to blue and ultimately to green. The entirety of the procedure was conducted in accordance with the methodology outlined by Jondoaghleboob (2019).

DPPH radical scavenging percentage: The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging percentage was assessed using an adapted approach based on Wu *et al*. (2003). For the DPPH reagent preparation, 0.004 g of the substance was dissolved in 100 ml of pure methanol. Following this, a blend of the methanolic extract solution (1 ml) and DPPH reagent (1 ml) was created and left to incubate for 30 min in a dark environment at room temperature. After the incubation period, the absorbance of the sample was measured at 517 nm. A control sample, containing all reagents except for the plant extract, was employed for comparative analysis.

$I\% = \left\{\frac{\text{Ablank}-\text{Asample}}{\text{Asample}}\right\} \times 100$, A blank: control sample (mixture of 1 ml of methanol with 1 ml of

Where, Ablank: Control sample without plant extract, Asample: Plant sample, I%: Inhibition of free radicals.

Total antioxidant capacity (TAOC): To assess the total antioxidant activity, the methodology outlined by Sun *et al*. (2011) with minor adjustments was followed. The TAOC reagent was prepared by combining 28 mM sodium phosphate (Na3PO4), 4 mM ammonium molybdate (N6H24Mo7O24), and 0.6 mM sulfuric acid (H2SO4). Then, 100 µl of the methanolic extract was added to 1 ml of the TAOC reagent, and the solution was thoroughly mixed using a vortex. Subsequently, the test tubes were placed in a water bath and incubated at 95 °C for 90 min. After allowing them to cool, the absorbance of the samples was measured and juxtaposed with a control sample that lacked the plant extract. To establish the standard curve, ascorbic acid (C6H8O6) was employed as the standard, utilizing the equation y = 0.0023x - 0.028. Finally, the outcomes were quantified in mg of ascorbic acid/g plant DM.

Iron ion reducing power (FRAP): The antioxidant activity was determined based on iron reducing power using the method described by Benzie *et al*. (1996). Briefly, approximately 0.1 g of the plant tissue was dissolved in 5 ml of distilled water and homogenized. The resulting mixture was incubated at room temperature for 30 minutes and filtered using Whatman filter paper. The filtrate was stored in the dark at low temperatures until use. The FRAP reagent was prepared by combining sodium acetate (CH3COONa3H2O), glacial acetic acid, 4,2 and 6-milliopriidyl-S-triazine (TPTZ), 37% hydrochloric acid, and iron chloride (FeCl3). To initiate the assay, 50 µl of the extract was mixed with 1.5 ml of FRAP reagent and vortexed. The sample was then incubated for 40 minutes at 40 °C. The absorbance of the sample was immediately recorded at 594 nm after cooling. Ammonium ferrous sulfate ((NH4)2 Fe (SO4)2) .6H2O was used to create the standard curve (y = 0.0017x - 0.2809). Finally, the results were reported as milligrams of ferrous ions per gram of the plant's dry weight.

Taxol: To extract taxane derivatives, approximately 1 g of dried and powdered needles was soaked in 10 ml of HPLC grade methanol and shaken for 16 hours. To enhance the extraction efficiency, the samples were then treated with ultrasound for 10 minutes. The resulting extracts were filtered and defatted using 5 ml of hexane and a decanter. Following defatting, taxane derivatives such as paclitaxel in the defatted sample were sequentially extracted using dichloromethane in three steps (5 ml each). The solvent was then eliminated using a rotary evaporator. To facilitate the HPLC analysis, the concentrated extract was dissolved in 1 ml of acetonitrile and subjected to filtration using a 0.25-micron syringe filter before being analyzed (Ahadi *et al.* 2018).

To quantitatively detect and evaluate Taxol in plant samples, a pure standard Taxol solution with a concentration of 1000 ppm was first prepared by dissolving the pure powder in HPLC grade methanol to reach the desired concentration. To establish the calibration curve, standard concentrations of 10, 20, 30, 40, 50, and 100 ppm were prepared from the base solution and injected into the HPLC machine.

For the analysis, a Merck Hitachi system (model 7100) equipped with Chrome-Gate software, a LaChrom pump, and a C18 column with a particle size of 5 micrometers, a length of 150 mm, and a diameter of 4.6 mm was utilized. A mobile phase of methanol, acetonitrile, and deionized water (40:40:20) with a flow rate of 1 ml/min was used. A wavelength of 230 nm, an injection rate of 20 µl, and a column temperature of 25 °C were the other characteristics of the HPLC system used for the analysis.

Molecular analysis

***DNA extraction and polymerase chain reaction (PCR)*:** To extract DNA from adult yew leaves, the Cetyltrimethyl ammonium bromide (CTAB) buffer method proposed by Huang *et al.* (2000) was used with slight modifications. The changes applied in this method included the removal of PVP and the use of activated charcoal instead. Also, in the modified method, ascorbic acid and DIECA were not used for the DNA extraction. The extracted DNA was evaluated for quantity and quality before conducting polymerase chain reaction (PCR) using inter-simple-sequence repeats (ISSR) markers listed in Table 9. For the PCR reaction, a final volume of 12 µl was used, consisting of 5 µl of PCR master mix (YTA company – 2x concentration), 5 µl of sterile distilled water, 1 µl of ISSR primers, and 1 µl of template DNA at a concentration of 20 ng.

Statistical analysis

In this study, a nested design with three replications was used. The data were analyzed using SPSS 19 software to perform analysis of variance and to compare the means using the LSD test at a confidence level of 0.95. Additionally, factor analysis was performed for morphological traits, and correlation coefficients were calculated to determine the relationships between phytochemical and morphological traits. To analyze the DNA data, each fragment was scored as either 0 or 1 for the presence or absence of DNA fragments, respectively. Then, NTSYS pc 2.02 software was used to calculate genetic distances to draw dendrograms based on the UPGMA method, and perform principal components (PCA) analysis. Finally, GenAlex (6.51) software was used to determine the amount of polymorphism among and within populations.

