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Original paper

Effect of nitrogen fertilization, cropping seasons and cutting on growth and nutritive value of vetiver

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Abstract Vetiver introduced as perennial grass crop in Mediterranean basin is tested for its potential fodder ability. Effects of nitrogen (N) fertilization, on growth, yield and forage quality parameters were assessed during two cropping seasons using a randomized complete block design with three replicates. Three N levels as ammonium-nitrate, were tested: 0, 30 and 60 kg N ha^{-1}. Results showed that biomass increases under maximum N rate compared to control. The improvements in fresh and dry weights were 39.61% and 257.14%, respectively. Second cutting increased DY by 14.70% compared to first cut. Forage yield increase was closely related to a rise of number of tillers by 24.7% under 60 kg N ha^{-1}. The increased tillers enhanced forage yield by 14% during cropping seasons. N produced a linear increase in crude protein under 30 and 60 kg N ha^{-1} of 40% and 63.8%, respectively. In addition, the increase of leaf cellulose content was less impacted by N fertilization allowing vetiver to be more digestible forage. The results showed that vetiver could be grown as a forage crop in Mediterranean areas. N fertilization since a low rate of 30 kg N ha^{-1} is sufficient to stimulate regrowth, increase biomass yield and nutritional value.

Keywords *Chrysopogon zizanioides*, dry yield, fodder, crude protein, Mediterranean climate.

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Introduction

Pasture and fodder crops are among the main world agriculture sowing species, occupying 70% of the world land agricultural area and providing 85% of ruminant protein needs [1,2]. In Tunisia, rangeland and permanent grasslands cover about 4.8 million ha and suffer from overgrazing and low productivity [3]. Forage crops are sown on approximately 321 000 ha representing only 6% of the arable agricultural area, leading to cover only19% of the livestock needs with 715 million UF [4]. Forage deficit is the result of several limiting factors such as water shortage, rainfall irregularities and frequent droughts [5,6], as well as empirical cultivation practices especially nitrogen (N) fertilization.

In Tunisia, *Avena sativa* is the main forage specie occupying 70% of total forage area [7]. Thus, several forage species have been introduced to primarily fill the forage deficit, such as *Atriplex nummularia*, *Accacia salicina* and *Erharta calycina*. Recently, vetiver (*Chrysopogon zizanioides)* native to the tropics and subtropics [8,9] has been introduced in Tunisia as a perennial grass species. *C. zizanioides* L. belongs to the Poaceae family. The vetiver adaptability to Mediterranean conditions characterized by increasing temperature and day length significantly increased plant height [10]. In the Mediterranean areas, these range of temperature and photosynthesis conditions are obtained mainly from April to September and are considered as optimum growth period.

The genus *C. zizanioides* is characterized by its extreme hardiness and adaptability to wide pedo-climatic conditions [11,12]. Moreover, the plant is used in many countries for water conservation, land stabilization, phytoremediation, and bioethanol production [13–15]. *C. zizanioides* is characterized by high levels of crude protein, carotene, lutein and high-quality edible herbage for cattle and goats, particularly in the vegetative growth stages [16]. Fresh yield, dry yield and crude protein are considered as the main components of forage quality for the grassland species [17].

Few studies highlighted the opportunities of cropping *C. zizanioides* in rainfed conditions through the evaluation of its N fertilization and cutting responses. The present study aimed to emphases the impact of N fertilization, cutting and cropping seasons on vetiver growth, yield and forage quality.

Materials and methods

Experimental site

A two-year field study was conducted from 2015 to 2017 at the experimental station of Agricultural Hight School of Mateur, Tunisia (37°03’15’’N., 9°37’11’’E., Altitude 20 m). This area is characterized by a Mediterranean sub-humid climate conditions.

The experiment was carried out on a silty-clay soil texture characterizing the exploited vetiver root depths. Prior to seeding, soil samples from each plot were taken and analyzed. The soil showed an average of 1.93% total organic carbon (TOC) and a low total organic nitrogen (TN) of 0.2%.

Cultural practices and Experimental design

The plants were obtained from 25 cm long vetiver cuttings, grown for one month in greenhouse on brown peat substrate. Vetiver field transplantation was released on July 15th, 2015 on a follow field. The experiment was arranged as Randomized Complete Block Design (RCBD) with three replications; 36 plants were placed in experimental units of 9 m^2 (3×3 m). The plots were constituted by four rows spaced by 0.5 m and plots spacing was 3 m. Before transplantation, a homogenization cut was released to keep plant height to 10 cm above the ground. A manual weeding was carried out during initial plant growth stages. All the plots were irrigated by sprinkling (3 bars) when evaporation was 70 mm from the surface of evaporation pan class “A”. Three level N fertilization as ammonium nitrates (33.5% N) were tested: (0, 30 and 60 kg N ha^1). The N treatments were spread manually after each cutting on a moist soil (20% humidity). After 21 days of plant growth, vetiver plants were cut. During each year, two cuts were applied separated by 21 days.

