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Development and application of sex-specific indel markers for *Hippophae salicifolia* based on third-generation sequencing

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Abstract

Hippophae salicifolia, a dioecious shrub endemic to the Himalayan region, holds substantial ecological and economic value. The lack of pre-flowering morphological traits for sex identification has long impeded efficient germplasm management and breeding efforts. In this study, we utilized third-generation sequencing technology to conduct whole-genome comparative analysis of known-sex individuals, identifying insertion/deletion loci significantly associated with sex. Based on these loci, we designed a specific primer pair, Hsa09. PCR amplification results demonstrated 100% accuracy in sex differentiation, with female individuals showing a single band and male individuals exhibiting a double-band pattern. Cross-species validation revealed limited applicability of Hsa09 in closely related *Hippophae* species, suggesting notable divergence in sex determination mechanisms within the genus. This study establishes the first molecular tool for early sex identification in *H. salicifolia*, overcoming the dependence on reproductive organ development in traditional methods. It provides essential technical support for understanding the evolutionary pathways of sex chromosomes and advancing sex-regulated breeding strategies within the *Hippophae* genus.

Clinical trial number

Not applicable.

Keywords *Hippophae salicifolia*, Sex determination, Molecular markers, Sex chromosomes

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Introduction

Hippophae salicifolia D. Don (Elaeagnaceae, genus *Hippophae*) is a deciduous tree or shrub primarily distributed along the southern slopes of the Himalayas, including Gyirong County and Cuona County in southern Tibet, China, as well as in regions of India, Nepal, and Bhutan [1]. Known for its exceptional ecological adaptability, this species can withstand harsh cold temperatures and nutrient-poor soils. It is commonly found in riparian zones, slopes, and shrublands, where it plays a critical role in soil conservation and ecosystem protection [2]. As a non-leguminous woody nitrogen-fixing plant, *H. salicifolia*

forms root nodules in symbiosis with *Frankia* bacteria, facilitating atmospheric nitrogen fixation. This process significantly enhances soil fertility and overall ecosystem productivity [3, 4]. In addition, its rapid growth and strong clonal propagation capacity make it an important species for ecological restoration, vegetation rehabilitation, and desertification control. The roots, stems, leaves, flowers, and fruits of *H. salicifolia* are rich in bioactive compounds, such as flavonoids, vitamins, and superoxide dismutase (SOD), which confer considerable nutritional and medicinal value. These compounds are widely used in pharmaceuticals and nutraceuticals for treating conditions such as coronary heart disease and tumors [4, 5].

Despite its significant ecological and economic value, research on the sex determination mechanisms of *H. salicifolia* remains limited. In angiosperms, approximately 6% of species are dioecious [6, 7], a condition that is widely distributed across various phylogenetic lineages. The evolutionary origins and molecular mechanisms underlying dioecy have long been important topics in plant biology. Studies indicate that species of the genus *Hippophae* are diploid and follow an XY-type sex determination system [4, 8]. However, natural populations of *Hippophae* frequently display biased sex ratios. For example, male individuals are significantly more prevalent than female individuals in populations of *H. rhamnoides* subsp. *turkestanica* and *H. tibetana* [8–10]. This skewed sex ratio may be linked to differences in environmental adaptability between the sexes: male plants tend to exhibit greater resilience under environmental stress, whereas female plants, with higher energy and resource demands for reproduction, show comparatively weaker adaptability [7, 11]. Nevertheless, the precise factors driving this sex ratio bias—whether genetic factors in seeds or post-germination environmental adaptation—remain unclear and warrant further investigation.

Currently, the understanding of the sex ratio bias and sex determination mechanisms in *H. salicifolia* remains unclear, partly due to the lack of effective sex identification methods. In species of the *Hippophae* genus, male and female plants cannot be reliably distinguished by morphological traits before flowering, and sexual maturity typically requires 3–4 years from seedling establishment [8, 12]. Moreover, the small size of their inflorescences and short flowering periods make accurate sex identification difficult, even during the reproductive phase. These factors limit the ability to study the adaptive differences between male and female plants and the mechanisms underlying sex determination in *H. salicifolia*. Therefore, the development of rapid and accurate molecular markers for early sex identification is of paramount importance.

