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Research Paper

Biomass accumulation, phenology and seed yield of *Trifolium alexandrinum* ecotypes evaluated in Central India

Acumulación de biomasa, fenología y rendimiento de semilla de ecotipos de Trifolium alexandrinum *en India central*

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Abstract

Berseem or Egyptian clover (*Trifolium alexandrinum*) comprises 3 ecotypes, Miskawi, Fahli and Saidi, with Miskawi being the most widely cultivated. The narrow genetic base coupled with low availability and utilization of genetic resources is hindering genetic improvement of Berseem in India. Exploitation of new and diverse sources of variation is essential for the genetic enhancement of the cultivated genepool of Berseem. In the present study 7 populations of the 3 *T. alexandrinum* ecotypes were evaluated over 2 years to analyze the patterns of biomass accumulation, phenology, nutritional value and seed yield. Results indicate that Fahli and Saidi populations accumulated higher biomass per unit area than the tested populations of Miskawi and were earlier maturing. While crude protein (CP) concentration in forage was higher for Miskawi, Fahli and Saidi ecotypes contained more than 17% CP at 50% flowering. Further, seed yields of Fahli and Saidi populations were significantly higher than those of Miskawi. It is possible that genetic improvement of cultivated populations of Miskawi could be achieved by incorporating genes for dry matter yield and seed yield from the populations of Fahli and Saidi ecotypes.

Keywords: Berseem, dry matter yields, ecotypes, genetic improvement, legumes.

Resumen

El bersín o trébol de Alejandría (*Trifolium alexandrinum*) comprende los ecotipos Miskawi, Fahli y Saidi, siendo Miskawi el más ampliamente cultivado. La estrecha base genética y la baja disponibilidad y utilización de recursos genéticos son un obstáculo para el mejoramiento genético de esta leguminosa en la India. Por tanto la explotación de nuevas y diversas fuentes de variación genética es esencial. En el presente estudio, 7 poblaciones de estos ecotipos de *T. alexandrinum* fueron evaluadas durante 2 años para analizar los patrones de acumulación de biomasa, fenología, valor nutritivo y rendimiento de semilla. Los resultados indican que los ecotipos Fahli y Saidi produjeron más biomasa por unidad de área y fueron más precoces que las poblaciones del ecotipo Miskawi. No obstante en muestras de plantas con 50% de floración, la concentración de proteína cruda en el forraje fue significativamente mayor en las poblaciones de Miskawi (20%) que en las de Fahli y Saidi (17.4 y 18.0%, respectivamente). Por otra parte, los rendimientos de semilla de los ecotipos Fahli y Saidi fueron significativamente mayores que los de las poblaciones de Miskawi. Los resultados sugieren que es posible mejorar los rendimientos de biomasa y la producción de semilla de las poblaciones cultivadas de Miskawi mediante la incorporación de genes procedentes de los ecotipos Fahli y Saidi.

Palabras clave: Bersín, ecotipos, leguminosas, mejoramiento genético, rendimiento de materia seca, trébol de Alejandría.

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Introduction

The genus *Trifolium* comprises more than 250 species and is widely distributed, being best adapted to mesic or humid environments, in soils of moderate to high fertility and slightly acid to alkaline pH. *Trifolium* species play a key role in soil improvement by providing biological nitrogen fixation and a source of green manure. Ten *Trifolium* species are used as the major forage legumes in tropical upland, Mediterranean and temperate regions of the world with the most important being white clover (*T. repens*), red clover (*T. pratense*) and Berseem or Egyptian clover (*T. alexandrinum*) (Zohary and Heller 1984). The eastern Mediterranean region possesses the greatest species diversity and is believed to be the center of origin of the genus *Trifolium* (Vavilov 1926). Berseem or Egyptian clover ($2n = 2x = 16$) is an important annual forage legume and has been introduced from the Mediterranean region to many countries like India, Pakistan, South Africa, USA and Australia.

Trifolium alexandrinum is divided into 3 different bio/ecotypes, Miskawi/Miscavi, Saidi and Fahl/Fahli. The Miskawi, Saidi and Fahli ecotypes differ in morphology, yield and regrowth ability after cutting. Miskawi can be cut 4–6 times in a season, while Saidi can be cut twice and Fahli only once. Fahli berseem is a low branching cultivar and is more adapted to dry areas than the other ecotypes (Suttie 1999; Hannaway and Larson 2018). This species is commonly grown in Egypt and displays high level of morphological and molecular variability compared with Miskawi (Hussain et al. 1977; Muhammad et al. 2014). Miskawi was introduced into India in 1904 and has been widely utilized since 1916 as a major winter fodder due to its multi-cut (4–8 cuts) nature, ability to provide fodder for a long duration (November–May), very high green fodder yields (up to 85 t/ha), good forage quality (20% crude protein), high digestibility (up to 65%) and good palatability (Narayanan and Dabadghao 1972).

