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Evaluating Species Diversity and Distribution using Ecological Niche Modeling and Diva-GIS in Red Clover (*Trifolium pratense* L.)- A Temperate Forage Legume

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ABSTRACT

Background: *Trifolium pratense* L. (red clover) is a temperate, leguminous pasture crop cultivated in the Himalayan region, primarily as a fodder source for livestock. Its ability to fix atmospheric nitrogen and function effectively as a cover crop enables it to thrive as a companion legume in grass-legume mixtures. Beyond its local utility, red clover holds global significance for fodder security and the conservation of agro-biodiversity in the present climatic regime.

Methods: The present study employed novel ecological niche modelling (ENM) to identify ecologically suitable regions for the cultivation of red clover in India for the first time. Additionally, DIVA-GIS software was used to analyse the diversity and distribution patterns of *Trifolium pratense* L. within a defined ecological framework.

Result: The study identified 26 districts across three Indian states/Union Territories as suitable for red clover cultivation and on-farm conservation: 16 districts in Jammu and Kashmir (J and K), 6 in Himachal Pradesh and 4 in Uttarakhand. Agronomic characterization of 26 red clover accessions collected from various locations in Jammu and Kashmir revealed notable variability in several traits, including plant height (34.8-64.6 cm), leaf length (22.8-65.4 mm), leaf width (13.7-45.5 mm), floret number per flower head (30.2-74.3) and dry matter yield per plant (18.0-49.4 g). Nutritional analysis also demonstrated variation in crude protein content (20.2-23.6%), neutral detergent fibre (48.3-54.2%) and acid detergent fibre (37.2-42.8%). DIVA-GIS analysis highlighted the existence of diversity-rich pockets in the southwestern region of Jammu and Kashmir. These areas represent valuable reservoirs of genetic diversity and could play a crucial role in conservation strategies while offering insights into the taxonomy, origin and evolution of the species in the Himalayan region.

Key words: Conservation, DIVA-GIS, Diversity, Ecological niche modelling, Forage legume, Red clover.

INTRODUCTION

Forage crops are vital for livestock nutrition, offering a balanced diet that promotes animal health, growth and productivity (Karthikeyan *et al.,* 2024; Ravi *et al.,* 2024). Grasses and cereal forages serve as key energy sources, supplying the carbohydrates required for maintenance and productive functions. In contrast, leguminous fodders such as alfalfa and clover are rich in protein, playing a critical role in muscle development, milk yield and overall growth (Kumar *et al.,* 2016; Shri Rangasami *et al.,* 2024). Perennial temperate forage crops are a vital component of livestock feeding systems, supplied in fresh (cut-and-carry), dried (hay), or conserved (silage) forms due to their rich nutritional content, including proteins, carbohydrates, vitamins and minerals (Singh *et al.,* 2023). These crops are extensively grazed and can also be stored for feeding during lean periods. Legume crops, encompassing key grain, pasture and agroforestry species, play a vital role in ensuring agricultural, food, nutritional and livelihood security for hill farmers (Pandey and Kumar, 2014; Takawale *et al.,* 2016). The significance of legume crops lies in their rich nutritional profile (Jat *et al.,* 2024), characterized by high protein content (17-50%), slow-digesting carbohydrates (0.4-55%), substantial dietary fiber (3-15%) and low fat levels (0.8-6.6%). Legumes rank third in global crop production, following cereals and

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oilseeds and serve as a crucial source of food, feed and fodder across both temperate and tropical climates (Kumar, 2021). Forage legumes, in particular, play a critical role not only in meeting the mineral and vitamin needs of livestock but also in enhancing soil fertility through nitrogen fixation, thereby reducing the need for synthetic fertilizers (Frame *et al.,* 1998; Rasmussen *et al.,* 2012; Carranca *et al.,* 2015; Kamboj and Nanda, 2018). In addition, their brightly coloured flowers attract pollinators, supporting biodiversity and honey production (Nicholls and Altieri, 2013).

