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IDENTIFICATION OF DIFFERENT VARIETIES OF *TRIFOLIUM REPENS* L. IN NEPAL

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ABSTRACT

Trifolium repens is an important fodder crop in subtropical and temperate pastures of Nepal where it plays an important role in pasture management. It was introduced in 1860 in Nepal from England and has since establishment in and outside of such pastures. The species has been known for exhibiting phenotypical differences but no attempt has so far been made for identification of its different variations. This is the first attempt where identification of varieties of *Trifolium repens* collected from different parts of Nepal was carried out. "Flower color and leaf markings" singly or in combination was found to be the chief indicators for varietal differentiation. Morphological study of each variety of *Trifolium repens,* collected from Kirtipur, Dhunche and Chandanbari was carried out. Highest and lowest number value and average highest total value of different morphological parameters, were measured. SPSS/PC+, one way analysis of variance at 0.05 levels showed significant variations in nodule size, stipule length, petiole length, petiolule length, leaflet size, head length, floret number, peduncle length, floret length, calyx length, anther size and ovule number among eight varieties growing at Kirtipur, six at Dhunche and six at Chandanbari. The morphological evidence on the basis of above identifying phenotypic characters of *Trifolium repens,* growing at Krtipur, Dhunche and Chandanbari helps to conclude that eight varieties of *Trifolium repens* were identified using these criteria (i.e. Intra-specific diversity) in Nepal.
Keywords: Identification, Varieties, Flower color, Leaf markings

INTRODUCTION

Trifolium repens L. (white clover) is one of the most important forage crops containing high protein and minerals than any other grasses and other species of *Trifolium.* It is a widely grown legume of cool subtropical, temperate pastures and Mediterranean areas of the world and commonly used in renovation of permanent pastures lacking legumes. It fixes atmospheric nitrogen and improves feed quality. This genus contains about 240 species (Shrestha, 2002). White clover is widely variable species, and mainly based on flower color and size of flowers and leaflets. Vacek et al. (1979) compared 24 and 41 cultivars respectively for shoot and flower morphology. Norris, 1985 compared 10 varieties for flower head morphology. Caradus et al. (1989) provide a new classification system for 100 cultivars from 24 countries grown at Palmerston north on the basis of their shoot morphology, leaf markings, cyanogenesis and flowering pattern. Plants vary greatly in degree of coloration of the petals, but no study of the genetics of this variation is known. Flower color variants deserve more attention in genetic studies as they offer some potential characters as useful markers (Williams, 1987). Shrestha et al. (2002) identified eight varieties of *Trifolium repens* L. in Nepal by Electrophoretic separation of leaf protein profiles.

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Origin and dissemination in Nepal

In Nepal *Trifolium repens* was first introduced in 1860 AD during the period of the Prime Minister Jung Bahadur Rana. It was introduced as a horse feed and was brought from England. The seeds were very hard and distributed everywhere in the lawn with the horse faeces. Under favorable condition, the plants bloomed as a beautiful white colored flower above the ground like snowfall. So it became an orchard plant in the beginning and then it got established in roadside, vegetable fields and in cultivated land of the Kathmandu valley. Later on it was widely spread as a forage crop. The fodder value of white clover was later recognized and the Livestock Development Program of the Ministry of Agriculture introduced and disseminated it in different parts of the country (Shrestha, 2002). White clover

was introduced as a ground fodder for pastureland in government as well as in private dairy farms at Langtang, Dhunche, Chandanbari, Pokhara, Pansy Khola, Jumla, Solokhumbu, Nuwakot (Trishuli) and Jiri area (Pande, 1997).

Systematic position of *Trifolium repens*:

Class	- Dicot
Subclass	- Polypetalae
Series	- Calciflorae
Order	- Rosales
Family	- Leguminaceae
Sub-family	- Papilonatae
Genus	- *Trifolium*
Species	- *repens*

Description

Trifolium repens (white clover), is an allo-tetraploid, from the cross of putative parents *T. pallescenes* and *T. occidentale* (Richards, 2011). It is a perennial as well as an annual herb. It is a C_3 plant. There is a great range in size of plants and plant parts. Root system shows a typical taproot as well as adventitious nature. Nodules are present on roots. Stolons have internodes and apical bud. Leaflets are three to ten in number. Leaflets are elliptical to obovate to heart-shaped, frequently with white or light green markings of crescent shaped or V-shaped. Inflorescence is racemose or umbellate head with white to pink flowers or florets. The whitish green calyx consists of 5 sepals. Polypetalous corolla, the typical papilionaceous set of five petals comprising a larger posterior standard folded round two lateral wings petals which in turn enclose two anterior keel petals. Androecium is diadelphos. Dehiscence is introse. Carpel lies at the center of the floret. Pollen grains are almost spherical. The ovules are campylotropous. Pod is with 2-5 seeds. Seed is ovoid to reniform and yellow to reddish-brown colored (Shrestha, 2002).

