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Research article

Assessment of water use efficiency in single and multi-cut lines of berseem (*Trifolium alexandrinum* L.)

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Abstract

Water use efficiency (WUE) and biomass yield were evaluated in released berseem varieties and breeding lines. The study included single-cut variety JBSC-1, three multi-cut varieties (Wardan, Bundel Berseem-2, Bundel Berseem-3), six single-cut lines (NU/7/5/15, NU/2/14/2015, NU/2/19/2015, NU/2/23/2015, Ex-8, FAO5), and four multi-cut lines (IL-16-1, IL-16-2, IL-16-3, IL-13-106), grown under pot conditions. Daily plant water requirements were recorded after 10, 20, and 40 consecutive days of irrigation, corresponding to 20, 30, and 40 days after sowing. Among multi-cut lines, IL-16-1 (3.4 g fwt plant^{-1} kg^{-1} water) and IL-13-106 (3.2 g fwt plant^{-1} kg^{-1} water) exhibited the highest WUE, whereas among single-cut lines, NU/2/19/2015 (12.8 g fwt plant^{-1} kg^{-1} water) and NU/2/14/2015 (12.2 g fwt plant^{-1} kg^{-1} water) showed superior performance. A significant positive correlation ($p < 0.05$, $p < 0.01$) was observed among all photosynthetic pigments. The levels of photosynthetic pigments, relative water content, and membrane stability index showed contrasting associations with green fodder yield, negative in multi-cut and positive in single-cut genotypes. Significant differences (p <0.05, p <0.01) were detected in plant height and dry fodder yield across the evaluated varieties. WUE, assessed on both a fresh and dry matter basis, exhibited a negative association. Notably, the single-cut variety JBSC-1 recorded the highest dry-matter-based WUE (0.23 g dwt plant^{-1} kg^{-1} water at 10 days) among all varieties tested.

Keywords: Berseem, Drought, Multi-cut, Single-cut, Water use efficiency.

Introduction

Drought is the primary environmental constraint limiting crop productivity under Indian agro-climatic conditions. Crop and forage species exposed to water stress exhibit a range of adaptive responses, the most common being stomatal closure and modulation of leaf area, which collectively reduce canopy-level water loss (Buckley, 2019). Improving agricultural water use efficiency (WUE) has therefore become a key priority, as drought remains one of the most significant factors limiting green fodder and grain yield across arid and semi-arid ecosystems worldwide. WUE, defined as the ratio of crop yield to the water consumed during growth (Waraich *et al*., 2008), is considered a valuable selection criterion for enhancing yield stability under dry environments (Buckley, 2019) and serves as a reliable indicator of plant performance (Dheeravathu *et al*., 2018).

Berseem or Egyptian clover (*Trifolium alexandrinum* L., 2n = 2x = 16) is an important annual, winter-season forage legume originally domesticated in the Mediterranean region and subsequently introduced to India, Pakistan, South Africa, the USA, and Australia. *T. alexandrinum* is broadly grouped into three distinct ecotypes: Miskawi (Miscavi), Saidi, and Fahli, which differ markedly in morphology, biomass productivity, and regrowth capacity after cutting. The Miskawi ecotype exhibits vigorous regrowth and supports 4-6 harvests per season, the Saidi type supports two cuts, and the Fahl (Fahli) type is single-cut, reflecting its limited regrowth ability (Singh *et al*., 2020). Berseem requires frequent irrigations to achieve multiple cuttings and high green fodder yield (Dheeravathu *et al*., 2017b). Consequently, forage yield is often reduced under inadequate rainfall or limited irrigation availability. Water deficit further suppresses chlorophyll synthesis, adversely affecting photosynthesis, growth rates, and ultimately fodder production (Singh *et al*., 2020; Dheeravathu *et al*., 2017b). Climate-resilient forage crops including grasses such as guinea grass, bajra–napier hybrids, and dinanath grass (Dheeravathu *et al*., 2021; Dikshit *et al*., 2020; Antony *et al*., 2021); legumes such as cowpea, berseem, clitoria, centrosema, and siratro (Dheeravathu *et al*., 2017a; 2017b; 2023); and forage cereals, particularly millets such as pearl millet, kodo millet, and sorghum (Dheeravathu *et al*., 2022; Dheeravathu and Vadithe 2024) have demonstrated high adaptability to climate change. Enhancing WUE through judicious water management and genetic improvement is therefore essential to sustain agricultural productivity in arid and semi-arid regions.

