Int.J.Curr.Microbiol.App.Sci (2019) *8*(1): 3028-3036

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 01 (2019)**
Journal homepage: http://www.ijcmas.com

Review Article https://doi.org/10.20546/ijcmas.2019.801.322

Genetic Improvement of Berseem (*Trifolium alexandrinum*) in India: Current Status and Prospects

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Keywords

Cross incompatibility, Genomic resources, Gene pool, Polyploidy, Wide hybridization

Article Info

Accepted:
26 December 2018
Available Online:
10 January 2019

A B S T R A C T

Berseem, a nitrogen-fixing, annual, multicut forage crop cultivated around 2 million hectares areas of northern, central and eastern parts of India. Berseem has variability for pollination behavior however variation for morphological and agronomic traits are scare, perhaps, because of initially introduction of crop with narrow genetic base. Genetic variability have been fortified through introduction of exotic materials, intra-interspecific hybridization, induction of polyploidy and mutation. ICAR-Indian Grassland and Fodder Research Institute maintain >900 accessions of *Trifolium* spp. Different genetic improvement programmes in India resulted with development of >15 cultivars apart from unique agro-morphological variants. Affinity of Berseem was tested with other species and suitable donors for introgression of genes especially for biotic stress were identified. By adopting embryo rescue technique, wide crosses of Berseem with *T. constantinopolitanum*, *T. apertum*, *T. resupitanum* and *T. vessiculosum* successfully developed and genes for biotic stresses and agro-morphological traits were incorporated. Longer duration, an important agronomic trait in Indian condition, has been induced through induction of mutation by physical mutagens. Induction of autopolyploidy by using coltchicine treatment made major breakthrough in berseem breeding in India by the development of high biomass producing cultivars. The future breeding strategies contemplate to intensification of gene pool through exotics from the centre of origin, increase of variability, development of genomic resources, development of inbreds, remodeling of breeding procedure as substantial points.

Introduction

The genus *Trifolium* from the tribe Trifolieae of the family Leguminosae (Fabaceae) is important for its agricultural value. A few of the 237 species of this large genus have actually been cultivated to date (Zohary and Heller, 1984), out of which 25 species are agriculturally important as cultivated and pasture crops (Lange and Schifino-Wittmann, 2000). Berseem or Egyptian clover (*Trifolium alexandrinum* 2n=2x=16) is commonly cultivated as winter annuals in the tropical and subtropical regions. Berseem, introduced in India from Egypt in 1904, started cultivation as a rotational crops at government cattle

farm, since 1910 its cultivation was taken up by cultivators (Das Gupta, 1943). Berseem has been established as one of the best *Rabi* (winter season) fodder crop in entire North West Zone, Hill Zone and part of Central and Eastern Zone of the country (Mehta and Swaminathan, 1965; Singh, 1988), occupy more than two million hectare (Pandey *et al.*, 2011). Berseem are popular due to its multicut (4-8 cuts) nature, providing fodder for a long duration (November to May), very high quantum of green fodder (85 t/ha) and better quality of fodder (20% crude protein), high digestibility (up to 65%) and palatability.

Pollination behavior

Understanding the natural mating behavior (self- or cross-pollination) is important for designing a suitable breeding strategy for genetic improvement of crop**.** The Berseem crop is a dilemma with regard to its self and cross pollination.

In Indian conditions, a number of reports on pollination in Berseem suggest that this crop is not self-sterile but tripping is essential for a good seed-set (Chowdhury *et al.*, 1966, Roy *et al.*, 2005). The crop is predominantly self-pollinated and shows wide diversity for self fertility and population with self compatible and self pollinating, self compatible requiring tripping, self incompatible with broad genetic base and self incompatible with narrow genetic base have been identified (Dixit *et al.*, 1988). Roy *et al.*, (2005) indicated considerable variation between different populations of Berseem for self-compatibility, together with a requirement of tripping for pollination and seed set, even in self-compatible lines. Extant of natural cross pollination was reported up to 4.73% by Beri *et al.*, (1985a) and seed setting were higher under un-caged condition against caged condition due to tripping mechanism done by honey bees (Beri *et al.*, 1985b)

