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Genetic Improvement of Temperate Grasses and Legumes in Indian Himalayan Region: A Review

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A B S T R A C T

Pastures in Indian Himalayas are based largely on annual and perennial temperate species that are rich in biodiversity but have less productivity. The main temperate grass and legume species prevalent are perennial ryegrass, tall fescue, festulolium hybrid, red and white clovers. Fescue grass consists of two agriculturally important forage crops, hexaploid tall fescue and diploid meadow fescue. Ryegrass contains two widely cultivated temperate species, Italian or annual ryegrass and perennial ryegrass. Both species are naturally diploid and are closely related to fescues. Festulolium hybrids have been developed by crossing fescue to ryegrass to combine the desirable traits of both species and broaden the genetic pool. Red clover contains an enzyme polyphenol oxidase (PPO) which reduces the losses of protein. Thus the genotypes/ lines containing high PPO are rich in protein and can be further used in the breeding programme. Breeding of white and red clover builds on the advantages of these species in terms of their impact on animal performance, fatty acid profiles and flavour. Further improvement in germplasm of temperate forage grasses can be made by linking of physiology and genomics, integration of genomics with classical breeding combined with appropriate phenotypic analysis of key traits will be essential for the development of improved forage cultivation.

Introduction

Middle hills occupy 35% of total grassland vegetation of Indian Himalayan region. This grassland vegetation includes cold temperate grasses, subalpine and alpine meadows of the great Himalayas (Rawat, 1998). Temperate areas are primarily situated between 2000-4500 m amsl with significant biodiversity. In India, Jammu & Kashmir, Himachal Pradesh, Uttarakhand, Sikkim and Arunachal Pradesh are the states where temperate/alpine pastures are abundant. Pastures in Indian Himalayas are based largely on annual and perennial temperate species that are rich in biodiversity but have less productivity. Forage crops are the major source of livestock economy but are vulnerable to some of the major constraints like overgrazing, anti-nutritional factors, drought and frost susceptibility. One of the main impediments in the improvement of livestock is the unavailability of qualitative and quantitative fodder which could only be overcome by growing high yielding fodder crops like fescue, ryegrass etc. to increase fodder production per unit area. These crops provide fodder not only for few months but round the year. Some of the fodder crops like fescues are tolerant to drought as well as cold. These desirable features can be introgressed to other related crops like ryegrass to enhance the efficiency and persistency of both the crops. Legume fodder like red clover contains a high amount of desirable proteins, but along with that they also contain some anti-nutritional factors causing harmful effect on fodder quality. White clover contains HCN that causes blotting in animals which is injurious to animal health. Since forages are the major source of livestock feed and contribute towards the increasing amount of milk and meat production, hence breeding efforts should be made to improve the potential of forage crops.

Major threats to temperate grasses and legumes

Overgrazing by livestock

When the land is exposed to intensive grazing for a prolonged period of time, there is complete damage to the vegetation and overgrazing occurs. Plants do not have enough time to recover to the proper height and restored carbohydrate reserves which results in erosion and degradation of the land.

Obnoxious weed

It is defined as a harmful or injurious weed that causes devastating loss to agriculture or livestock and is harmful to human health. They are poisonous to both humans as well as animals.

Low persistence

Despite being perennial species, they start behaving as annuals or biennials and this reduces their productive potential in the long run.

Low Productivity

Since temperature is low in the temperate climate, it affects overall yield of fodder grass. During the winter season growth of temperate grass is extremely affected by chilling temperature. The amount of photosynthesis is also reduced by the low availability of light.

The advantages of temperate grasses over sub-tropical grasses

Temperate grasses have higher nutritive value i.e. higher dry matter digestibility, due to lower lignin content.

The optimum temperature for growth of temperate grasses is about 20°C while for sub-tropical grasses it is much higher (25-35°C). Consequently, temperate grasses grow actively in winter when sub-tropical grasses are dormant.

There is a cold zone where the persistence of most sub-tropical grasses is adversely affected by the combination of cold, wet soils and frosts. The temperate perennial grasses are persistent and highly productive in this area. As a result, there is a large potential for temperate perennial grasses to be grown widely in the medium to high rainfall areas of this cold zone, which is largely used for growing pastures with only a small proportion under crop.