Increasing soil salinity, deteriorating water quality, rising atmospheric CO_2 levels, and elevated temperatures pose further challenges for forage productivity by altering soil health and affecting plant physiological and biochemical processes. Drought reduces the area under fodder crops and contributes to fodder scarcity, especially during lean periods. Improving water productivity remains a critical strategy for enhancing food and fodder production under limited water availability (Rosegrant *et al*., 2002). Escalating energy costs further necessitate efficient water resource management. It is essential for farmers to optimize the use of every available unit of water, whether derived from irrigation or rainfall; however, livestock farmers often do not adopt specialized practices for forage and pasture management during drought.

Although substantial research has been conducted to optimize crop water use, genetic improvement of transpiration efficiency (TE) has progressed slowly, primarily due to the lack of reliable and high-throughput screening techniques for assessing genetic variability in WUE across germplasm, segregating populations, and inbred lines. Therefore, the present study was undertaken to standardize an effective screening methodology and evaluate both multi-cut and single-cut berseem lines for high water use efficiency.

Materials and Methods

Site description and design: The experiment was conducted during the rabi seasons of 2018–19 and 2019–20 at the ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India (25°45′ N, 78°58′ E; 243 m above MSL). The study was laid out in a completely randomized block design (CRBD) with three replications under pot culture conditions. Seeds were sown in plastic pots (22 cm height × 14 cm width) filled with 4 kg of a soil mixture comprising dry soil, sand, and farmyard manure in a 2:1:1 ratio. Pots were saturated with water and allowed to drain overnight to attain field capacity (FC). Three empty pots (without seeds) were maintained to estimate evaporative loss, while three additional pots (with seeds) per variety were used for determining initial plant biomass. A polyvinyl chloride (PVC) tube (10 cm length × 2.2 cm diameter) with a perforation near the lower end was inserted vertically into each pot to facilitate water application at the root zone. To minimize direct soil evaporation, a thin polythene mulch was placed on the soil surface. After seedling emergence, plants were thinned to four seedlings per pot, and soil moisture was maintained at 80% available soil moisture (ASM). Daily plant water use was recorded at 20, 30, and 40 days after sowing (DAS) for ten consecutive days.

Experimental material: During the 2018-19 *Rabi* season, four multi-cut breeding lines *viz*., IL-16-1, IL-16-2, IL-16-3, IL-13.106; six single-cut breeding lines *viz*., Nu/7/5/15, Nu/2/14/2015, Nu/2/19/2015, Nu/2/23/2015, FAO-5 and EX-8 with three multi-cut released varieties *viz*., Wardan, Bundel Berseem-2 (BB2) and Bundel Berseem-3 (BB3); were evaluated. During the 2019-2020 *Rabi* season, multi-cut varieties *viz*., Wardan, BB2, and BB3, and single-cut variety, JBSC-1 were used in the study.

Determination of water use efficiency (WUE): Water use efficiency was calculated following Dheeravathu *et al*. (2018). At the beginning of each measurement cycle, all pots were saturated with water and allowed to drain for 4 to 6 days, stabilizing moisture at 80% ASM. Initial biomass was quantified by uprooting plants from three pots per genotype. Pots were weighed daily (between 9:00 and 11:00 AM), and the amount of water lost (transpiration + evaporation) was replenished to maintain 80% ASM. Water was supplied through PVC tubes to ensure uniform moisture distribution across the root zone.

Cumulative water transpired (CWT) =Cumulative water added (CWA) on a daily basis - Cumulative evaporative loss (CEL)

Water use efficiency (WUE) (g kg^{-1} or L^{-1}) =

$$\frac{\text{Final dry weight} - \text{Initial dry weight}}{\text{Cumulative water transpired}}$$

Estimation of physiological parameters: Physiological traits, including chlorophyll content, carotenoids, membrane stability index (MSI), relative water content (RWC), and total plant water content (TPWC), were determined using fully expanded leaves (third leaf from the top). Using the acetone method, green leaf samples (200 mg fresh weight) to extract chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (total Chl), and carotenoids (Car). Additionally, 100 mg leaf samples were used to determine the membrane stability index (MSI) according to the method of Premachandra *et al*. (1990). The relative water content (RWC) of 100 mg leaf samples was measured using Weatherley's (1950) method. The total plant water content was calculated as outlined in Dheeravathu *et al*. (2018). These parameters were calculated using the formulae given below-

Relative water content (RWC%): = [(Fresh weight -Dry weight)/ (Total weight - Dry weight] x 100

