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## Evaluation of *Desmanthus* genotypes for high green forage yield and improved nutritional quality

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### Abstract

A perennial leguminous shrub, *Desmanthus virgatus* has great potential as a high-protein ruminant feed. Because it provides an excellent combination of crude protein, digestibility, and biomass output, its usage as a forage crop is becoming increasingly popular for sustainable livestock production. Identifying genotypes with higher green forage yield and improved nutritional quality is necessary to meet the growing demand for high-quality forage. A field experiment was conducted to evaluate suitable genotypes for the higher green forage yield and better nutritional quality during the year 2023-24 at the farm of All India Coordinated Research Project on Forage Crops & Utilization at Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, involving six genotypes of *Desmanthus virgatus* in a Randomized Block Design with four replications. Results revealed that VTDV-4 produced the highest green forage yield during the first cut of the six *Desmanthus* genotypes evaluated in this study. However, the VTDV-1 and VTDV-2 genotypes had better nutritional quality than the other genotypes. Moreover, VTDV-2 produced the highest overall green forage yield (38.7 t ha<sup>-1</sup>) across multiple harvests and had the potential for sustained and abundant forage production. These results imply that selecting genotypes based on green forage quality and yield will aid in developing superior *Desmanthus* cultivars and meet the need for high-quality forage.

**Keywords:** *Desmanthus virgatus*, forage, green forage yield and nutritional quality

### Introduction

The total livestock population of India is 535.78 million, nearly 20% of the global livestock population; however, it accounts for only 2.3% of the world's land area (GoI, 2022) <sup>[7]</sup>. It leads in cattle (16%) and buffalo (55%) populations and has the second-largest goat (20%) and fourth-largest sheep (5%) populations globally (ICAR, 2009) <sup>[10]</sup>. The National Commission on Agriculture (1976) suggested that at least about 16.5 million hectares (i.e., 10% of the cultivable land) be under high-yielding forage crops to meet the requirements for green forage for the increasing livestock population (Singh *et al.*, 2011) <sup>[26]</sup>. However, only 8.3 million hectares (4% of the total cropped area) are under fodder cultivation, resulting in a significant 61.1% deficit in green fodder supply. The livestock population is projected to grow at 1.23% annually, and with current growth rates in forage resources, the country is projected to face an 18.4% shortfall in green fodder and a 13.2% shortfall in dry fodder by 2050 (ICAR-IGFRI, 2013) <sup>[11]</sup>. One of the main reasons for this shortfall is the low yield of green forage from cultivated fodder.

Livestock productivity must be enhanced to attain the desired annual agricultural growth rate of 4%. The country's livestock production is increasing, with meat and milk consumption predicted to grow at 2.8% and 3.3% per annum, respectively. This growth will require a steady supply of fodder. With the world's largest livestock population, India relies heavily on animal husbandry, especially where crop farming yields lower returns. High-yielding and nutritious green fodder for effective livestock management should be provided (Mynavathi *et al.*, 2017) <sup>[16]</sup>. Presently, the availability of green fodder, dry fodder, and concentrates is 734.2, 326.4, and 61 million tonnes, respectively, against the required 827.19, 426.1, and 85.78 million tonnes, resulting in deficits of 11.2%, 23.4%, and 28.9% (Roy *et al.*, 2019) <sup>[22]</sup>. Therefore, one of the key challenges

to livestock productivity is the insufficient supply of quality fodder (Shekara *et al.*, 2019) <sup>[23]</sup>.