Measured parameters and data analysis

Plant height (H) and leaf chlorophyll content (Ch) were weekly measured on nine plants per treatment for 21 days. After three weeks of plant growth and to ovoid edge effects, five plants per plot in the two middle rows were cut and weighed. The nitrate content (Nc) was determined on 20 g of fresh leaves cut into thin strips and then ground until juice is extracted, which is then analyzed with nitrat-chek No_3^- meter (LAQUA twin, HORIBA).

The leaves (L) and stems (S) of each plant were separated and weighed. The leaf per stem ratio (L/S) is then calculated. Leaves and stems were separately cut into 1 cm layers dried at 60°C for 48 h to calculate ratio of leaves dry matter per stems dry matter (DM_l/ DM_s). The forage yield (kg/m^{-2}) was estimated randomly using 0.5 m^2 quadrates on each plot.

Dried samples were used to analyze plant nutritive components. The dried matter content of the entire plant (DM_p) was obtained by keeping the fresh samples at 80°C in forced air oven till constant weight. Ashes (Ash) was determined after burning the samples in muffle furnace at 550°C for 10 h while the organic matter (OM) is than calculated from the weight loss. The crude protein (CP) was carried out by micro-kjeldhal digestion [18]. Crude cellulose (Cel) was determined using FIBERSAC procedures outlined by

Table 1. Physical and chemical properties of the experimental field soil.

Depth (cm)	pH	C (%)	SI (%)	S (%)	TOC (%)	TN (%)	$CaCO_3T$ (%)	$CaCO_3act$ (%)
0-30	8.3	22.5	57.3	17.3	1.9	0.2	20.1	10.1
30-60	8.4	21.6	57.3	18.3	2.08	0.21	20.9	9.8
60-90	8.5	18.5	52	16.1	1.83	0.21	19.9	10.1

C: clay, SI: silt, S: sand, TOC: total organic carbon, TN: total organic nitrogen, CaCO3T: total limestone, $CaCO_3act$: active limestone

ANKOM method (AOCS procedures Ba 6a-05). Extractible ether was determined using Soxhlet (ISO 6492:1999). The calcium content of plants (Ca) was determined by SAAF method (ISO6869:2000), and the concentration of phosphorous (P) were estimated by spectrophotometric method (ISO 6491:1998).

Collected data were subjected to analysis of variance using the GLM procedure. The treatment means differences were compared using Tukey HSD test ($P < 0.05$). Pearson correlation coefficient was determined for all the measured components for two cropping seasons and two cutting under three N treatments. The stepwise analysis was released upon vetiver fresh forage yield (FY) as the most important forage yield components as dependent variable the choice of predictive variables is carried out by an automatic procedure. All statistical analysis were released using Statistica 12.0 (TIBCO Software).

Results

Impact of weather growth conditions on vetiver growth

As a perennial crop, vetiver growth started in March with a maximum during summer season characterized by increased temperature reaching a mean temperature of 27.3°C in August. The growth is completely stopped in winter where minimum mean temperature reached 11.3°C in January. The average annual rainfall (30 years, 1984-2014) was 547 mm, of which over 70% occurred between November and March. During the two cropping seasons 2015-2016 and 2016-2017, precipitation was 17.9% and 22.12% below the thirty's year average. The maximum values of evapotranspiration were recorded during July and August, with an average of 208.1 mm and 201.7 mm, respectively (Figure 1).

Agronomic parameters

Vetiver growth

Statistical analysis showed significant effect ($P < 0.01$) of N fertilization, cropping seasons (Cs), cutting (Cut), N×Cs, and Cs×Cut interactions on vetiver growth parameters as plant height (H) and number of tillers per plant (Nt) (Table 2).

The maximum H and Nt were obtained under 60 kg N ha^{-1} with respective increase of 31.64% and 31.64% compared to control (0 kg N ha^{-1}). H reached its maximum after two cropping seasons (113.73 cm) and in Cut1 (114.7 cm). The same trend of increase was observed for Nt after two cropping seasons reaching 36.07 tillers per plant in the second cropping season of 2016-2017. Meanwhile, maximum Nt was noted after Cut2 with an increase of 16.85% compared to Cut1 (Figure 2, A).