Membrane stability index (MSI%): MSI = [1 - (C1/C2)] * 100
Total plant water content (TPWC%) = TPWC% = [(Fresh weight - Dry weight)/ Dry weight] x 100
Chlorophyll 'A' (mg g^{-1} fw) = [(12.7 x A_{663}) -(2.69 x A_{645})] x V/ 1000 x W
Chlorophyll 'B' (mg g^{-1} fw) = [(22.9 x A_{645}) -(4.68 x A_{663})] x V/ 1000x W
Total chlorophyll (mg g^{-1} fw) = [(8.02 x A_{663}) +(20.2 x A645)] x V/ 1000x W
Total carotenoids (mg g^{-1} fw) = [(A_{480} +(0.114 x A_{663})] – (0.638XA645) x V/ 1000 x W
where, A=Absorbance, V=Volume (ml), w= Weight (g)

Biomass and related traits: Four plants per line were harvested at 40 DAS to determine initial biomass. At the end of the experimental period, another set of four plants per pot was harvested. Shoots were oven dried at 65°C to constant weight (approximately 72 hours) and weighed with an electronic balance. The increase in dry weight during the experiment was estimated as the difference between the whole-plant dry weights at the start and end of the experiment. Plant height was recorded at 20 and 40 DAS for multi-cut lines and at 40 DAS for single-cut lines.

Statistical analysis: Data were analyzed using Microsoft Excel and SAS 9.3. Treatment means were compared using Duncan's Multiple Range Test (DMRT) at the 5 and 1% significance levels.

Results and Discussion

This study aimed to evaluate both single and multi-cut berseem released varieties as well as breeding lines, focusing on a key trait that significantly influences plant growth and yield.

Plant height: Plant height varied significantly among the evaluated lines (p <0.05). Among the seven multi-cut lines, the variety Wardan recorded the maximum height (46 cm), while the lowest height was observed in the IL-13-106 (10 cm). In the single-cut group, FAO-5 showed the maximum height (55 cm) and IL-16-8 exhibited the lowest height (36 cm) (Table 1). Overall, single-cut berseem lines were taller than the multi-cut lines, and data revealed that plant height and dry matter production per plant of berseem are significantly influenced by different varieties (p <0.05).

Relative water content and membrane stability index: Relative water content (RWC) and membrane stability index (MSI) are established indicators of abiotic stress tolerance (Dheeravathu *et al*., 2021). Among multi-cut, BB2 had the highest RWC (86%), while BB3 had the lowest (79%). MSI ranged from 86% in IL-16-2 to 72% in BB3 (Table 1). In single-cut, RWC varied from 82% (FAO-5) to 73% (Nu/7/5/15), while MSI ranged from 83% (Nu/2/14/2015) to 74% (Nu/2/23/2015).

Table 1. Plant height and different physiological parameters in multi and single cut berseem lines

Traits/ lines	PH	Chl a (mg)	Chl b (mg)	Total Chl (mg)	Chl a/b ratio	Car (mg)	MSI (%)	RWC (%)	WUE
Multi cut									
WARDAN	46 ± 0.94	1.29	0.49	1.78	2.65	0.11	75 ± 0.94	78 ± 1.89	3.0 ± 0.24
BB2	42 ± 1.89	1.7	0.59	2.3	2.87	0.14	85 ± 2.36	86 ± 0.47	1.7 ± 0.12
BB3	42 ± 0.94	1.28	0.48	1.76	2.67	0.11	72 ± 1.89	79 ± 1.41	1.5 ± 0.09
IL-13-106	10 ± 0.47	1.46	0.57	2.03	2.57	0.14	82 ± 0.47	85 ± 0.47	3.2 ± 0.05
IL-16-1	28 ± 0.94	0.95	0.38	1.33	2.54	0.1	84 ± 1.89	83 ± 2.60	3.4 ± 0.05
IL-16-2	40 ± 1.41	1.03	0.4	1.43	2.6	0.1	86 ± 1.41	81 ± 1.89	2.4 ± 0.09
IL-16-3	45 ± 1.41	1.07	0.45	1.52	2.43	0.07	83 ± 0.94	80 ± 1.89	2.6 ± 0.10
Single cut									
FAO-5	55 ± 1.41	1.27	0.45	1.72	2.91	0.11	77 ± 0.94	82 ± 1.89	9.5 ± 0.12
EX-8	47 ± 1.88	1.48	0.49	1.97	3.25	0.11	82 ± 2.83	77 ± 2.83	6.4 ± 0.14
NU/2/14/15	46 ± 0.47	1.26	0.45	1.71	2.90	0.11	80 ± 0.94	73 ± 0.82	12.1 ± 0.52
NU/2/19/2015	46 ± 2.35	1.35	0.52	1.87	2.71	0.14	83 ± 0.94	81 ± 2.36	12.8 ± 1.42
NU/2/23/2015	38 ± 1.88	1.03	0.41	1.44	2.50	0.12	76 ± 2.13	76 ± 1.89	7.9 ± 0.12
IL-16-8	36 ± 1.41	1.09	0.42	1.51	2.63	0.09	74 ± 1.19	78 ± 2.83	9.8 ± 0.17