*Desmanthus virgatus* (L.) Willd., also known as hedge lucerne, is a perennial shrub legume and capable of frequent cutting; it is a valuable forage legume that can be safely fed to both ruminants and non-ruminants. It is favored by cattle for its palatable green fodder and sufficient crude protein content (Radhakrishnan *et al.*, 2007; Deepthi *et al.*, 2013) <sup>[21, 4]</sup>. Additionally, it enhances soil health by fixing atmospheric nitrogen. Hedge Lucerne is a unique species with limited research dedicated to improving its genetic makeup. Consequently, efforts to enhance its green fodder yield and quality must be intensified. There is a pressing need to introduce variability in *Desmanthus* to boost fodder quality and supply to meet growing demands. This research aimed to identify a suitable genotype of *Desmanthus* with high green forage yield and better nutritional quality to help in their selection for further breeding programmes.

## Materials and Methods

### Experimental site

The experiment was conducted at the All India Coordinated Research Project on Forage Crops & Utilization experimental site (20°15' N latitude and 85°52' E longitude), Odisha University of Agriculture and Technology, Bhubaneswar, from September 2023 to April 2024. The site is in a subtropical zone with warm, humid summers and mild winters. The soil of the experimental plot was sandy-loam, acidic (pH =4.1-5.3), and the EC of the soil was 0.025 dSm<sup>-1</sup>. The organic carbon in the soil was 0.54%, and the available N, P, and K were 85, 38.5, and 180 kg ha<sup>-1</sup>, respectively.

### Experimental details

The experiment was conducted from September 2023 to April 2024 in a Randomized Block Design (RBD) with six genotypes (VTDV-1, VTDV-2, VTDV-3, VTDV-4, VTDV-5, and VTDV-6) and four replications. Each plot size was 4 x 3 m. These genotype names are provisional and are used for convenience in the research project, as these genotypes are still under trial. Their official nomenclature and release as varieties will be provided upon completion of the trial.

The field was thoroughly ploughed and levelled, and farmyard manure at 5 t ha<sup>-1</sup> was applied. The seeds were sown on 10 September 2023 in a 50 cm line spacing at a seed rate of 20 kg ha<sup>-1</sup>. Initially, the field was irrigated immediately after sowing to ensure good seed germination. The soil was irrigated every 5-7 days during the first few weeks, depending on rainfall and soil moisture levels. After the plants were established, the frequency was reduced to every 10-14 days. The entire amount of fertilizers was applied as a basal dose on 25 days after sowing (DAS) @ 25 kg N, 40 kg P<sub>2</sub>O<sub>5</sub>, and 20 kg K<sub>2</sub>O ha<sup>-1</sup>. The first harvest was done at 90 DAS, while the second and third were done at 150 and 210 DAS, respectively.

### Observations Recorded

#### Leaf chlorophyll, carotenoid content and SPAD value

The leaf chlorophyll and carotenoid content were determined using the method outlined by Arnon (1949) <sup>[1]</sup>. The second leaf from the top of the five plants in each plot was used for this analysis. The chlorophyll and carotenoid content of the leaf was expressed as mg g<sup>-1</sup> fresh weight (FW) of the leaf. The soil plant analysis development (SPAD) value of the leaf is commonly used to measure leaf greenness and indirectly shows the crop nitrogen status. It was measured using the SPAD-502 Konica

Minolta chlorophyll meter at 90 days after sowing.

### Biochemical parameters and green forage yield

Five leaves from each replicated plot were randomly selected at each cutting and used to estimate various biochemical parameters of six genotypes. The mean values for each cutting were used in the statistical analysis and are presented in this paper.

The nitrogen content in the leaves was estimated using Kjeldahl's method, as Barbano *et al.* (1990) <sup>[2]</sup> outlined. The crude protein content of the plant sample was determined by multiplying the nitrogen content by a factor of 6.25 (Dubetz and Wells, 1968) <sup>[5]</sup> and expressed as a percentage for each genotype. Phosphorus content in leaves was quantified through the Vanado-molybdate method of Jackson (1967) <sup>[12]</sup>, employing a UV-VIS spectrophotometer (Shimadzu UV-19001) at λ=420 nm after an appropriate dilution of an aliquot from the acid-digested extract. Potassium in the acid-digested leaf samples was measured with a flame photometer (Systronics Flame Photometer 128) following suitable dilution, following the method described by Page *et al.* (1982) <sup>[17]</sup>.

