Breeding strategies to improve fodder legumes with special emphasis on clover and medics

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In India, Forage has a unique integration of agriculture and animal industry in diversified rich cultural environment. Greater part of rural economy in India relies much upon mixed farming system, a well-knit combination of crop production and livestock rearing. In India, grazing-based livestock husbandry plays an important role in the rural economy as around 50% of animals depend on grazing. Pasturelands over an area of 12 Mha constitute the main grazing resources that are available. Nearly 30 pastoral communities in hilly or arid/semi-arid regions in northern and western parts of India, depend on grazing-based livestock production. Nomadic pastoralism, a traditional form of human-livestock-grassland interaction, is still predominant in the drylands of western India, the Deccan Plateau, and in the mountainous reaches of the Himalayas. (Roy and Singh, 2013).

Indian sub-continent is endowed with a rich genetic diversity of forage plant species which is largely due to diverse eco-climatic conditions and a variety of habitats and niches. Indian gene centers also hold rich diversity in native grasses and legumes, which have potential to become important components of pastures and rangelands. Rich genetic diversity of both cultivated and rangeland species include trees, browse species and shrubs. Major forage genera with biodiversity include legumes like *Desmodium, Lablab, Stylosanthes, Vigna, Macroptelium, Centrosema* etc.; grasses like *Bothriochloa, Dichanthium, Cynodon, Panicum, Pennisetum, Cenchrus, Lasiurus, etc* ; browse plants such as *Leucaena, Sesbania, Albizia, Bauhinia, Cassia, Grewia etc*. These genera form integral part of feed and fodder resources of the country.

Early researchers focused on the widely recognized species that farmers were actually using and had certain traditional knowledge about heir cultivation, flowering, seeding and time of grazing or utilization, etc. These valuable forage and pasture plants were already adopted into domestication by farmers and graziers. Clayton (1983) suggests that "it is likely that all useful species are already known to the herdsman", Much of the early tropical pasture legume breeding was aimed at domestication of a "tropical white clover", i.e. a perennial, persistent, high quality legume that would form stable associations in permanent grass/legume pastures (Hutton, 1965; Casler and vanSanten, 2010) .

Forage breeding presents certain difficulties which are commonly not observed in the cultivated grain or legume crops. Forage breeding, requires a diversity of specialized, professional support and multidisciplinary approach involving several disciplines, viz., plant breeding, genetics, agronomy, pathology, nematology, entomology, physiology, biotechnology and animal nutrition, etc. A thorough understanding of species relationships, chromosomal constitution, genome structure, putative parentage and extent of possible gene exchange/ recombination and nature of polyploidy is also required for the breeding program.

Animal products are an integral and important component of the food supply chain across the globe. With increasing awarenss about health and increasing purchasing power, the demand of dairy products and meat is going to increase in the near and distant future. This becomes more important in the tropical countries especially in growing economy like India, where the demand of diary product couples with increasing urbanization and purchase power. With increasing global population especially in the Asia, and with an increasing global population production levels are set to increase. Ruminant animals are valuable in their ability to convert a fibre-rich forage diet into a high-quality protein product for human consumption.

Unique problems associated with range legumes: Forage crop improvement has its own limitations. Many aspects related to forage breeding, plant genetic resources, plant protection measures, forage quality, palatability and seed production need to be appropriately addressed adopting an integrated approach. The difficulties arise from the diversity in pollination of the different species, irregularities in fertilization and seed setting, the perennial nature of most forage species. There are certain unique problems associated with the range grasses and legumes. Only a few forage crops have been domesticated. Most of the pasture crops still possess traits of wild plants that include seed shattering, small seed size, seed dormancy, relatively slow germination rates, etc. In most of the cases we have very little knowledge about the basic biology of the species.

Some of the problems include

- overlapping of vegetative and reproductive growth phases, uneven pod setting, non-synchronous maturity and seed shattering in forage legumes
- Inherent heterozygosity as most forage species are cross pollinated.
- Self incompatibility limiting the extent to which they may be inbred.
- Small floral parts, making artificial hybridization tedious.
- Poor seed producers, or produce seed with low viability as well as inherently low seedling vigor and competitive ability. Many forage species produce weak seedlings and stands are not easily established.
- Strains may perform differently with different systems of grazing management
- Persistence of perennial forage crops is not as a single trait, but rather as a complex of traits dependent on various factors, such as disease, insects, abiotic stresses, or management stress
- Most forages are long-lived perennials and many years are required to evaluate persistence and productiveness of new strains.
- Fertility barriers of one sort or another are very common in tropical forage breeding, owing to the wild nature of the species and inadequate knowledge of inter- or intraspecific variation.
- Inadequate germplasm base