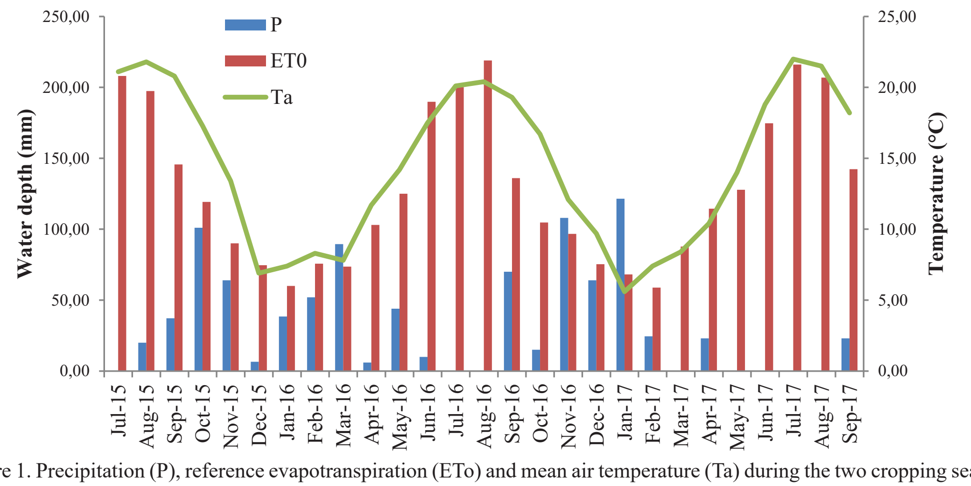

Figure 1. Precipitation (P), reference evapotranspiration (ETo) and mean air temperature (Ta) during the two cropping season assays.

Results showed a significant effect ($P < 0.01$) of N, Cs, Cut and Cs×Cut on Plant fresh weight (FW_p) (Table 3). Maximum N fertilization (60 kg N ha^{-1}) increased FW_p and DM_p respectively by 55.17% and 12.36% compared to control. FW_p increased significantly after Cut2 by 20.42% and after two years cropping by 31.85%.

Moreover, the L/S ratio was affected significantly by N, Cs and N×Cs. Maximum L/S (1.91) was observed under control conditions and was reduced since 30 kg N ha^{-1} (Table 2).

Results showed a significant effect ($P < 0.01$) of only Cs on the ratio of leaves dry matter per stems dry matter (DM_l / DM_s). The maximum leaf growth was observed in the first cropping season of 2015-2016 with an increase of 3.98%.

Vetiver chlorophyll and nitrate content

Results showed a significant effect of N ($P < 0.01$) and Cs×Cut ($P < 0.05$) on Leaf chlorophyll content, estimated by SPAD (Ch). The maximum SPAD measured three weeks (21 days) after each cutting, was reached since 30 kg N ha^{-1} (Table 2).

Leaf nitrate content (Nc) showed significant ($P < 0.01$) effects on the interactions N×Cs, N×Cut and Cs×Cut. The maximum Nc was reached at maximum N fertilization rate with 73.14% increase compared to control. The second cutting (Cut2) and cropping season (2016-2017) were among a respective increase of 53.9% and 57.76% compared to control. The same trend of increase was observed for Nc under Cs, Cut and N fertilization. Maximum Nc increase was observed under maximum N input in second cropping season in Cut2 (Figure 2, B).

Vetiver forage yield

The analyzed data showed that fresh forage yield (FY) was significantly ($P < 0.01$) affected by N, Cs, Cut, N×Cut, N×Cs and Cs×Cut (Table 2). N and N×Cs showed significant effects ($P < 0.01$) on dry forage yield (DY). FY and DY increased under maximum N fertilization rate respectively by 39.62% and 252.11% compared to control. The same trend was noted for Cut2 with 13.2% and 14.74% increase respectively for FY and DY, compared to Cut1. The second Cs (2016 - 2017) was higher for both vetiver forage yield as FY and DY by 53.81% and 60.71%, respectively.

Both yield components as (FY and DY) increased with rising N rates, cropping seasons and number of cutting.

Vetiver forage quality

Vetiver forage quality components were evaluated after each cutting. The results showed that cellulose content (Cel) was under the significant effects ($P < 0.01$) of N, Cs, Cut, N×Cut, N×Cs and Cs×Cut (Table 2).

Increasing N rates was associated with Cel increase reaching 14.18% in average under 60 Kg N ha^{-1} compared

Table 2.Effects of three nitrogen rates (N), cropping seasons (Cs) and cutting (Cut) on vetiver height (H, cm), number of tillers per plant (Nt), fresh weight per plant (FW_p, Kg), ratio of leaves fresh weight per stem fresh weight (L/S ratio), plant dry matter content (DM_p, %), ratio of leaves dry matter per stems dry matter (DM_l/DM_s), fresh forage yield (FY, kg m^{-1}), dry forage yield (DY, kg m^{-1}), leaf chlorophyll content estimated by SPAD (Ch), leaves nitrate content (Nc, ppm), leaf cellulose content (Cel, %), extractible ether (EE, %), organic matter (OM), ashes (Ash, %), calcium (Ca, %), phosphorus (P, %) and crude protein (CP, %).