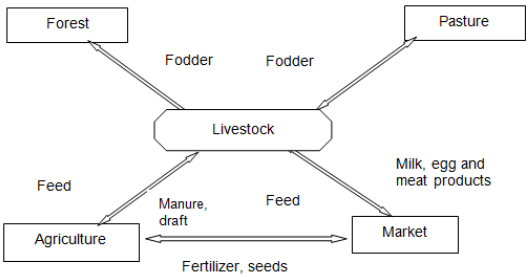
Habitat

Trifolium repens grows usually in moist places. It grows in grasslands, roadsides, open pasture, woods, especially in lawns, along trails and meadows. It has become naturalized in high hills or temperate regions of Nepal. It needs fertile soil for the growth and also grows under the canopy of trees such as *Cinnamomum camphora, Callistemon linaries and Abies spectabilis*.

Economic value of *Trifolium repens*

Livestock directly contributes to about 30% of the GDP of Nepal (FAO, 2005). Especially in the northern hills and mountain districts of Nepal, livestock makes a very important component of the livelihood system of the local populations. It provides the manure and draft power that is so essential in hill agriculture. Livestock provides milk, meat, hide, and wool. These are not only consumed by the households but are also an important source of cash income from the local market (see Fig 1). Nepal has one of the largest livestock populations of the world per arable land (Shrestha, 1992). Livestock population in Nepal is nine times larger than the carrying capacity of the land (Rajbhandari and Shah, 1981). Livestock in these areas and most part of Nepal depend on fodder from forest and grassland leading to degradation of such areas.

Fig. 1: *Farming system web in Nepal hills*:



White clover plays a very important role in well-established and properly managed pastures. Better milk production cheese, wool, meat, skin, hair, eggs, feathers, nails, horns, hoofs, muscles has been reported from cows, yaks, sheep and *chauri* grazing on white clover due to presence of higher proportion of crude protein. White clover is also a valuable constituent in swards for conventional or organic free-range systems of poultry (big sized eggs) or pig husbandry (Lampkin, 1990), deer farming (Stevens et al., 1992), daily and annual live weight gains in beef production, growing and fattening lambs and for horse or husbandry breeding enterprises.

T. repens growing in high hill regions particularly helps in soil conservation by its adventitious roots coming from the nodes of the stolon and spreading and slowing the movement of surface water, reducing runoff and holding the soil (Collins and Rhodes,

1991). On high mountains, 30% cover of clovers deposits about 100 Kg nitrogen / ha in the soil due to the nodulation by *Rhizobium trifolii* (Pueppke, 1991). These characters make clovers suitable to be used as bio-fertilizers, which enhance the fertility status of the soil (Hopkins et al., 2004; Ryoko, 2005).Their capacity for nitrogen fixation and high protein content reduces the need for external inputs of fertilizers and concentrate feeds. The high feed quality of forages legumes leads to improved manure and compost with further beneficial consequences for livestock production (Peters et al., 2001).Pure-sown or in mixture with grass, white clover is a popular cover crop used in agriculture (Miguel, 1987), road-bank seedlings and in orchards. White clover is also used as an understory to supply the nitrogen requirements of a cereal crop benefiting from both current nitrogen fixation and previously fixed nitrogen. Apart from livestock products they can also contribute to weed control and pest and disease reduction on other crops.

Flowers of the clovers are the "lovers of bees". White clover is pollinated by bees, bumble bees, butterflies, moths and flies (Love, 2004). The bees for making honey prefer the nectar produced from the flowers of clover. Importance of honey as an income-generating commodity for the rural poor and its medicinal value make it a valuable material to be promoted. Thus, white clover is important in bee keeping and it is recommended for enhancement of honey production (Crane et al., 1984). White clover is an important feed for rabbit in the production of rabbit fur or angora and rabbit meat. White clover contains higher minerals, especially sodium, chlorine, phosphorous, molybdenum and vitamin C. So, it can also be used as edible as well as medicinal purpose for human beings.

OBJECTIVE

Proper livestock and pasture / range management including introduction and management of suitable fodder / forage species is one possible method for improving the status of pastureland. Its importance is increasing further as grass/ clover swards became an economically viable and environmentally friendlier option to heavily N-fertilized grass swards**.** Improved breeds are becoming increasingly popular in hills requiring different management systems for the livestock. Popularity of improved breed and the need to reduce degradation of forest and pasture land also means need for a better management of the pasture land. One of the promising fodder / forage plant being tested in Nepal is "white clover". The objective of the study was to find out appropriate morphological characters to identify the different varieties of *Trifolium repens* for a better management of the pastureland in different parts of Nepal.

MATERIALS AND METHODS

Site selection: Kirtipur (1245-1330m) represents a subtropical location in Kathmandu district, Dhunche (1962-2012m) represents a sub-temperate to temperate and Chandanbari (3400-3500m) represents temperate to sub-alpine location in Rasuwa district, were selected as the research sites.

Morphological study: Morphological study of *Trifolium repens* collected from Kirtipur, Dhunche and Chandanbari was carried out. Six individual plants of each variety were taken for study. Highest and lowest number value and average highest total value of different morphological parameters, were measured from different varieties of *Trifolium repens* at Kirtipur, Dhunche and Chandanbari. Occulometer, Camera Lucida and Photography were also used during morphological study. The morphological study included 19 different parameters, which are shown in Table 3, 4 and 5.

Statistical analysis: SPSS/PC+, statistical software package were used to carry out one way analysis of variance at 0.05 levels among different varieties of *Trifolium repens* (Gupta and Kapoor, 1984).