Results and Discussion

Morphological diversity

There were significant differences among yew populations for all morphological traits examined, as detailed in Tables 3 and 4. These differences show that there can be a high level of diversity in terms of morphological traits in the yew populations studied. The mean value of morphological traits (Table 4) showed that the longest needles (27.53 mm) were found in the population collected from the site of Pooneh Aram. Another remarkable trait that showed a vast range of diversity in the yew populations is the number of needles at the last 10 cm of the stem, ranging from 44.44 to 67.33. Similar findings regarding variation in morphological traits have been reported in related studies. For instance, Dempsey *et al.* (1999) identified a range of needle lengths from 14 to 34 mm in their investigation. Likewise, Dempsey and Hook (2000) observed variations in needle length (ranging

from 7.7 to 22.4 mm) and width (ranging from 1.3 to 3 mm) across the studied accessions. In the case of *Taxus baccata*, Zarek (2007) noted needle lengths ranging from 15.4 to 36.6 mm and widths ranging from 1.8 to 3.1 mm. Furthermore, Vessella *et al.* (2013) reported that needle length and width in the *Taxus baccata* tree spanned from 10.6 to 21.5 mm and 1.8 to 3.6 mm, respectively. Leaf morphology holds significance as an indicator for plant taxonomy, but is influenced notably by environmental factors (Xie *et al*. 2009). Altitude, as indicated by Royer *et al.* (2008), can distinctly impact leaf size, with higher altitudes correlating to smaller leaves. Devaney *et al.* (2015) emphasized that needle morphology is more closely linked to plant size and age. Additionally, a plant's growth stage determines its photosynthetic capability, leading to variations in needle dimensions (length, width, thickness, and surface area) contingent on light exposure. Notably, young plants in low light conditions tend to exhibit larger leaf surface areas compared to those in well-lit conditions.

Table 3. Analysis of variance of morphological traits in the studied Yew populations.

Measured traits	F	P-value	df	Mean squares
Needle length	3.042	0.018	9	10.304*
Needle width	6.372	0.000	9	0.129**
Ratio of Needle length/Needle width	4.02	0.005	9	2.442**
Needle thickness	9.816	0.000	9	0.010**
Needle area	2.426	0.047	9	0.015*
Number of needles at the last 10 cm of the stem	4.993	0.001	9	153.094**
100-needle fresh weight	3.500	0.009	9	0.258**
100-needle dry weight	2.857	0.024	9	0.182*

*,**Significant at 0.05 and 0.01 probability levels, respectively.

Principal component analysis

The study employed an average of eight distinct morphological traits to conduct principal component analysis, aiming to discern the traits exerting the most significant influence on diversity. The analysis yielded three components with values surpassing 1, collectively accounting for 74.19% of the overall variance (as detailed in Table 5). The primary component emerged as the most impactful, contributing to 30.7% of the total variance. The second and third components elucidated 23.9% and 19.6% of the total variance, respectively. Within the first component, fresh weight per 100 needles, dry weight per 100 needles, and needle thickness exhibited the most pronounced positive coefficients, standing at 0.93, 0.89, and 0.83, respectively. The second component highlighted needle length and the ratio of

needle length to needle width, with coefficients of 0.86 and 0.68, respectively. As for the third component, it was associated with needle width and needle surface, characterized by coefficients of 0.92 and 0.64, respectively. Although boasting the highest positive coefficient effects, the third component played a comparatively smaller role in influencing diversity.

Table 4. Mean value of morphological traits in the studied Yew populations.

Population	Traits							
	Needle Length (mm)	**Needle width (mm)**	**Needle length/ needle width**	**Needle Thickness (mm)**	**Needle Area (cm^2)**	**Number of needles at the last 10 cm of the stem**	**100- needle fresh weight (g)**	**100-needle dry weight (g)**
AZ	26.42±0.79^{ab}	2.80±0.08^{abc}	9.42±0.26^{ab}	0.54±0.02^{a}	0.49±0.13^{a}	51.99±6.88^{ced}	1.88±0.61^{a}	1.26±0.46^{ab}
BK	23.99±2.53^{bcd}	2.88±0.29^{ab}	8.41±1.58^{bc}	0.45±0.03^{b}	0.40±0.09^{abc}	44.44±2.52^{e}	1.36±0.20^{bcd}	0.92±0.12^{bc}
SDZ	23.69±2.59^{bcd}	2.3d9±0.15^{d}	9.89±0.43^{a}	0.47±0.05^{b}	0.39±0.10^{abc}	54.99±6.06^{bcd}	1.69±0.27^{ab}	1.05±0.32^{abc}
DA	26.44±2.21^{ab}	2.60±c0.08^{cd}	10.13±0.61^{a}	0.48±0.01^{b}	0.46±0.08^{ab}	48/44±2.54^{ed}	1.64±0.12^{abc}	1.11±0.08^{ab}
PA	27.53±1.01^{a}	2.64±0.05^{c}	10.42±0.22^{a}	0.48±0.02^{b}	0.29±0.02^{c}	50.77±3.86^{ced}	1.69±0.27^{ab}	1.23±0.19^{ab}
AT	25.36±1.31^{abc}	3.03±0.10^{a}	8.35±0.43^{bc}	0.47±0.03^{b}	0.46±0.08^{ab}	58.88±7.18^{abc}	1.94±0.60^{a}	1.25±0.11^{ab}
TGN	22.58±0.79^{cd}	2.66±0.07^{bc}	8.46±0.06^{bc}	0.42±0.03^{b}	0.33±0.05^{bc}	47/55±1.95^{ed}	1.07±0.20^{d}	0.65±0.13^{c}
DSH	25.56±0.52^{bcd}	3.02±0.05^{a}	8.45±0.22^{bc}	0.47±0.00^{b}	0.52±0.03^{a}	49.33±1.45^{ed}	1.82±0.03^{a}	1.35±0.10^{a}
SHK	23.95±1.17^{bcd}	2.95±0.05^{a}	8.10±0.31^{bc}	0.31±0.02^{c}	0.44±0.03^{ab}	61.66±8.37^{ab}	1.20±0.12^{cd}	0.65±0.19^{c}
MP	21.66±3.18^{d}	2.71±0.18^{bc}	8.05±1.58^{c}	0.44±0.04^{b}	0.40±0.03^{abc}	67.33±8.18^{a}	1.75±0.24^{ab}	1.11±0.42^{ab}