In recent years, molecular marker technology has been extensively applied for sex identification in dioecious plants, such as *Actinidia chinensis* [13], *Carica papaya* [14], and *Spinacia oleracea* [15]. By detecting genetic variations in sex-specific genomic regions, re-sequencing offers an effective approach for early sex determination [8, 16]. In the *Hippophae* genus, several sex-specific molecular markers have been developed; however, their applicability is often restricted to specific populations or geographic regions, which limits their generalizability and reliability [8, 16–21]. For instance, Zeng et al. developed sex-specific markers for *H. tibetana* and *H. gyantsensis* through whole-genome resequencing of distinct populations, enabling accurate sex differentiation based on PCR amplification banding patterns [8, 16]. However, these markers are species-specific and fail to cross-amplify in other *Hippophae* species, highlighting the complexity of sex chromosome evolution within the genus. This limitation may be attributed to the use of second-generation sequencing data in previous studies, where the short read lengths hindered the identification of large insertions and deletions (InDels), thereby reducing the robustness of the markers.

For long-lived woody species such as *H. salicifolia*, which require several years to reach sexual maturity, molecular markers are particularly valuable. They allow for sex identification at the seedling stage, thus optimizing cultivation strategies, improving breeding efficiency, and reducing planting costs [8, 12]. With the development of re-sequencing technologies, research on plant sex determination has entered a new phase. Whole-genome resequencing of male and female individuals allows for the precise detection of single nucleotide polymorphisms (SNPs) and InDel variations, which facilitates the development of more accurate sex-specific markers [22]. Third-generation sequencing (TGS) technologies, with their longer read lengths, lower costs, and higher accuracy, provide excellent data support for the development of sex markers in species like *Hippophae*. These technologies enable the identification of larger InDels, improving both the universality and accuracy of the markers.

In this study, we analyzed TGS data from sex-confirmed *H. salicifolia* individuals to screen and develop molecular markers capable of accurately distinguishing between male and female plants. Additionally, we have validated the cross-species applicability of these markers within the *Hippophae* genus, with the goal of creating a genus-wide sex identification toolkit. Our findings are expected to provide new insights into the sex determination mechanisms of *H. salicifolia*, advance breeding and propagation research for *Hippophae* species, and offer theoretical and technical support for ecological restoration and the development of economically important crops.

Materials and methods

Sample collection and DNA extraction

Samples of *Hippophae salicifolia* were collected from two natural populations in the Tibet Autonomous Region, China. The Lebu Valley population is located in Cuona County (CN; 27°55′25.98″ N, 91°48′49.62″ E; elevation: 3,265 m), and the Gyirong Valley population is situated in Gyirong County (JL; 28°23′56.05″ N, 85°21′52.09″ E; elevation: 2,875 m). Based on long-term field investigations conducted by our research team, the regions where these two populations are located represent the only confirmed distribution areas of *H. salicifolia* within China. Geographical coordinates and elevation data were recorded using a handheld GPS device. During fieldwork, the sex of each plant was determined based on floral morphology. To ensure genetic diversity among the samples, 18 female and 18 male individuals were randomly selected from each population, yielding a total of 72 individuals. A minimum horizontal distance of 100 m was maintained between any two sampled individuals to avoid collecting clonal ramets from the same maternal plant.

Fresh leaf tissues were rapidly desiccated in sealed containers using color-indicating silica gel and transported to the laboratory, where they were stored at -20 °C for preservation. Voucher specimens representing both sexes have been deposited in the Herbarium of the College of Ecology and Environment, Tibet University, under accession numbers LQ20240672 (female) and LQ20240673 (male). Genomic DNA was extracted from dried leaf tissues using a modified cetyltrimethylammonium bromide (CTAB) protocol [23]. DNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Samples were considered of sufficient quality if the A260/A280 ratio ranged between 1.8 and 2.0 and the A260/A230 ratio exceeded 2.0. All qualified DNA samples were stored at -20 °C for subsequent PCR amplification experiments.

Sequencing data alignment

The third-generation HiFi sequencing data for both female (SRR31560253) and male (SRR30829845) *H. salicifolia* individuals were retrieved from the NCBI SRA database (https://www.ncbi.nlm.nih.gov/), while the female reference genome was obtained separately from figshare (https://doi.org/10.6084/m9.figshare.27987659.v2). The sequencing reads were aligned to the reference genome using minimap2 v2.28 [24] with default parameters. The resulting alignments were converted to BAM format using samtools v1.19 [25] for subsequent analysis.