While Berseem produces high quality forage in India, aspects requiring improvement are dry matter (DM) yields from early cuts, initial vigor, extended vegetative growth and resistance to stem rot and root rot disease complexes (Malaviya et al. 2004b). However, owing to extensive genetic drift and natural selection over time and space, the genetic base of this crop in India is narrow (Verma and Mishra 1995) and needs to be broadened for targeted traits using different breeding approaches like hybridization, mutation, etc.

There are many species in the secondary and tertiary gene pool of *T. alexandrinum* L. such as *T. apertum* Bobrov, *T. meironense* Zohary & Lerner, *T. resupinatum* L., *T. constantinopolitanum* Ser. and *T. vesiculosum* Savo possessing genes for wide adaptability and resistance to biotic and abiotic stresses (Putiyevsky and Katznelson 1973; Malaviya et al. 2004a). However, cross-incompatibility barriers and linkage drags limit their exploitation for cultivar improvement (Putiyevsky and Katznelson 1973; Malaviya et al. 2018). For the development of interspecific hybrids with these species, the embryo rescue technique is needed (Malaviya et al. 2004b; Kaur et al. 2017). However, the primary gene pool of Berseem needs to be exploited before addressing the interspecific hybrids, which are coupled with problems like linkage drag.

Detecting and exploiting genetic variation in biomass accumulation and phenology is of great importance for increasing Berseem yield as well as development of plant ideotypes for different cropping systems. Therefore, we conducted an investigation to characterize a selection of *T. alexandrinum* lines for targeted traits that could be utilized for genetic improvement of this crop by breeders in the future.

Materials and Methods

Genetic material and location of experiment

Seven lines (populations) of *T. alexandrinum* comprising cvv. Wardan, Bundel Berseem-2 and Bundel Berseem-3 from Miskawi, JHBF-1 and JHBF-2 from Fahli, and JHBS-1 and JHBS-2 from Saidi ecotypes were used. The experiment was conducted for 2 consecutive years during winter (November–April) at the Central Research Farm of the ICAR-Indian Grassland and Fodder Research Institute (25°31' N, 78°32' E; 237 masl), Jhansi, India. The experimental site is characterized by a semi-arid climate with extreme temperatures during summer (43–46 °C) and winter (as low as 2 °C). The soil was deep, moderately well drained, and brown to dark grayish brown with fine loamy texture. Nitrogen (20 kg N/ha), phosphorus (60 kg P/ha) and farmyard manure (30 t/ha) were applied at sowing. The design was a completely randomized block (CRBD) with 3 replications. Each line was planted (second week of November) in 4 × 3 m plots with 10 rows of plants/plot. Line to line distance was maintained at 30 cm. In each plot equal plant populations were maintained by planting of equal numbers of viable seeds. Immediately after sowing a very light irrigation was applied followed by 2 light irrigations at 7 day intervals and 9 subsequent irrigations at intervals of 12–15 days. Meteorological data for the experimental period showed that in the first winter season (November 2016–April 2017) total rainfall was as low as 5.6 mm and 5.0 mm in the second winter season (November 2017–April 2018), while the mean monthly minimum and maximum temperatures during those 6 months were 12.2 and 29.4 °C, respectively, for 2016/17, and 16.6 and 29.8 °C, respectively, for 2017/18.

Data recording and statistical analyses

During the growing period, biomass yields (kg DM/ha) were measured on 4 occasions, viz. 45 days after sowing (DAS), 60 DAS, 75 DAS and at 50% flowering by clipping 2 rows (2.4 m^2) of each plot at 6 cm above ground level. Immediately after harvest, fresh forage yield was determined using a portable balance. A 500 g sample of fresh forage was taken from each plot and dried at room temperature without direct exposure to sunshine. When samples showed equal weight on 3 successive days, the weight recorded was considered as approximate dry weight and DM yields were determined. Dried samples of similar growth stage (50% flowering) material were ground for estimating the nutrient parameters in the 2016/17 cropping season. Crude protein (CP) percentage was estimated as per procedures of AOAC International (2005). Fiber fractions, namely neutral detergent fiber (NDF) and acid detergent fiber (ADF), were determined following the detergent method of Van Soest et al. (1991). Days to initiation of flowering, 50% flowering and maturity were recorded from planting date. Plant height (cm) and leaf:stem ratio were recorded on 5 plants in each plot at the 50% flowering stage. Plant height was measured from soil surface to tip of flower. For estimation of leaf:stem ratio (fresh weight) leaves and stems of clipped plants were hand-separated and weighed immediately. Seed yields (kg/ha) were recorded on open-pollinated plots and thousand-seed weights (g) were assessed.