Abbreviations: AZ: Abshar Ziarat, BK: Baran Koh, SDZ: Sefid Dareh Ziarat, DA: Dal Aram, PA: Pooneh Aram, AT: Afra Takhteh, TGN: Tarkat Gahan Nama, DSH: Dareh Shor, SHK: Shast Kola, MP: Mohavate Pardis; Means with different letters in each column are significantly different at 5% probability level, using the LSD test..

Principal component analysis

The study employed an average of eight distinct morphological traits to conduct principal component analysis, aiming to discern the traits exerting the most significant influence on diversity. The analysis yielded three components with values surpassing 1, collectively accounting for 74.19% of the overall variance (as detailed in Table 5). The primary component emerged as the most impactful, contributing to 30.7% of the total variance. The second and third components elucidated 23.9% and 19.6% of the total variance, respectively. Within the first component, fresh weight per 100 needles, dry weight per 100 needles, and needle thickness exhibited the most pronounced positive coefficients, standing at 0.93, 0.89, and 0.83, respectively. The second component highlighted needle length and the ratio of needle length to needle width, with coefficients of 0.86 and 0.68, respectively. As for the third component, it was associated with needle width and needle surface, characterized by coefficients of 0.92 and 0.64, respectively. Although boasting the highest positive coefficient effects, the third component played a comparatively smaller role in influencing diversity.

Table 5. Results of the principal component analysis using eight morphological traits in yew

Traits	Coefficients of the traits in principal components		
	1	**2**	**3**
Needle length	0.324	0.860	0.017
Needle width	-0.061	-0.042	0.921
Needle length / Needle width	0.290	0.688	-0.611
Needle thickness	0.831	0.222	0.140
Needle area	0.371	0.028	0.643
Number of needles at the last 10 cm of the stem	0.031	-0.752	0.109
100- needle fresh weight	0.934	-0.101	0.146
100-needle dry weight	0.898	0.014	0.132
Eigen value	2.767	2.148	1.763
Percentage of variance	30.739	23.872	19.587
Percentage of cumulative variance	30.739	54.611	74.194

Cluster analysis based on morphological characteristics

The needle's morphological attributes were leveraged to conduct cluster analysis on the yew populations, leading to the identification of five clusters within the 30 samples from 10 distinct populations (Figure 1). This analysis underscored the absence of a discernible relationship between observed morphological diversity and geographical proximity. This was evident as individuals within the same populations were situated in disparate groups or subgroups, showcasing substantial intra-population diversity in morphological traits. Similarly, analogous research has explored the morphological diversity of other plants, such as *Festuca arundinacea* and *Lavandula stachys*. In these cases, cluster analysis and principal component analysis were also employed, uncovering clusters and components wielding noteworthy impacts on diversity (Afkar *et al.* 2009). Such studies have postulated that the observed diversity might be shaped by habitat conditions or the genetics inherent to the scrutinized populations. Likewise, needle morphological diversity discerned within yew populations in this current study may be attributed to the interplay of both genetic and environmental factors, reflecting a parallel pattern of influence.

Phytochemical diversity

Table 6 presents the results of the analysis of variance for phytochemical traits among yew populations. The analysis revealed significant differences among populations for DPPH, FRAP,

TAOC, and total alkaloid content. Conversely, for traits such as taxol, total phenols, and total flavonoids, no statistically significant differences were observed among yew populations.