RESULTS

Field observation and collections: During the general field survey, eight varieties of *Trifolium repens* from Kirtipur and six varieties each from the Dhunche and Chandanbari areas were observed and collected as shown in Table 2 and Photograph.

Identification: Various varieties collected from three different study areas were identified

based on flower color and leaf markings ('V' marking) and expressed as a combination of both characters as shown in Table 1 and Photograph.

Morphological studies: Morphological studies at Kirtipur, Dhunche and Chandanbari were tabulated in Table 3, 4 and 5, where, Analysis of variance among eight varieties of *Trifolium repens* at Kirtipur showed significant andnon-significant p-value for different parameters (Table 3). Similarly 6 varieties found in Dhunche showed significant and non-significant values for their morphological characters as shown in Table 4.Also in Chandanbari, the existing six varieties of white clover have been studied for their morphological characters as well as the variance among six varieties as given in Table 5.

Comparative study among Kirtipur, Dhunche and Chandanbari: As mentioned in field observation and collection, due to absence of two varieties of *Trifolium repens* such as Light pink colored flower with marked leaf and Light pink colored flower with non-marked leaf from Dhunche and Chandanbari, these were not included in comparative account. The significant parameters among six varieties in three sites were tabulated in Table 6. Leaflet size was the common significant parameter among six varieties of white clover growing at Kirtipur, Dhunche and Chandanbari.

Table 1: Identification of different varieties of *Trifolium repens*

Flower color	Leaf marking	Name of varieties	Symbol
White	Marked	White flower with marked leaf	WML
White	Non-marked	White flower with non-marked leaf	WNML
White with pink tinged	Marked	White with pink tinged flower and marked leaf	WPML
White with pink tinged	Non-marked	White with pink tinged flower and non-marked leaf	WPNML
Pink	Marked	Pink flower with marked leaf	PML
Pink	Non-marked	Pink flower with non-marked leaf	PNML
Light pink	Marked	Light pink flower with marked leaf	LPML
Light pink	Non-marked	Light pink flower with non-marked leaf	LPNML

Table 2: White clover varieties recorded at four locations from Kirtipur, Dhunche and Chandanbari

Location	Altitude (masl)	Variability	Total
Area at Central Department of Botany, T.U. Kirtipur (Dry, open area)	1300	1. White flower with marked leaf 2. White flower with non-marked leaf 3. White with pink tinged flower and marked leaf 4. White with pink tinged flower and non-marked leaf 5. Pink flower with marked leaf 6. Pink flower with non-marked leaf 7. Light pink flower with marked leaf 8. Light pink flower with non-marked leaf	8
Dhunche (Open area)	1962-2012	1. White flower with marked leaf 2. White flower with non-marked leaf 3. White with pink tinged flower and marked leaf 4. White with pink tinged flower and non-marked leaf 5. Pink flower with marked leaf 6. Pink flower with non-marked leaf	6
Chandanbari (Open area)	3400-3500	1. White flower with marked leaf 2. White flower with non-marked leaf 3. White with pink tinged flower and marked leaf 4. White with pink tinged flower and non-marked leaf 5. Pink flower with marked leaf 6. Pink flower with non-marked leaf	6

Table 3: Comparative morphological study among eight varieties of *Trifolium repens* at Kirtipur