Trifolium is a significant genus within the legume family, comprising over 200 species, though only a few are widely cultivated (Abberton and Thomas, 2011). Among these, red clover (*Trifolium pratense*) and white clover (*T. repens*) have been extensively used to improve temperate pastures and boost soil nitrogen levels. Red clover is a key forage species found in diverse habitats such as forests, meadows, orchards, cultivated lands and wastelands. Native to Eurasia and first domesticated in Europe, it is known for moderate persistence, providing good forage yields for about 3 to 6 years in pure stands. Cytogenetically, red clover is a diploid species (2n = 2x = 14) with a heterozygous nature due to gametophytic self-incompatibility (Taylor, 1982).

Ecological niche modelling (ENM) has become an effective tool for understanding species-climate relationships and predicting potential shifts in species distribution. The MaxEnt (maximum entropy) algorithm is widely used for this purpose, offering valuable insights for cultivation planning under current and future climate scenarios (Phillips, 2006). DIVA-GIS, an open-source software, is also frequently applied in biodiversity and spatial analysis. Given the increasing global focus on food and fodder security and the conservation of genetic resources, species like *Trifolium* offer promising opportunities for sustainable agriculture in temperate and sub-temperate regions.

The present study aims to assess the ecological resilience and diversity of red clover (*Trifolium pratense* L.) in the northwestern Himalayan region of India. Using Ecological Niche Modelling and DIVA-GIS, this research evaluates 26 districts across three states/Union Territories to identify suitable areas for cultivation and promote on-farm conservation of this valuable forage legume.

MATERIALS AND METHODS

Data recording

A total of 26 accessions of Red clover collected from diverse ecological niches of Jammu and Kashmir were evaluated at ICAR- IGFRI Regional Station, Srinagar, Jammu and Kashmir (33°59′ N latitude, 74°47′ E longitude, 1640 m above mean sea level) consecutively for two years (Table 1). The recommended packages of practices were followed to raise a good crop. Sowing was done with (30 cm × 20 cm) crop geometry. Each genotype was sown in 3 rows of 3m length. The observations on six quantitative traits *viz.,* plant height (cm), leaf length (mm), leaf width (mm), floret number per flower head, 100 seed weight (mg), dry matter per plant (g) were recorded on five random plants. Nutritional parameters like crude protein % (CP), neutral detergent fibre % (NDF), acid detergent fibre % (ADF) were also analysed following standard procedures (Van soest, 1991; AOAC, 2000). Statistical analysis was done using MS Excel to work out descriptive statistics and variability patterns and Statistical Package for the Social Sciences (SPSS, version 19) for carrying out multivariate analysis of quantitative traits.

MaxEnt and DIVA-GIS analysis

MaxEnt software version 3.3.3k downloaded from www.cs.princeton.edu/~schapire/maxent was used for ecological niche modelling of red clover. Using 11 selected red clover evidence points which were surveyed during exploration missions organised by the Regional Research Station, Srinagar (J and K), ICAR- Indian grassland and fodder research institute (IGFRI), MaxEnt runs were performed. The red clover evidence points were recorded using global positioning system (Garmin GPS-12). World clim (WC) database (http://www.worldclim.org), provides interpolated global climate surfaces using latitude, longitude and altitude as independent variables and represents long-term (1950-2000) monthly means of maximum, minimum, mean temperatures and total rainfall. For future climates, we used global circulation model outputs from the Intergovernmental panel on climate change, Fourth assessment report (IPCC, 2007). Default settings were used in MaxEnt so that the complexity of the models varied depending upon the number of data points used for model fitting. The ASCI-file generated by the MaxEnt run for current and future climatic situations of red clover were imported to grid file using DIVA-GIS software version 7.5 (Hijmans *et al.,* 2012). The grid layer generated for each current and future climate was overlaid on India shapefile using DIVA-GIS and analysed. Diversity analysis of red clover plant traits was analysed using data and analysis menu bars on the DIVA-GIS window.