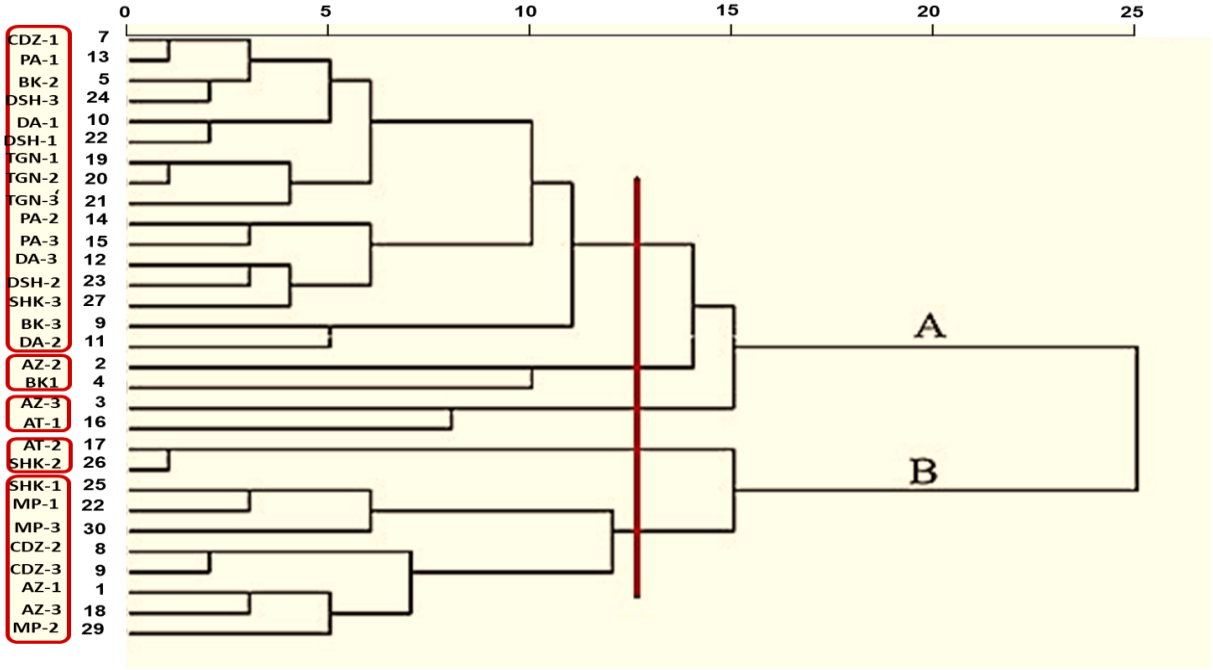

Figure 1. Dendrogram from the cluster analysis of studied yew samples using eight morphological traits; Abbreviations are mentioned in Table 1.

Table 7 displayed the range of taxol across the studied yew populations, spanning from 0.041 to 0.068 mg/g of plant dry weight, but the differences were not significant. This may be attributed to the similarity in climatic conditions across the investigated areas. Earlier research on yew plants by Ghorbanli and Delavar (2001) revealed variations in taxol concentration among different parts of the tree, with leaves and roots displaying the highest taxol content and branches having the lowest concentration. They proposed that environmental factors might influence the taxol content in different parts of the tree. Therefore, alongside genetic diversity and environmental conditions, the plant's organs can act as a third factor influencing taxol accumulation. In this study, only the taxol content in branches was examined, and no significant variation among the accessions was observed. Furthermore, the ability of Taxus species to produce taxol can also vary; for instance, Jondoaghleboob *et al.* (2019) found that the amount of taxol in the leaves of *T. brevifolia* was one-third of that of *T. baccata*. Rahmati *et al.* (2017) reported estimated taxol concentrations of 0.062 mg/g dry weight in the leaves of *T. baccata* and 0.036 mg/g dry weight in *T. brevifolia*. Among different yew plant organs, it seems that the leaves are the primary contributors to taxol production. Additional factors

influencing taxol production include plant age, light exposure, moisture levels, temperature, soil fertility, and microorganisms (Shao *et al.* 2020).

The phenol content exhibited a range from 5 to 7.9 mg of gallic acid per gram of plant dry weight, while the flavonoid content varied from 0.63 to 0.98 equivalents of quercetin per gram of plant dry weight. However, as stated above, the difference among populations was not significant for the phenol content in our experiment (Table 7). The accumulation of phenylpropanoid compounds is known to fluctuate under the influence of organ type and growing season. Dubravina *et al.* (2005) noted significantly higher phenolic compound concentrations in yew collected during summer compared to winter, with the concentrations of 46.3 and 27 mg/g dry weight, respectively. A similar trend was observed for the flavonoid content, with concentrations of 19.2 and 13 mg/g dry weight during summer and winter, respectively. Dubravina *et al.* (2005) further highlighted that phenolic and flavonoid content in yew is substantially higher during the growing season compared to winter. Similarly, Guleria *et al.* (2013) reported a phenolic content as high as 70 mg gallic acid per gram of plant dry weight in yew needles. However, research findings have reported considerable diversity in phenol and flavonoid content, partially attributable to genetics, ecological conditions (such as soil acidity, texture, mineral composition, rainfall, light, temperature, wind, altitude, slope direction, and latitude), and sampling sources (Ghasemnezhad *et al.* 2021).

The quantified alkaloid content within the studied populations displayed significant variation, with the "Pooneh Aram" population exhibiting the highest alkaloid percentage (4.52%) and "Shast Kola" recording 2.67%. Factors influencing plant alkaloid content include soil fertility and water availability, among others (Roshandel and Jamei 2015). While soil texture and organic matter (Table 1) along with precipitation (Table 2) might suggest higher alkaloid content in yew plants from "Shast Kola" compared to "Pooneh Aram," it is important to note that altitude from the sea level significantly affects secondary metabolite accumulation. Studies have indicated that the alkaloid content is directly linked to altitude and soil nitrogen and phosphorus content (Dilamghani *et al.* 2008). Therefore, altitude likely accounts for the unexpected outcomes observed in the alkaloid content of the studied populations. Results from other studies on different plant species, such as horseradish, revealed that environmental factors influencing secondary metabolite accumulation vary depending on the plant species (Shaukat Yari *et al.* 2014).

