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Evaluation of White Clover (*Trifolium repens* L.) Germplasm for Different Agro-Morphological Traits Diversity in Mid-Himalayan Region

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ABSTRACT

White clover (*Trifolim repens* L.) is one of the most nutritious and widely grown forage legumes in the world. It is also a common component of cool season perennial grass pastures in Indian Mid-Himalayas. Being an important component of temperate grassland, diminutive efforts have been made in genetic improvement of white clover through comprehensive evaluation of germplasm collection. In present study, total 258 germplasm accessions of white clover were characterized under field conditions for the genotypic variation in twenty five agro-morphological traits. Characterized germplasm showed sufficient variation among the populations for qualitative traits, biomass yield and seed yield as revealed by range and coefficient of variation. Association analysis showed that dry matter yield was positively correlated with seed yield components traits. Principal component analysis revealed that first seven principal components accounted more than 60% of total variation. Out of 258, ten high biomass producing populations were selected and further evaluated for biomass yield, crude protein and seed yield potential. Average biomass yield of three populations *viz.,* RRCPL-13 (15.74 q/ha/year), RRCPL-19 (17.25 q/ha/year), RRCPL-27 (18.79 q/ha/year) were better than the check variety Palampur Composite-1 (14.99 q/ha/year). Results of this study will help in designing future strategy in white clover improvement program for the development of new varieties.

Key words: Exotic germplasm, Forage quality, Mid-Himalayas, Temperate grassland.

INTRODUCTION

The genus *Trifolium* commonly called clovers comprises of 237 species including annuals and perennials (Zohary and Heller 1984). Out of which, 25 species are agriculturally important which are cultivated as pasture crops (Lange and Schifino-Wittmann 2000). Among the pasture species, White clover (*Trifolium repens* L.) is one of the most nutritious and widely grown (3-4 Mha) forage legumes in the world (Mather *et al*., 1996). In India, it is a common component in cool season perennial grass pastures in Himachal Pradesh and Jammu and Kashmir. White clover is a predominant legume sown in most grazed temperate pastures as it improves forage quality *vis a vis* sustainability of grazed swards by providing up to 400 kg N/ha in annually through nitrogen fixation (Crush 1987). Apart from nitrogen fixation and high forage quality, white clover is an ideal companion legume in most grass swards due to its stoloniferous growth and phenotypic plasticity (Woodfield and Caradus 1994). Besides, it can also withstand in severe defoliation.

Being a predominant component of sub-temperate and temperate flora in India, consistence efforts have not been made towards the genetic improvement of white clover in India. Unpredictable seed yield and lack of persistence are the disadvantages associated with it (Frame and Newbould 1986). This calls for need to improve it so that farmers can adopt it. The ICAR-Indian Grassland and Fodder Research Institute maintains >900 accessions of *Trifolium* spp. including white clover (Singh *et al.,* 2019). For effective utilization of conserved germplasm in breeding programme needs to be focused for identification of valuable alleles/traits through characterization and evaluation (Cameron 1983, Singh *et al.,* 2014, Dikshit *et al.,* 2014). Therefore, the present investigation was undertaken to estimate the extent of genetic variability among the existing white clover germplasm lines which includes existing collections, old introductions (exotic collections) and others selections in order to identify useful genetic diversity for different agro-morphological traits for future breeding programme in white clover.

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MATERIALS AND METHODS

Plant materials and location of experiment

Genetic materials consisted of 258 accessions of white clover which includes 211 indigenous accessions and 47 exotic collections were taken for study. Plant materials were planted at ICAR-Indian Grassland Fodder Research Institute, Himachal Pastureland, Palampur, Himachal Pradesh (latitude 32.6°N, longitude 76.3°E, altitude 1,290 m, annual rainfall 2000- 2200 mm) in augmented block design (Federrer 1956) with 9 blocks containing 31 genotypes in each with 29 test entries and two check entries (cv. Palampur composite-1 and RRCP-L-118). Last (9th) block was represented by 24 test accessions. In each block the checks were allotted randomly. Each accession was represented by a plot size of 1×1 meter dimensions with 5 lines. Out of 258 accessions, ten high biomass producing populations were selected and planted in randomized block design with 3×3 meter plot size in three replications. The plants were spaced planted for optimal expression of traits.