RESULTS AND DISCUSSION

Agronomic characterization

Mean values of various agronomic and nutritional data recorded during the present study have shown significant differences among various accessions. Significant variability was observed for various quantitative traits within the accessions studied (Table 1).

Plant height varied from 34.8 cm (IC-0622345) to 64.6 cm (IC-0615770), floret number per flower head from 30.2 (IC-0622340) to 74.3 (IC-0622358), 100 seed weight from 0.132 (IC-0622345) to 0.178 mg (IC-0622358), dry matter per plant from 18.0 g (IC-0622345) to 49.4 g (IC-0622358), leaf length from 22.82 mm (IC-0622340) - 65.42 mm (IC-0622358), leaf width varied from 13.7 mm (IC-0622340) to 45.5 mm (IC-0622358). A high coefficient of variation was

observed in leaf width (33.35), dry matter (26), leaf length (19.89), floret number per flower head (19.92), plant height (16.64) and less coefficient of variation was observed in forage quality parameters. Crude protein (%) showed a range of (20.21-23.64%), NDF (48.35-54.23) and ADF (37.17-42.78). Significant variation was also observed in 'V' marking, in some accessions, conspicuous in some whereas less prominent in others as well as absent in some (Plate 1).

Table 1: Variability observed for different traits in red clover germplasm.

Acc. no.	100 seed weight (mg)	Plant height (cm)	Floret no./ flower head	Leaf length (mm)	Leaf width (mm)	Dry matter (g)	CP (%)	NDF (%)	ADF (%)
IC-0622381	0.173	53.4	65.5	54.4	37.5	47.6	23.64	51.28	37.45
IC-0622375	0.172	62.8	74.3	46.4	23.4	47.6	21.34	50.53	39.34
IC-0615770	0.178	64.6	68.2	65.4	45.5	49.4	22.64	51.51	40.29
IC-0622364	0.164	42.5	53.6	45.5	21.4	39.5	21.33	48.39	37.17
IC-0622358	0.176	50.5	60.4	48.8	22.8	49.1	20.21	49.24	38.68
IC-0622377	0.174	54.8	45.4	57.6	38.8	48.1	23.62	49.79	38.57
IC-0615772	0.164	52.6	58.7	42.2	20.6	40.9	22.32	49.49	38.27
IC-0615781	0.156	54.8	62.5	46.8	20.4	38.5	22.21	49.35	38.28
IC-0615787	0.152	52.5	54.4	41.4	18.8	38.5	22.22	50.16	39.38
IC-0622340	0.135	37.8	45.2	28.2	13.7	23.9	22.32	52.44	41.18
IC-0622373	0.174	64.5	73.6	59.4	40.4	38.1	21.61	48.35	37.28
IC-0622374	0.162	58.6	72.4	49.8	21.7	37.1	20.72	48.39	37.17
IC-0622345	0.132	34.8	30.2	22.8	15.6	18.0	23.42	50.75	39.48
IC-0622378	0.146	45.6	50.4	43.5	23.5	21.7	23.21	49.68	38.68
IC-0622384	0.145	45.4	42.5	38.6	22.4	21.2	21.12	49.95	38.77
IC-0622408	0.148	52.4	54.6	42.2	14.4	21.3	22.43	50.49	39.27
IC-0622417	0.152	50.2	52.2	48.2	22.6	37.1	22.21	49.41	38.19
IC-0622423	0.158	55.6	58.8	43.2	19.4	38.0	21.61	49.43	38.21
IC-0615792	0.156	56.8	60.7	45.4	20.4	37.3	22.21	48.65	37.28
IC-0615793	0.146	48.2	38.5	42.6	20.2	26.4	22.62	49.39	38.17
IC-0615794	0.154	58.6	68.3	42.6	22.6	35.7	23.09	50.61	39.23
IC-0622400	0.152	56.4	73.2	43.2	22.4	35.2	22.11	50.71	39.49
IC-0622402	0.154	56.4	58.6	42.4	21.8	35.7	21.62	54.23	42.78
IC-0622335	0.148	50.8	54.4	38.6	18.5	33.7	21.31	50.19	38.97
IC-0622349	0.142	42.4	52.2	35.4	25.6	28.9	20.62	50.24	38.88
IC-0622354	0.146	45.6	48.4	39.5	30.7	30.1	21.42	49.54	39.12
Mean	0.156	50.8	56.8	44.4	24.0	35.3	22.05	50.08	38.84
Max	0.178	64.6	74.3	65.42	45.5	49.4	23.64	54.23	42.78
Min	0.132	34.8	30.2	22.82	13.7	18.0	20.21	48.35	37.17
CV (%)	8.17	16.64	19.92	19.89	33.35	26.00	4.15	2.59	3.23
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001



