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RESEARCH PAPER

Morphological and physiological traits for yield in lucerne (*Medicago sativa* L.) under irrigated condition

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Abstract: A field experiment was laid out in RBD at SRRS, ICAR-IGFRI, Dharwad, from December 2019 to January 2021 to screen lucerne genotypes under irrigated conditions. Morphological traits like plant height and the number of branches were higher in genotypes A2×WC(D-4), Anand-2, and ACC45/2010 under irrigated conditions. A2×WC(D-4) and Anand-2 also maintained good persistence, resulting in higher fresh biomass. A significant correlation was noticed between fresh biomass and an increase in the number of branches at harvest and persistence (r = 0.63* and 0.63* respectively). Anand 2 recorded the highest chlorophyll and carotenoid content. Photosynthetic rate and conductance recorded a significant correlation with fresh biomass (r = 0.51* and 0.71** respectively)under irrigation. At 12 MAS, the crown diameter was prominent in varieties that recorded high biomass *viz.,* Anand-2, A2×WC(D-4), and ACC45/2010. Hence, the morphophysiological traits like the number of branches during the juvenile phase, persistence percentage, photosynthesis and conductance, and crown diameter at 12 MAS can be used for screening lucerne genotypes for high biomass under irrigated conditions.

Keywords: Biomass, Crown diameter, Lucerne, Photosynthesis

Introduction

Lucerne (*Medicago sativa* L.), the queen of forage crop, has significant economic value and is widely cultivated worldwide. In India, lucerne is grown in Punjab, Maharashtra, Uttar Pradesh, Gujarat, and Tamil Nadu in over one million ha with the productivity of 60-130 tons per ha. Nutritionally, lucerne is known to have 65-72 % digestible carbohydrates and about 12-24 % crude protein, and this quality is known to be maintained nearly constant throughout the year (Ferreira *et al.*, 2015). Lucerne also contains minerals like phosphorus, magnesium, and calcium and vitamins like A and D. However, total energy content is high, and it helps save the amount spent on concentrates by farmers. Lucerne is used as a concentrated feed for milking cows as it got high protein content and high digestible fibre content. Apart from cattle, lucerne is also fed to sheep, goats, and rabbits (Heuze *et al*., 2013). Lucerne plants are hairless with many stems originating from the crown, and the leaves are trifoliate with leaflet length greater than width. During harvest, the destruction of shoot buds in the crown region hinders further regrowth (Hamidi and Safarnejad, 2010). Flowers of lucerne are compact racemes with purple floret. Pods are coiled spirally with two to five kidney-shaped yellow or brown seeds.

In India, Lucerne has grown mainly for a ‘cut and carry’ system, whereas, in pastures, it is grown for grazing. Under the cut and carry system, lucerne is grown under irrigated conditions for obtaining high biomass. In India, indigenous and introduced germplasm of lucerne is used to select high yielding genotypes suitable for the particular region under irrigated conditions. This study was undertaken to assess the morphological and physiological parameters required for selecting lucerne genotypes for high biomass under irrigated conditions.

Material and methods

An experiment was conducted at Southern Regional Research Station, ICAR- Indian grassland and fodder research institute (IGFRI), Dharwad, from December 2018 to March 2020 to screen lucerne genotypes under irrigated conditions. Irrigation was provided once in 10 days up to field capacity. As the seeds were tiny, sowing was done at shallow depth manually on the soil’s surface at a spacing of 30 x10 cm. The recommended dose of fertilisers as per AICRP (20:80:0 kg ha^{1}) was applied at the time of sowing and after every harvest. The first harvest was taken 60 DAS, and after that, every 25 days, the crop was harvested, and fresh biomass was recorded. The biomass harvested at 25-day intervals was added up, and the average was taken to express it in t/ha/cut.