Parameters	WML	WNML	WPML	WPNML	PML	PNML	LPML	LPNML	p-value
Root Length (cm)	4.17 ± 1.4	3.57 ± 1.5	3.87 ± 2.7	4.13 ± 3.8	3.03 ± 1.11	1.63 ± 0.8	3.17 ± 1.0	3.17 ± 2.9	#
Nodule No./root	4 ± 1.73	4 ± 2	8.33 ± 4.9	5 ± 3.68	3.33 ± 4.2	3.33 ± 3.5	5.67 ± 2.3	0.33 ± 0.6	#
Nodule size (sq.mm)	1.73 ± 1.0	1.04 ± 0.9	1.50 ± 0.8	0.38 ± 0.1	0.22 ± 0.22	0.62 ± 0.5	0.32 ± 0.0	0.01 ± 0.0	0.0198 *
Int stolon Length (cm)	2.63 ±0.3	2.43 ± 1.0	1.7 ± 1.1	1.97 ± 0.1	2.27 ± 0.12	1.27 ± 0.3	1.66 ± 0.3	1.8 ± 0.6	#
Int stolon Breadth (cm)	0.21 ±0.0	0.22 ± 0.1	0.15 ± 0.1	0.18 ± 0.01	0.15 ± 0.1	0.17 ± 0.03	0.15 ± 0.0	0.18 ± 1.1	#
Stipule Length (cm)	0.73 ±0.1	0.93 ± 0.1	0.67 ± 0.1	0.63 ± 0.11	0.9± 0.1	0.63 ± 0.1	0.72 ± 0.1	1 ± 0	0.0002 *
Petiole Length (cm)	8.6 ± 0.6	8.77 ± 2.6	10.7 ± 1.3	6.87 ± 1.6	6.5 ± 0.71	1.77 ± 0.31	7.57 ± 2.3	7.67 ± 0.9	0.0002 *
Petiole Breadth (cm)	0.1 ± 0	0.12 ± 0.0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.08 ± 0.01	0.09 ± 0.0	0.1 ± 0	#
Petiole Length (cm)	0.16 ±0.01	0.10 ± 0.0	0.11±0.01	0.1 ± 0	0.15 ± 0	0.06 ± 0.01	0.15 ± 0	0.1 ± 0	0.0001 *
Leaflet size (sq.mm)	1.40 ±0.3	2.04 ± 0.8	1.46 ± 0.4	0.76 ± 0.1	0.99 ± 0.2	0.24 ± 0.01	1.22 ± 0.3	1.20 ± 0.3	0.0018 *
Head Length (cm)	1.13 ±0.13	1.27 ± 0.4	0.77 ± 0.3	1.13 ± 0.2	1.53 ± 0.52	0.8 ± 0.2	1.07 ± 0.1	1.4 ± 0.1	0.0347 *
Head Breadth (cm)	1.13 ±0.4	1.77 ± 0.2	1.37 ± 0.6	1.23 ± 0.5	1.47 ± 0.4	1.1 ± 0.1	1.13 ± 0.2	1.3 ± 0.4	#
Floret No. per head	43.67 ±5.1	63.67 ± 8	37.33 ±4.0	50.7 ± 8.1	42.33 ± 1.5	37.67 ±11	36.33 ± 13	54.67 ±10	0.0109 *
Peduncle Length (cm)	12.53± 1.5	13.23 ±3.7	13.23 ±4.7	10.83 ± 1.6	13.53 ± 2.8	4.97 ± 1.1	14.07 ± 3	16.9 ±1.2	0.0043 *
Peduncle Breadth (cm)	0.14 ± 0.04	0.76 ± 1.1	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.13 ± 0.01	0.10 ± 0.0	0.12 ±0.0	#
Floret Length (cm)	0.63 ± 0.04	0.58 ±0.0	0.55 ± 0.1	0.47 ± 0.0	0.5 ± 0	0.53 ± 0.1	0.38 ± 0.0	0.58 ±0.0	0.0001 *
Calyx Length (cm)	0.43 ± 0.04	0.42 ±0.1	0.35 ± 0.1	0.3 ± 0.0	0.37 ± 0.0	0.38 ± 0.1	0.3 ± 0	0.5 ± 0	0.0009 *
Anther size (sq.mm)	0.14 ± 0.1	0.17 ±0.0	0.15 ± 0.0	0.13 ± 0.0	0.07 ± 0.0	0.16 ± 0.01	0.13 ± 0.0	0.06 ±0.0	0.0003 *
Ovule No, per floret	4 ± 0	4.33 ±1.5	4 ± 0	4 ± 0	3.33 ± 1.2	5.33 ± 0.6	1.67 ± 0.6	3.33 ±1.2	0.0051 *

Table 4: Comparative morphological study of six varieties of *Trifolium repens* at Dhunche

Parameters	WML	WNML	WPML	WPNML	PML	PNML	p-value
Root length (cm)	4.73 ± 2.45	4.9 ± 2.69	5.2 ± 5.48	4.93 ± 4.22	4.67 ± 4.62	2.83 ± 1.04	#
Nodule/root	8 ± 2.65	6 ± 4.58	1.33 ± 1.16	15 ± 16.46	3.67 ± 3.79	5.33 ± 3.06	#
Nodule size (sq. mm)	0.53± 0.37	0.9 ± 0.59	0.67 ± 0.72	1.01 ± 1.2	0.48 ± 0.54	0.77 ± 0.41	#
Int stolon length (cm)	1.33 ± 0.29	1.87 ± 0.64	2.27 ± 0.99	1.73 ± 0.40	1.87 ± 0.32	0.73 ± 0.25	#
Int stolon breadth (cm)	0.16 ± 0.03	0.18 ± 0.03	0.18 ± 0.03	0.2 ± 0.0	0.63 ± 0.75	0.17 ± 0.03	#
Stipule length (cm)	0.77 ± 0.12	0.9 ± 0.26	1.1 ± 0.17	0.73 ± 0	1.03 ± 0.15	0.63 ± 0.03	0.0252 *
Petiole length (cm)	4.63 ± 0.35	6.43 ± 1.6	4.73 ± 1.12	5.13 ± 0.15	5.13 ± 1.01	2.67 ± 0.76	0.0119 *
Petiole breadth (cm)	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	#
Petiolule length (cm)	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	#
Leaflet size (sq. cm)	1.16 ± 0.43	1.39 ± 0.33	1.37 ± 0	0.78 ± 0.13	0.82 ± 0.23	0.43 ± 0.06	0.0371 *
Head length (cm)	1.03 ± 0.25	1.63 ± 0.12	0.97 ± 0.15	1.33 ± 0.35	1.4 ± 0.17	1.2 ± 0.26	0.0383 *
Head breadth (cm)	1.5 ± 0	1.57 ± 0.12	1.4 ± 0.15	1.47 ± 0.50	1.53 ± 0.35	1.3 ± 0.2	#
Floret No. per head	38.3 ± 2.89	47.3 ± 11.24	50.7 ± 9.02	36.7 ± 12.58	36 ± 9.64	33 ± 4.58	#
Peduncle length (cm)	8.6 ± 1.4	11.9 ± 2.66	10.3 ± 9.02	6.5 ± 2.52	10.1 ± 1.91	5.23 ± 0.01	0.0466 *
Peduncle breadth (cm)	0.11 ± 0.01	0.12 ± 0	0.17 ± 0.03	0.1 ± 0	0.09 ± 0.01	0.11 ± 0.01	0.0006 *
Floret length (cm)	0.63 ± 0.12	0.7 ± 0.05	0.53 ± 0.03	0.58 ± 0.08	0.68 ± 0.03	0.53 ± 0.08	0.0431 *
Calyx length (cm)	0.42 ± 0.03	0.47 ± 0.06	0.36 ± 0.01	0.4 ± 0.05	0.4 ± 0	0.38 ± 0.03	0.0458 *
Anther size (sq. mm)	0.16 ± 0.03	0.09 ± 0.02	0.12 ± 0.01	0.15 ± 0.04	0.13 ± 0.03	0.11 ± 0.02	#
Ovule number/ floret	4.67 ± 1.15	3 ± 0	3.37 ± 0.58	3.67 ± 0.58	4 ± 0	3 ± 0	0.0321 *