Data were analyzed using PROC GLM (SAS Institute 2011). Mean data for populations were compared using the t-test. Shapiro-Wilk's test was used for normality of residual effect and homogeneity of variance was tested using Levene's test. Crop growth rate (CGR) was measured as dry biomass (kg/ha/d) accumulated in different growth stages using the following formula:

$$CGR = (W2 - W1)/(T2 - T1)$$

where:
W1 = Dry biomass at T1 of the period (kg/ha);
W2 = Dry biomass at T2 of the period (kg/ha);
T1 = Date at the start of the period; and
T2 = Date at the end of the period.

Results

Analysis of variance for biomass yield (green and dry) at 4 different growth stages, plus phenology and seed yields of the 7 different Berseem populations (lines) belonging to 3 different ecotypes was conducted to determine the variability between populations and years and any interactions. Population × year interactions were rarely significant so main effects only are presented. The majority of the diversity was attributable to differences between lines rather than between years. Year effects were significant only for days to initiation of flowering, 50% flowering and maturity, as well as seed yields.

Biomass yields (green and dry) of the populations belonging to Fahli and Saidi ecotypes were greater than those of Miskawi at all growth stages (Tables 1 and 2) and accumulated progressively with time irrespective of ecotype (Figure 1). At 75 DAS Fahli and Saidi ecotypes yielded 5.80 t DM/ha, while the Miskawi ecotype yielded 4.16 t DM/ha. Number of days to initiation of flowering, 50% flowering and maturity differed significantly between ecotypes with Fahli<Saidi<Miskawi (P<0.05; Table 3) with the main difference being between Fahli

Table 1. Fresh forage yield (kg green herbage mass/ha) of Berseem ecotypes and populations in two cropping seasons at different growth stages in Jhansi, India.

Ecotype	Population	Growth stage											
		45 days after sowing			60 days after sowing			75 days after sowing			50% flowering		
		Yr I	Yr II	Mean	Yr I	Yr II	Mean	Yr I	Yr II	Mean	Yr I	Yr II	Mean
Fahli	JHBF-1	11,278	10,978	11,128	26,320	26,220	26,270	38,617	38,285	38,451	39,083	38,301	38,692
	JHBF-2	11,952	11,651	11,801	25,775	25,675	25,725	35,677	35,361	35,519	36,177	35,453	35,815
	Mean	11,615	11,315	11,465	26,048	25,948	25,998	37,147	36,823	36,985	37,630	36,877	37,254
Saidi	JHBS-1	11,194	10,894	11,044	24,549	24,449	24,499	35,805	35,502	35,653	36,538	35,807	36,172
	JHBS-2	11,699	11,399	11,549	25,947	25,847	25,897	35,225	34,930	35,077	36,925	36,186	36,555
	Mean	11,447	11,147	11,297	25,248	25,148	25,198	35,515	35,216	35,365	36,732	35,997	36,364
Miskawi	Wardan	8,439	8,039	8,239	18,141	17,941	18,041	23,700	23,477	23,588	24,133	26,547	25,340
	Bundel Berseem-2	8,953	8,553	8,753	18,536	18,336	18,436	24,546	24,314	24,430	25,113	27,624	26,368
	Bundel Berseem-3	8,993	8,592	8,792	18,799	18,599	18,699	24,847	24,615	24,731	25,330	27,863	26,596
	Mean	8,795	8,395	8,595	18,492	18,292	18,392	24,364	24,135	24,250	24,859	27,345	26,101
Overall mean		10,358	10,015	10,187	22,581	22,438	22,510	31,202	30,926	31,064	31,900	32,540	32,220
LSD (P<0.05)		1,902	1,902	1,219	1,959	1,959	1,255	2,223	2,131	1,420	2,370	2,370	1,519

Table 2. Dry herbage mass (kg DM/ha) of Berseem ecotypes and populations in two cropping seasons at different growth stages in Jhansi, India.