Morphological observations like plant height, number of branches, fresh and dry weight were recorded at every harvest. Persistence was calculated as the number of plants per unit area and the number of components, counted between the 3rd and initial harvest. The crown diameter was measured by rounding a thread around the crown region and later measured the thread’s length (cm) using a scale. The leaf weight ratio was calculated as a ratio of the leaf dry weight to the leaf area and expressed in mg cm-^{2}. Chlorophyll content was estimated by the DMSO (Dimethyl sulfoxide) method of Hiscox and Israelstam (1979), including the improvements suggested by Barnes *et al.*(1992) and Wellburn (1994). Total sugars were estimated at the second harvest by the phenol sulfuric acid

Table 1. Plant height and number of branches in Lucerne genotypes under irrigation

Genotypes	Plant height (cm)	Persistence at the juvenile phase (%)		Number of branches at third cut the juvenile stage	Increase/Decrease in number of branches (%)at
A2xWC (T-5)	49.0^{abc}	87.51	(9.35)	10.88^{def}	-86.89
A2xWC (D-5)	50.0^{ab}	88.90	(9.42)	15.88^{a}	+94.60
RL-88xWC (T-4)	49.0^{abc}	96.79	(9.84)	10.33^{efg}	-80.85
RL-88xWC (D-4)	42.6^{de}	86.65	(9.39)	9.33^{g}	-89.38
A2xWC (T-4)	44.6^{bcde}	93.76	(9.70)	9.66^{fg}	-79.95
A2xWC (D-4)	39.6^{ef}	100.00	(9.99)	11.33^{cde}	+103.33
ACC 45/2010	48.6^{abc}	93.55	(9.67)	12.55^{bc}	+111.17
Raj 22/2007	47.6^{bcd}	87.11	(9.32)	11.11^{de}	+108.20
Anand-2	54.0^{a}	93.76	(9.69)	13.33^{b}	+91.57
RL-88	41.0^{ef}	82.84	(9.10)	10.22^{efg}	-73.18
ACC 23/2010	37.0^{f}	96.86	(9.84)	12^{cd}	+129.79
ACC 38/2010	43.6^{cde}	87.86	(9.35)	10.55^{efg}	-93.03
S.Em. ±	1.83	1.83	0.43		
C.D. at 5%	5.37	5.37	1.12		

method (Dubois *et al.,* 1956). Photosynthesis and conductance were measured using IRGA LI6400XT with a leaf chamber area of two (2) cm^2. The light intensity used was 1200 µ moles m^{-2} s^{-1}. Functional leaf was inserted into the leaf chamber, and the readings were logged when photosynthesis and conductance, values were positive and stable.

Statistical analysis was done using WASP-2 (Web Agri stat package2.0) hosted by CARI, Goa (www.ccari.res.in).The CD values were calculated wherever the F test was significant and denoted using Duncans Multiple range test (DMRT). The correlation was calculated using Graph pad prism 8.4.3 (686) and presented as a heat map.

Results and discussion

Lucerne, a perennial crop, exhibits varying growth characteristics related to genotype, environment, and management practices. Lucerne is grown under irrigated cut and carry and pasture system. It is crucial to consider morphological and physiological characters to select genotypes suitable for a given ecosystem. Among the genotypes, Anand-2 (8.95 & 2.06 t/ha/cut), ACC 45/2010 (6.99 & 1.81t /ha/cut) and A2 x WC(D-5) (6.84 &1.69 t/ha/cut) recorded the highest fresh and dry biomass respectively (Table 2). Further, Anand -2 recorded the highest plant height (54 cm) and was minimum (37 cm) in ACC 23/2010 (Table 1). A significant increase in branches was observed in ACC23/2010 and ACC 45/2010 (Table 1). Persistence indicating the number of plants surviving after each harvest was highest in A2 x WC (D-4). The number of branches and persistence were positively correlated significantly to fresh and dry biomass (r = 0.63* and 0.69*, Fig 2), respectively.