A nutritional index was developed to identify genotypes with better nutritional quality. It was based on multiple nutritional traits, assigning scores based on their deviation from the mean ± SEM. Six parameters, viz., dry matter, crude protein, phosphorus content, potassium content, crude fiber content, and ash content, were used for the scoring. The genotype having a higher value than (Mean + SEM) in respect of a trait was scored as '2', having a value in between (Mean - SEM) and (Mean + SEM) was scored as '1' and having a lower value than (Mean - SEM) was scored as '0' (Swagatika, 2023) <sup>[27]</sup>. Finally, the scores for all traits were summed to obtain the nutritional index.

The crude fibre content was determined using a muslin cloth, as described in the Weende or proximate method (Henneberg and Stohmann, 1859) <sup>[9]</sup>, an official method of the Association of Official Analytical Chemists (AOAC).

To determine ash content, crucibles were dried in a hot-air oven to about 600°C, cooled, and their weights (without samples) were recorded. About 5 g of the dried sample was placed in the crucibles. Then, the crucibles were heated in a muffle furnace at 600°C for 5 hours until only white ash remained. The crucibles were weighed after cooling in a desiccator to determine the ash content using the following formula.

$$\% \text{ Total ash content} = \frac{(\text{Wt of empty crucible} + \text{ash}) - \text{Wt of empty crucible}}{(\text{Wt of empty crucible} + \text{sample}) - \text{Wt of empty crucible}} \times 100$$

Green forage yield (GFY) was measured after harvesting at three cuttings per unit area. The harvests from all three cuts were combined to determine the high-yielding genotype.

### Statistical Analysis

The data on individual characteristics were analyzed using analysis of variance (ANOVA) for a randomized block design (RBD), as described by Panse and Sukhatme (1985) <sup>[18]</sup>. Duncan's multiple range test (DMRT) was used to assess differences among the treatment means at the 5% significance level.

### Results and Discussion

#### Variation in leaf greenness, chlorophyll and carotenoid contents in *Desmanthus* genotypes

Genotype VTDV-1 had the highest SPAD value (Table 1). However, there were no significant differences in SPAD values

among VTDV-1, VTDV-2, and VTDV-6. The lowest SPAD value was recorded in the VTDV-5 genotype. The leaf chlorophyll and carotenoid content were highest in the VTDV-1 genotype during the first cutting. The lowest chlorophyll content was observed in the VTDV-3 genotype, which was not significantly different from VTDV-5. The lowest carotenoid content in leaves was found in the VTDV-4 genotype.

Chlorophyll content has been positively correlated with photosynthetic activity, which in turn affects forage yield and nutritional value (Kumar *et al.*, 2012) [14]. Similarly, carotenoids, which contribute to antioxidant activity, are vital for enhancing the nutritive value of forage plants (Singh *et al.*, 2015) [24]. A relationship between SPAD values and nutritional quality has been established: higher SPAD values often indicate higher chlorophyll content, leading to improved plant vitality and forage quality (Gamon *et al.*, 1997) [6].

#### Variation in nutritional quality in *Desmanthus* genotypes

The leaf nitrogen content was highest in VTDV-1 and was statistically similar to that of VTDV-2, VTDV-3, and VTDV-6. However, low N content was observed in the VTDV-4 and VTDV-5 genotypes (Table 2). Similarly, the highest phosphorus content was found in VTDV-1, which was significantly higher than all other genotypes. VTDV-4 and VTDV-5 had significantly higher leaf potassium content than the other genotypes (Table 2). High to moderate nutrient (NPK) content makes the genotype suitable for forage purposes. Considering different parameters (mentioned in the earlier section), out of the six genotypes evaluated, VTDV-1, VTDV-2, and VTDV-6 had high nutritional index values, making them nutritionally superior to other genotypes.