As plants constantly interact with stressors, particularly oxidative stress, various antioxidant systems become active. Consequently, relying on a single method might not suffice to accurately measure a plant's antioxidant activity (Baba *et al.* 2015). Thus, this study adopted three methods (DPPH, TAOC, and FRAP) to comprehensively assess the plant extract's ability to inhibit free

radicals. The assessment of antioxidant activity unveiled significant disparities among yew populations for all three utilized methods (Table 6). According to Table 7, DPPH free radical scavenging percentages ranged from 25.4 to 48.6%. The highest TAOC level recorded was 7.5 mg of ascorbic acid per gram of plant dry weight, while the lowest was 2.7 mg. The FRAP antioxidant activity spanned from 14.8 to 21.9 mmol of ferrous ions per gram of dry plant weight. The potential of plants to generate free radicals, interact with various stressors such as herbivores, fungi, microorganisms, salinity, drought, air pollutants, and heavy metals. Wild plants in natural environments encounter more stress agents than those cultivated in controlled conditions. Therefore, defense compound production in wild plants is typically more robust (Ghorbanzadeh *et al*. 2020). According to this perspective, observed differences in the production of chemical compounds (i.e., phenols, flavonoids, and alkaloids) as well as the capacity to inhibit free radicals among the studied populations could be influenced by the distinct environmental conditions of each habitat. It's worth noting that loamy soil texture, high acidity, and low lime percentage are associated with better antioxidant performance (Khalasi Ahvazi *et al.* 2016).

Table 6. Analysis of variance of phytochemical traits of yew populations.

Traits	F	P-value	df	Mean squares
Taxol	0.901	0.542	9	0.001
Total phenols	1.992	0.096	9	2.840
Total flavonoids	1.237	0.32	9	0.052
Total alkaloids	2.969	0.020	9	0.257*
DPPH	2.814	0.026	9	177.604*
TAOC	3.405	0.011	9	15.864*
FRAP	4.770	0.002	9	6.341**

*and **Significant at 5% and 1% levels of probability, respectively; DPPH: 2,2-diphenyl-1-picrylhydrazyl; TAOC: Total antioxidant capacity; FRAP: Iron ion reducing power.

Correlation coefficients among phytochemical traits

Total phenol content was found to be positively and significantly correlated with the flavonoid content (Table 8). One would anticipate that phenols, acting as electron donor compounds, would exhibit a positive association with antioxidant activity. However, the present study did not identify a substantial correlation between total phenol content and antioxidant activity. Several factors contribute to the absence of a direct relationship between phenolic compounds and antioxidant potential. The choice of solvent and the solubility of phenolic compounds in that solvent, the

molecular structure of phenolic compounds, polymerization of phenols, and their interactions with other compounds are pivotal determinants that can yield varying outcomes concerning the antioxidant activity of phenolic compounds (Kopjar *et al.* 2015). Adhikari *et al.* 2020) conducted a study on the chemical composition of *Taxus wallichiana*, revealing a positive correlation among total phenols, total flavonoids, biologically active compounds, altitude, and antioxidant activity. In another study on cloves, Saboora *et al.* (2013) reported a negative correlation between antioxidant activity and phenol content in cloves, even though they employed different measurement methodologies. Hence, establishing a direct relationship between the plant active compounds and antioxidant activity can prove challenging due to the intricate nature of secondary compounds present in plants. In contrast to total phenol content, the alkaloid content exhibited a positive and significant correlation with antioxidant activity using the DPPH, FRAP, and TAOC. Gan *et al.* (2017) noted a positive correlation between iron-reducing alkaloid content (FRAP) and the hydroxyl radical averting capacity.

Table 7. Mean of phytochemical traits in the studied yew populations.

Population	Taxol (mg/g)	Total phenols (mg gallic acid/g dry weight)	Total flavonoids (mg quercetin/g dry weight)	Total alkaloids (%)	DPPH (%)	TAOC (mg ascorbic acid/g dry weight)	FRAP (mg ammonium iron (Ⅱ) sulfate/g dry weight)
AZ	0.031±0.019^{a}	7.63±1.10^{a}	0.94±0.10^{a}	3.83±0.65^{abc}	30.62±11.58^{bc}	2.66±0.54^{d}	14.84±1.52^{c}
BK	0.068±0.018^{a}	6.83±1.54^{a}	0.91±0.49^{a}	3.95±0.34^{ab}	39.51±3.43^{ab}	3.86±0.58^{d}	15.17±0.30^{bc}
CDZ	0.046±0.042^{a}	6.44±1.77^{a}	0.98±0.11^{a}	4.14±0.88^{ab}	31.69±13.68^{ab}	5.24±2.21^{d}	15.45±4.10^{b}
DA	0.045±0.030^{a}	7.85±0.95^{a}	0.78±0.15^{a}	3.85±0.87^{abc}	38.61±8.74^{ab}	3.82±0.98^{cd}	16.36±0.83^{bc}
PA	0.049±0.043^{a}	5.42±0.27^{a}	0.68±0.07^{a}	4.85±0.60^{a}	48.57±6.06^{a}	2.87±0.20^{d}	15.59±1.38^{c}
AT	0.019±0.006^{a}	5.05±0.07^{a}	0.72±0.31^{a}	3.17±0.20^{bc}	33.04±2.05^{bc}	5.45±1.01^{bc}	16.3±2.57^{b}
TGN	0.014±0.025^{a}	6.9±2.84^{a}	0.63±0.15^{a}	4.23±0.44^{ab}	43.78±4.46^{ab}	4.64±0.68^{b}	19.55±1.76^{bc}
DSH	0.030±0.025^{a}	6.05±0.91^{a}	0.67±0.06^{a}	4.45±0.72^{a}	44.56±7.30^{ab}	4.69±0.75^{cd}	16.86±0.28^{bc}
SHK	0.044±0.041^{a}	7.83±0.19^{a}	0.95±0.13^{a}	2.67±0.23^{c}	25.42±3.45^{c}	7.54±1.94^{a}	21.94±5.54^{a}
MP	0.054±0.009^{a}	6.75±2.63^{a}	0.77±0.27^{a}	4.52±0.97^{a}	45.23±9.75^{ab}	5.78±0.92^{bc}	18.83±6.04^{ab}

Abbreviations: AZ: Abshar Ziarat, BK: Baran Koh, SDZ: Sefid Dareh Ziarat, DA: Dal Aram, PA: Pooneh Aram, AT: Afra Takhteh, TGN: Tarkat Gahan Nama, DSH: Dareh Shor, SHK: Shast Kola, MP: Mohavate Pardis; Means with different letters in each column are significantly different at 5% probability level using the LSD test (for all traits, except taxol content, total phenols, and tolal flavonoids, which were not significantly affected by the population type in the analysis of variance in Table 6; DPPH: 2,2-diphenyl-1-picrylhydrazyl; TAOC: Total antioxidant capacity; FRAP: Iron ion reducing power.