Table 5: Comparative morphological study among six varieties of *Trifolium repens* at Chandanbari

Parameters	WML	WNML	WPML	WPNML	PML	PNML	P-value
Root length (cm)	4.07 ± 1.02	5.1 ± 1.42	5.0 ± 2.1	7.37 ± 2.38	6.67 ± 2.41	6.43 ± 2.67	#
Nodule/root	9.67 ± 2.31	7.0 ± 3.61	8.67± 6.35	6.6 7 ± 2.52	8.33 ±1.53	8.67 ± 5.03	#
Nodule size (sq.mm)	1.3 ± 0.1	0.74 ± 0.57	1.03 ± 0.42	0.83 ± 0.06	0.8 ± 0.62	1.21 ± 0.60	#
Int stolon length (cm)	1.53 ± 0.42	1.1 ± 0.36	1.63 ± 0.32	1.3 ± 0.5	1.63 ± 0.67	1.23 ± 0.4	#
Int stolon breadth (cm)	0.14 ± 0.1	0.18 ± 0.03	0.18 ± 0.03	0.2 ± 0	0.18 ± 0.03	0.17 ± 0.03	#
Stipule length (cm)	0.83 ± 0.25	0.87 ± 0.06	0.87 ± 0.06	0.8 ± 0.17	0.97 ± 0.21	0.77 ± 0.12	#
Petiole length (cm)	4.37 ± 3.06	6.47 ± 1.29	4.1 ± 0.85	4.5 ± 1.0	4.73 ± 1.04	3.0 ± 0.7	#
Petiole breadth (cm)	0.11 ± 0.04	0.12 ± 0	0.1 ± 0	0.1 ± 0	0.09 ± 0.01	0.1 ± 0.01	#
Petiolule length (cm)	0.09 ± 0.01	0.13 ± 0.03	0.1 ±0	0.09 ± 0.01	0.08 ± 0.03	0.1 ± 0	#
Leaflet size (sq. cm)	0.83 ± 0.45	1.5 ± 0.17	1.23 ± 0.32	0.7 ± 0.2	1.0 ± 0.2	0.6 ± 0.1	0.0114 *
Head length (cm)	1.47 ± 0.80	1 .0 ± 0.3	1.17 ± 0.15	1.33 ± 0.61	1.1 ± 0.4	1.03 ± 0.23	#
Head breadth (cm)	1.77 ± 0.49	1.2 ± 0.26	1.67 ± 0.58	1.4 ± 0.26	1.5 ± 0.3	1.07 ± 0.06	#
Floret No. per head	53.7 ± 17.61	34.3 ± 5.13	48.3 ± 2.89	47.7 ± 6.66	33.7 ± 9.29	36.7 ± 4.93	#
Peduncle length (cm)	6.73 ± 3.47	12.7± 9.29	6.5 ± 3.04	5.9 3 ± 5.07	4.93 ± 2.10	3.97 ± 0.55	#
Peduncle breadth (cm)	0.12 ± 0.03	0.1 ± 0	0.13 ± 0.06	0.17 ± 0.06	0.1 ± 0	0.09 ± 0	#
Floret length (cm)	0.63 ± 0.15	0.67 ± 0.21	0.67 ± 0.12	0.8 ± 0.17	0.63 ± 0.15	0.6 ± 0.17	#
Calyx length (cm)	0.38 ± 0.03	0.42 ± 0.08	0.45 ± 0.05	0.45 ± 0.09	0.43 ± 0.08	0.4 ± 0.09	#
Anther size (sq. mm)	0.18 ± 0.01	0.17 ± 0.04	0.16 ± 0.04	0.1 5 ± 0.02	0.17 ± 0.02	0.12 ± 0.01	#
Ovule number/ floret	3.33 ± 2.08	4.33 ± 1.15	3.67 ± 0.58	4.67 ± 0.58	4.67 ± 1.15	2.33 ± 1.15	#

Table 6: Significant values of morphological parameters among Kirtipur, Dhunche and Chandanbari