Germplasm management

In India, National Bureau of Plant Genetic Resources (NBPGR), New Delhi is the nodal organization for exchange, quarantine, collection, conservation, evaluation and the systematic documentation of plant genetic resources. It has introduced >500 accessions of *Trifolium* spp from different countries and maintained in long term storage conditions. The Indian Grassland and Fodder Research Institute (IGFRI), Jhansi is a National Active Germplasm Site for the systematic management and utilization of germplasm wealth of forage crops including agro-forestry trees maintains >900 accessions of *Trifolium* spp. For effective utilization and maintenance of conserved germplasm, IGFRI has catalogue the information on different qualitative, quantitative and origin place of all 594 accessions. To assist the utilization of Berseem germplasm by curators/scientists throughout the international plant genetic resources network, IGFRI has developed descriptor list of Berseem (Roy *et al.*, 2009). Many of the Berseem germplasm having unique characteristics have been generated (Singh *et al.*, 2017) and registered at NBPGR, New Delhi (Table 1).

Breeding approches

Berseem is an introduced crop in India and one of the most important drawbacks in genetic improvement of Berseem is lack of genetic variability (Verma and Mishra, 1995; Roy *et al.*, 2004; Malaviya *et al.*, 2005; Malaviya *et al.*, 2007). Variability in the existing gene pool of Berseem has been induced in through mutation, polyploidization and interspecific hybridization. Different genetic improment programmes by utilizining breeding approches like selection, polyploidy and mutation leads to the development of >15 varieties for different berseem growing regions of India (Table 2). High biomass

production potential alongwith extended growth period and resistance to biotic stresses specially root rot and stem rot are the main target traits has to be improved genetically.

Inter-specific hybridization

Initially, the aim of interspecific hybridization was to clarify the closest relatives of *T. alexandrinum. T. alexandrinum* (2*n* = 16) was successfully hybridized with *T. berytheum* (2*n* = 16) and *T. salmoneum* (2*n* = 16) and found the most probable parent. Recently, efforts has been put into using this approach with the aim of improving *T. alexandrinum*'s resistance to biotic and abiotic stresses, tolerance to soil alkalinity and length of the vegetative period. Genes for wide scale adaptability and disease resistance widely distributed in several wild species of *Trifolium* (Table 3) could not be incorporated into the present day cultivars because of interspecific incompatibility barrier which are common among other *Trifolium* species also. Embryo culture has been effectively used in developing interspecific hybrids of Berseem with *Trifolium apertum* (Malaviya *et al.,* 2004), *T. constantinopolitanum* (Roy *et al.,* 2004), *T. resupinatum* (Kaushal *et al.,* 2005) and *T. vesiculosum* (Kaur *et al.,* 2017). Progenies of interspecific hybrids showed introgression of various desirable traits, including late flowering and resistance to root rot and stem rot diseases.

Ploidy manipulation

A major breakthrough in Berseem breeding in India was achieved through induction of polyploidy. The work on polyploidyzation of Berseem genome was started with the aim to induce grater leaf and stem size (Mehta and Swaminathan, 1957; Sikka *et al.,* 1959). Autotetraploid induced by using coltchicine treatment, and selection at tetraploid level resulted the development of first Berseem variety 'Pusa Giant' with more fodder production and good regeneration capacity, uniform and higher yield throughout the season than diploid varieties released for general cultivation in India (Metha and Swaminathan, 1965). Another big achievement in polyploidy breeding was achieved at IGFRI, Jhansi by developing an autotetraploid variety namey 'Bundel Berseem-3' through colchiploidy followed by recurrent single plant selection followed with mass selection. It is released for north east zone, Bihar Orissa, WB and eastern UP.