Abioitc stress tolerance: Abiotic stress is defined as environmental conditions that reduce growth and yield below optimum levels. Plant responses to abiotic stresses are dynamic and complex. They are both reversible and irreversible. Abiotic stresses also play an important role in limiting the persistence of some forage species. The complex plant response to abiotic stress involves several genes and biochemical-molecular mechanisms, The genes include three major categories (Wang et al., 2003):

- those that are involved in signaling

cascades and in transcriptional control, such as MyC, MAP kinases and SOS kinase, phospholipases , and transcriptional factors such as HSF, and the CBF/DREB and ABF/ ABAE families

- those that function directly in the protection of membranes and proteins, such as heatshock proteins (Hsps) and chaperones, late embryogenesis abundant (LEA) proteins, osmoprotectants, and free-radical scavengers
- those that are involved in water and ion uptake and transport such as aquaporins and ion transporters

Systems biology approach to abiotic stress: In the post-genomic era, comprehensive analyses using three systematic approaches or omics have increased our understanding of the complex molecular regulatory networks associated with stress adaptation and tolerance. It includes **'transcriptomics'** (for the analysis of expression profiles of RNAs); **'metabolomics'** (to analyze a large number of metabolites) and **'proteomics'** (protein and protein modification profiles to understand regulatory networks (Cramer et al. 2011)

Forage Quality: Genetic improvement of forage crops for desirable quality traits is the most cost-effective mechanism for increasing the nutritional value. Even a small increase in digestibility results in appreciable improvements in animal performance and productivity. Improvement in IVDMD result from desired changes in chemical and morpho-anatomical traits. Such genetic changes if successfully incorporated and being both genetically and environmentally stable need only one time investment in case of perennial species. The positive correlation of quality traits with yield, biotic and abiotic stress tolerance should also be kept in mind while breeding with this objective. It is quite a tedious and nearly impossible job to get a cultivar with all the desired traits. However, but accumulation of as much possible desired genes by suitable conventional and modern breeding tools will certainly result in a long lasting variety.

Anti-nutritional Factors: Many types of plant compounds and structures can be detrimental to utilization of forage crops by livestock, largely by reducing palatability, digestibility, intake, and/or health and fitness of livestock. These are chemical in nature and include toxins, estrogenic compounds, alkaloids. Saponins, tannins, estrogens, etc. While tannins are known to cause reductions in palatability of some legumes, they are also thought to play an important role in binding soluble proteins in the rumen, helping to protect ruminants against bloat. Genetic variability and relatively simple inheritance patterns have been demonstrated for many of these compounds, which are fairly amenable to selection.

Biotechnological tools:

Significant advances in biotechnological tools including transgenic and omics techniques have equipped the scientists with options to tailor the plants according to need. The improvement of forage crops through biotechnological approach has started in late eighties has made remarkable headway at the global level. Biotechnological approach offers opportunities for creation of novel variations in forages which as such are not possible through conventional methods. The various means of creating variation in forage grasses and achievement are somaclonal variation, somatic hybridization, genetic transformation etc. Artificial introduction of some foreign gene in the plant genome is genetic transformation. Insertions of genes may be by chemical, electrical, physical or micro-projectile transfer. A number of techniques such as embryo rescue, micro-propagation, androgenic haploid plant production and creation of novel variations help at one or more steps involved in conventional breeding methods. These techniques save time and energy required for conventional methods. Many interspecific hybrids in *Trifolium* involving *T. alexandrinum* as female and *T. constantinopolitanum*, *T. apertum, T. resupinatum* as female has been achieved. Genetic diversity using molecular markers have been studied in *Trifolium Stylosanthes, Medicago* etc. (Malaviya et al., 2005; Chandra et al., 2006, 2010, 2011, Chandra 2009; Chandra and Kaushal, 2009)).

Some of the important forage pasture species

***Stylosanthes* :** The genus Stylosanthes (Fabaceae) comprises approximately 40 species (Kirkbride and de Kirkbride 1985)., distributed in the tropical, subtropical and temperate regions areas of America, Africa, and Southeast Asia. It can be grouped into two subgeneric sections, sectt. Styposanthes and Stylosanthes. Most species are diploid (2n = 20) but polyploid species (2n = 40 and 2n = 60) also exist. Six species, namely *S. scabra, S. Seabrana, S. hamata, S. guianensis*, *S. humilis* and *Stylosanthes viscosa*, are predominantly used as fodder legume in humid to semi-arid tropics of India. These are very popular and have been widely adapted due to their ability to restore soil fertility, improve soil physical properties, and provide permanent vegetation cover as well as to provide nutritious fodder.