Identification of sex-specific loci

We followed the strategy proposed by Zeng et al. [8, 16] to identify sex-associated loci by comparing sequence alignments between male and female individuals, with a focus on detecting sex-specific InDels. Given that *H. salicifolia* follows a diploid XY sex determination system and that the reference genome was derived from a female plant, we examined alignment discrepancies between male and female samples at homologous loci. To minimize the impact of individual genetic variation—since only two individuals were sequenced—and based on previous findings suggesting that chromosome 2 may function as the sex chromosome in *Hippophae*, we focused our analysis on this chromosome. Alignment results were manually verified using the Integrative Genomics Viewer (IGV) [26], and male-specific long InDels on chromosome 2 were selected as candidate regions for primer design.

Sex-specific primer design

Flanking sequences (1,000 bp upstream and downstream) of the target variants were extracted from the reference genome using SeqKit v2.6.1 [27]. Candidate sequences were evaluated for their suitability for primer design based on the criteria proposed by Zeng et al. [8], with a focus on selecting fragments with balanced nucleotide composition and no highly repetitive elements within 500 bp of the target region. Primers were batch-designed using Primer3 v2.3.7 [28], with careful consideration to avoid mismatches, dimers, and hairpin structures. Primer specificity was confirmed by BLASTn v2.5.0 [29] against the reference genome. High-specificity primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd.

Validation of primer specificity

To validate the specificity of the primers, we initially tested them on six *H. salicifolia* individuals from two populations, including three females and three males (CN population: 2 females, 1 male; JL population: 1 female, 2 males). Primers that showed clear differentiation between sexes in this preliminary screening were subsequently tested on additional sex-confirmed individuals—specifically, six females and six males from each population (CN and JL)—to assess their consistency and applicability. Finally, primers that remained reliable in distinguishing sexes were further validated on an expanded set of samples, comprising 12 females and 12 males from each population, resulting in a total of 48 *H. salicifolia* individuals used for the final validation.

PCR reactions were performed in a 30 μL total volume, containing 1× Taq Mix (Vazyme), 50–100 ng genomic DNA, 0.3 μM of each primer, and nuclease-free water to volume. The PCR program included an initial denaturation at 95 °C for 3 min; followed by 35 cycles of 95 °C for 15 s, 58 °C for 15 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min. PCR products were separated on

Table 1 Sex-specific marker information for *H. salicifolia*. The deletion at the variant site corresponding to Hsa09 results in two products being amplified in male samples, while only one product is amplified in female samples. The product lengths, highlighted in bold, represent those generated in both male and female individuals

Marker	Primer sequences (5′ to 3′)	Tm (℃)	Product length (bp)	Variant position
Hsa09	F: CAAAATGCA-CAACTCCCTTTTTCG R: AACCACACAAGATTTTGAT-GCCAT	55	**650**|**576**	Chr02: 32,637,380

a 2% regular agarose gel (Agarose, Regular; Sangon Biotech, Order No. A620014).

Cross-species applicability testing

To evaluate primer universality within the *Hippophae* genus, PCR amplification was performed on sex-validated individuals of four species: *H. tibetana*, *H. neurocarpa*, *H. rhamnoides* subsp. *sinensis*, and *H. gyantsensis*. Detailed sampling information for each species is presented in Supplementary Table 1. A total of six male and six female individuals were randomly selected for testing in each species.

Results

Screening and preliminary validation of sex-specific markers

Through a detailed screening of long InDels variants across the *Hippophae salicifolia* genome, 59 sex-specific loci were identified on chromosome 2, exhibiting significant divergence between female and male individuals. Based on these loci, 20 candidate primer pairs were designed and subjected to preliminary screening through PCR amplification and agarose gel electrophoresis. The initial results demonstrated that two primer pairs, Hsa04 and Hsa09, showed potential for distinguishing between the sexes (Supplementary Fig. 1).

To further validate the reliability of these primers, testing was expanded to a larger cohort. The results showed that Hsa09 consistently and accurately differentiated sexes, while Hsa04 exhibited variable amplification patterns under different PCR conditions and failed to distinguish reliably between male and female plants (Supplementary Fig. 2). Detailed information regarding the design of Hsa09 is provided in Table 1. Visualization of the Hsa09-associated locus revealed complete alignment of sequencing reads from female individuals with the reference genome. In contrast, approximately 50% of male reads showed a 74-bp deletion at this locus (Fig. 1), strongly confirming the reliability of Hsa09 as a sex-specific molecular marker.