Plate 1: Variability of leaf size and 'V'mark in red clover.

Probably, the present study would be the first of its kind analysing the morphological variability of red clover in India. However, its genetic diversity has been reported by few researchers around the world (De vega *et al.,* 2015; Jones *et al.,* 1997). Although, a similar study was carried out with comprehensive characterization as well as comparative evaluation of white clover germplasm based on a number of agromorphological traits in mid-hills of Himachal Pradesh, India (Singh *et al.,* 2021). Climate variables play a pivotal role in determining the genetic structure of plant populations (Greene *et al.,* 2004). Dendrogram based on the average linking methods grouped 26 accessions into three clusters (Fig 1). Cluster I consist of two accessions (IC-622345 and IC-622340), cluster-II consists of 04 accessions (IC- 622377, IC-622381, IC-622373 and IC-615770), cluster-III can be grouped into sub-cluster-IIIA with 06 accessions (IC-622408, IC-622378, IC-615793, IC-622384, IC-622354 and IC-622394), cluster-III B with 14 accessions comprises of IC-622375, IC-622374, IC-622400, IC-615794, IC-622358, IC-622417, IC-622364, IC-622335, IC-622402, IC-615787, IC-622423, IC-615772, IC-615792 and IC-615781. The results are in conformity with the studies conducted in other legume crops by Goudar *et al.* (2017) and Wankhade *et al.* (2017) and Makwana *et al.* (2024).

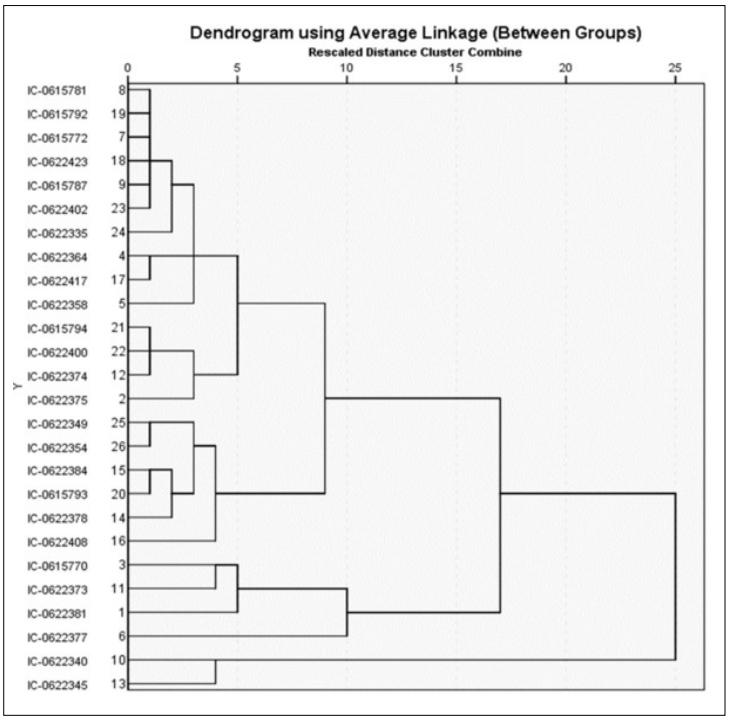


Fig 1: Dendrogram using average linkage method between groups performed on plant traits.