Crude protein content was highest in VTDV-1, followed by VTDV-6 and VTDV-2. However, these three genotypes had similar crude protein content in the leaves (Fig. 1). Lower crude protein content was observed in the VTDV-4 and VTDV-5 genotypes. The variation in crude protein content may be due to the genotypes' genetic makeup. This result aligns with the findings of Jayaprakash *et al.* (2016) [13], who also observed significant variation in crude protein content among *Desmanthus* accessions. Radhakrishnan *et al.* (2007) [21] found the highest crude protein content (22.7%) in *Desmanthus* among the three fodder leaves/shrubs studied. Similarly, Gunasekaran *et al.* (2013) [8] and Pasupathi *et al.* (2015) [19] reported crude protein contents of 18.9% and 20.3%, respectively, for *Desmanthus*. However, the *Desmanthus* genotypes studied here had lower crude protein content, ranging between 11.2 to 14.8%.

The lowest crude fibre content was observed in VTDV-1, followed by the significantly similar VTDV-6 and VTDV-2 genotypes (Fig. 2). The highest crude fibre content was observed in the VTDV-5 genotype. The high crude fiber content in

VTDV-5 and VTDV-4 suggests that these genotypes may be less digestible than VTDV-1, which had the lowest fiber content. This pattern mirrors the observations of Singh and Chauhan (2017) [25], who found that higher crude fiber content generally correlates with reduced palatability and digestibility in fodder crops.

The lowest ash content was found in VTDV-1, which was significantly different from other genotypes (Fig. 3). The highest ash content was observed in VTDV-4, which was significantly greater than other genotypes. Lower ash content is preferred because it reduces the indigestible portion of the forage, thereby enhancing its quality (Jayaprakash *et al.*, 2016) [13]. Dash (2024) [3] found that heritability for ash content was relatively high (77.9%), suggesting that this trait is genetically influenced and could be targeted in breeding programmes. Several researchers have reported that variation in crude fiber and ash content among *Desmanthus* genotypes may be attributed to differences in their genetic makeup and nutrient uptake efficiency. (Queiroz *et al.*, 2021; Muttappanavar *et al.*, 2022) [20,15].

#### Green forage yield (GFY) of *Desmanthus* genotypes

Based on the combined data from all three cuttings, the green forage yield of different genotypes was evaluated to identify the most productive genotype. In the first cutting for green forage, the VTDV-4 genotype produced the highest forage yield among the other genotypes. During the second and third cuttings, VTDV-2 gave the highest forage yield (Fig. 4). The total yield across the three cuts ranged from 19.74 to 38.68 t ha<sup>-1</sup>. Genotype VTDV-2 exhibited the highest total forage yield, with a cumulative yield of 38.68 t ha<sup>-1</sup>, followed by VTDV-3 (35.13 t ha<sup>-1</sup>) and VTDV-6 (33.79 t ha<sup>-1</sup>). In contrast, VTDV-1 showed the lowest total yield (19.74 t ha<sup>-1</sup>), considerably lower than the other genotypes. The results suggest that VTDV-2 is likely the best genotype for green forage yield, making it a promising candidate for selection in forage breeding programs. Out of the six genotypes evaluated, VTDV-2, VTDV-3, VTDV-4 and VTDV-6 had high green forage yield, making them superior for forage yield to other genotypes. Genotypes with greater biomass and larger leaves yield higher green forage, as shown in the VTDV-2 genotype (Dash, 2024) [3]. Singh and Chauhan (2017) [25] also observed that higher biomass is typically associated with increased forage yield in sorghum varieties.

Based on the first cut, the VTDV-1 *Desmanthus* genotype, which had high crude protein and phosphorus contents and low crude fiber and ash contents, was found to be superior in nutritional quality but produced the lowest green forage. Moreover, when all three cuts and nutritional quality were considered, the VTDV-2 genotype was significantly superior to the other genotypes evaluated in this study.