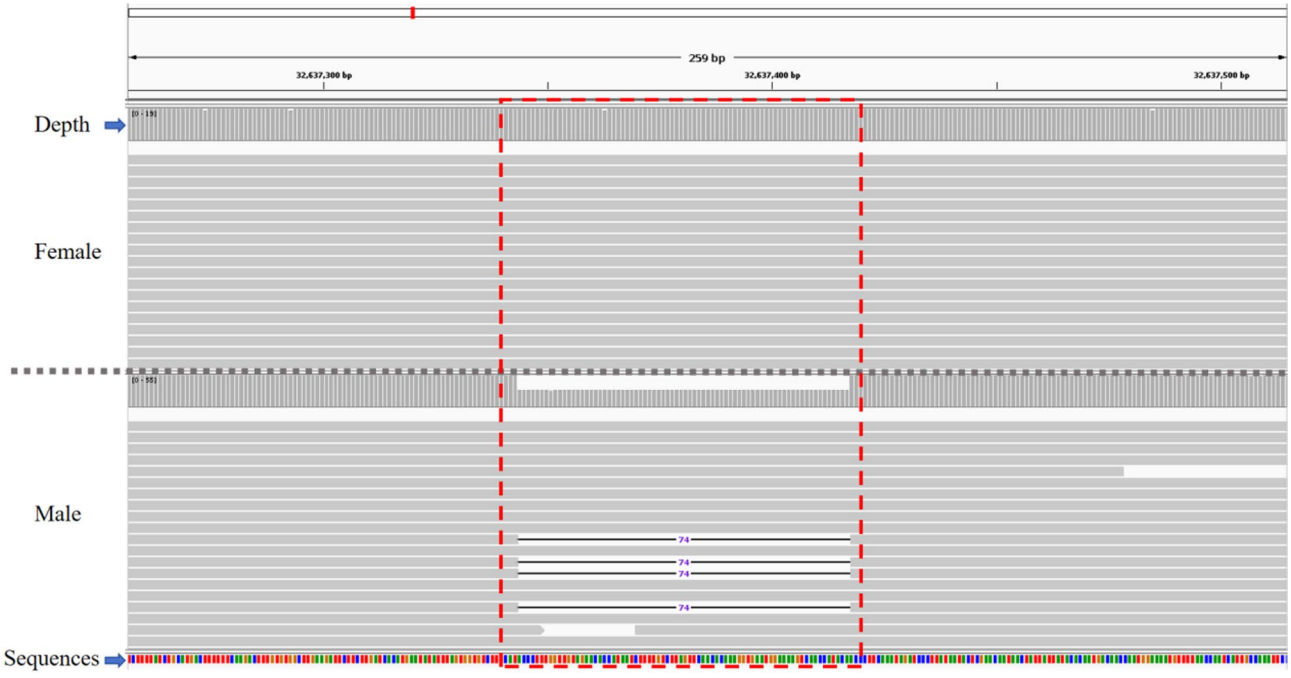

Fig. 1 Visualization of the third-generation sequencing data alignment of male and female *H. salicifolia* individuals to the reference genome chromosome 2. In the region marked by the red dashed box, all reads from the female individual align completely in the Hsa09 primer amplification area, while approximately 50% of the sequences from the male individual show a 74 bp deletion