Major temperate grasses and legumes are grown in Himalayan regions

Fescue

The genus *Festuca* is one of the largest in Gramineae. It comprises of some 450 species (Clayton and Renvoize, 1986) that range from diploid (2n=2x=14) to dodecaploid (2n= 12x=84) in chromosome number (Smarda and Stancık, 2006). The genus *Festuca* contains two agriculturally important forage crops, hexaploid tall fescue *F. arundinacea* (2n=6x=42) and diploid meadow fescue *F. pratensis* (2n=2x=14). Other fescues of some importance are red fescue, *F. rubra* (2n=6x=42 or 8x=56), and sheep fescue, *F. ovina* (2n=4x=28). The agriculturally undeveloped species, giant fescue *F. gigantea* is important because it produces a mass of very large soft leaves of high nutritive value. Tall fescue is the most important forage species worldwide of the *Festuca* genus. It is indigenous to Europe and also occurs naturally on the Baltic coasts throughout the Caucasus in western Siberia and extending into China (Jauhar, 1993). It is a long-lived perennial, cool season, high yielding and drought tolerant bunch grass. It is valued for its better-stockpiling properties, tolerate hot dry conditions, frost conditions, pasture pest attack and to produce leafy green herbage over the summer. Typically this species of grass has a long growing season and ranges between 2 to 4 feet tall in seed head stage. It spreads through rhizomes. New emerging leaves are rolled in the bud with no prominent ligules which make this a key identification feature of tall fescue. The upper leaf surface is dull with distinct veins running on it, whereas the upper surface is smooth with rough edges.

Genetics

Majority of tall fescue cultivars are allohexaploid with 42 chromosomes. The genomic constitution of hexaploid tall fescue consists of two groups of chromosomes originated from Meadow fescue (*Lolium pratensis*) designated PP and tetraploid fescue (*Festuca arundinacea* var. *glaucescens*), designated $G_1G_1G_2G_2$. Tall fescue has a large genome i.e. 5.27-5.83×10^6 kb. It is predominantly cross-pollinated species with a high level of self-incompatibility. It is highly heterozygous and heterogeneous.

Traits of Interest in Tall Fescue Breeding

Drought tolerance

Most of the tall fescue species are tolerant to drought but, this trait is correlated with traits. Johnson and Yangyang (1999) were successful in selecting for decreased C^{13} isotope discrimination which has been associated with increased water use efficiency but gives low forage yield.

Digestibility

Primary limit to animal performance in tall fescue is endophyte toxicity. Endophyte is a fungus *(Neotyphodium coenophialum*) that lives inside the tall fescue plant in a symbiotic relationship. Host plant becomes resistance to insect attack, drought and moisture stress. Animals feeding on fescue grass infected with endophyte suffer from fescue toxicity and fescue foot. This reduces the ability of animals to cool themselves and also causes lameness and weight loss. Some of the earlier varieties of tall fescue (Alta, Kentucky-31) contained endophyte. New strains of endophytes have been identified that contain less or no amount of toxic alkaloids. Nontoxic endophyte cultivars can improve animal weight gains by as much as 60-100% (Bouton *et al.,* 2002; Hopkins and Alison, 2006). Endophyte-free cultivars are Alta, Kenhy, and KY-31. Increased digestibility is expected to enhance these gains further and will remain an important breeding objective to develop early maturity varieties.

Increased palatability

Palatability of tall fescue declines as it is allowed to over-mature. Cultivars such as Lubrette, Adora, Quantum, and Advance were selected for leaf softness in an effort to improve intake and palatability. Increasing palatability of tall fescue when it grows tall is another important aspect.

Quality traits

Under grazing conditions, crude protein and dry matter digestibility (DMD) start increasing in spring as well as with the onset of the autumn season whereas start decreasing in summer.

In the mid-hill Himalayan conditions the dry matter, NDF, ADF and hemicellulose content increased while crude protein, IVDMD, leaf stem ratio and oxalate decreased with an increase in the age of plants.

Tall fescue grass retains maximum nutritive value during the month of March and April (Katoch *et al.,* 2012).