Cluster analysis based on the phytochemical factors

The outcomes of the cluster analysis, as depicted in Figure 2, provided insights into the shared characteristics and distinctions both among and within the investigated populations. While

geographical proximity was not the sole determinant of phytochemical diversity, the cluster analysis divided the samples into six distinct groups. Notably, samples from the same population frequently found themselves in separate groups or subgroups, underscoring significant disparities in chemical compounds across the analyzed samples. This variance in chemical compounds aligns with the findings of other studies. For instance, a phytochemical diversity investigation of essential oils from 10 *Achillea millefolium* species indicated the division of the species into three and four groups before and after flowering, respectively (Fayyaz *et al.* 2021). This study highlighted diversity among Iranian Yarrow species in terms of secondary metabolite production. Similarly, Jafarpour *et al.* (2018) assessed the phytochemical and antioxidant diversity among 18 Salvia species and observed their classification into three groups based on cluster analysis. It seems that the generation and accumulation of secondary compounds in plants are substantially influenced by various factors, including climate, soil composition, and other environmental conditions.

Table 8. Correlation coefficients among phytochemical traits of yew populations.

Trait	Taxol	Total phenols	Total flavonoids	Total alkaloids	DPPH	FRAP	TAOC
Taxol	1						
Total phenols	0.063	1					
Total flavonoids	0.128	0.523**	1				
Total alkaloids	0.189	0.275	0.111	1			
DPPH	0.191	0.263	0.24	0.875**	1		
FRAP	0.031	0.218	0.032	0.402*	0.256	1	
TAOC	0.152	0.227	0.151	0.376*	0.231	0.708**	1

*,**Significant at 5% and 1% probability levels, respectively; DPPH: 2,2-diphenyl-1-picrylhydrazyl; TAOC: Total antioxidant capacity; FRAP: Iron ion reducing power.

Genetic diversity based on ISSR markers

The current study underscored the strong reproducibility of ISSR markers in assessing the genetic diversity of yew populations, a fact supported by the substantial count of scorable DNA fragments and the high percentage of polymorphisms observed across the studied populations (Figure 3). Collectively, the study generated 335 DNA fragments from a pool of 30 samples collected across 10 populations from 10 distinct regions. Within the spectrum of markers employed, UBC835 emerged as the most prolific, yielding 99 fragments, while ISSR-15 contributed the lowest count of 45 fragments (Table 9). On average, each marker produced 67 distinct, well-defined fragments spanning a size range of 250 to 1500 bp.

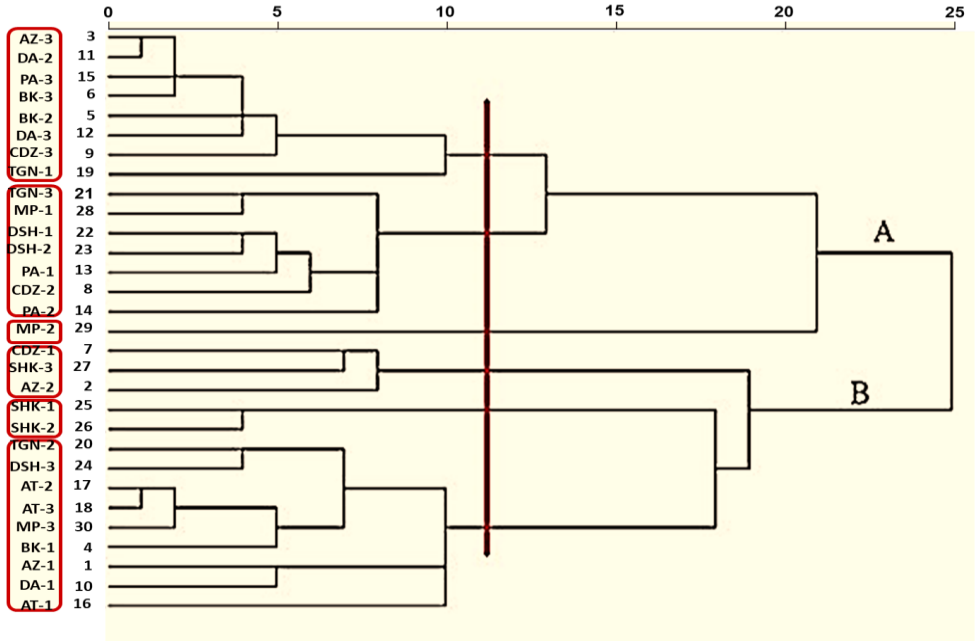

Figure 2. Dendrogram from the UPGMA cluster analysis of 30 yew samples based on the phytochemical traits; Abbreviations are mentioned in Table 1.

Polymorphism can be defined as the multiple phenotypic forms of a character attributable to the alleles of a single gene or the homologs of a single chromosome (Ahmadi *et al.* 2012). The detected polymorphism level depends on the types of primers and the genetic divergence of species (Zallaghi *et al.* 2020). The selection of ISSR primers was grounded in the previous understanding that these markers are not influenced by environmental factors in comparison to certain other morphological and biochemical markers. Moreover, a notable advantage of ISSR markers is their independence from pre-existing sequencing information, making them highly accessible for use (Rahimi *et al.* 2019; Ghorbanzadeh *et al.* 2021). Table 9 furnishes pertinent data regarding five chosen primers encompassing the number of polymorphic fragments, total fragment number, gene location, annealing temperature, and polymorphism information content (PIC). This information was provided to facilitate the comprehensive assessment of genetic diversity within yew populations.