SN	Kirtipur	P- value	Dhunche	P- value	Chandanbari	P-value
1	Stipule length	0.00283	Stipule length	0.02518	Leaflet size	0.01138
2	Petiole length	0.00009	Petiole length	0.01189		
3	Petiolule length	0.00038	Leaflet size	0.03708		
4	Leaflet size	0.00256	Head length	0.03825		
5	Floret number	0.00508	Peduncle length	0.04656		
6	Peduncle length	0.02371	Peduncle breadth	0.00057		
7	Floret length	0.00198	Floret length	0.04306		
8	Anther size	0.00516	Calyx length	0.0458		
9			Ovule number/ floret	0.03206		

Photos

Photo 1: WML

Photo 2: WNML

Photo 3: WPML

Photo 4: WPNML

Photo 5: PML

Photo 6: PNML

Photo 7: LPML

Photo 8: LPNML

Photographs showing eight varieties of *Trifolium repens*

DISCUSSION

Identification: Morphological diversity within a species of *Trifolium repens L.* was studied at Kirtipur, Dhunche and Chandanbari. The geographic distribution of *Trifolium repens L.* shows that it is widely adapted to the regions from subtropical to sub-alpine and has a wide altitudinal range from 1200 m to 4000 m in Himalayan region of Nepal (Shrestha and Ghimire, 2001).

Flower color and leaf marking singly or in combination were found the chief indicators. Flower color and leaf marking (white V-marking on the leaf) have been reported to be reliable indicators of varietal differences by Chesneaux, 1972 and William, 1987. It revealed the difference among the varieties of white clover with different flower color, i.e., the variability was found in the flower color. Flower color variants deserve more attention in genetic studies as they offer some potential characters as useful varietal markers (Williams, 1987). There is also a significant variation in white "V" leaf markings and without the leaf markings found in white clover. Harper (1977) and Burdon (1980) also studied the genetic basis of variation in white leaf marking. Charles (1968), Cahn and Harper(1976) had done a study in white clover plants that without white leaf markings, under sheep grazing, survived better than marked plants. They had suggested that genetic diversity of leaf markings might be maintained by preferential selection by sheep grazing of leaves with the most common marking. Chesneaux, 1972 compared 19 varieties of white clover for leaf markings and cyanogenesis.

On the basis of identification given in Table.1, eight varieties of *Trifolium repens* were identified from dry and open area at 1300 m altitude at T.U, Kirtipur. However, from other study areas namely Dhunche and Chandanbari, only six varieties of it were identified (Tables 2). Light pink color flower with marked as well as non-marked leaf varieties were present at kirtipur, while both of these were absent at Dhunche and Chandanbari. Van Treuren et al. (2005) concluded that polymorphism in white clover might be due to selection in the development of varieties.

Morphological variations: White clover growing at three different areas namely Kirtipur, Dhunche and Chandanbari as shown in Table 3, 4, and 5 revealed that these varieties were morphologically or phenotypicaly different from each other.

The longest root length was found in WPNML type growing in low moisture content at Kirtipur, Dhunche and Chandanbari. White clover in a more acidic soil (pH 4.5) of Dhunche and Chadanbari had as many as twice the nodules than the plants growing in less acidic soil (pH 6.5) of Kirtipur. So, low pH seems stimulating to the nodulation in white clover (Sugawara and Stanley, 1992). Inter node stolon and stipule showed marked variation in its length and breadth. WPML at Kirtipur and WNML at Dhunche and at Chandanbari were the tallest plants among other types. The height of the plant depends upon the length of the petiole because the plant is a runner in nature. White clover from Kirtipur and Chandanabari shows variations in petiole breadth among different varieties, whereas it was found to be of the same size in all varieties of white clover growing at Dhunche. The ultimate thickness attained by a petiole varies with genotype and environment (Thomas, 1987).

Leaflet size was found biggest in WNML and smallest in PNML, as compared to other varieties growing at Kirtipur, Dhunche and Chandanabri, because WNML was also a tallest plant having biggest leaflet size. However, the smallest leaflets of PNML are due to the presence of shortest petiole length so and smallest in plant size. It grows in dry places. Leaflet size varies greatly with genotype and environment (Denne, 1996). Leaflet size may vary markedly with cultivar and it decreases with increased grazing pressures (Jones and Kersten, 1992). Thus leaflet size was one of the important characters for the screening of the various types of *Trifolium repens*. Hawkins (1959) has determined the variation in leaf size within individual plants. Leaflet size was well correlated with other shoot morphological character that has been shown elsewhere (Hawkins, 1959) and for this reason leaflet size has been used to classify cultivars (Rhind et al., 1979; Lehmann et al., 1984).

The longest head length was found in WNML variety growing at Dhunche among different sites, e.g., Kirtipur, Dhunche and Chandanbari. The comparison between head length and calyx length shows close relationship in between

them. The widest head breadth of WNML at Kirtipur type showed the higher number of florets per head. Norris, 1985 compared 10 varieties for flower head morphology. When peduncle length and petiole length were compared, it seems that there was a close relation in between them. On an average, the peduncle length and petiole length was longest in WNML and shortest in PNML In this way these are also important characters to identify the above types of *Trifolium repens.* The peduncle breadth was widest in WNML variety growing at Kirtipur. Peduncle breadth and petiole breadth also showed close relationship between them.