	H	Nt	FW_p	L/S ratio	DM_p	DM_l/DM_s	Ch	Nc	FY	DY	Cel	EE	OM	Ash	Ca	P	CP
Nitrogen rates (N)																	
0 kg N	87.65 c	24.29 c	1.16 c	1.91 a	12.46 c	2.71 a	31.38 b	676.79 c	2.07 c	0.14 c	25.47 c	1.89b	84.57 a	15.43 b	0.29c	0.287a	9.32c
30 kg N	106.77 b	26.89 b	1.56 b	1.76 b	13.46 b	2.47 b	38.84 a	852.14 b	2.57 b	0.28 b	27.06b	2.86a	82.87b	17.13 a	0.37b	0.268b	13.05b
60 kg N	115.39 a	30.29 a	1.80 a	1.69 b	14 a	2.4 b	43.12 a	1171.79 a	2.89 a	0.50 a	29.68 a	2.95a	82.59 b	17.41 a	0.43a	0.249c	15.27a
Cutting (Cut)																	
Cut 1	114.7a	26.87b	1.42b	1.73a	13.24a	2.57a	37.42a	757.63b	2.50b	0.34a	26.86b	2.63a	82.82b	17.18a	0.37a	0.280a	12.49a
Cut 2	91.85b	31.40a	1.71a	1.70a	13.58a	2.54a	38.09a	1166a	2.83a	0.39a	26.97a	2.52b	83.86a	16.14b	0.36a	0.257b	12.60a
Cropping seasons (Cs)																	
2015-2016	92.82b	22.20b	1.35b	1.69a	13.13a	2.61a	37.2a	746.30b	2.10b	0.28b	27.04b	2.58a	82.88b	17.12a	0.37a	0.269a	12.81a
2016-2017	113.73a	36.07a	1.78a	1.74a	13.68a	2.51a	39.6a	1177.33a	3.23a	0.45a	27.78a	2.56a	83.80a	16.20b	0.36a	0.268a	12.28a

Means followed by different letters are significantly different ($P<0.05$) according to Tukey HSD test.

to control (Table 2). This trend was more pronounced in Cut2 during second cropping season. In addition, cutting increased vetiver cellulose accumulation by 7.3% in Cut2 compared to Cut1 (Figure 2, C).

Results showed a significant effect ($P < 0.01$) of all studied factors and the interaction of N×Cut×Cs on the ashes (Ash) (Table 2). N fertilization increased vetiver Ash content with a maximum in Cut1 during the first cropping season (2015-2016). In average, Ash increase by 12.81% under 60 kg N ha^{-1}compared to control. The maximum increase was observed under Cut1 (6.44%) and in first Cs (5.67%).