Ecotype	Population	Growth stage											
		45 days after sowing			60 days after sowing			75 days after sowing			50% flowering		
		Yr I	Yr II	Mean	Yr I	Yr II	Mean	Yr I	Yr II	Mean	Yr I	Yr II	Mean
Fahli	JHBF-1	1,447	1,394	1,420	3,624	3,666	3,645	6,016	6,120	6,068	7,173	7,006	7,089
	JHBF-2	1,557	1,502	1,529	3,522	3,601	3,561	5,648	5,748	5,698	7,292	7,121	7,206
	Mean	1,502	1,448	1,475	3,573	3,634	3,603	5,832	5,934	5,883	7,233	7,064	7,148
Saidi	JHBS-1	1,423	1,371	1,397	3,494	3,312	3,403	5,749	5,641	5,695	7,139	6,972	7,055
	JHBS-2	1,473	1,420	1,446	3,704	3,651	3,677	5,798	5,690	5,744	6,591	6,437	6,514
	Mean	1,448	1,396	1,422	3,599	3,482	3,540	5,774	5,666	5,720	6,865	6,705	6,785
Miskawi	Wardan	821	766	793	2,664	2,607	2,635	4,197	4,143	4,170	4,367	5,019	4,693
	Bundel Berseem-2	887	830	858	2,631	2,576	2,603	4,225	4,171	4,198	4,332	5,241	4,786
	Bundel Berseem-3	906	848	877	2,678	2,622	2,650	4,151	4,098	4,124	4,249	5141	4,695
	Mean	871	815	843	2,658	2,602	2,629	4,191	4,137	4,164	4,316	5,134	4,725
Overall mean		1,216	1,162	1,189	3,188	3,148	3,168	5,112	5,087	5,100	5,878	6,134	6,005
LSD (P<0.05)		270	266	171	249	482	311	333	456	259	586	567	371

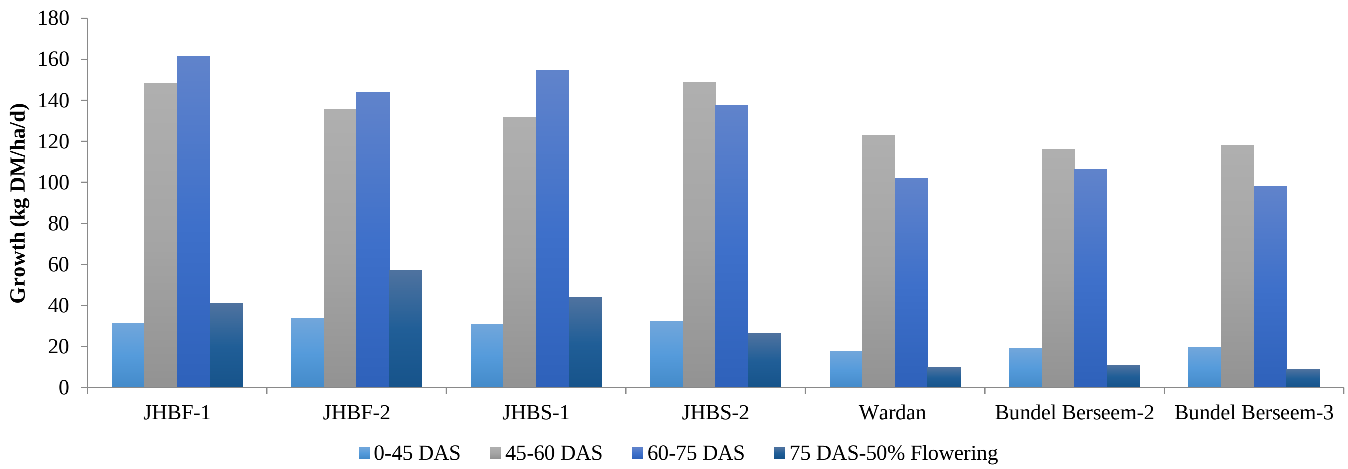

Figure 1. Dry biomass accumulation of Berseem populations at different growth stages in Jhansi, India. DAS = days after sowing.

Table 3. Flowering phenology of Berseem ecotypes and populations in two cropping seasons at different growth stages in Jhansi, India.