Ryegrass

The genus *Lolium* contains two widely cultivated temperate species, *Lolium multiflorum* (Italian or annual ryegrass) and *L. perenne* (perennial ryegrass). They are characterized by a bunch of growth habits.

It is native to Europe, Asia, and northern Africa. It is naturally diploid (2n=14) and is closely related to fescues. It should not be confused with rye (*Secale cereale*), which is a grain crop.

Lolium multiflorum

It is commonly known as Australian ryegrass, short rotation ryegrass and westerwolds ryegrass. It differs from *L. perenne* in its spikelet which has a long bristle at the top and its stem is round rather than folded. It is mainly grown as silage or an ornamental grass.

Lolium perenne

It is commonly known as English grass or winter grass, tufted and hairless with a bunching type of growth habit. The leaves are dark green in colour on the lower surface having a smooth and glossy texture with prominent parallel veins on the upper surface.

Genetics

Comparisons of the relative positions of RFLP probes CDO545, RZ144, R2869 and C764 in *L. perenne* and rice indicated a region of synteny between *L. perenne* L7 and rice chromosome 6 which covered the region of the rice genome containing the Hd3 locus (Yamamoto *et al.,* 1998). Additionally, QTLs for heading date have also been identified on chromosome 7 of wheat and barley (Laurie *et al.*, 1995; Worland *et al.*, 1998; Borner *et al.*, 2002) to which *Lolium* is more closely related than to rice.

Traits of Interest in Ryegrass Breeding

Disease resistance

The occurrence and severity of rust diseases caused by *Puccinia graminis* and *P. coronata* are decreased in perennial ryegrass cultivated for forage if grown in mixture with other grass species (Roscher *et al.,* 2007).

Resistance to crown rust in perennial ryegrass appears to be controlled by both major and minor genes (Kimberg, 1999).

Herbicide tolerance

The economic value of perennial rye grass seed diminishes when it is mixed with the seed of Quack grass which acts as a weed. Perennial ryegrass is tolerant to Assure II (Herbicide) but this herbicide effectively controls quack grass.

Winter Hardiness

Initially, NK 200 was the key source for winter hardiness but it was poor in quality and susceptible to crown rust. This trait of winter hardiness was transferred from NK 200 to perennial ryegrass.

Lodging resistance

Seventeen QTLs related to lodging resistance have been detected for six traits in Italian ryegrass. Out of these QTLs, qHD6, qHD7, qCPR6, qCPR1 and qCPR5-1 had high lod score.

Festulolium hybrid

Festulolium is an intergeneric hybrid between *Festuca* and *Lolium* species. This hybridization provides plant breeder with greater opportunity to introgress the important traits from *Festuca* to *Lolium* and vice versa.

The hybrids generated from the cross between diploid species have low fertility which can be restored by giving colchicine treatment followed by doubling the chromosome numbers.

Partially male and female fertile F_1 hybrids can also be obtained by crossing autotetraploid of *Lolium* sp. and *F. pratensis*.

The first amphidiploid *Festulolium* cultivars Elmet (*L. multiflorum* × *F. pratensis*) and Prior (*L. perenne* × *F. pratensis*) were bred at the Welsh Plant Breeding Station, in the early 1970s (Thomas and Humphreys, 1991).

Introgression in *Lolium/Festuca*

Ryegrasses have excellent productivity and more digestible to the ruminant animal. However, several of the fescues, though lacking some of the qualities of ryegrass, are superior in other respects. It has long been our wish to combine the complementary characteristics of ryegrass and fescue. Humphreys *et al.,* (2005) hybridized *Festuca arundinacea* var. *glaucescens* accession Bn 354-4 (2n=4x=28) with Bb 2264-2, a genotype from a synthetic autotetraploid *Lolium* cultivar to transfer the drought resistance from *Festuca* into *Lolium* and the results were analyzed by cytological studies.

Trifolium

The clover genus *Trifolium* has 250–300 species (Zohary and Heller, 1984; Ellison *et al.,* 2006) about 10% of which are used as forage plants in commercial agriculture. Clovers, like most legumes, are co-evolved complexes of the plant, symbiotic bacteria *(Rhizobium leguminosarum* var. *trifolii*), fungi and insect pollinators.