Although possessing fewer gene loci in comparison to other primers, the ISSR-2 primer produced the greatest number of fragments, albeit accompanied by the lowest PIC. Utilizing the acquired data, the number of polymorphic gene loci for each population was computed, as outlined in Table 10.

To increase the efficiency of breeding programs, especially to reduce the number of crosses and gain a better understanding of the diversity, plant breeders usually use multivariate statistical methods such as cluster analysis and principal component analysis to group the evaluated germplasm based on the measured traits (Zare-Kohan *et al.* 2021). The outcome of the cluster analysis applied to the studied samples unveiled six distinct groups, as showcased in Figure 4. It's noteworthy that these

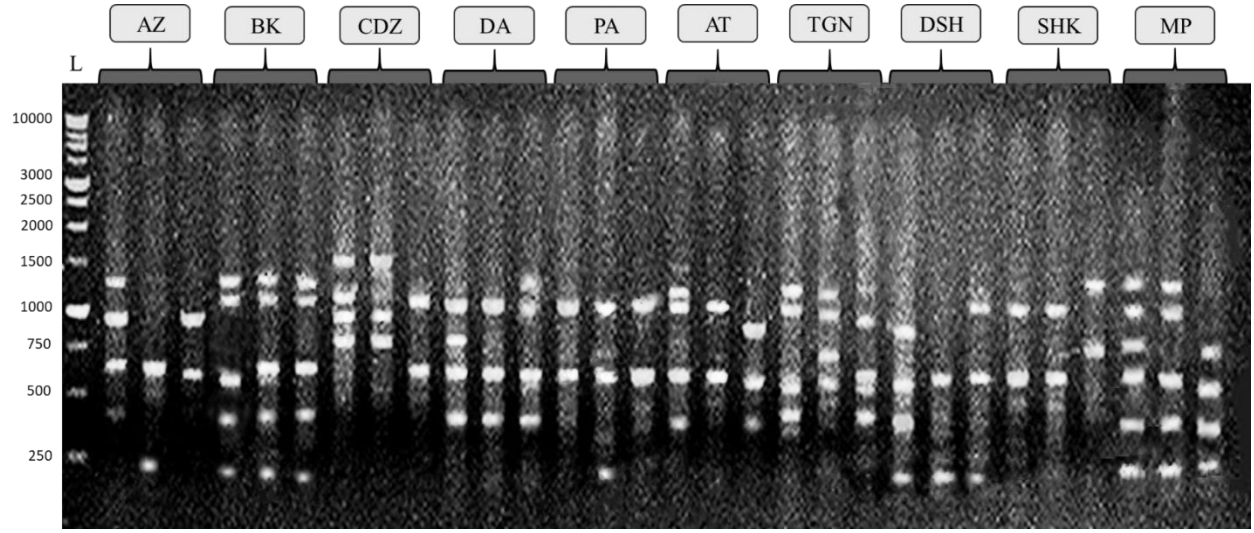

Figure 3. Band patterns of the UBC835 primer in the 1.5% agarose gel; Abbreviations are mentioned in Table 1.

Table 9. Characteristics of selected ISSR primes in the study of genetic diversity of yew populations.

Primer name	Sequence	Number of polymorphic bands	Gene loci	Number of bands	Annealing temperature	PIC
ISSR-2	GAGAGAGAGAGAGAGAGAC (Koshhal Sarmast *et al.* 2018)	3	3	80	50.2	0.159
ISSR-15	*GAGAGAGAGAGAGAGAC* (Koshhal Sarmast *et* al. 2018)	5	5	45	49.6	0.333
UBC807	AGAGAGAGAGAGAGAGT (Ghorbanzadeh *et al*. 2021)	4	4	64	54.3	0.393
UBC807	ACACACACACACACACG (Ghorbanzadeh *et al*. 2021)	5	5	47	51.9	0.324
UBC835	AGAGAGAGAGAGAGAGYC (Ghorbanzadeh *et al*. 2021)	8	8	99	54.6	0.344

PIC: Polymorphic information content

Table 10. Percentage of polymorphic gene loci in each yew population.

	Population										Average
	AZ	BK	CDZ	DA	PA	AT	TGN	DSH	SHK	MP	
Polymorphic gene loci (%)	6	8	5.33	7.33	4	6.66	8.66	8.66	8.66	4	7.20

Abbreviations are mentioned in Table 1.

results highlighted the absence of a significant relationship between molecular and geographical diversity. Intriguingly, samples procured from a single region often found themselves situated in separate groups or subgroups (Figure 4), underscoring a pronounced level of genetic divergence among the examined samples within populations. This phenomenon could be attributed to several factors, such as heightened gene flow, facilitated by easily dispersed pollen and seeds, or the presence

of microclimates within the collection zones. As reported by Koshhal Sarmast *et al.* (2018), this scenario might arise from the dioecious nature of the yew and its cross-pollination behavior, both contributing to an increased gene flow. According to Su *et al.* (2001) and Meloni *et al.* (2006), the existence of large diversity within yew populations may be caused by the existence of high degrees of gene flow and heterozygosity in the species. Another study also highlights that genetic diversity in the wild *T. baccata* population mirrors ongoing gene exchange between populations (Gargiulo *et al.* 2019). While the genetic diversity of *T. baccata* in the Hyrcanian forest stands relatively high, it is anticipated that over time, inbreeding depression could have been intensified due to the accumulation of detrimental alleles, exacerbating the process of extinction. This process might be propelled by human activities, habitat fragmentation, the age of trees, low regeneration rates, and the presence of substantial geographical barriers, which results in a considerable reduction in gene flow across habitats (Hematzadeh *et al.* 2023). On the other hand, the adaptability of *T. baccata* may be constrained by limited gene dispersal within the species (Chybicki and Oleksa 2018). However, it's crucial to acknowledge that a more in-depth exploration is required to comprehend the potential impact of microclimates on the measured characteristics.