Specific leaf weight is the indicator parameter that gives the thickness of the leaf, which infers tolerance to any stresses. RL-88 recorded the highest SLW, *i.e.,* 4.15 mg cm^{-2}, followed by Raj 22/2007, ACC 45/2010, and A2 × WC (T-4), whereas the lowest was observed in A2 × WC (T-5),3.65mg cm^{-2} (Table 3). Among genotypes, Anand-2 (1.33 µg ml^{-1}), RL-88 (1.31 µg ml^{-1})

Table 2. Fresh and dry biomass in Lucerne genotypes under irrigation

Genotypes	Fresh biomass (t/ha/cut)	Dry biomass (t/ha/cut)	Crown diameter at 12 MAS (cm)
A2xWC (T-5)	4.83^{cde}	1.08^{fg}	14.3^{e}
A2xWC (D-5)	6.84^{bc}	1.69^{abc}	19.9^{cd}
RL-88xWC (T-4)	5.68^{bcde}	1.46^{bcdef}	16.7^{de}
RL-88xWC (D-4)	4.37^{de}	1.20^{ef}	20.1^{cd}
A2xWC (T-4)	5.63^{bcde}	1.42^{cdef}	22.9^{bc}
A2xWC (D-4)	7.55^{ab}	1.81^{abc}	22.8^{bc}
ACC 45/2010	6.99^{abc}	1.81^{ab}	24.4^{b}
Raj 22/2007	4.28^{e}	0.70^{g}	23.5^{bc}
Anand-2	8.95^{a}	2.06^{a}	29.9^{a}
RL-88	4.84^{cde}	1.24^{def}	22^{bc}
ACC 23/2010	6.40^{bcd}	1.61^{bcd}	21.96^{bc}
ACC 38/2010	5.80^{bcde}	1.47^{bcdf}	20.26^{cd}
S.Em. ±	0.71	0.13	1.23
C.D. at 5%	2.09	0.38	3.63

and ACC 23/2010 (1.30 µg ml^{-1}) recorded highest and RL-88 × WC (D-4) recorded lowest total chlorophyll content (0.93 µg ml^{-1}) in irrigated condition (Table 3). The highest carotenoid content under irrigation (1126.66 µg ml^{-1}) was recorded in RL-88, ACC 38/2010, Anand-2, and ACC 45/2010. The lowest carotenoid was recorded in RL-88 × WC (D-4); 664.92 µg ml^{-1} (Table 3). When irrigated, genotype, A2 × WC (T-5) recorded the highest total soluble sugars, followed by ACC 23/2010, A2 × WC (D-5), and RL-88 × WC (T-4), lowest was recorded in genotype ACC 38/2010 (26.74 mg/g; Table 3)

Under irrigated conditions, the genotype Anand-2 recorded maximum photosynthetic rate (31.66 µ moles CO_2 m^{-2} s^{-1}), and genotype A2 × WC (T-4) recorded minimum photosynthetic rate (21.66 µ moles CO_2 m^{-2} s^{-1}). The genotype RL-88 × WC (D-4) and RL-88 × WC (T-4) recorded the highest (24.04 µ molesCO_2 m^{-2} s^{-1}) and the lowest (10.16 µCO_2 m^{-2} s^{-1}) photosynthetic rate respectively. The cultivar Anand-2 and A2 × WC (D-5) recorded the highest conductance, and the

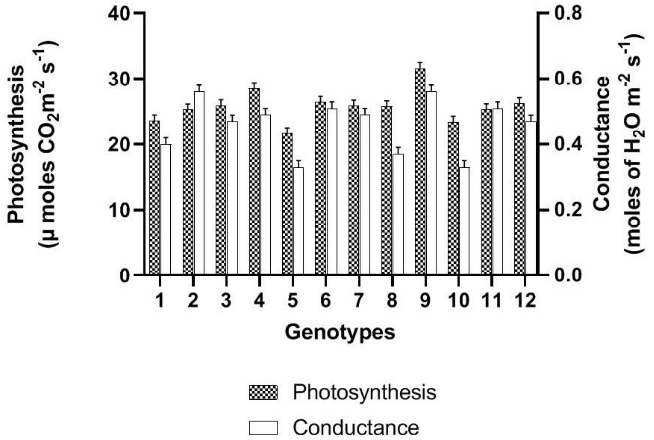

Fig 1. Photosynthesis and Conductance observed in *Medicago sativa* genotypes under irrigation. Numbers 1-12 denotes genotype A2xWC (T-5), A2xWC (D-5), RL-88xWC (T-4), RL-88xWC (D-4), A2xWC (T-4), A2xWC (D-4), ACC 45/2010, Raj 22/2007, Anand-2, RL-88, ACC 23/2010, ACC 38/2010 respectively

lowest was recorded in A2 × WC (T-4) and RL-88 (0.33 moles of H_2O m^{-2} s^{-1}); Fig 1