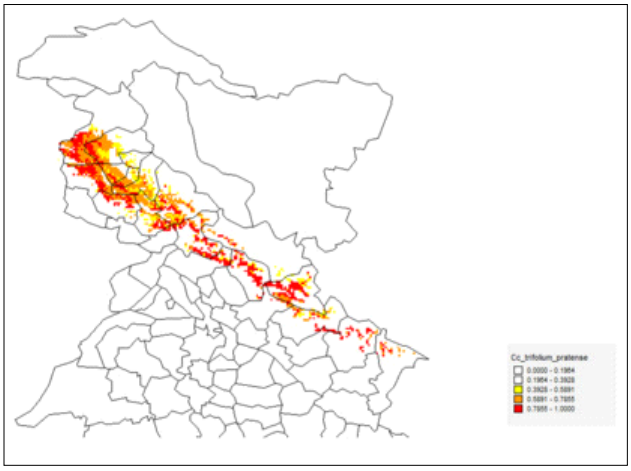


Fig 2: Ecological niche model generated for red clover (current climate).

MaxEnt analysis

We have used MaxEnt for generating Ecological Niche Models based on current and future climatic grids. Maximum entropy (Maxent) as a niche modelling approach has been used for making predictions or inferences from incomplete information. The species distribution models generated for red clover based on current and future climate grids have been presented (Fig 2 and 3) respectively. The probability distribution has been calculated as the sum of each weighted variable divided by a scaling constant to ensure that the probability value ranges from 0 to 1. The program starts with a uniform probability distribution and works in cycles adjusting the probabilities to maximum entropy. It iteratively alters one weight at a time to maximize the likelihood of reaching the optimum probability distribution. The information available about the target distribution of red clover often presents itself as a set of real-valued variables, called 'features' and the constraints are that the expected value of each feature should match its empirical average (Phillips *et al.,* 2006).

The potential regions identified for red clover genetic resource management in Kashmir valley, Himachal Pradesh and Uttarakhand have been provided (Table 2).

A total of 21 districts were identified in three states/ UTs of India covering 11 districts inthe UT of J and K, 06 in Himachal Pradesh and 04 in Uttarakhand, respectively, for cultivation and *on-farm* conservation of red clover. Table 3 gives an estimation of relative contributions of the environmental variables to the MaxEnt models of current and future climatic conditions. Bioclimatic variables *viz*., temperature annual range (bio 7), precipitation of coldest quarter (bio 19), precipitation of driest month (bio14), minimum temperature of the coldest month (bio6), precipitation of wettest quarter (bio 16) contributed maximum to the current climate model with percentage values of 46.6, 9.9, 9.3, 5.1, 5.1 respectively (Table 2). Interestingly, the niche model for future climate governed by the bioclimatic variables, Mean temperature of warmest quarter (bio 10), temperature annual range (bio 7), precipitation of wettest month (bio 13), precipitation of driest month (bio 14), precipitation of coldest quarter (bio 19) with percentage values of 33.2, 18.5, 14.2, 8.9 and 12.6 respectively.

MaxEnt is considered as the most accurate model performing extremely well in predicting occurrences with other common approaches (Hijmans and Graham, 2006), especially with incomplete information. Such modelling of red clover geographic distributions based on locality factors provides an important information on the ecology, conservation biology and invasive-species management (Welk*et al.,* 2002; Peterson and Shaw, 2003). The MaxEnt modelling approach can be used in its present form for many applications with presence-only datasets and merits further research and development (Sivaraj *et al.,* 2016). MaxEnt as a successful prediction distribution has been used by many researchers earlier, coral reefs (Tittensor *et al.,* 2009); forests (Carnaval and Moritz, 2008), rare plants (Williams *et al.,* 2009) and many other species (Elith *et al.,* 2006). The availability of detailed environmental databases, comprising ofcheap and efficient computer technology, has driven a rapid increase in predictive modelling of species environmental requirements and geographic distributions. The potential impact of climate changes on terrestrial production (especially agricultural crop) varies spatially and depends on crop-specific biophysical constraints (Kumar

Table 2: Potential regions identified for red clover (*Trifolium pratense* L.) genetic resources management.