Ecotype	Population	Flowering stage								
		Days to initiation of flowering			Days to 50% flowering			Days to maturity		
		Yr I	Yr II	Mean	Yr I	Yr II	Mean	Yr I	Yr II	Mean
Fahli	JHBF-1	91	93	92	105	106	105	121	123	122
	JHBF-2	92	94	93	104	105	104	123	126	125
	Mean	92	94	93	104	105	105	122	124	123
Saidi	JHBS-1	97	99	98	105	109	107	121	134	128
	JHBS-2	96	97	97	104	109	106	123	136	130
	Mean	96	98	97	104	109	107	122	135	129
Miskawi	Wardan	116	118	117	128	129	128	161	162	162
	Bundel Berseem-2	114	117	115	126	129	128	162	163	163
	Bundel Berseem-3	123	123	123	138	140	139	168	171	169
	Mean	117	119	118	130	133	132	164	165	165
Overall mean		104	106	105	116	118	117	140	145	142
LSD (P<0.05)		1.9	2.0	0.7	2.4	1.6	0.8	1.8	1.6	0.6

plus Saidi and Miskawi. This difference increased as the stage of flowering advanced, the difference being 21–25 days at initiation of flowering and 36–42 days at maturity. Seed yields of Fahli and Saidi ecotypes exceeded those of Miskawi (750 vs. 414 kg/ha) as did 1,000-seed weights (P<0.001; Table 4).

Both Fahli and Saidi ecotypes were significantly taller (P<0.001) than Miskawi ecotype (Table 5) but were less leafy as reflected in the lower leaf:stem ratios. Crude protein concentration differed (P<0.05) between ecotypes, being highest in the Miskawi populations, but variation within ecotypes was non-significant (Table 5).

Variation in NDF and ADF concentrations between and within ecotypes was non-significant.

Table 4. Seed yields and 1,000-seed weights of Berseem ecotypes and populations in two cropping seasons in Jhansi, India.

Ecotype	Population	Seed yield (kg/ha)			1,000-seed weight (g)		
		Yr I	Yr II	Mean	Yr I	Yr II	Mean
Fahli	JHBF-1	803	767	785	3.65	3.64	3.65
	JHBF-2	730	730	730	3.65	3.61	3.63
	Mean	767	748	758	3.65	3.63	3.64
Saidi	JHBS-1	770	690	730	3.67	3.66	3.67
	JHBS-2	783	767	775	3.62	3.67	3.65
	Mean	777	728	753	3.65	3.67	3.66
Miskawi	Wardan	473	420	447	2.15	2.21	2.18
	Bundel Berseem-2	477	467	472	2.16	2.18	2.17
	Bundel Berseem-3	330	320	325	3.14	3.15	3.15
	Mean	427	402	414	2.48	2.51	2.50
Overall mean		624	594	609	3.15	3.16	3.16
LSD (P<0.05)		69	63	43	0.07	0.04	0.03

Table 5. Plant height, leaf:stem ratio and nutritive quality parameters of Berseem ecotypes and populations at 50% flowering in Jhansi, India.

Ecotype	Population	Plant height (cm)	Leaf:stem ratio	CP (%)	ADF (%)	NDF (%)
Fahli	JHBF-1	114.3	0.7	17.4	35.5	47.4
	JHBF-2	111.0	0.8	17.3	38.0	46.5
	Mean	112.7	0.8	17.4	36.7	47.0
Saidi	JHBS-1	109.0	0.7	17.8	34.7	44.9
	JHBS-2	109.0	0.7	18.2	34.9	45.9
	Mean	109.0	0.7	18.0	34.8	45.4
Miskawi	Wardan	90.3	0.9	19.9	34.7	44.3
	Bundel Berseem-2	88.3	0.9	20.5	33.7	44.7
	Bundel Berseem-3	81.0	0.8	19.6	34.7	44.4
	Mean	86.6	0.9	20.0	34.4	44.5
Overall mean		100.4	0.8	18.7	35.2	45.4
LSD (P<0.05)		18.7	0.1	1.7	2.5	7.6

CP = crude protein; ADF = acid detergent fiber; NDF = neutral detergent fiber.