PH: Plant height (cm); Chl a: Chlorophyll a; Chl b: Chlorophyll b; Total Chl: Total chlorophyll; Chl a/b: Chlorophyll a/b ratio; Car:

RWC and MSI showed positive correlations with biomass yield in multi-cut lines, while negative correlations were observed in single-cut lines (Table 2). This aligns with earlier findings that plants maintaining high RWC and MSI under stress are more tolerant (Bangar *et al.*, 2019; Rahimi *et al.*, 2021). Total plant water content (TPWC) was highest in BB3 (88.71%), while the lowest TPWC was recorded in JBSC-1 (82.91%) (Table 3). Dry fodder content (%) was maximum in JBSC-1 (17.1%), followed by Wardan (16.5%), whereas BB3 recorded the lowest (11.3%) (Table 4).RWC, MSI, and TPWC were generally higher in high-WUE lines, consistent with Dheeravathu *et al.* (2021).

Chlorophyll and carotenoid content: Chlorophyll and carotenoid contents varied significantly across lines (Table 1). In the multi-cut group, chlorophyll a ranged from 1.07 to 1.70 mg g^{-1} FW, and chlorophyll b from 0.38 to 0.59 mg g^{-1} FW. In single-cut lines, chlorophyll a ranged from 1.03 to 1.48 mg g^{-1} FW, and chlorophyll b from 0.41 to 0.52 mg g^{-1} FW. Carotenoids ranged from 0.01 to 0.14 mg g^{-1} FW in multi-cut lines and 0.09 to 0.14 mg g^{-1} FW in single-cut genotypes. The highest carotenoid content (0.14 mg g^{-1} FW) was observed in BB2, IL-13-106, and Nu/2/14/2015. Chlorophyll pigments are critical for photosynthesis and often decrease under drought stress, leading to reduced growth (Dheeravathu *et al.*, 2021). In this study, high chlorophyll and carotenoid concentrations were associated with higher WUE, indicating better physiological efficiency under water-limited conditions.

Fresh weight, dry weight and water use efficiency: Among multi-cut lines, IL-16-1 (3.4 g FW $plant^{-1}$ kg^{-1} water) and IL-13-106 (3.2 g FW $plant^{-1}$ kg^{-1} water) exhibited the highest WUE. The variety Wardan (3.0 g FW $plant^{-1}$ kg^{-1} water) recorded the highest WUE among released multi-cut varieties (Table 1).In the single-cut group, NU/2/19/2015 (12.8 g FW $plant^{-1}$ kg^{-1} water) showed maximum WUE, followed by NU/2/14/2015 (12.1 g FW $plant^{-1}$ kg^{-1} water). The single-cut variety JBSC-1 recorded the highest WUE (0.23 g DW $plant^{-1}$ kg^{-1} water), while multi-cut varieties showed comparatively lower WUE. Among the multi-cut released varieties, Wardan had the highest WUE, followed by BB2, with BB3 recording the lowest (Table 4). These findings align with Singh *et al*. (2020), who reported that Mescavi ecotype multi-cut varieties accumulate less biomass than the single-cut Fahli ecotype variety.

A positive correlation was observed between plant height, plant water content, green fodder yield, and dry fodder yield. Plant water content showed a negative relationship with dry matter content. A significant negative correlation was found for WUE between green fodder yield and dry fodder content (p <0.05; p <0.01) (Table 5). Low-WUE lines exhibited lower RWC, MSI, chlorophyll, and carotenoid levels, whereas high-WUE lines showed higher values for these parameters (p <0.05). Photosynthetic pigments were significantly and positively correlated with each other (p <0.01). In multi-cut lines, photosynthetic pigments, RWC and MSI showed negative correlations with green fodder yield, while in single-cut genotypes, these parameters showed positive correlations with biomass yield (Table 1). Two lines, IL-13-106 (9.0 g FW $plant^{-1}$) and NU/2/14/2015 (18 g FW $plant^{-1}$), produced low biomass yet exhibited high WUE consistent with findings by Sevanayak *et al*. (2017b) and Dheeravathu *et al.* (2018). These Mescavi-type lines, along with the high WUE single-cut variety, may serve as valuable donors for breeding programmes targeting enhanced WUE under limited water conditions.