Mutation breeding

Major constraints in genetic improvement of Berseem are narrow genetic base of the crop coupled with cross incompatibility barriers (Malaviya *et al.,).* Efforts have been made to generate variation in the existing gene pool through mutation by using physical or chemical mutagens (Sindhu and Mahindiratta 1976: Jatasra *et al.,* 1980; Shukla and Tripathi, 1984). Major success was achieved by induction of longer duration mutant in Mescavi variety through gamma ray treatment (Sohoo *et al.,* 1985). These longer duration mutant in the form of BL-22 a variety released in 1988 for temperate and north west zone; BL-180 released in 2006 for cultivation in north-west zone of India. Longer duration (flowering in May-June) is the important agronomic trait in Indian condition. Incorporation of this trait in Berseem variety for additional cut of green fodder during the scarcity period of summer months may be achieved.

Biotic and abiotic stress tolerance

Berseem cultivars are susceptible to diseases like root rot (*Rhizoctonia solani* and *Fusarium semitactum*), stem rot (*Scferotinia trifoliorum*), leaf blight (*Epicocum* sp.) powdery mildew (*Oidium* sp.) and downy mildew (*Perenospora trifolif*) (Bhaskar *et al.,* 2002).

Table.1 Novel genetic stock of Berseem (*Trifolium alexandrinum*) registered with NBPGR, New Delhi

s.no.	Trait	Ploidy level	Year	INGR number
1	Purple leaf and flower	Diploid (2n=2X=16)	2005	NGR05017
2	Pentafoliate Berseem Penta 1	Diploid (2n=2X=16)	2009	INGR 09045
3	Pentafoliate	Tetraploid (2n=4X=32)	2012	INGR 12010
4	Black seeded	Diploid (2n=2X=16)	2012	INGR 12009
5	Self incompatible	Tetraploid (2n=4X=32)	2012	INGR 12011
6	Self compatible	Diploid (2n=2X=16)	2012	INGR 12012
7	Blackseeded pentafoliate	Diploid (2n=2X=16)	2016	INGR 15026

Table.2 Berseem varieties released/notified in India

S. No.	Variety	Breeding method	Year of release/ notification	Institution responsible for the development	Area of adaptation
1.	Mescavi	Selection	1975	CCS HAU, Hisar	Entire growing area
2.	Pusa Giant	Polyploidy breeding	1975	IARI	Entire growing area
3.	BL 1	Selection	1978	PAU, Ludhiana	Punjab, H.P., Jammu
4.	Wardan	Selection	1982	IGFRI, Jhansi	Entire growing area
5	Jawahar Berseem 1 (JB 1)	Selection	1981	JNKVV, Jabalpur	Central India, central and north western zones
6	BL-10	Mutation breeding	1985	PAU, Ludhiana	Punjab, Haryana, H.P., Jammu
7.	BL 22	Mutation breeding	1988	PAU, Ludhiana	Sub-temperate, hill regions of North India
8.	BL 2	Selection	1989	PAU, Ludhiana	Punjab, Haryana, H.P., Jammu, Western UP, Uttrakhand
9.	UPB 10	Composite Selection	1993	GBPUAandT, Pantnagar	North-west India
10.	Bundel Berseem 2	Mass Selection	1997	IGFRI, Jhansi	Central, North-west zone
11.	Bundel Berseem 3	Polyploidy breeding	2001	IGFRI, Jhansi	North-east , Eastern region
12.	JB-5	Polyploidy breeding	2005	JNKVV, Jabalpur	Central, North-west zone
13.	BL 42	Mutation breeding	2003	PAU, Ludhiana	North-west India
14.	BL 180	Mutation breeding	2006	PAU, Ludhiana	North-west India
15	Hisar Berseem 1 (HFB 600)	Selection	2006	CCS HAU, Hisar	North-west India
16.	JBSC-1	Selection	2017	IGFRI, Jhansi	Central, North-west zone