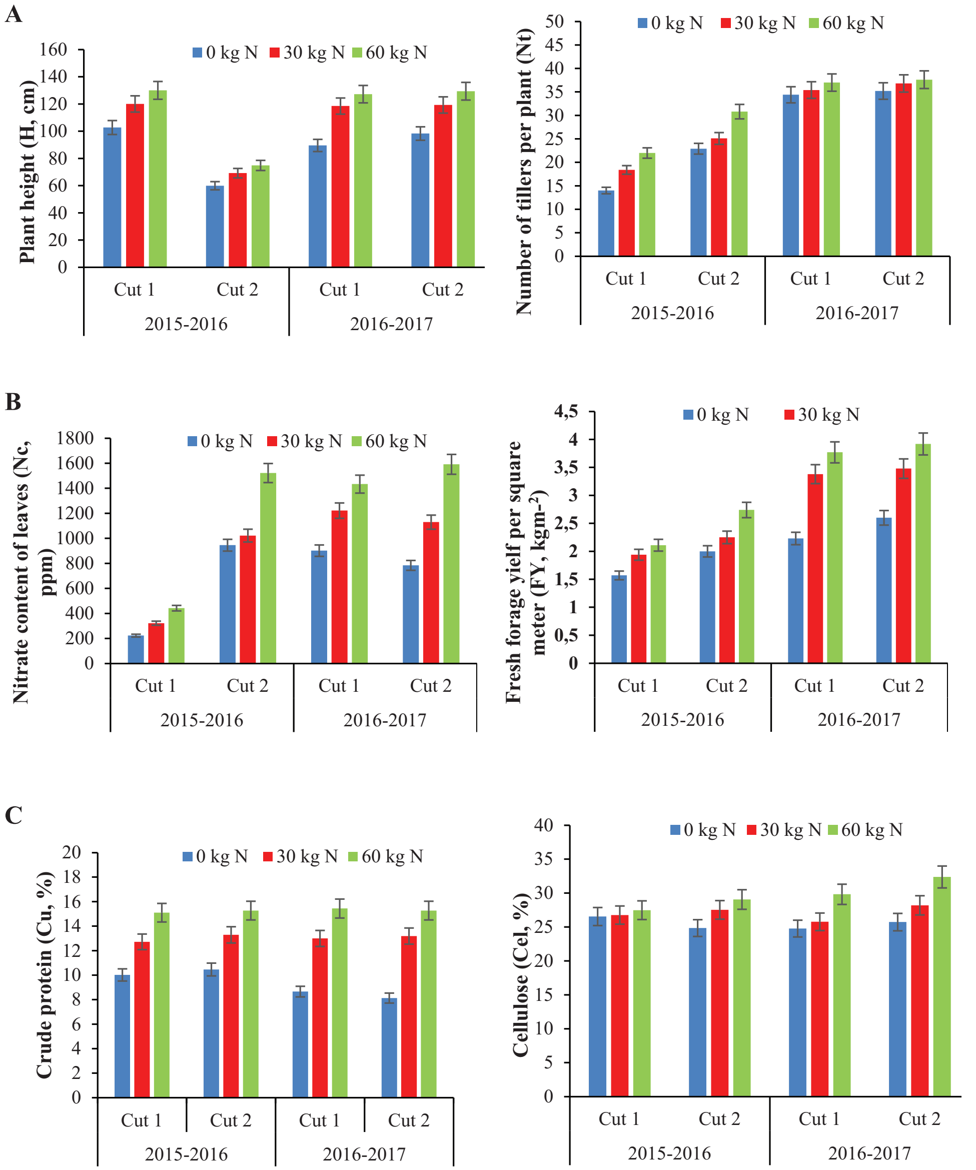

Figure 2. Variation of main morphologic (A), physiologic (B) and forage quality parameters (C) of vetiver grown during two cropping seasons (2015-2016 and 2016-2017), under three nitrogen fertilization rates (0, 30 and 60 kg N ha-1) and two cuttings (MW1 and MW2). A: plant height (H) and number of tillers per plant (Nt). B: Nitrate Content of leaves (Nc) and Fresh forage yield (FY); C: Crude protein content (CP) and cellulose leaf content (Cel).

The analyzed data showed crude protein content (CP) was significantly ($P < 0.01$) affected by N, Cs, Cut, N×Cs, and Cs×Cut (Table 2). N fertilization resulted in a significant and linear increase in crude protein content. This increase was maintained during the two growing seasons. Maximum values were recorded at 60 kg N ha^{-1}, with an increase of 67% over the control. On the other hand, in unfertilized plots, there was a drop of 22% in the second year. The lowest value was recorded during the Cut2 (Figure 2, C).

Results showed a significant effect ($P < 0.01$) of all studied factors and interaction N×Cut×Cs on the organic matter content (OM) (Table 2).

N input has decreased the OM content of vetiver. This decrease was more marked under 60 kg N ha^{-1}. The minimum OM was recorded during Cut1 in first cropping seasons with 81.67%, the Maximum was observed during the Cut2 in the second cropping seasons for the control with 85.85%.

N and N×Cut interaction showed a significant effect ($P < 0.01$) on leaves calcium content (Ca) of vetiver (Table 2). Ca in vetiver leaves increased with a maximum of 48% under 60 kg N ha^{-1} over the control. Moreover, variations between the cuts were only observed under 60 kg N ha^{-1} (Data not Shown).

Vetiver phosphorus (P) content is significantly ($P < 0.01$) affected by N and Cs. P decreased with N fertilization. Maximum decrease (13.24%) was observed under 60 kg N ha^{-1} (Table 2).

Vetiver extractible ether (EE) content is significantly ($P < 0.01$) affected by N and Cs. The maximum EE content was registered during the first cropping season, since 30 kg N ha^{-1} with an increase of 51.32% compared to control (Table 2).