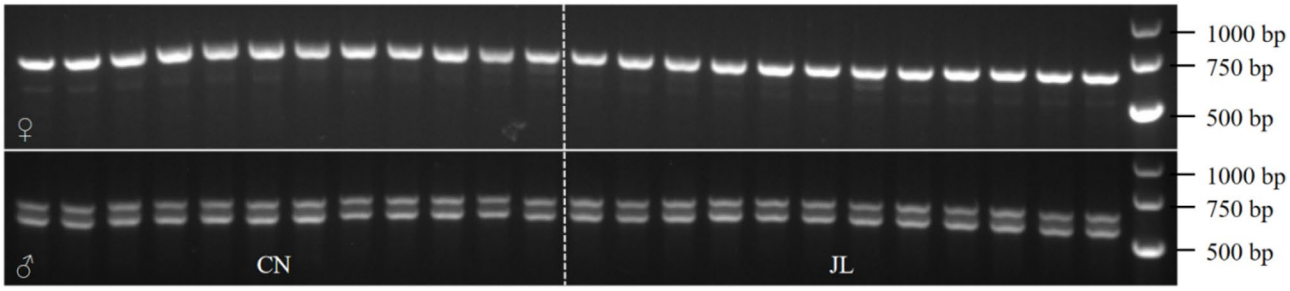

Fig. 2 PCR amplification results of Hsa09 for 48 *H. salicifolia* samples from two populations. The symbols ♀ and ♂ represent female and male samples, respectively. CN and JL represent samples collected from Cuona and Gyirong, respectively. The original gel electrophoresis images obtained using the Hsa09 primers are presented in Supplementary Fig. 3

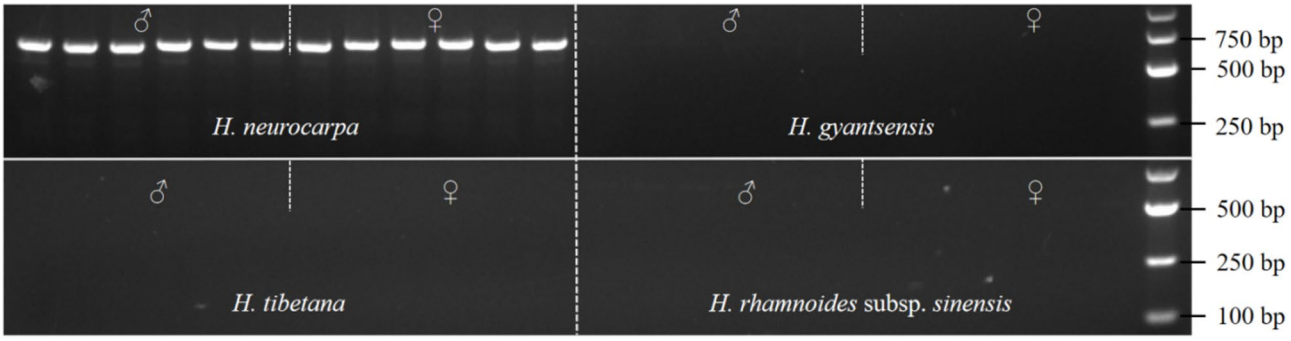

Fig. 3 PCR amplification results of Hsa09 in four other *Hippophae* species. The symbols ♀ and ♂ represent female and male samples, respectively. The original gel electrophoresis images obtained using the Hsa09 primers are presented in Supplementary Fig. 4

Validation of sex-specific marker accuracy

To comprehensively assess the applicability and accuracy of Hsa09 across two *H. salicifolia* populations, PCR amplification was performed using an expanded set of samples (24 females, 24 males), under the same conditions as those used in the preliminary screening. The results confirmed that Hsa09 could reliably distinguish between male and female individuals. In males, the primers amplified both the X and Y alleles: the X allele generated a 650-bp product, while the Y allele produced a shorter 576-bp fragment due to a 74-bp deletion. Consequently, male samples exhibited two distinct bands (650 bp and 576 bp), whereas female samples, possessing only X alleles, showed a single 650-bp band (Fig. 2).

Cross-species applicability of sex-specific primers

The cross-species applicability of Hsa09 was evaluated in four other *Hippophae* species: *H. neurocarpa*, *H. tibetana*, *H. rhamnoides* subsp. *sinensis*, and *H. gyantsensis*. Amplification succeeded only in *H. neurocarpa*, where both sexes produced a single 650-bp band, making sex differentiation impossible. No amplification occurred in the other three species (Fig. 3). These results suggest that the Hsa09 primer developed for *H. salicifolia* exhibits limited cross-species applicability within the *Hippophae* genus. This observation is consistent with the findings of Zeng et al. (2024b), further supporting the high species specificity of sex-linked markers in this group. It also suggests that sex determination mechanisms may evolve divergently among different *Hippophae* species.