Trait evaluation

White clover accessions were examined for five qualitative traits *viz.,* plant habit, leaf colour, leaflet shape, leaflet margin and corolla colour and twenty quantitative traits *viz.,* plant height (cm), mid leaflet length (cm), mid leaflet width (cm), petiole length (cm), number of stolons, stolon length (cm), number of nodes/stolon, internode length (cm), days to 50% flowering, number of heads/plant, diameter of head (cm), number of florets/head, length of floret (cm), peduncle length (cm), number of seed/floret, number of seed/head, total green fodder yield (q/ha), total dry matter yield (q/ha), 1000-seed weight (g), seed yield (q/ha). Ten high biomass producing populations were evaluated for biomass yield, crude protein and seed yield potential for two consecutive years. Forage yield was determined by harvesting of each plot at early flowering stage. A random sample of 0.50 kg biomass from each plot were dried at 65°C until the weight was constant to measure the dry matter and nitrogen content for calculation of the dry biomass yield and crude protein content in all the treatments. Crude protein was determined by applying the micro-Kjeldahl method (Eastin 1978).

Data analysis

To describe the variability among the accessions, several simple univariate statistics including means, ranges and variance were used which were obtained for each trait based on the accessions. Coefficients of variation (CV%) was also calculated from the variance components and the overall means for all the investigated traits. Clustering of accessions based on the morphological traits was carried out using ‘Fastcluster’ package of R statistical software (version 3.4.4) with squared Euclidean distance as a measure of dissimilarity and incremental sums of squares (Ward 1963) as a grouping strategy. Correlations were calculated on each trait of germplasm lines. Data of all characters were standardized to a mean of zero and variance of one and Principal component analysis (PCA) was done to investigate the importance of different traits in explaining multivariate polymorphism in white clover accessions. First, second and third principal component axes scores were plotted to aid visualization of germplasm accessions.

RESULTS AND DISCUSSION

Frequency distribution of 258 germplasm accessions of white clover based on five qualitative traits (Fig 1) and analysis of the basic data collected on the 20 continuously varying traits (Table 1) showed wide variation for most of the evaluated traits in white clover germplasm. Maggs Kölling *et al*. (2000), Prosperi *et al*. (2006) and Morris (2009) also used univariate statistics for analyzing the variability in germplasm of different crops. The results of descriptive statistical analysis showed that characters *viz*., seed yield (0.10-2.81 q/ha), dry matter yield (0.01-3.93 t/ha) and green fodder yield (0.11-32.70 t/ha) are the highly variable, plant height (1.08-15.94 cm), petiole length (0.77-13.06 cm), internode length (0.33-5.16 cm), number of heads/plant (0.64-12.35), number of seeds/head (14.58-261.60) are