Correlation analysis of agronomic and forage quality parameters

Relationship between all agronomic and forage quality parameters were evaluated using Pearson's correlation analysis. FY under 60 kg N ha^{-1} were positively correlated with FW_p, DM_p, Nc and DY. A negative correlation was found between Ch and $FY_{60\ kg\ N\ ha^{-1}}$ (r=-0.507). Positive correlation showed between $FY_{30\ kg\ N\ ha^{-1}}$ and FW_p, Nc, and DY. While, $FY_{0\ kg\ N\ ha^{-1}}$ was negatively correlated with DM_l / DM_s (r=-0.573), EE (r=-0.847) and CP (r=-0.604). In the other hand, the lowest rate of nitrogen (0 kg N ha^{-1}) were positively correlated with Cel and Ca. A positive correlation was showed between Nt and three level of nitrogen fertilization with respectively, $FY_{0\ kg\ N\ ha^{-1}}$ (r=0.681), $FY_{30\ kg\ N\ ha^{-1}}$ (r=0.799) and $FY_{60\ kg\ N\ ha^{-1}}$ (r=0.755) (Table 4).

Nt, FW_p, Nc, FY was positively correlated with DY under 30 and 60 (kg N ha^{-1}). A negative correlation was observed between $DY_{0\ kg\ N\ ha^{-1}}$ and Nt (r=-0.769), L/S ratio (r=-0.733) and Cel (r=-0.617). While, $DY_{0\ kg\ N\ ha^{-1}}$ was positively correlated with EE (r=0.529) and CP (r=0.891) (Table 4).

$CP_{0\ kg\ N\ ha^{-1}}$ was positively correlated with DM_l/DM_s (r=0.508), DY (r=0.891) and EE (r=0.680). While a negative correlation was noted between $CP_{0\ kg\ N\ ha^{-1}}$ and Nt (r=-0.771), L/S ratio (r=-0.550), FY (r=-0.604), Cel (r=-0.693). A negative correlation was noted between EE and P under $CP_{30\ kg\ N\ ha^{-1}}$ with respectively (r=- 0.17) and (r=-0.506) (Table 4).

Stepwise analysis

Stepwise analysis was released upon vetiver fresh forage yield (FY) most important forage yield components as dependent variable and DY, DM_p, DM_S, DM_l and FW_s as independent variables.

For FY under 0, 30 and 60 kg N ha^{-1}, the independent variable that was first chosen by the model was DY followed by DM_p. Both parameters accounts for 99% of the FY despite N fertilization (Table 3). Then, DM_s and FW_s are chosen by the model even if their contribution is low.

Table 3. Multiple linear regression (stepwise) explaining fresh forage yield (FY) variation within nitrogen fertilization rate as a dependent variable, and all measured parameters as independent variables.

Treatments	Variable chosen	R^2
$FY_{0\ kg\ N\ ha^{-1}}$	DY	0.97**
	DY, DM_p	0.99**
	DY, DM_p, DM_s	0.99**
$FY_{30\ kg\ N\ ha^{-1}}$	DY	0.95**
	DY, DM_p	0.99**
$FY_{60\ kg\ N\ ha^{-1}}$	DY	0.98**
	DY, DM_p	0.99**
	DY, DM_p, DM_l	0.99**
	DY, DM_p, DM_l, FW_s	0.99*

* $P < 0.05$; ** $P < 0.01$.

Discussion

Agronomic parameters

In the present investigation, N fertilization impacted growth and development of vetiver during the two cropping seasons (2015-2016) and (2016-2017). Plant height increased linearly with N increased rates. Similar results were highlighted by Mondyagu et al. [19] for *C. zizanioides*, where 200 mg N l^{-1} lead the plant height to reach a maximum of 105.0 cm under controlled conditions. For forage species as oats, 100 kg N ha^{-1} as urea increases height by 33% [20].

On the other hand, growth has also been affected by temperatures. During the second year and after mowing, plant height was significantly reduced in all three treatments, regardless the N rates. This decrease is attributable to low temperatures recorded from October to November ranging from 16.7 to 12.1°C. Temperature is one of the main factors driving growth kinetics of perennial forage species [21,22].

Table 4. Correlation coefficients for the relationships between fresh yield (FY), dry yield (DY) and crude protein (CP) under three nitrogen rates (N) and all measured parameters as vetiver height (H, cm), number of tillers per plant (Nt), fresh weight per plant (FW_p, Kg m^{-1}), ratio of leaves fresh weight per stem fresh weight (L/S), plant dry matter content (DM_p, %), ratio of leaves dry matter per stems dry matter (DM_l/ DM_s), fresh forage yield (FY, kg m^{-1}), dry forage yield (DY, kg m^{-1}), leaf chlorophyll content estimated by SPAD (Ch), and leaves nitrate content (Nc, ppm), cellulose content (Cel), extractible ether (EE), organic matter (OM), ashes (Ash), calcium (Ca), phosphorus (P) and crude