Discussion

Hippophae salicifolia plays a vital ecological role in fragile regions such as the Qinghai-Tibet Plateau, contributing significantly to ecosystem protection while also holding substantial economic value. Its remarkable environmental adaptability and efficient clonal propagation make it a key species in ecological restoration, vegetation rehabilitation, and desertification control [2, 3]. Moreover, the fruit of *H. salicifolia* is rich in bioactive compounds, such as flavonoids and vitamins, highlighting its potential in the development of pharmaceuticals and nutraceuticals [4, 5]. However, as a dioecious woody species, sex identification in *H. salicifolia* presents significant challenges. Pre-flowering morphological traits are insufficient for sex differentiation, and the prolonged juvenile period (3–4 years from germination to flowering) substantially limits its application in breeding programs and industrial development [8, 12].

In this study, we utilized TGS data to identify long InDel variants distinguishing male and female *H. salicifolia* plants, leading to the development of a sex-specific molecular marker, Hsa09. Validated across a larger sample cohort, Hsa09 enables accurate and rapid sex

identification at various developmental stages. Compared to traditional methods such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP), our approach of screening genome-wide variants offers a more efficient strategy for developing sex-specific markers [6]. The advantage of long-read sequencing provided by TGS data enables the identification of larger insertions/deletions, presenting the potential to develop sex-specific markers applicable across the entire *Hippophae* genus. However, despite the initial design of 20 candidate primers, only Hsa09 proved reliable. This limitation is likely due to the small sample size of sequenced individuals, where many identified sex-specific loci may reflect inter-individual variation rather than true sex-linked differences.

The successful development of a sex-specific marker on chromosome 2 aligns with previous findings in *H. tibetana* and *H. gyantsensis* [8, 16, 30], further supporting the hypothesis that chromosome 2 harbors the sex-determining region in *Hippophae*. The evolution of plant sex chromosomes typically involves the gradual accumulation of sex-determining genes and differentiation of sex-linked regions on specific chromosomes [6]. The role of chromosome 2 in *H. salicifolia* sex determination suggests that this chromosome likely contains key sex-regulatory genes or regulatory regions. Thus, further investigation of the genomic structure and functional annotations of chromosome 2 in *Hippophae* would provide new insights into the molecular mechanisms of sex determination and contribute significantly to the study of plant sex chromosome evolution.

Despite the successful development of a reliable sex-specific marker for *H. salicifolia*, the applicability of this marker across other *Hippophae* species was limited. In our tests on *H. tibetana*, *H. rhamnoides* subsp. *sinensis*, *H. neurocarpa*, and *H. gyantsensis*, the developed primers failed to distinguish male and female plants in these species. This finding underscores the high complexity of sex determination mechanisms within the *Hippophae* genus, indicating that different species may possess distinct sex-determining genes or regulatory regions. Similar patterns have been observed in other dioecious plants. For instance, sex-specific markers in *Actinidia chinensis* and *Diospyros kaki* may work effectively in certain cultivars or populations, but they do not exhibit cross-species applicability [31, 32]. This discrepancy is likely due to differences in the evolutionary rates of sex chromosomes across species, with accelerated mutations and recombination in the sex-determining regions leading to variation in sex-specific loci. Therefore, the development of universal sex-specific markers for the entire *Hippophae* genus remains a significant challenge, requiring further comparative genomics and functional studies across different species.

The sex-specific molecular marker developed in this study holds significant promise for applications in breeding and ecological restoration. By enabling rapid and precise sex identification at the seedling stage, breeders can optimize the female-to-male ratio based on specific objectives, such as enhancing fruit yield or improving ecological resilience. For instance, increasing the proportion of female plants in fruit production systems can substantially boost yields, while prioritizing male plants in ecological restoration efforts may enhance population stress tolerance under challenging environmental conditions. Moreover, this molecular marker reduces cultivation costs and accelerates breeding efficiency. Traditional methods require years of waiting for flowering to determine sex, whereas molecular screening at the seedling stage can identify the required male and female plants early on, saving both time and resources and promoting the rapid growth of the *Hippophae* industry.

However, this study also has certain limitations. First, the species-specific nature of the developed sex-specific marker restricts its applicability across the *Hippophae* genus, limiting its potential use in genus-wide breeding programs. Second, although sex-associated loci were identified on chromosome 2, the absence of a male reference genome prevented fine mapping of the sex-determining region (SDR) and functional analysis of candidate genes. Future research could leverage high-quality male genome assemblies and transcriptomic data to further elucidate the structure and function of the sex-regulatory elements on chromosome 2. Additionally, expanding the sample size of TGS data and integrating various molecular biology and genomics techniques, along with functional gene analysis, will allow for further identification and validation of sex-determining genes. These efforts will provide new insights into the sex biology of *Hippophae* and other dioecious plants.