**Table 1:** Variations in SPAD values, leaf chlorophyll and carotenoid contents (mg g<sup>-1</sup> FW) of different genotypes of *Desmanthus virgatus* at first cutting (90 DAS)

| Genotypes | SPAD value | Leaf chlorophyll content (mg g <sup>-1</sup> FW) | Leaf carotenoid content (mg g <sup>-1</sup> FW) |
|-----------|------------|--------------------------------------------------|-------------------------------------------------|
| VTDV-1    | 42.2a      | 2.51a                                            | 0.08a                                           |
| VTDV-2    | 38.8a      | 1.11b                                            | 0.03c                                           |
| VTDV-3    | 36.1b      | 0.91c                                            | 0.04b                                           |
| VTDV-4    | 33.7c      | 1.23b                                            | 0.02d                                           |
| VTDV-5    | 30.4c      | 1.00c                                            | 0.03c                                           |
| VTDV-6    | 40.1a      | 1.30b                                            | 0.03c                                           |

FW: fresh weight; Columns with different letters denote significant ( $P \leq 0.05$ ) differences between the genotypes by Duncan's multiple range test (DMRT)

**Table 2:** Leaf NPK contents and nutritional index in forage of different genotypes of *Desmanthus virgatus*

| Genotypes | Nitrogen content (%) | Phosphorus content (%) | Potassium content (%) | Nutritional index |
|-----------|----------------------|------------------------|-----------------------|-------------------|
| VTDV-1    | 2.38a                | 0.42a                  | 1.7b                  | 10                |
| VTDV-2    | 2.34a                | 0.36b                  | 1.7b                  | 10                |
| VTDV-3    | 2.14a                | 0.31c                  | 1.8b                  | 3                 |
| VTDV-4    | 1.85b                | 0.28cd                 | 2.2a                  | 4                 |
| VTDV-5    | 1.59b                | 0.27d                  | 2.0a                  | 2                 |
| VTDV-6    | 2.37a                | 0.31c                  | 1.8b                  | 8                 |

Columns with different letters denote significant ( $P \leq 0.05$ ) differences between the genotypes by Duncan’s multiple range test (DMRT)

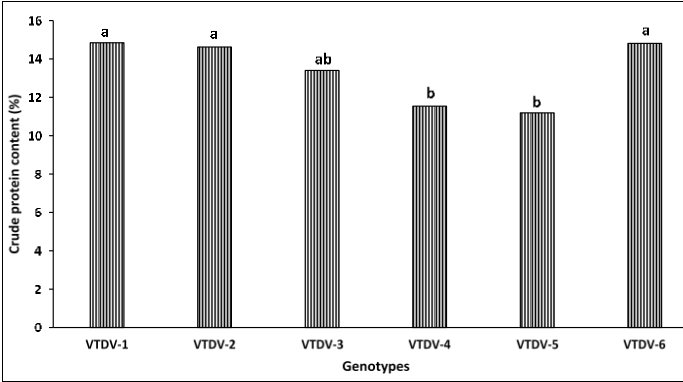


**Fig 1:** Crude protein content (%) in different genotypes of *Desmanthus virgatus*. Bars with different letters denote significant ( $P \leq 0.05$ ) differences between the genotypes by Duncan’s multiple range test (DMRT)