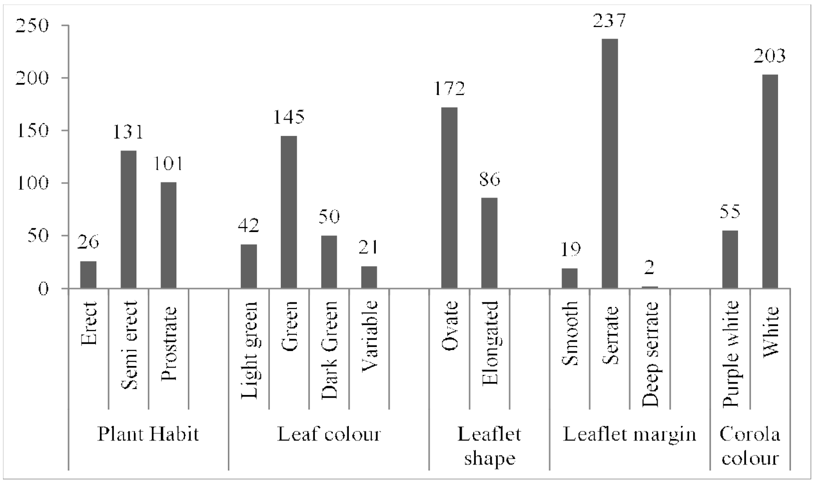

Fig 1: Frequency distribution of qualitative traits among the 258 germplasm accessions of white clover.

moderately variable and days to 50% flowering (102-141 days), 1000-seed weight (0.35-0.75 g), floret length (0.58-1.20 cm) and diameter of head (1.08-4.32 cm) are least variable traits based on their respective CV percentage value. These significant differences could be attributed to the diverse genetic composition of the population comprising of dissimilar germplasm accessions. The wide range of variation observed in agro-morphological characters offers an immense scope in evaluation and selection of desirable populations for their utilization in future white clover breeding programme. A lot of genetic variability has been reported both within and between white clover populations for biomass and seed yield contributing traits (Caradus *et al*. 1989).

The variability reported in the seed yield, green fodder yield and dry matter yield could directly be exploited for pasture improvement of mid Himalayan region. Days to 50% flowering and thousand seed weight are important traits for survival through different maturity times and initial establishment of seedling in pasture land, respectively. Therefore, introduction/collection of germplasm which are variable for these traits will enhance white clover breeding programme. Further, genetic diversity in these traits can be introgressed through wide hybridization, mutation or other genetic modification.

Correlation analysis

Pearson's correlation coefficients for twenty traits, among the possible 190 correlation combinations, 49 character pairs

Table 1: Descriptive statistics of agro-morphological traits in 258 germplasm of white clover.

Traits	Mean±SE	Variance	Kurtosis	Skewness	Range	CV%
Plant height (cm)	5.16±0.16	6.55	1.49	0.91	1.08-15.94	49.63
Leaflet length (cm)	1.04±0.02	0.08	0.19	0.70	0.48-1.92	27.01
Leaflet width (cm)	0.84±0.02	0.11	-0.07	0.11	0.15-1.80	39.07
Petiole length (cm)	4.54±0.14	5.14	1.35	1.04	0.77-13.06	49.93
Number of stolons	6.29±0.14	5.30	9.29	2.07	1.92-21.06	36.58
Stolon length (cm)	8.11±0.16	6.85	2.32	0.81	2.50-21.06	32.27
Nodes/stolon	7.02±0.16	6.43	2.53	0.97	1.34-17.28	36.10
Internode length (cm)	1.26±0.04	0.35	6.82	1.59	0.33-5.16	46.70
Days to 50% flowering	117.68±0.55	78.80	-0.55	0.12	102-141	7.54
Number of heads/plant	4.02±0.11	3.07	5.05	1.70	0.64-12.35	43.56
Diameter of head (cm)	1.97±0.02	0.15	9.18	1.59	1.08-4.32	19.40
Number of florets/head	46.33±0.83	176.69	2.55	0.81	16.00-110.09	28.69
Floret length (cm)	0.85±0.01	0.01	-0.25	-0.02	0.58-1.20	14.11
Peduncle length (cm)	8.91±0.17	7.67	1.99	0.85	2.81-18.79	31.07
Number of seeds/floret	2.20±0.04	0.50	0.25	0.26	0.16-4.56	32.06
Number of seeds/head	88.58±2.37	1454.35	1.05	0.76	14.58-261.60	43.05
1000 seed weight (g)	0.57±0.00	0.00	-0.07	-0.22	0.35-0.75	11.23
Seed yield (q/ha)	0.37±0.03	0.19	4.99	1.96	0.10-2.81	117.31
Green fodder yield (t/ha)	6.14±0.36	33.92	3.66	1.81	0.11-32.70	94.84
Dry matter yield (t/ha)	0.74±0.04	0.47	3.08	1.68	0.01-3.93	92.56