Conclusion

This study successfully developed Hsa09, a molecular marker for accurate sex identification in *H. salicifolia* using third-generation sequencing data, addressing the long-standing challenge of early sex identification. This marker provides a powerful tool for its application in breeding and ecological restoration. Our findings localized the sex-specific loci to chromosome 2, supporting the hypothesis that chromosome 2 may function as the sex chromosome in *Hippophae*, thereby laying the groundwork for future mechanistic studies. However, the limited cross-species applicability of this marker highlights the complexity of sex determination mechanisms within the *Hippophae* genus. The development of universal sex-specific markers remains a significant challenge. Future research should focus on expanding the sample size, incorporating multi-species genomic data,

and utilizing advanced sequencing technologies to further explore sex-determining genes or regulatory regions. These efforts will contribute to refining the theoretical understanding of sex chromosome evolution and drive innovations in *Hippophae* cultivation and ecological research.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06725-1.

Supplementary Material 1

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Author contributions
Z Z and J W: Conceptualization, Supervision, Methodology, Resources, Investigation, Validation, and Writing—original draft preparation. S Z, X T, N T, Z S, and J Z: Data collection, Formal analysis, and Data curation. W Z and L Q: Critical revision of the manuscript, Writing—review and editing.All authors read and approved the final manuscript.

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Data availability
The datasets generated and/or analysed during the current study are described in the "Materials and Methods" section of this article. The sequencing data used in this study were obtained from the NCBI SRA database https://www.ncbi.nlm.nih.gov/, and the female reference genome is available on figshare https://doi.org/10.6084/m9.figshare.27987659.v2.

Declarations

Ethics approval and consent to participate
In this study, the research samples are *H. salicifolia*, which is not listed as a protected species in China. All plant materials were collected under the approval of relevant local authorities and in strict accordance with the regulations and guidelines set forth by institutional, national, and international bodies. No specific permits were required for the collection of these samples, and the procedures used ensured compliance with all relevant legislation.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Ranjana S, Goyal A, Jena G, Tikoo K, Bansal AK, Singh IP, Jachak SM. Hippophae salicifolia D. Don, a fascinating medicinal plant: an update on its traditional medicinal uses, ethnopharmacology and phytochemistry. Curr Tradit Med. 2023;9(2):19–32.
2. Basistha BC, Sharma NP, Lepcha L, Arrawatia ML, Sen A. Ecology of Hippophae salicifolia D. Don of temperate and sub-alpine forests of North Sikkim Himalayas—a case study. Symbiosis. 2010;50:87–95.
3. Khan A, Myrold DD, Misra AK. Molecular diversity of Frankia from root nodules of Hippophae salicifolia D. Don found in Sikkim. Indian J Microbiol. 2009;49:196–200.
4. Nybom H, Ruan C, Rumpunen K. The systematics, reproductive biology, biochemistry, and breeding of sea Buckthorn—A review. Genes. 2023;14(12):2120.
5. Suryakumar G, Gupta A. Medicinal and therapeutic potential of sea Buckthorn (Hippophae rhamnoides L). J Ethnopharmacol. 2011;138(2):268–78.
6. Peng D, Wu Z. Progress on sex determination of dioecious plants. Biodivers Sci. 2022;30(3):21416.
7. Renner SS, Müller NA. Plant sex chromosomes defy evolutionary models of expanding recombination suppression and genetic degeneration. Nat Plants. 2021;7(4):392–402.
8. Zeng Z, Wang R, Wang J, Chen Y, Wang Y, Song Z, et al. Development and validation of sex-linked molecular markers for rapid and accurate identification of male and female Hippophae Tibetana plants. Sci Rep. 2024;14(1):19243.
9. Ali A, Kaul V. Seabuckthorn—a valuable resource of the cold desert (Ladakh). J Plant Dev Sci. 2012;4(2):151–5.
10. Lu J, Ma ZQ, Gao PF, Fan BL, Sun K. Changes in the Hippophae Tibetana population structure and dynamics, a pioneer species of succession, to altitudinal gradients in the Qilian mountains, China. Chin J Plant Ecol. 2022;46(5):569.
11. Razumova OV, Alexandrov OS, Bone KD, Karlov GI, Divashuk MG. Sex chromosomes and sex determination in dioecious agricultural plants. Agronomy. 2023;13(2):540.
12. Jain A, Kumar A, Sharma PC. Repertoire of molecular markers and their applications in Seabuckthorn. InThe Seabuckthorn genome 2022 (pp. 187–212). Cham: Springer International Publishing.
13. Guo D, Wang R, Fang J, Zhong Y, Qi X. Development of sex-linked markers for gender identification of Actinidia arguta. Sci Rep. 2023;13(1):12780.
14. Bui TL, Truong NT, Do TK. The application of molecular marker in Papaya sex determination: from the laboratory to the field. Sci Hortic. 2024;327:112872.
15. She H, Xu Z, Zhang H, Li G, Wu J, Wang X, et al. Identification of a male-specific region (MSR) in Spinacia oleracea. Hortic. Plant J. 2021;7(4):341–6.
16. Zeng Z, Wang J, Tian Z, Norbu N, Chen Y, Chen J, et al. Development of sex-specific molecular markers for early sex identification in Hippophae gyantsensis based on whole-genome resequencing. BMC Plant Biol. 2024;24(1):1187.
17. Sharma A, Zinta G, Rana S, Shirko P. Molecular identification of sex in Hippophae rhamnoides L. using isozyme and RAPD markers. Stud China. 2010;12:62–6.
18. Korekar G, Sharma RK, Kumar R, Meenu, Bisht NC, Srivastava RB, et al. Identification and validation of sex-linked SCAR markers in dioecious Hippophae rhamnoides L.(Elaeagnaceae). Biotechnol Lett. 2012;34:973–8.
19. Chawla A, Kant A, Stobdan T, Srivastava RB, Chauhan RS. Cross-species application of sex linked markers in H. salicifolia and H. tibetana. Sci Hortic. 2014;170:281–3.
20. Das K, Ganie SH, Mangla Y, Dar TU, Chaudhary M, Thakur RK, et al. ISSR markers for gender identification and genetic diagnosis of Hippophae rhamnoides Ssp. Turkestanica growing at high altitudes in Ladakh region (Jammu and Kashmir). Protoplasma. 2017;254:1063–77.