State/UT	District
Jammu and Kashmir (J and K)	Kupwara, Poonch, Rajauri, Udhampur, Baramula, Budgam, Pulwama, Anantnag, Shopian, Kulgam, Doda, Srinagar, Kathua, Ganderbal, Baramulla, Bandipora
Himachal Pradesh	Chamba, Kangra, Kinnaur, Shimla, Lahul and Spiti, Kullu
Uttarakhand	Pithoragarh, Chamoli, Uttar Kashi, Almora

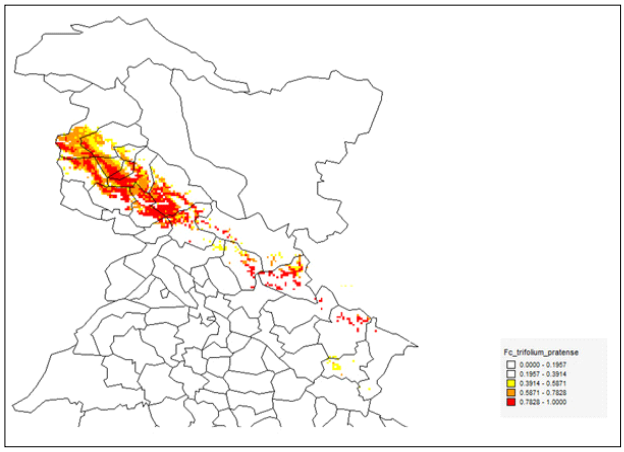


Fig 3: Ecological niche model generated for red clover (future climate).

et al., 2021). Due to increase in animal population worldwide, the demand for fodder sources would increase in the changed climate regime. As the extensive adaptation of red clover would prove vital in meeting food security, use of MaxEnt method is highly warranted in preserving the important forage species. The potential regions identified using MaxEnt analysis would be providing new insights in red clover genetic resources in Kashmir valley and in the states of Himachal Pradesh and Uttarakhand.

Table 3: Estimates of relative contributions of the environmental variables to the MaxEnt models (Current and future) for red clover (*Trifolium pratense* L.).

Variable	Per cent contribution	
	Current climate	Future climate
Temperature annual range (bio7)	46.6	18.5
Precipitation of driest month (bio14)	9.3	8.9
Mean temperature of coldest quarter (bio11)	1.5	0.2
Precipitation of wettest month (bio13)	4.0	14.2
Precipitation seasonality (bio15)	4.2	2.9
Mean temperature of wettest quarter (bio8)	1.6	1.1
Minimum temperature of coldest month (bio6)	5.1	0
Annual precipitation (bio12)	2.6	1.6
Precipitation of driest quarter (bio17)	4.2	0.9
Mean temperature of warmest quarter (bio10)	2.2	33.2
Annual mean temperature (bio1)	0.7	0
Precipitation of wettest quarter (bio16)	5.1	0.5
Mean diurnal range (bio 2)	0.7	0
Isothermality (bio 3)	2.1	0
Precipitation of coldest quarter (bio19)	9.9	12.6
Mean temperature of driest quarter (bio 9)	0.2	5.4
Precipitation of warmest quarter (bio18)	0	0
Temperature seasonality (bio 4)	0	0
Maximum temperature of warmest month (bio 5)	0	0