White clover

White clover (*Trifolium repens*) commonly known as Dutch clover or Ladino clover is the most common cultivated clover species worldwide (Ellison *et al.,* 2006).

It belongs to the family Fabaceae and native to Europe, Middle East, and North Africa. It is a herbaceous, perennial, low growing plant with heads of whitish flowers often with a tinge of pink or cream that may come on with the ageing of the plant. The leaves are trifoliate, smooth and elliptic to egg-shaped with long petiole.

It often forms a mat with the stems creeping as much as 18 cm in a year and rooting at the nodes. The leaves form the symbol known as shamrocks. It improves the nutritional value of pasture by increasing its protein and mineral content (Reid and Strachan, 1974) and also improves its digestibility and palatability (Jones and Roberts, 1991).

Table.1 DNA contents and breeding system in *Lolium* species

S. No.	Species	Mode of breeding	Habit	DNA content (pg)
1	*L. perenne*	Outbreeder	Perennial	4.15 (Evans *et al.*, 1972)
2	*L. multiflorum*	Outbreeder	Biennial	4.31(Rees and Jones, 1967)
3	*L. rigidum*	Outbreeder	Annual	4.33 (Hutchinson *et al.*, 1979)
4	*L. canariense*	Outbreeder	Annual	4.23 (Hutchinson *et al.*, 1979)
5	*L. temulentum*	Inbreeder	Annual	6.23 (Hutchinson *et al.*, 1979)
6	*L. remotum*	Inbreeder	Annual	6.04 (Hutchinson *et al.*, 1979)
7	*L. persicum*	Inbreeder	Annual	6.35 (Hutchinson *et al.*, 1979)
8	*L. subulatum*	Inbreeder	Annual	5.49 (Hutchinson *et al.*, 1979)

Table.2 Chromosome numbers and DNA contents of some *Festuca* and *Lolium* species (Kopecky *et al.*, 2008)

Species	Common name	Chromosome number	DNA content (pg)
Festuca arundinacea	Tall fescue	2n=6x=42	6.05
F. arundinacea var. *glaucescens*	-	2n=4x=28	4.28
Festuca gigantea	Giant fescue	2n=8x=56	7.23
Festuca mairei	Atlas fescue	2n=4x=28	3.95
Festuca pratensis	Meadow fescue	2n=2x=14	2.20
Festuca scariosa	-	2n=2x=14	2.68
Lolium perenne	Perennial ryegrass	2n=2x=14	2.08
Lolium multiflorum	Italian ryegrass	2n=2x=14	4.10

Table.3 *Festulolium* cultivars developed in Europe and USA (Yamada *et al.*, 2005)

Type	Hybrid combination	Cultivar name	Country	Year
Amphidiploid	*L. multiflorum* × *F. pratensis*	Perun	Czech Republic	1991
		Rakopan	Poland	2001
	F. pratensis × *L. multiflorum*	Paulena	Germany	1995
		Punia	Lithuania	1997
		Agula	Poland	2002
	L. perenne × *F. pratensis*	Prior	U.K.	1973
		Spring Green	USA	2001
Introgression	*L. multiflorum* × *F. arundinacea*	Kenhy	USA	1977
		Johnston	USA	1983

Genetics

Most of the White clover cultivars are naturally tetraploid (2n=4x=32) species with disomic (amphidiploid) inheritance (Williams *et al.*, 1998). The diploid ancestors of white clover are *T. pallescens* and *T. occidentale.*

The genome size of white clover has been reported as 0.559 pg (Vizintin *et al.*, 2006).

Traits of interest in white clover breeding

Pest and Disease Tolerance

White clover is susceptible to many pests and diseases however, the wild relatives of white clover are resistance to most of the diseases i.e. *T. uniflorum* (2n=32) is resistance to grass grub (*Costelytra zealandica*) due to nutritional quality of the roots, absence of feeding stimulants or the production of feeding deterrents (Dymock *et al.*, 1989), *T. nigrescens* and *T. ambiguum* is resistant to southern root-knot nematode (*Meloidogyne incognita*) (Pederson and Windham, 1989). So, the resistance of *T. uniflorum, T. nigrescens* and *T. ambiguum* could be utilized in *T. repens* by hybridization. Till now, an interspecific hybrid between *T. uniflorum* × *T. repens* has some problems. No seed formation takes place from both *T. uniflorum* × *T. repens* and *T. repens* × *T. uniflorum* crosses, but there is observed development of embryos after hybridization of compatible genotypes (Evans, 1962). With the use of embryo rescue, successful production of seedlings from each cross takes place.