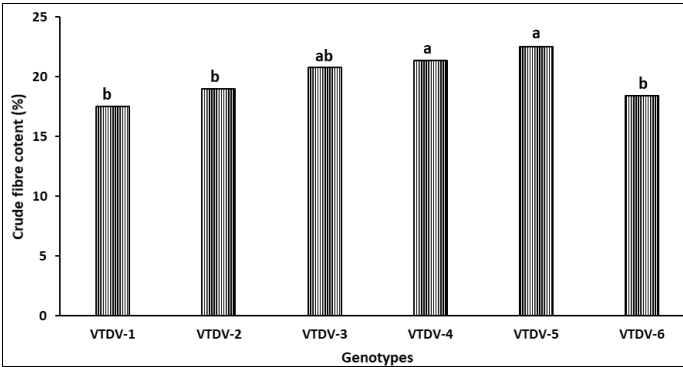


**Fig 2:** Crude fibre content (%) in different genotypes of *Desmanthus virgatus*. Bars with different letters denote significant ( $P \leq 0.05$ ) differences between the genotypes by Duncan’s multiple range test (DMRT)

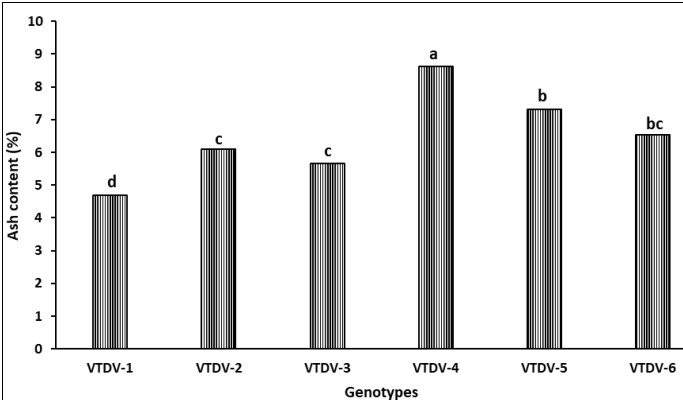


**Fig 3:** Ash content (%) in different genotypes of *Desmanthus virgatus*. Bars with different letters denote significant ( $P \leq 0.05$ ) differences between the genotypes by Duncan’s multiple range test (DMRT)

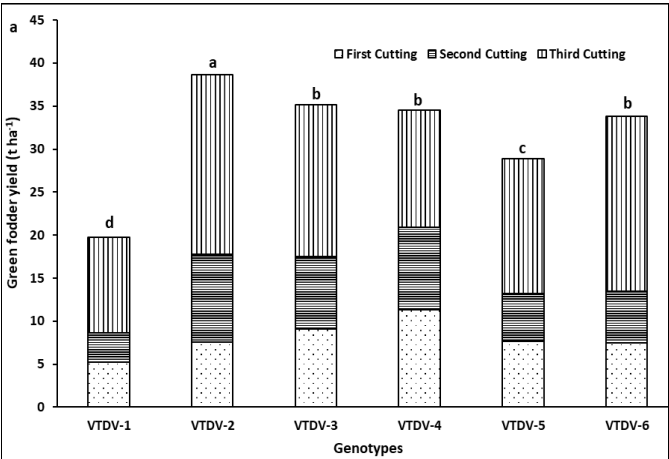


**Fig 4:** Green forage yield on different cuttings of *Desmanthus genotypes*. Bars with different letters denote significant ( $P \leq 0.05$ ) differences between the genotypes by Duncan’s multiple range test (DMRT)

Conclusion

This study demonstrates significant variability in nutritional quality and green forage yield among the six *Desmanthus* genotypes evaluated. Among the first-cut *Desmanthus* genotypes, VTDV-4 stands out as the best-performing for green fodder yield. VTDV-1 and VTDV-2 are the preferred genotypes for farmers prioritizing nutritional quality due to their higher crude protein content and lower crude fiber and ash content. Moreover, VTDV-2 performed exceptionally well, delivering the highest overall green forage yield across multiple harvests, demonstrating its potential for sustained, abundant forage production. The choice of genotype will depend on whether the goal is to maximize yield or improve forage quality. The results suggest that selecting genotypes based on their yield and nutritional attributes can enhance forage production and quality, improving livestock nutrition. This study will contribute to the ongoing efforts of the breeding program to improve India's forage crop productivity and quality.

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