Table.3 Desirable characters in Berseem ecotypes and wild *Trifolium* species

Species	Gene pool	Chromosome Number (2n)	Desirable characters	References
***T. alexandrinum* ecotype Mescavi**	Primary	2n=16	Annual, multicut, highly productive, crude protein, high digestibility and palatability, basal branching	Malaviya et al., 2004
***T. alexandrinum* ecotype Fahli**	Primary	2n=16	Annual, single cut, self compatible, stem branching	Singh *et al.*, 2015
***T. alexandrinum* ecotype Saidi**	Primary	2n=16	Annual, 2-3 cut, stem and basal branching	
T. berytheum	Secondary	2n=16	-	Putiyevksy and Katznelson, 1973
T. salmoneum	Secondary	2n=16	-	Putiyevksy and Katznelson, 1973
T. apertum	Secondary	2n=16	Annual, profuse basal branching, late flowering, resistance against root rot and stem rot, high protein content	Putiyevksy and Katznelson, 1973; Malaviya et al., 2004
T. meironense	Secondary	2n=16		Putiyevksyand Katznelson, 1973
T. resupinatum	Tertiary	2n=16	Root rot and stem rot resistance, soil alkalinity tolerance	Bhaskar et al., 2002; Kaushal et al., 2005
T. constantinopolitanum	Tertiary	2n=16	Profuse basal branching, resistance against root rot and stem rot	Roy et al., 2004
T. vesiculosum	Tertiary	2n=16	Lateness, disease resistance	Malaviya et al., 2004

Berseem cultivar 'Bundel Berseem-3' developed through polyploidy is moderately tolerant to the root rot and stem rot disease. Beseem is highly sensitive to drought conditions as it decreased plant fresh and dry matter yield (Sevanayak *et al.*, 2017).

Berseem cultivars and inbreds derived from interspecific hybrids were evaluated under drought stress condition and drought tolerance lines identified (Shipra *et al.*, 2010, Sevanayak *et al.*, 2017). Further, different species of *Trifolium* from secondary and tertiary gene pool are known to be resistance against various biotic and abiotic stresses (Table 3) and could be utilized for introgression of desirable genes by utilizing advanced molecular techniques.

Biotechnological approach

Biotechnological approaches offering alternative and effective tools for genetic improvement of crop plants. Utilization of biotechnological approaches in genetic improvement by genetic transformation and other means requires efficient method for plant regeneration via tissue culture using different parts of plant. Protocol for in vitro plant regeneration from meristematic tissue and the establishment of regenerable callus culture have been developed in Berseem and related species viz., *Trifolium glomeratum*, *T apertum*, *T resupinatum* (Kaushal *et al.*, 2004, Kaushal *et al.*, 2006). Embryo rescue technique has been effectively utilized to overcome the problems of post fertilization

barriers in interspecific crosees of Berseem with *Trifolium apertum*, *T. constantinopolitanum*, *T. resupinatum* and *T. vesiculosum* (Malaviya *et al.,* 2004; Kaushal *et al.,* 2005; Roy *et al.,* 2004; Kaur *et al.,* 2017). Limited availability of genomic resources in Berseem hampered the utilization of molecular markers in genetic improvement programme. Therefore, molecular markers were developed and validated in Berseem (Verma *et al.,* 2017, Chandra 2011). Genetic diversity in Berseem and related *Trifolium* species were studied by using isozymes (Malaviya *et al.,* 2005) and molecular markers (Kalia *et al.,* 2009).

Future prospects and conclusion

Berseem being an important forage crop providing nutritional security to the animals by producing high quantum of quality green forage, also contributing to the sustainability of rice-wheat cropping system. Being an introduced crop in India, genetic improvement in this crop is hampered by narrow genetic base and lack of variability in desirable traits. Further introduction of germplasm from its origin place, development of interspecific hybrids and induction of mutations will further contribute in broadening the genetic base of berseem. Desirable variants developed through interspecific hybridization and mutation could be utilized in development of improved cultivars. Development of inbreds and further establishment of heterotic pool will help in development high biomass producing synthetic and composite population and hybrids. There is a scope to further strengthen the genomic resources by developing more SSR markers, molecular linkage map and mapping of forage quality and biomass contributing traits which could be utilized to speed up conventional breeding programme going on in different research institutes/universities.

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How to cite this article:

Tejveer Singh, A. Radhakrishna, D. Seva Nayak and Malaviya, D.R. 2019. Genetic Improvement of Berseem (*Trifolium alexandrinum*) in India: Current Status and Prospects. *Int.J.Curr.Microbiol.App.Sci.* 8(01): 3028-3036. doi: https://doi.org/10.20546/ijcmas.2019.801.322