Genotypic and phenotypic variations in varieties interact with soil moisture levels to yield above ground matter which is the economic part of the lucerne. Interaction of moisture with below-ground matter or root system can influence the dry matter partitioning to shoots. When moisture is not deficit under irrigated conditions, the genotypes must be chosen based on specific phenological and physiological characteristics to obtain a high yield in lucerne. This study observed that the top three performing genotypes (Anand 2, ACC 45/2010, and A2 x WC (D-5) recorded the highest branches. The number of branches per plant significantly correlated with yield (r = 0.63*) and is a vital yield deciding factor in lucerne (Annicchiarico *et al*., 2013). Type I (branches from the axillary buds on stem remaining after the previous harvest) and Type II (the branches from the bud on tap root crown) branches bear leaves and increase biomass. At 12 MAS, the high crown diameter was recorded in the high yielding genotypes (Table 2). A genotype having more branches or increasing the number of branches after every harvest and large crown diameter produces more leaves and yields biomass under irrigated conditions (Baldissera *et al*., 2013). However, there was no correlation between plant height and fresh biomass. The correlation of morphological parameters with fresh biomass indicates that dry matter partitioning towards increasing the number of branches is more beneficial for yield than increasing plant height in lucerne under irrigated conditions. One of the critical factors in lucerne growing under any management practice is the population stand. Population or number of plants in a unit area decides the yield per unit area. In this study, all the top-yielding genotypes under irrigation had more than 90 % persistence The number of plants surviving per unit area or persisting after each harvest dictates the genotype's biomass potential in lucerne. Persistence and phyllochron are factors that decide productivity, longevity, and quality of lucerne in Australia, Newzealand, Argentina, and Brazil (Hoppen *et al.,* 2022).

Among the physiological parameters, it was observed that photosynthesis and conductance significantly correlated with fresh biomass and was recorded as a critical parameter that influenced biomass production. The stomatal conductance indirectly indicates the number of open stomata on the leaf surface. Conductance was correlated significantly with biomass in lucerne genotypes (r= 0.71**, Fig 2). Conductance is related to the WUE as stomates are the path for entry of CO_2 and transpiration. Water is not restricted under irrigated conditions, so genotypes with more conductance are photosynthetically biomass efficient. More photosynthesis ensures high dry matter and high biomass. Chlorophyll, SLW, and total sugars were not correlated to fresh and dry biomass (Table 3 and fig 2).

Under pasture conditions, lucerne is an indeterminate type, with slow initial ariel growth and fast underground growth until grazed or crushed by animals. These reserve pools in the underground organs increase the plant's ability to uptake mineral