Discussion

This study has shown that Saidi and Fahli ecotypes of Berseem clover have distinct advantages over Miskawi ecotype in terms of early growth following planting and overall yield in the first growth cycle to flowering. DM yields from Saidi and Fahli ecotypes in the 100 days following planting were 6,800–7,100 kg DM/ha compared with 4,700 kg DM/ha for the Miskawi ecotype, i.e. a 47% increase in yield at 50% flowering. Not only did the Saidi and Fahli ecotypes demonstrate this DM yield advantage over Miskawi but also they did so in a much shorter time as days to 50% flowering were much shorter in the former ecotypes (106 vs. 132 days, respectively). In situations where the window of opportunity for growing these forages is limited to about 3 months, the Saidi and Fahli ecotypes would seem to be the varieties to choose. While CP% in Miskawi populations was higher than that of the Saidi and Fahli ecotypes (20 vs. 17.4 and 18.0%, respectively), the DM yield advantage for the latter ecotypes would offset the small quality benefit.

In fact the Fahli ecotype is grown as a catch crop in Egypt and represents about 20% of the total area sown to Berseem preceding major summer crops. It has high nutritional value and is very palatable in addition to being highly productive (Muhammad et al. 2014). In India, Fahli Berseem could be planted to utilize the gap of 60–70 days between two main crops or in areas where fields are fallowed after harvesting of the rice crop. Rice fallow land represents a huge resource that could be utilized for fodder/nutritional security through planting of high-growth-rate ecotypes of Berseem (Fahli/Saidi), while ensuring sustainability of land resources with the cereal-legume rotational cropping system.

Seed yield potential of cultivated varieties of the Miskawi ecotype proved very low. Owing to its low productivity and greater demand for fodder during the lean period of summer, commercial seed production of clover is not highly successful in India (Vijay et al. 2017). The present study showed that seed yield potential of Fahli and Saidi ecotypes exceeded that of Miskawi. There is possible scope to increase seed yield potential of the cultivated Miskawi ecotype by transfer of genes from Fahli and Saidi. The poorer seed yields of Bundel Berseem-3 relative to Wardan and Bundel Berseem-2 may be due to the tetraploid nature of Bundel Berseem-3.

Seed size is a widely accepted measure of seed quality and an important seed yield component in crop species (Egli et al. 1987). Increased seed size has been positively associated with germination, seedling height, root length, primary leaf size and seedling weight, crop performance and yield potential in different species (Chandra Babu et al. 1990; Bretagnolle et al. 1995; Assis et al. 2018). Seed sizes of Fahli and Saidi ecotypes were much larger than Miskawi, although within Miskawi populations variation existed for seed size. This may be due to ploidy differences, because cultivars Wardan and Bundel Berseem-2 are diploid and the bold-seeded Bundel Berseem-3 is a polyploid ($2n = 4x = 32$) variety (Pandey and Roy 2011). Effects of ploidy on seed size have been reported in other species (Scott et al. 1998; Miller et al. 2012).

Conclusions

Both Saidi and Fahli ecotypes of Berseem seem to have distinct advantages over the Miskawi ecotype with more rapid establishment, more rapid growth and earlier maturity. In situations where short-term crops are needed to fill winter feed gaps, they would seem to be the varieties of choice. The slower establishment of Miskawi ecotype could possibly be improved through genetic methods.

Genetic improvement of Berseem crops in India has not been pursued in the past due to lack of variability for targeted traits in the Miskawi gene pool of *T. alexandrinum*. Researchers have attempted to exploit the secondary and tertiary gene pools of Berseem, which is associated with problems like cross-incompatibility barriers and linkage drags. Our investigation has shown that genetic variability for many agronomic traits exists at the ecotype level in the primary gene pool of Berseem. It is possible that genes present in one ecotype could be transferred successfully into other ecotypes of Berseem without biotechnological tools as needed in interspecific hybridization. Both Fahli and Miskawi ecotypes could be used as parents to develop mapping populations. Such a mapping population could be utilized in future for the development of linkage maps and to map a range of quantitative traits including days to flowering, regrowth potential, dry matter yields etc. Due to their rapid maturation and high biomass accumulation rate, Fahli and Saidi ecotypes could be used as catch crops during the gap between *kharif* (July–October) and *rabi* (November–April) crops in different cropping systems. In some areas where land is fallowed after harvesting of a rice crop, single-cut Berseem could be grown on residual moisture from the rice crop.

Every year India imports a huge quantity of Berseem seed from abroad. For self-sufficiency of Berseem seed, the low productivity of Miskawi varieties could be improved through a seed yield improvement program utilizing Fahli and Saidi ecotypes as parent populations for introduction of traits to enhance seed yield. More sources of germplasm must be targeted to increase diversity beyond the variability available in the Berseem genepool currently available in India to improve the cultivar development program.

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