	H	Ch	Nt	FW_p	L/S ratio	DM_l/ DM_s	Nc	DM_p	FY	DY	Cel	EE	Ash	OM	P	Ca	CP
$FY_{0\ kg\ N\ ha-1}$	0,1	-0,1	,779**	0,5	-0,2	-,742**	0,5	0,4	1,0	,989**	,765**	-,799**	-0,5	0,5	-0,2	-0,3	-,678**
$DY_{0\ kg\ N\ ha-1}$	0,0	-0,1	,768**	,567*	-0,2	-,726**	,534*	,551*	,989**	1,0	,776**	-,772**	-,569*	,569*	-0,2	-0,2	-,622*
$CP_{0\ kg\ N\ ha-1}$	-0,4	-0,1	-,772**	-0,2	-0,2	,658**	-0,2	0,1	-,678**	-,622*	-,680**	,675**	0,5	-0,5	0,4	,572*	1,0
$FY_{30\ kg\ N\ ha-1}$	0,405	-0,178	,799**	,756**	0,317	-0,071	,704**	0,174	1	,980**	-0,160	-0,015	-0,295	0,295	0,172	-0,080	0,005
$DY_{30\ kg\ N\ ha-1}$	0,336	-0,167	,848**	,819**	0,285	-0,067	,764**	0,356	,980**	1	-0,176	0,002	-0,235	0,235	0,117	-0,118	0,111
$CP_{30\ kg\ N\ ha-1}$	-0,356	-0,091	0,382	0,354	-0,476	-0,398	0,343	0,450	0,005	0,111	0,030	-,617*	-0,160	0,160	-,506*	0,164	1
$FY_{60\ kg\ N\ ha-1}$	0,115	-,507*	,755**	,721**	-0,217	-0,179	,708**	,814**	1	,991**	-0,236	0,053	-0,198	0,198	0,039	0,025	0,037
$DY_{60\ kg\ N\ ha-1}$	0,074	-0,473	,729**	,709**	-0,242	-0,233	,726**	,878**	,991**	1	-0,226	0,034	-0,136	0,136	0,034	0,001	0,048
$CP_{60\ kg\ N\ ha-1}$	-0,075	0,009	0,235	0,130	0,482	0,411	0,158	0,076	0,037	0,048	-0,204	-0,257	0,027	-0,027	0,050	-0,172	1

**: $P< 0.01$; *: $P<0.05$; Ns: not significant.

Vetiver is a tropical specie adapted to areas with temperatures ranging from 21°C to 44.5°C [23,24]. In Mediterranean, temperatures varying between 21°C and 29°C stimulates optimal vetiver vegetative growth [10].

A significant decrease in height was recorded in the control during the second cropping season. Such result could be attributed to the significant increase of the number of tillers/plant. The same observation was registered in forage crop such as *Megathyrsus maximus* [25]. Indeed, vetiver is a species known for its high tillering potential [26]. Moreover, vetiver well known as phytoremediation plant can uptake all soil nutrients content [27], to built-up its aerial biomass during the first cropping season, leading to poor soil during the second year of growth.

N have growth effect during vetiver first cropping year which is the installation growth stage for perennial crops. During spring growth restart, the N stored in vetiver long and developed roots from the first cropping season [29], are among the reborn of vegetative aerial part. The same observation was registered in forage crop such as *Lolium perenne* L. [30], *Cichorium intylus* [31] as well as grassland [32,33]. After last cutting concomitant with low temperatures, the suppression of photosynthetic tissues and resulting decrease of CO_2 assimilation are among a lack of available carbon to sustain regrowth [30]. Moreover, such conditions available impact negatively N mineral absorption and assimilation during the growth recovery stage [33].

N supply induced increase in vetiver fresh yield m^{-2}. This increase was maintained during the two growing seasons with a maximum reached in the second year across N rates. The same results were obtained for perennial forage species as *Brachiara brizantha* [34] *Phalaris arundinacea* [35], *Miscantus* [36] and *Miscantus×giganteus* [37]. In addition, N positively impacted FY components as fresh weight/plant and number of tillers/plant. Those results are in complete consistency of Lee et al. [38] studies on *Miscantus×giganteus and Panicum virgantum*. Moreover, studies on *Panicum virgantum* reached similar conclusions, emphasizing the importance of tiller/plant density as a selection criterion for increasing biomass production [39,40].

Interaction of N fertilization×Cs raised vetiver DY. N positive impact on forage crops dry matter production was well documented on *Leymus chinensis* grasslad [42], Sorghum bicolor [43,43] and hybrid Sorghum×Sudan [44]. Vetiver optimum DY appears to require lower N fertilization rate (60 kg N ha^{-1}) compared to other forage crops as Maize (*Zea maïs*) which need 150 to 300 Kg N ha^{-1} to reach maximum DY [45,46]. This fact is partially attributed to vetiver root N uptake ability from deep and large exploited soil volume. Thus, vetiver as phytoremediation species, can uptake soil mineral elements N [19], heavy metals [47], Radiocesuim [48] and crude-oil from contaminated soil [49]. In fact, vetiver use its long and developed roots as a Sink for all harmful components as for heavy metals leading to accumulate up to 24.5 mg kg^{-1} plomb of roots dry matter [50]. Moreover, foliar nitrate (Nc) levels increase as N fertilization rates and was found to be correlated to DY as reported for perennial ryegrass (*Lolium perenne*) [51-52].