Floret number per head was highest in WNML growing at Kirtipur. Similarly, the breadth of head was also found to be widest in WNML variety. Highest floret number might be due to widest head breadth. It is found that there is a close relation in between width of head and number of florets/head. Highest floret number per head at Chandanbari might be due to highest moisture content of soil. Flowering is reduced by water stress, which affects the production of seed number and its weight (Foulds, 1978). Except WPML and WPNML, all other varieties such as WML, WNML, PML, PNML, LPML and LPNML maintained the production of florets per flower head at three study areas during the study years. The floret length or the petal size was longest in WPNML growing at Chandanbari among all other varieties of *T. repens*. The floret length is longer in higher elevation than in lower elevation (Giri, 1992).

Calyx length was longest in WNML growing at Dhunche among the other varieties. Long calyx might help in the protection of other floral parts from cold and mist. Another size was found to be biggest in WPNML variety growing at Kirtipur. It also showed higher production of pollen grains, which is directly related to a higher production of fertile seeds and consequently to viable plants. The ovule number was found highest in PNML growing at Kirtipur than those growing at Dhunche and Chandanbari. It might indicate a high production of fertile seeds and consequently to viable plants. It has been revealed that the production of ovule and seed were not correlated.

Statistical analysis: The analysis of variance showed the common significant morphological characters such as nodule size, stipule length, petiole length, petiolule length, leaflet size, head length, floret number, peduncle length, floret length, calyx length, anther size and ovule number per root etc. among eight varieties of *Trifolium repens* growing at Kirtipur, probably indicate the variations among themselves. Similarly significant variations were also seen among different varieties at Dhunche and Chandanbari. Leaflet size was one of the key points to show the presence of diversity in *Trifolium repens* because it was the common significant parameter found at Krtipur, Dhunche and Chandanbari (Table 6).

CONCLUSIONS

This identification of white clover using "Flower color and Leaf markings" is the first attempt to identify the varieties of *Trifolium repens* in Nepal. On the basis of morphological data, we can conclude that *Trifolium repens* is a polymorphic plant. The highest value for each variable (eight varieties) on morphological parameters of *Trifolium repens* growing at Kirtipur, Dhunche and Chandanbari, asserts towards the conformity of varieties of *Trifolium repens* naturally grown in Nepal. The salient morphological features are:

WML: Biggest nodule size; longest stolon internode, petiolule length and widest head breadth.

WNML: Longest head length and calyx length; largest leaflets size; widest head and peduncle breadth and highest number of floret per head.

WPML: Longest stipule and petiole length.

WPNML: Longest root and floret length; highest nodule number; biggest anther size and widest petiole breadth.

PML: Widest stolon breadth and longest peduncle length.

PNML: Highest ovule number (most of the parameters were lowest)

LPML: Longest petiolule and peduncle length

LPNML: Longest stipule, peduncle, head and calyx lengths.

The morphological evidence on the basis of above identifying phenotypic characters of *Trifolium repens,* growing at Kirtipur, Dhunche and Chandanbari helps conclude that eight varieties of *Trifolium repens* (i.e. Intra-specific diversity) are present in Nepal. These varieties are very important for the development and management of pastureland in Nepal. Ability to discern varietal differences and then their habitat requirement will help pasture managers to apply appropriate variety of *Trifolium repens* to match site specific requirements of the pasture area to get the most out of their investment.

REFERENCES

Burdon JJ, 1980. Intra specific diversity in a natural population of *Trifolium repens*. J. Ecol. 68: 737-744.

Cahn MG and Harper JL, 1976. The biology of leaf mark polymorphism in *Trifolium repens* L. 2. Evidence for the selection of leaf marks by rumen fistulated sheep. Heredity.37: 327-333.

Caradus JR, MacKay AC, Woodfield DR, Van den Bosch J and Wewala S, 1989.Classification of a world collection of white clovers. Euphytica. 42:183-196.

Charles AH, 1968.Some selective effects operating on white and red clover swards.J. British Grassland Soc.23: 20-25.

Chesneaux MT, 1972. Possibilities of Checking Varietal Identity of White clover (*Trifolium repens* L.) in Controlled Conditions.Ann. de Lamelioration des Plantes. 22: 311-319.

Collins RP and Rhodes I, 1991. Genetic Variation in cold tolerance and spring growth in white clover, In: White clover Development in Europe. Meeting of FAO Sub-Network on Lowland pastures and Fodder crops, Polcenigo, Italy, Oct. 1990.pp.11-14.

Crane E, Walker P and Day R, 1984. Directory of Important World Honey Sources, International Bee Research Association U. K. Publication.pp. 300-301.

Denne MP, 1996. Leaf development in *Trifolium repens*. Botanical Gazette. 127: 202-210.

FAO, 2005. Livestock Sector Brief Nepal. Livestock Information, Sector Analysis and Policy Research. AGAL**.**

Foulds W, 1978. Response to soil moisture supply in three leguminous species. New Phytologist. 80: 535-545.

Giri N, 1992. Cytological and Biochemical Investigation in some *Trifolium* spp. PhD dissertation, Patna University, India.

Gupta SC and Kapoor VK, 1984.Fundamentals of Applied Statistics. Sultan Chand and sons Publishers, 23, Daryaganj, New Delhi-110002.