Table 3. Physiological traits of Lucerne genotypes under irrigationd

Genotypes	Specific leaf weight (mg cm^{-2})	Total chlorophyll (µg g^{-1} fresh wt^{-1})	Carotenoid content (µg g^{-1} fresh wt^{-1})	Total soluble sugars (mg/g)
A2xWC (T-5)	3.67[d]	1.13[cd]	1005.34[cd]	84.01[a]
A2xWC (D-5)	3.69[d]	1.11[d]	944.28[e]	61.45[b]
RL-88xWC (T-4)	3.84[c]	1.09[de]	846.64[f]	49.15[c]
RL-88xWC (D-4)	3.88[bc]	0.93[f]	664.92[g]	38.70[cd]
A2xWC (T-4)	3.96[bc]	1.04[e]	961.41[de]	38.24[d]
A2xWC (D-4)	3.92[bc]	1.05[e]	1031.45[bc]	39.58[cd]
ACC 45/2010	3.99[b]	1.18[bc]	1071.59[ab]	48.22[cd]
Raj 22/2007	3.99[b]	1.21[b]	1023.67[bc]	39.95[cd]
Anand-2	3.97[b]	1.33[a]	1098.00[a]	42.45[cd]
RL-88	4.15[a]	1.31[a]	1126.66[a]	42.51[cd]
ACC 23/2010	3.84[c]	1.30[a]	998.38[cde]	81.95[a]
ACC 38/2010	3.95[bc]	1.22[b]	1099.82[a]	26.74[e]
S.Em. ±	0.04	0.01	19.08	3.57
C.D. at 5%	0.12	0.06	55.97	10.48

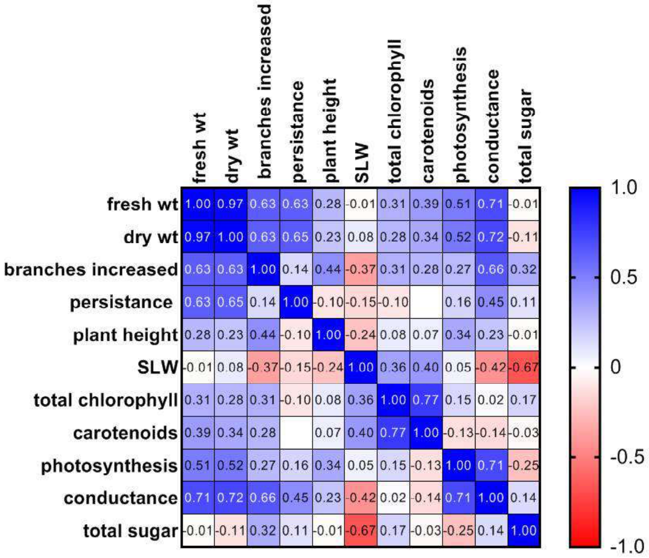

Fig 2: Heat map of Pearson's correlation coefficients of morphological and physiological parameters in lucerne genotypes grown under irrigated conditions. N= 12 at 5% P=0.55 at 1% P= 0.68. SLW is specific leaf weight

nutrients and water from below ground regions and the nutrients and water available in the shallow layers (Annicchiarico, 2007). There are no restrictions or deficits for plant growth. Its luxurious indeterminate growth and increased dry matter partitioning to below ground crown regions produce more branches and leaves despite flower initiation, resulting in high biomass under the pasture system.

In lucerne,80% of the root biomass is located in the top 50 cm of soil (Trubacheva *et al.,* 2019). So irrigation to wet 50 cm of soil can ensure a high yield in lucerne. Lucerne genotypes establish and flourish with a tendency to store more in the below-ground regions. However, the roots do not elongate more than 40-50 cm due to the repeated harvesting but result in a very thick crown region (Annicchiarico *et al*., 2013). The crown region having a higher diameter contained more growth reserve requiring elements like starch and various forms of sugars. Anand 2, the highest yielder, had the highest crown diameter and highest number of branches and biomass. Due to larger crown storage organs, the significant reserve pool permits faster shoot regrowth after harvest and increases the number of branches after harvest (Table 1; Fig 2). Anand 2 also had the highest photosynthetic rate and conductance, resulting in more biomass. A genotype that shows a steady increase in the number of branches, persistence, high photosynthetic rate, and conductance in each harvest is suitable for irrigated cut and carry system.

Conclusion

Biomass produced in lucerne is much higher in irrigated than in rainfed conditions. Under the irrigated cut and carry system, the extended root system is a waste of dry matter utilisation as water is available in shallow depth soils with no moisture deficit stress. The study revealed that for selecting lucerne genotypes to obtain higher biomass under irrigated cut and carry system, genotypes with high conductance, photosynthesis and higher number of branches combined with high persistence could be the vital selection criteria, primarily when selection is conducted during the juvenile phase of lucerne.

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