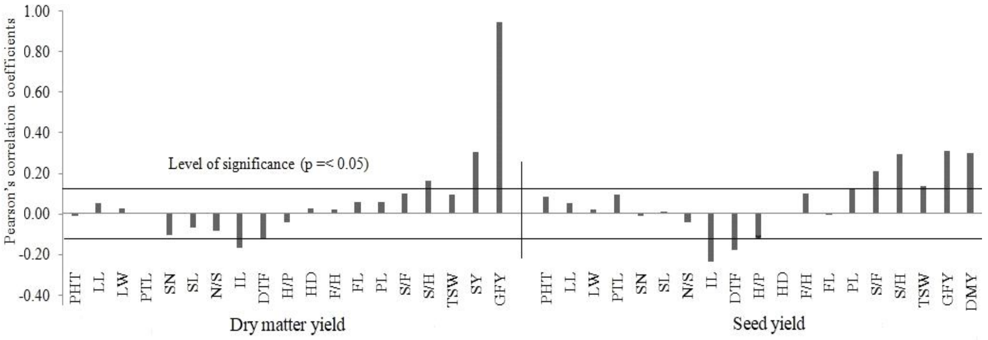

Fig 2: Pearson's correlation coefficients of dry matter yield and seed yield with agro-morphological traits among white clover germplasm accessions.

PHT= Plant height (cm), LL= Leaflet length (cm), LW= Leaflet width (cm), PTL= Petiole length (cm), SN= Number of stolons, SL= Stolon length (cm), N/S= Number of nodes/stolon, IL= Internode length (cm), DTF= Days to 50% flowering, H/P= Number of heads/plant, HD= Diameter of head (cm), F/H= Number of florets/head, FL= Floret length (cm), S/F= Number of seeds/floret, S/H= Number of seeds/head, TSW= 1000-Seed weight, SY= Seed yield (q/ha), GFY= Green fodder yield (t/ha), DMY= Dry matter yield (t/ha).

showed significant correlation either in positive or negative direction. The positive association of dry matter yield with green fodder yield, seed yield and TSW and negative association with internode length and days to 50% heading. Shivade *et al*. (2011) also reported positive association of dry matter yield and seed yield in black gram. Seed yield showed positive association with peduncle length, number of seeds per floret, number of seeds per head, green fodder yield and dry matter yield and negative association with number of heads per plant, internode length and days to 50% heading (Fig 2). The positive association between seed yield and number of florets per inflorescence was also reported by Jahufer and Gawler (2000) in white clover and Rao (2016) in groundnut. The correlations among others agro-morphological traits were also recorded. Number of stolons showed high positive association with stolon length. Plant height showed positive significant association with petiole length, leaflet length and width, peduncle length,

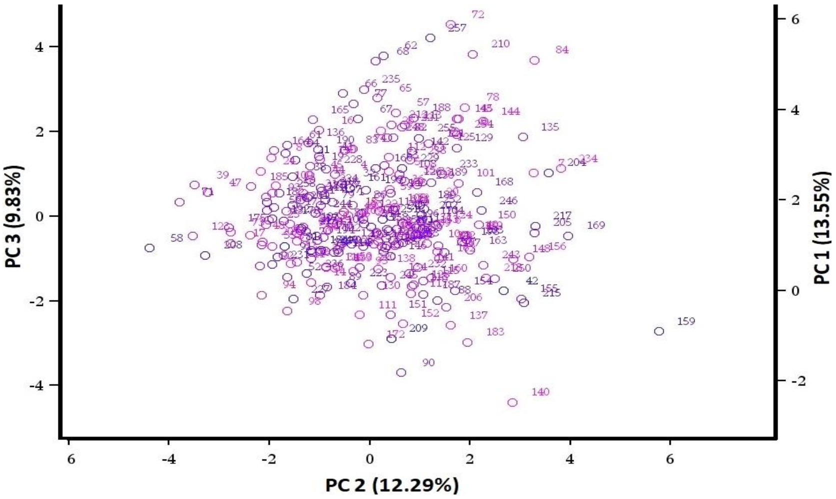

Fig 3: Scatter plot showing principal component analysis (PCA) of 258 white clover germplasm based on 20 agro-morphological traits.