Harper JL, 1977.Population Biology of Plants. U. K. Academic Press, pp. 892.

Hawkins RP, 1959. Botanical characters for the classification and identification of varieties of white clover. J. Nat. Inst. Agri. Bot. 8:675-682.

Hopkins A, Wang Z and Mian R, Sledge M and Barker RE, 2004.Molecular Breeding of Forage and Turf. Kluwer Academic Publishers, Dordrecht, Netherlands.

Jones RM and Kersten SMM, 1992.Plant Resources of Southeast Asia. No. 4. Forages. Pudoc Scientific Publishers, Wageningen.

Lampkin N. 1990. Organic Farming. Farming Press Books, IPSWICH.

Love J, 2004.St Kilda National Nature Reserve. A World Apart, SNH Publications

Lehmann J, Briner HU, Meister E and Joggi D, 1984.New varieties of white clover in the 'list of recommended varieties of grass and clovers'. Mitteilungen für die Schweizerische Land Wirtschaft. 32: 96–101.

Miguel AA, 1987. Agroecology, West View Press. II Publications, London.

Norris IB, 1985. Flowering of contrasting white clover varieties in relation to temperate in controlled environments. Ann. Bot. 56: 317-322.

Pande RS, 1977. Fodder and Pasture Development in Nepal. Udaya Research and Development Services (p).Ltd. Kathmandu, Nepal.

Peters M, Horne P, Schmidt A, Holmann F, Kerridge PC, Tarawali SA, Schultze-Kraft R., Lascano CE, Argel P, Stür W, Fujisaka S, Müller-Sämann K and Wortmann C, 2001.The role of Forages in Reducing Poverty and Degradation of Natural Resources in Tropical Production Systems, AGREN, 17, 1-12.

Pueppke SG, 1991. Biological Nitrogen Fixation Associated With Rice Production,

Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, Bombay, Calcutta. pp 25.

Rajbhandari HB and Shah SB, 1981.Trends and projections of livestock production in the hills. In: Nepal's experience in hill agriculture development. Ministry of Food and Agriculture, Kathmandu, Nepal.

Rhind JMLC, Smith A, Goodenough DCW, Morrison ARJ and Ellis JI, 1979. Cultivar evaluation of forage crops, July 1979. Pietermaritzburg, South Africa, Cedara College of Agriculture and Research Institute.p 14.

Richards R, 2011. A survey of the genetic diversity in populations of white clover,Trifoliumrepens with a focus on South-western Europe.MSThesis in Plant Molecular Biology, Massey University, Palmerston North, New Zealand.

Ryoko H, 2005. Ecogeographic and genetic survey of white clover (*Trifolium repens*L.) on St Kilda.MS Thesis in Conservation and Utilization of Plant genetic resources, University Of Birmingham.

Shrestha AM (Malla) and Ghimire GPS, 2001. Variability in white clover: Relationship with Plant and Soil Nitrogen. In Proceedings, Environment and Agriculture: Biodiversity, Agriculture and pollution in South Asia, Ecological Society (ECOS), Kathmandu, Nepal. pp. 120-124.

Shrestha AM (Malla), 2002. The Study on the Diversity of *Trifolium repens* L. *(white clover)* in Nepal. PhD Dissertation, Central Department of Botany, T.U. Kirtipur, Kathmandu, Nepal.

Shrestha AM, Ghimire GPS and Manandhar SP, 2002. Identification of different varieties of *Trifolium repens* L. by electrophoretic separation of leaf protein profiles. Ecoprint. 9(1): 50- 56.

Shrestha RK, 1992. Agro-ecosystem of Midhills, pp. 7-26. In: Sustainable Livestock Production in the Mountain Agro-ecosystem of Nepal. J.B. Abington (Ed.). FAO Animal Production and Health Paper 105.FAO.

Stevens DR, Drew K, Laas F and TurnerJD, 1992. Dear Production from ryegrass and tall fescue-based pastures. In proceedings, New Zealand Grassland Association. 54: 23-26.

Sugawara K and Stanley HD,1992.The effect of low pH growth medium on nodulation and nitrogen fixation of alfalfa (*Medicago sativa* L.) and white clover (*Trifolium repens* L.).J. Jap. Soc. Grassland Sci. 38 (1): 53-62.

Thomas RG, 1987. The structure of the mature plant. Vegetative growth and development. Reproductive development, In: M. J. Baker and W. M. Williams (Ed), White clover.CAB international, U.K. pp. 1-124.

Vacek V, Palau D, Turz V and Zapletalová I, 1979. Recent information from investigation of the white clover (*Trifolium repens* L.) gene pool. Sborníkredeckýchpracívýzkumného a slechtitelskéhoüstavuPícninárského v Troubsku u Brna (1978/79). pp. 109–119.

Van Treuren R, Bas N, Goossens PJ, Jansen J and Van Soest LJ. 2005. Genetic diversity in perennial ryegrass and white clover among old Dutch grasslands as compared to cultivars and nature reserves.Mol. Ecol.14:39-52.

Williams WM, 1987. Adaptive variation; White clover taxonomy and biosystematics; Genetics and breeding. In: M. J. Baker and W. M. Williams (Ed), White clover.CAB international, U.K. pp. 300-401.