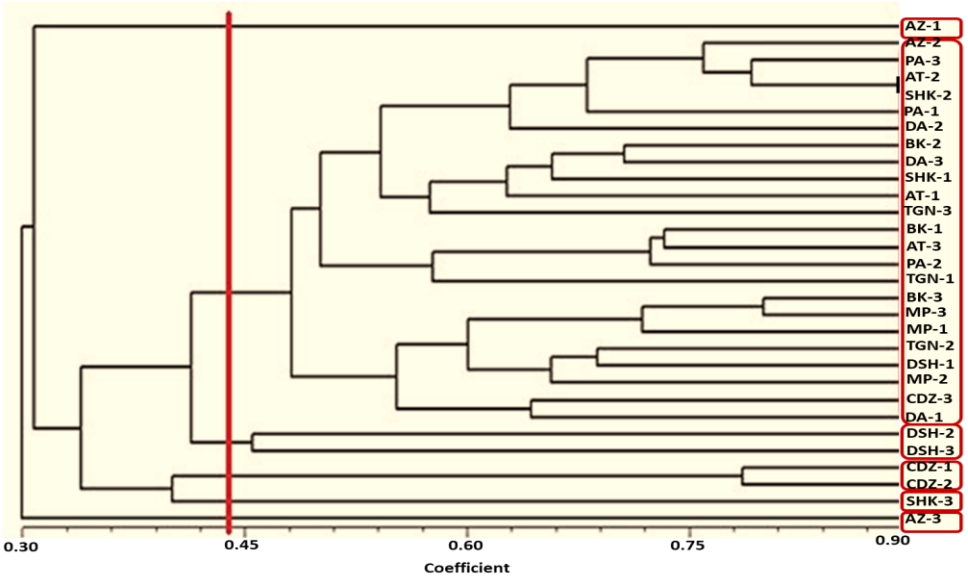

Figure 4. Dendrogram from the UPGMA cluster analysis of 30 yew samples using five ISSR markers; Abbreviations are mentioned in Table 1.

A pivotal result stemming from this study is the remarkable intra-population diversity that surfaced among the examined populations. The result of the analysis of molecular variance to determine the genetic variation among and within the populations is shown in Table 11. The overarching genetic diversity was predominantly attributed to within-population diversity,

comprising a substantial 84% as compared with the smaller fraction of 16% related to among-population diversity (Table 11). Komárková *et al*. (2022) carried out a study on the diversity of yew trees utilizing SSR markers and reached the same conclusion. They found a 7% genetic variation among the populations and a 93% genetic variation within the populations. Maroso *et al.* (2021) confirmed high genetic diversity of yew germplasm within the context of the Iberian Peninsula.

As the observed diversity in our study doesn't precisely correspond with geographical distance, it's reasonable to assume that a multitude of factors contributed to this level of diversity. These factors might encompass robust gene flow, germplasm transfer spanning regions, mixing of seeds and pollens, and the presence of heterozygosity (Aguinagalde *et al.* 2005). Chybicki *et al.* (2011) investigated the diversity of yew populations through AFLP and SSR markers, revealing a substantial level of variation. They further proposed that inbreeding could significantly differ among populations, with potential contributing factors such as establishment history (number of individuals), size, density, and the isolation level of a population. Future research endeavors should focus on enhancing genetic diversity by deploying various measures, such as mutation, migration, sexual recombination, and gene flow enhancement, to counteract the extinction trajectory faced by this endangered plant. The application of ISSR markers in this study has unveiled a good gene exchange dynamic among the studied yew populations, underscoring the pivotal role of cross-pollination in preserving the observed diversity. This can serve as the foundation for nurturing the genetic diversity of yew populations, thereby safeguarding the species for generations to come.

Table 11. Analysis of molecular variance based on ISSR markers in 10 yew populations.

Source of variation	df	Sum of squares	Mean squares	Component variance	Percentage of variance	Value	P-value
Among populations	9	51.13	5.68	0.694	16	0.162	0.002
Within populations	20	72	3.60	3.60	84		

Conclusion

This investigation has revealed a substantial level of diversity within the studied yew populations, encompassing morphological, chemical, and genetic characteristics. Cluster analysis using the ISSR markers and the morphological and phytochemical traits showed that the diversity among yew populations appeared to deviate significantly from the geographical proximity, with samples from the same population frequently diverging into separate clusters or subgroups, underscoring the marked within-population diversity. This can be attributed to the robust gene flow, heterozygosity, shared

origins, and the transfer of germplasm among the studied yew populations. These revelations cast a spotlight on the critical need to safeguard and preserve the species, particularly within Golestan province and more broadly throughout Iran, due to its endangered status. Moreover, pinpointing the unique attributes of each population, whether for utilization in diverse industries, propagation, or cultivation, could galvanize efforts to extend the species range and fend off the looming specter of extinction.

In summary, the findings gleaned from this research hold substantial ramifications for the conservation and management of yew populations in the region, signaling a clarion call for strategic actions to ensure the enduring survival of this invaluable species.

Conflict of Interest

No conflict of interest exists for all participating authors.

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