Table 2: Eigen values and Eigenvectors of the important principal components (PC) for variation among the 258 germplasm accessions of white clover using 20 agro-morphological traits.

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Plant height (cm)	0.202	0.311	0.416	-0.111	-0.264	-0.067	0.063
Leaflet length (cm)	0.105	0.068	0.369	0.097	0.429	0.002	0.009
Leaflet width (cm)	0.057	0.270	-0.159	0.167	-0.224	-0.400	0.297
Petiole length (cm)	0.191	0.235	0.391	-0.162	-0.383	0.069	0.027
Number of stolons	-0.036	0.243	0.003	-0.017	0.054	0.549	-0.182
Stolon length (cm)	-0.005	0.470	-0.241	0.206	-0.124	0.120	0.028
Nodes/stolon	-0.087	0.392	-0.283	0.280	-0.038	0.179	0.073
Internode length (cm)	-0.207	0.240	-0.045	0.236	0.301	-0.038	-0.003
Days to 50% flowering	-0.162	0.044	0.006	-0.042	0.094	0.161	0.642
Number of heads/plant	-0.052	0.143	-0.021	0.097	-0.175	-0.364	-0.521
Diameter of head (cm)	0.094	0.161	0.260	0.151	0.139	-0.250	0.090
Number of florets/head	0.206	0.183	-0.318	-0.256	0.110	-0.308	-0.006
Floret length (cm)	0.100	0.118	0.294	0.161	0.347	-0.167	0.148
Peduncle length (cm)	0.168	0.233	0.106	0.011	0.066	0.287	-0.227
Number of seeds/floret	0.298	0.120	-0.113	-0.329	0.229	0.076	0.007
Number of seeds/head	0.368	0.127	-0.274	-0.333	0.280	-0.084	0.026
1000 seed weight (g)	0.121	-0.100	-0.052	-0.120	-0.325	0.087	0.315
Seed yield (q/ha)	0.362	-0.050	-0.070	-0.043	-0.063	0.166	-0.039
Green fodder yield (t/ha)	0.430	-0.205	-0.074	0.440	-0.033	0.060	0.023
Dry matter yield (t/ha)	0.428	-0.208	-0.074	0.439	-0.036	0.061	0.025
Eigen value	2.709	2.458	1.968	1.617	1.434	1.271	1.092
Percent of variance explained	13.55	12.29	9.84	8.08	7.17	6.35	5.46
Cumulative percent of total variance explained	13.55	25.83	35.67	43.76	50.92	57.28	62.74

floret length, number of seed per floret and head diameter. Petiole length showed positive association with peduncle length and number of seeds per floret and negative association with internode length. Significant correlation coefficient of dry matter yield with green fodder yield and seed yield showed that these characters may be successfully used as selection criteria in improving both forage production potential and grain yield simultaneously. Jahufer and Gawler (2000) also advocated the improvement in seed yield and biomass yield simultaneously by selecting germplasm for superior agronomic and herbage yield attributes along with high seed yield.