Introgression of drought tolerance trait from Caucasian clover

It is more drought tolerant than white clover which may be conferred by its rhizomes. Marshall *et al.*, (2001) developed Backcross hybrids between white clover and Caucasian clover, using white clover as the recurrent parent and found that when drought stress is induced, white clover was the least able to withstand drought than Backcross hybrids.

Genetic diversity in white clover

The genetic diversity of 28 white clover accessions was studied by Sharma *et al.*, (2005) and grouped into seven clusters showing a high level of genetic divergence. Molecular variation of the higher order was resolved in the exotic accessions to compare to the indigenous germplasm. This suggests the need to introduce this germplasm in the Indian white clover gene pool to increase the genetic diversity.

Molecular markers

In collaboration with Plant Biotechnology Centre, Victoria, Australia, IGER have developed the first molecular genetics map of white clover and have delineated the genetic control of important agronomic traits. Additionally, fatty acid profiling of the individual genotypes is underway to identify genetic loci underpinning variation in individual fatty acids.

Red clover

It is a high yielding forage legume with high nutritional value and protein content. It helps in fixing atmospheric nitrogen and is native to Europe, Western Asia, and northwest Africa but planted and naturalized in many other regions also. It belongs to the family Fabaceae. It is a herbaceous, short-lived perennial plant with variable size and 20-80 cm tall.

Genetics

Red clover is a diploid species with the diploid chromosome number 14. It is heterozygous due to its gametophytic self-incompatibility system.

Advantages of Red Clover

Long productive season. It will continue to grow throughout the summer whenever moisture is available. It can tolerate heat and drought much better than white clover.

Seedling vigour of red clover is excellent and much better than any other clover or alfalfa, making it easy to get good stands.

It has high forage yield potential.

It is adapted to a wide range of soils.

Traits of interest in Red clover breeding

Enhanced grazing tolerance

Current varieties lack the ability to withstand overgrazing by the cattle. Enhanced grazing tolerance of these varieties will allow the farmers to have more productivity of red clover and fewer chances of crop failure.

Pest and disease resistance

Eelworm/stem nematode is a major pest of red clover that causes swollen and distorted stems and/or flower with short and stunted plants. A major disease of red clover is clover rot caused by *Sclerotinia* sp., both of these causes huge loss of biomass production in red clover.

Quality traits

Low level of phyto-oestrogen

Red clover varieties have reduced levels of the major phyto-oestrogen. These compounds can reduce fertility, particularly in ewes and a variety with lower level would increase the versatility of the crop. The variety Aber Ruby has low levels of the phyto-oestrogens that at higher levels cause reduced fertility in ewes.

Polyphenol oxidase

Following cellular damage, PPO catalyzes the conversion of phenols to quinones that bind to proteins, protecting them from early degradation and preventing subsequent formation of polluting ammonia. The action of this enzyme is to reduce the losses of protein during silage making. This results in increased protein utilization by ruminant animals.

Polyunsaturated fatty acids

It is another beneficial feature of red clover. There is clear evidence that meat and milk from animals fed on diets high in red clover (typically silage) contains more of those Polyunsaturated fatty acids which is beneficial for human health.

Selection for increased levels of these compounds in leaves will provide germplasm that will be used to test the effectiveness of this approach in raising levels in animal products.

Analyzing gene expression changes underpinning key traits

Gene expression studies are in progress involving a network of collaborations and a range of approaches to identify the key genes involved in nodule and root senescence. The effects of root and nodule death on legume persistency, soil structure, composition and leaching are not well understood but are likely to have a significant impact on agro-ecosystems. Individual nodules show visible signs of early senescence but recover from this temporary stress with no apparent loss of function.

Future Prospectus

A key consideration for the future will be the harnessing of the power of genomics, linking of physiology and genomics.

Integration of genomics with classical breeding combined with appropriate phenotypic analysis of key traits will be essential for the development of improved forage cultivation.

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