***Medics*:** Medicago, a genus of Leguminosae, includes annual and perennial forage species important for both temperate and tropical environments. Lucerne (*Medicago sativa* L.) is a native to South west Asia as indicated by occurrence of wild types in the Cancasus and in mountainous region of Afghanistan, Iran. It is a perennial species cultivated in many countries including India as a winter multi-cut forage. *M. sativa* complex , comprises of several members at the same ploidy level (e.g., *M. falcata, M. media* and *M. glutinosa*), which freely intercross, without any hybrid sterility in the F1 or later generations (Lesins and Lesins 1979).

It is generally agreed that the basic chromosome number for the genus Medicago are x = 7 and x = 8. Perennial species are mainly tetraploids (2n = 4x = 32) outcrossing, however diploid (2n = 2x = 16) and hexaploid (2n = 6x = 48) cytotypes have also been reported. The cultivated forms probably arose in western persia and then spread to become widely cultivated throughout Asia, Europe, America and widely distributed in temperate regions. commonly know as rijka in northern India. It is having two cultivated species, viz.; *sativa* and *falcata*. Lucerne is an autotetraploid and highly cross-pollinated crop. Lucerne growing area include India, Australia, France, Japan, USA, Russia, Bulgaria. In India, it is grown in Maharashtra, Gujarat, Andhra Pradesh, Karnataka, Tamil Nadu, Haryana, Madhya Pradesh, Rajasthan, Punjab. The major breeding objectives in the crop include Vigorous tall growing plants, better tillering having quick regeneration potentialities; A good balance between the seed and forage yield; Persistence of stands in perennial good Nutritive value and Palatability. The Future strategies should include development of cold and drought hardy lucerne with degree of persistence for pasture and meadows, increasing genetic base, high seed production, stress tolerance, diseases and pest resistance etc.

Weevil (*Hypera postica* Gyll.) infestation is one of the major problem in the tropics. The pest remains active during December to March in parts of India where temperature is low and the incidence is maximum in February. After screening 197 global germplasm encompassing 50 *Medicago* species for weevil tolerance, 22 lines representing 13 species were identified where leaf damage was B15% (P B 0.05). (Chandra, 2009).

Two accessions of *M. tenoreana* were observed closest to Indian lucerne cultivars. The rich variability revealed can be used as potential resource for transferring genes across species. Due to post fertilization barrier, interspecific hybridization is difficult so we need to use biotechnological tools like ovule-embryo culture and electroporation. Tolerance for weevil has been reported in several annual species such as glandular haired *M. scutellata* and genotypes of *M. prostrata* (Chandra *et al.* 2006; Chandra and Pandey 2008)

Three families of proteinase inhibitors, namely, serine, cysteine (thiol) and aspartic (carboxyl) were examined for their inhibitory effects on growth and development of Indian alfalfa weevil (Coleoptera: Curculionidae). Larval leaf feeding, survival, pupation and adult emergence were significantly decreased by pHMB, (p-hydroxy-mercuribenzoic acid), cystatin and E-64 (trans-epoxysuccinyl-L-leucylamido-(4guanidino)-butane) belonging to cysteine class of proteinases, at a concentration of 0.1 and 0.5%. (Chandra and Pandey, 2008)

Clovers**:** The genus *Trifolium* of tribe Trifolieae Leguminosae (Fabaceae), commonly called clovers, includes 237–290 annual and perennial species, of which about 20 are important as cultivated and pasture crops. The important perennial pasture clovers *T. repens* (white clover), *T. hybridum* (alsike clover), *T. pratense* (red clover), and *T. ambiguum* (Caucasian clover) are widely distributed in the temperate and subtemperate regions of the world. The annual types *T. alexandrinum* (Egyptian clover or Berseem), *T. resupinatum* (Persian clover or Shaftal), and *T. subterraneum* (subterranean clover) are commonly cultivated as winter annuals in the subtropical regions such as Egypt, India, Pakistan, Turkey, and the Mediterranean countries. Mediterranean region is considered as one of the main centers of distribution of the genus and also of domestication and breeding. Self-incompatibility is prevalent in the genus, controlled by a single, multiallelic gene expressed gametophytically in the pollen. In Egyptian clover, pollination studies have indicated existence of several groups having different pollination mechanism (Roy et al. 2005). Interspecific hybridization in the genus *Trifolium* by conventional crossing techniques has been largely unsuccessful. So embryo culture technique was used to obtain new combinations though interspecific hybrids. Embryo culture has been effectively used in developing interspecific hybrids . Many interspecific hybrids in *Trifolium* involving *T. alexandrinum* as female and *T. constantinopolitanum*, *T. apertum, T. resupinatum* as female has been achieved (Roy et al, 2004, 2011; Malaviya et al., 2004, Kaushal et al., 2005). These have resulted in creation of diversity for future selection by the breeders.

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