Principal component analysis

The principal component analysis was erective in that the first seven principal components accounted for 62.74% of the total variation among the 258 germplasm accessions of white clover (Table 2). Scatter plot of white clover germplasm (Fig 3) based on 20 agronomic traits showing that first three principal components explained about 35% of total variation. According to Johnson and Wichern (2002) it is possible to decide the importance of traits in the different principal components. Accordingly, in the first principal component, number of seeds/head, green fodder yield, dry matter yield and seed yield were the most important traits contributing to variation that explained 13.55 per cent of total variance. In the second principal component, which describe 12.29% of total variance originated mainly from stolon length, nodes/stolon, plant height and leaflet width. Similarly, the plant height, petiole length, leaflet length, number of florets/head and floret length constituted a large part of total variance among white clover germplasm explained by the third principal component. In the fourth principal component, which describe 8% of total variance originated mainly from green fodder yield, dry matter yield, number of seeds/floret and number of seeds/head.

Cluster analysis

The cluster analysis placed 258 white clover germplasm into two clusters (Fig 4). Cluster one was included 85 accessions while cluster two included remaining 173 accessions. Out of total 47 exotic accessions that was introduced from United Kingdom, 16 accessions were included in first cluster and remaining 31 were included in cluster second. Similarly, indigenous collections were also distributed in both clusters. The distribution of germplasm accessions in the study indicated that the geographical origin did not have any bearing on clustering pattern. The genotypes within the same cluster although formed specific clusters but were collected from different places, which indicated that the geographical distribution and genetic divergence did not follow the same trend. Murty and

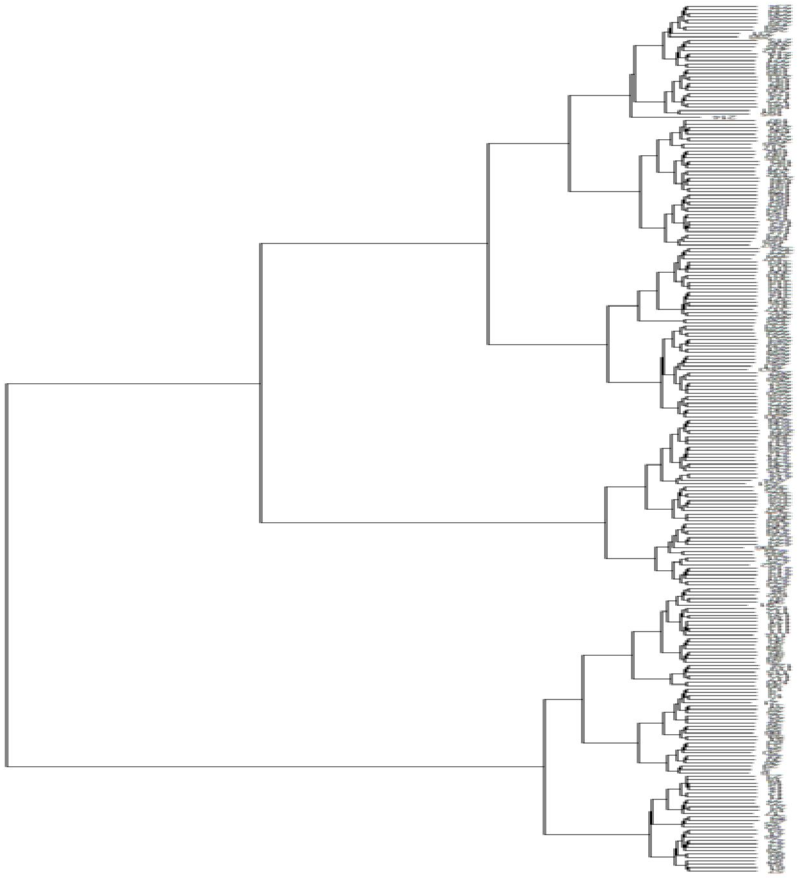

Fig 4: UPGMA cluster diagram showing relationship among 258 white clover germplasm accessions.

Table 3: Performance of ten selected white clover populations in two years.