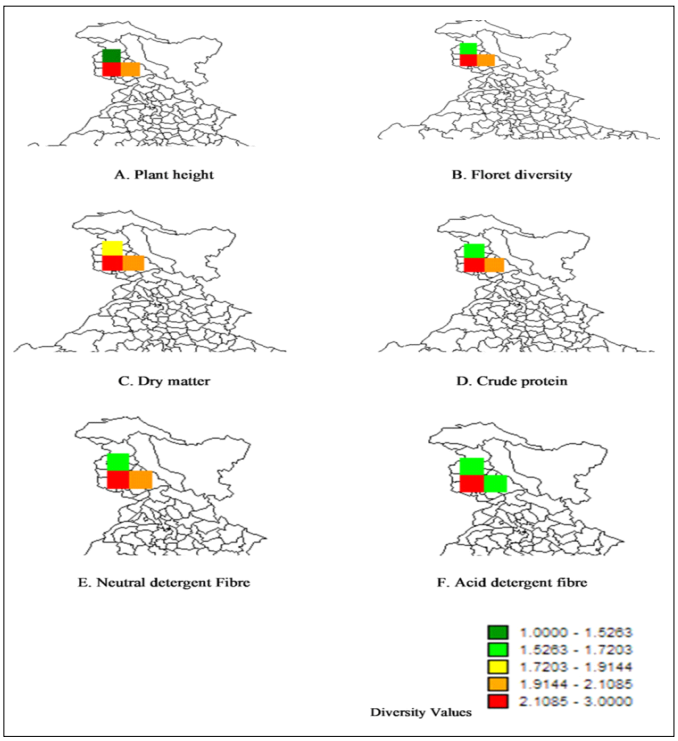


Fig 4: DIVA-GIS diversity analysis for red clover.

DIVA-GIS diversity-analysis

DIVA-GIS diversity analysis was performed for the selected plant traits *viz*., plant height, floret, dry matter, crude protein, neutral detergent fiber and acid detergent fiber (Fig 4). Diversity rich pockets exist in Anantnag, Kupwara, Badgam districts of J and K as derived from the DIVA-analysis diversity maps. High diversity values ranging from 2.1-3.0 were recorded for the phenotypic traits of red clover germplasm collected from J and K. A better perception of the red clover genetic diversity distribution is essential for its conservation and use. Hence, diversity-rich pockets are to be identified for covering maximum diversity that will help us in determining the conservation strategy and will improve our understanding of the taxonomy and the origin and evolution. The study showed a greater diversity in South-Western parts of J and K, indicating that this region is an important habitat for planning on-farm conservation. GIS mapping has been successfully used in assessing biodiversity and in identifying areas of high diversity in several crops, like *Phaseolus* bean (Jones *et al.,* 1997), wild potatoes (Hijmans and David, 2001), horsegram (Sunil *et al.,* 2008), linseed (Sivaraj *et al.,* 2009), blackgram (Babu Abraham *et al.,* 2010), medicinal plants (Varaprasad *et al.,* 2007) and agrobiodiversity (Varaprasad *et al.,* 2008).

CONCLUSION

Among the 26 accessions collected and evaluated, IC-0615770 and IC-0622358 showed superior performance across multiple agronomic and quality parameters, making them highly promising candidates for forage improvement programs. Ecological niche modelling identified 26 districts across Jammu and Kashmir (16), Himachal Pradesh (6) and Uttarakhand (4) as suitable zones for red clover cultivation under current and future climate scenarios. Notably, bioclimatic variables such as temperature annual range, precipitation of the driest month and minimum temperature of the coldest month were found to be the most influential environmental factors determining the species' distribution. DIVA-GIS-based diversity analysis revealed diversity-rich pockets in the southwestern parts of Jammu and Kashmir, particularly Anantnag, Kupwara and Budgam districts. Further molecular marker-based diversity studies (*e.g.* SSRs, SNPs) are recommended to validate and deepen our understanding of genetic diversity and population structure. Identified high-performing accessions should be evaluated under multi-location trials across varying altitudes and stress conditions (drought, cold, pests) to develop climate-resilient cultivars.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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