21. Zhou W, Wang Y, Zhang G, Luan G, Chen S, Meng J, et al. Molecular sex identification in dioecious Hippophae rhamnoides L. via RAPD and SCAR markers. Molecules. 2018;23(5):1048.
22. Varshney RK, Terauchi R, McCouch SR. Harvesting the promising fruits of genomics: applying genome sequencing technologies to crop breeding. Plos Biol. 2014;12(6):e1001883.
23. Aboul-Maaty NA, Oraby HA. Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. Bull Natl Res Cent. 2019;43(1):1–0.
24. Li H. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics. 2018;34(18):3094–100.
25. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and samtools. Bioinformatics. 2009;25(16):2078–9.
26. Thorvaldsdóttir H, Robinson JT, Mesirov JP. Integrative genomics viewer (IGV): high-performance genomics data visualization and exploration. Briefings Bioinf. 2013;14(2):178–92.
27. Shen W, Le S, Li Y, Hu F. SeqKit: a cross-platform and ultrafast toolkit for FASTA/Q file manipulation. PLoS ONE. 2016;11(10):e0163962.
28. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG. Primer3—new capabilities and interfaces. Nucleic Acids Res. 2012;40(15):e115.
29. Chen Y, Ye W, Zhang Y, Xu Y. High speed BLASTN: an accelerated megablast search tool. Nucleic Acids Res. 2015;43(16):7762–8.
30. Wang R, Wu B, Jian J, Tang Y, Zhang T, Song Z, et al. How to survive in the world's third Poplar: insights from the genome of the highest altitude Woody plant, Hippophae Tibetana (Elaeagnaceae). Front. Plant Sci. 2022;13:1051587.
31. Guo DD. Validation of Kiwifruit sex molecular markers in Actinidia arguta. J Fruit Sci. 2019;36(5):549–56.
32. Zhang PX, Yang SC, Liu YF, Zhang QL, Xu LQ, Luo ZR. Validation of a male-linked gene locus (OGI) for sex identification in persimmon (Diospyros Kaki Thunb.) and its application in F1 progeny. Plant Breeding. 2016;135(6):721–7.

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