Population	GFY (t/ha) I Yr.	GFY (t/ha) II Yr.	DMY (t/ha) I Yr.	DMY (t/ha) II Yr.	CP %	Seed yield (Kg/ha)
RRCPL 10	12.75	10.66	2.02	1.92	20.24	315.67
RRCPL 13	17.20	14.28	3.09	2.75	18.86	455.88
RRCPL 19	18.44	16.06	3.31	3.10	21.94	464.88
RRCPL 27	19.25	18.33	3.69	3.61	20.17	484.50
RRCPL 99	17.09	16.49	3.07	2.97	17.88	245.13
RRCPL 17	14.24	13.65	2.08	2.01	20.15	306.50
RRCPL 20	10.62	8.76	1.78	1.55	21.23	267.00
RRPCL 21	9.98	10.50	1.67	1.72	21.45	255.67
RRCPL 166	11.86	10.65	2.12	1.89	20.96	302.34
Palampur composite 1	15.25	14.73	2.68	2.47	19.64	362.35
CV%	8.24	12.96	8.17	12.83	4.56	7.6
LSD (5%)	2.07	2.9	0.35	0.52	1.58	45.66

GFY= Green fodder yield, DMY= Dry matter yield, CP= Crude protein.

Arunachalam (1966) also mention that genetic drift and selection in different environment could cause greater diversity than geographical distance. Further more, there was a free exchange of seed material among the different regions. As a consequence, the trait constellation might be associated with particular region and in nature loose their individuality under human interference. However, in some cases, effect of geographical origin influenced clustering in white clover (Caradus *et al.* 1989). So, geographic distribution was not the sole criterion of genetic diversity.

Evaluation of selected high biomass producing populations

Out of 258 populations, ten high biomass producing populations including Palampur Composite-1 were selected and further evaluated for two years for biomass yield, seed yield and crude protein yield (Table 3). Out of selected ten populations, three populations *viz*., RRCPL-13 (15.74 q/ha/year), RRCPL-19 (17.25 q/ha/year), RRCPL-27 (18.79 q/ha/year) were better than the check variety Palampur Composite-1 (14.99 q/ha/year) in biomass and seed yield production. The selected populations could carry the favorable alleles for biomass yield, seed yield and forage quality. Therefore these selected populations could be utilized either in varietal development programme directly or further improvement through recurrent selection.

CONCLUSION

The present investigation was conducted to carry out a comprehensive characterization as well as comparative evaluation of white clover germplasm based on 25 agro-morphological traits. On the basis of results, it can be concluded that agro-morphological traits can be effectively used for identification, documentation and grouping of white clover populations. Traits *viz.,* seed yield, dry matter yield and green fodder yield showed high variability and days to 50% flowering, 1000-seed weight, floret length and diameter of head showed least variability in the evaluated germplasm. Principal component analysis showed that out of 20 principal components, first seven components controlled 62.67% of total variability and highest contribution towards this variability is made by green fodder yield, dry matter yield, seed yield, stolon length, plant height and leaflet size. Results of multivariate analysis showed sufficient variability in biomass yield and seed yield for exploitation in varietal development programme through different breeding techniques. Variability in biomass yield was exploited through selection in form of ten high biomass producing populations. Further, selected populations meticulously evaluated and three populations *viz.,* RRCPL-13, RRCPL-19 and RRCPL-27 were selected for high biomass yield, seed yield and crude protein content. This study helps in determination of the extent of genetic diversity for different traits that are present in the currently available breeding materials to classify them into groups and to identify the appropriate germplasm. Results of this study will also help in choosing right parents based of genetic diversity for the development of new varieties. Three populations selected for high biomass, seed yield and quality could directly be tested in varietal development programme. The present collection of white clover germplasm was found deficient in high 1000-seed weight and variability in days to flowering, floret length and leaf size. This warrants addition of new germplasm accessions possessing these characteristics/ variability to the present germplasm collection. Although, diversity assessment at agro-morphological level has been done precisely but there are further scope to validate the findings through diversity analysis at molecular level as molecular markers based genetic diversity analysis helps for more effective assessment of genetic relationship among the genotypes.

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