Forage quality

CP as well as quality components depends on soil N content [53], issued from mineral and/or organic origins. Vetiver were grown under the low organic matter content (OM) (1.8-2%) which limits mineralization and thus available N. Results showed that N fertilization rates improved vetiver forage quality mainly for crude protein content (CP). Same observations were registered by Coblenz et al. [54], on oat showing 37% increase of CP under 80 kg N ha^{-1} and by Oliveira et al. [55], on *Megathyrsus maximus* showing 55.7 % increase of CP under 50 kg N ha^{-1}. In fact, N is the main constitutive component of plant proteins with an average of 1.5% of shoot dry matter [56]. This positive effect has been widely described in literature as for brome grass (*Bromus diandrus*) [56], reed canary grass (*Phalaris arundinacea*) [35], timothy (*Phleum pratense*) [57], and for oats (*Avena sativa*) [58]. Increased forage grass CP content promotes ruminant's ingestion. In fact, low CP limits the efficiency of microbial digestion, as well as the level of PDI intake [59].

Moreover, N fertilization increased vetiver shoot Cel content. The increase of Cel under N fertilization was previously reported for oat [60], and sorghum [61]. It's clear that N induced both proteins and cellulose biosynthesis process. Several authors have already described the relationships between the processes of protein and cellulose biosynthesis in plants as for *Lepidium sativum* [62], *Picea abies* [63], *Arabidopsis thaliana* [64-66]. At the anthesis, sorghum bicolor cellulose and hemicellulose contents varied between 20.5% - 27.5% and 18.7% - 23.2% respectively [67]. Moreover, cropping seasons, growth stages and cuts positively impacted Alfalfa crude protein content in leaves and stems as well as crude fiber content in stems [68].

N fertilization increased both leaves / plants ratio and plant dry matter content (DM_p). Meanwhile, results showed that N decreased ratio of fresh weight and dry matter leaves/stems. Those results are due in part to vetiver perennial growth kinetics influenced by its old and new tissues composition. In fact, perennial plants, as *Lollium perenne*, have very complex carbon distribution models due at the presence of carbon in old tissues [69]. Thus, allocation and reallocation of carbon in plants are strongly correlated to Source-Sink relationship mainly under stress conditions [70]. Increase in total leaves are considered as essential forage crops aptitude. Leaves had higher digestibly, lower fiber content and higher protein content [71]. At grazing, the total ruminant's ingestion is strongly correlated with green leaves biomass [72].

Forage mineral composition is dependent on plant growth stage and mineral fertilization [73,74]. The absorption of mineral elements must adjust to kinetic of development of new plant tissues and therefore to the absorption and metabolism dynamics of nitrogen and carbon [68]. Thus, ashes content (Ash) increased under N fertilization rates as already reported for *Sorghum bicolor* with 10% increase under 100 kg N ha^{-1} [75]. However, for other species as *Sudan grass* no significant effect was found for N fertilization on leaves mineral content [76].

Results showed that N fertilization promoted calcium shoot accumulation and decreased total phosphorus content. It's well documented that Ca and P content decreased with plant growth [77]. N fertilization for fodder species as Bermuda grass, increased Ca and P levels [78]. In fact, N rate of 448 kg ha^{-1} allowed an increase of P and Ca contents. The same trend of ca increase was observed for vetiver under only 60 kg N ha^{-1}. These facts indicate the ability of vetiver to offer high level of ca and mineral to feed qualitatively livestock.

Conclusion

Vetiver presents agronomic interest for the Mediterranean areas, due to its adaptability and very high yield potential compared to other forage crops. This two-year study proved that using the same technical package of other summer perennial forage crops as N fertilization rates, growing seasons and mowing significantly improved growth, yield and nutritional quality the vetiver and those since 30 Kg N ha^{-1}. Maximum N rate of 60 Kg N ha^{-1} allowed vetiver to reach optimum development and spring regrowth after the winter dormant growth stage, as well as after each mowing. Compared with other fodder grasses such as sorghum or corn, the N requirements of vetiver are fairly contained, limiting production costs and N pollution. The introduction of this species into the fodder production systems of Mediterranean countries could have an agronomic and ecological interest.

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Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constituted as a conflict of interest regarding the publication of this paper.

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