Table 2. Correlation coefficient among the morpho-physiological and biochemical traits of diverse group of berseem multi and single cut lines

Traits	PH	FW	Chl a	Chl b	Total Chl	chl a/b	Car	RWC	MSI
PH		-0.541	-0.255	-0.258	-0.255	-0.230	-0.337	-0.599	-0.245
FW	0.051		-0.305	-0.295	-0.303	-0.305	-0.334	0.059	0.252
Chl a	0.486	0.496		0.996**	0.996**	0.903**	0.925**	0.567	-0.067
Chl b	0.327	0.697	0.922**		0.998**	0.862*	0.927**	0.509	-0.132
Total Chl	0.456	0.549	0.549	0.549		0.894**	0.926**	0.553	-0.083
chl a/b	0.590	-0.065	0.704	0.704	0.638		0.824*	0.776*	0.228
Car	0.125	0.779*	0.639	0.848*	0.696	-0.029		0.476	-0.233
RWC	0.427	-0.150	-0.024	0.058	-0.006	-0.148	-0.079		0.655
MSI	0.421	-0.213	0.285	0.194	0.268	0.338	-0.064	0.142	

Correlation coefficient value: Upper diagonal in multicut lines and lower diagonal in single cut lines; *(p <0.05);**(p <0.01); FW: Fresh weight; PH: Plant height; Chl a: Chlorophyll a; Chl b: Chlorophyll b; Total Chl: Total Chlorophyll; Chl a/b: Chlorophyll a/b ratio; Car: Carotenoids; RWC: Relative water content; MSI: Membrane stability index

Table 3. Plant height and water use efficiency, plant water content and dry fodder content (%) in single and multi-cut berseem varieties (on dry weight basis)

Varieties	Plant height (cm)	CW A (kg)	CE L (kg)	CWT (kg/ plant)	A = Initial dry wt/pot	B=Final dry wt/pot	C(B-A) Actual dry weight gain/pot	WUE =(FWT/ Total water used per pot)	WUE=Dry weight / plant (g kg^{-1})
Wardan	26.33b	0.13	0.06	0.07	0.07	0.2	0.13	1.90	0.19b
BB2	32.00a	0.14	0.06	0.07	0.05	0.18	0.13	1.77	0.18b
BB3	28.33b	0.13	0.06	0.07	0.04	0.14	0.10	1.44	0.14c
JBSC-1	28.83ab	0.13	0.06	0.07	0.06	0.22	0.16	2.27	0.23a
Mean	28.87								0.19
CV	6.54								7.04
CD (0.05)	3.55								0.02

CWT: Cumulative water transpired; Means followed by the same letter (s) in column (s) are not significantly different (P<0.05), where letter 'a' represents the least value, or Different letters indicate statistically significant differences (P<0.05)

Table 4. Water use efficiency (fresh and dry weight basis), plant water content and WUE ratio in single and multicut berseem varieties

Varieties	WUE= Fresh weight/plant (g kg^{-1})	WUE=Dry weight /plant (g kg^{-1})	Plant water content (%)	WUE ratio
Wardan	1.15	0.19	83.48	16.5
BB2	1.26	0.18	85.98	14.0
BB3	1.27	0.14	88.71	11.3
JBSC-1	1.33	0.23	82.91	17.1

WUE: Water use efficiency

Table 5. Correlation among different parameters upon water use efficiency in berseem varieties

Traits	PH	TPWC (%)	WUE_F	WUE_D
TPWC(%)	0.24	0.56	0.05	-0.21
WUE_F		0.20	-0.90	-1.00**
WUE_D			0.26	-0.11
DFC				0.93**

**(P<0.01); PH: Plant height; TPWC: Total plant water content; WUE_F: Fresh weight per plant; WUE_D: Dry weight per plant; DFC: Dry fodder content

Conclusion

Among the multi-cut lines IL-16-1, IL-13-106, and the variety Wardan were identified as high-WUE performers. In the single-cut group, the line NU/2/14/2015 and the variety JBSC-1 showed superior WUE. Although IL-16-1 and IL-13-106 exhibited lower biomass production, their consistently high WUE makes them suitable candidates for developing water-efficient berseem varieties. The single-cut variety JBSC-1, characterized by high WUE and higher dry matter content, can be recommended for cultivation in low-water cropping systems. The identified high-WUE liness particularly IL-16-1, IL-13-106, and JBSC-1, have strong potential for use in breeding programmes aimed at enhancing drought tolerance and water productivity in berseem, thereby contributing to climate-resilient forage production. Further research employing molecular and physiological approaches may help elucidate the mechanisms responsible for the superior WUE of single-cut genotypes compared with multi-cut types.

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