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# *Trifolium mutabile* as New Species of Annual Legume for Mediterranean Climate Zone: First Evidences on Forage Biomass, Nitrogen Fixation and Nutritional Characteristics of Different Accessions

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**Abstract:** The present study evaluated the forage production, nitrogen fixation and the qualitative characteristics of different accessions of *Trifolium mutabile*, a new species of annual clover, collected in southern Italy. Forage traits were assessed by harvesting plants at the vegetative stage (stem elongation) and the subsequent regrowth at the flowering stage (inflorescence emergence-main shoot). From results, significant differences were found among the accessions of *T. mutabile* in terms of forage biomass production (from 5.1 to 8.2 t ha$^{-1}$ dry matter), capacity of nitrogen fixation (58.2–76.8% Ndfa) and forage nutritional characteristics. Besides the high forage yield, the investigated accessions showed favourable values of production and quality, representing also worthy germplasm for selection programs as well as the application for possible plant cultivar registration. Moreover, it is interesting to underline that *T. mutabile* may represent a valuable alternative to commonly cultivated annual clover species due to its prolonged vegetative cycle. However, further investigations are needed to assess the self-reseeding capacity of *T. mutabile* when utilized as pasture species.

**Keywords:** annual clover; N fixation; biomass production; forage

## 1. Introduction

Annual clovers are the basis of production systems in the Mediterranean area. Historically, the most widespread clovers are berseem (*Trifolium alexandrinum* L.), crimson clover (*Trifolium incarnatum* L.), Persian clover (*Trifolium resupinatum* L.), squarrosum clover (*Trifolium squarrosum* L.), arrowleaf clover (*Trifolium vesiculosum* L.) and subterranean clover (*Trifolium subterraneum* L.) in many countries [1–3]. *T. mutabile* (Turkish clover) is an endemic species of the Mediterranean area [4], which was described for Dalmatia. In the wild it is widespread in the western Balkans (Dalmatia, Greece, Albania) and in Southern Italy, from Campania to Sicily [5], where it is represented by *T. mutabile* var. *gussoneanum*, endemic to Southern Italy. The same species has been previously evaluated by Hoveland and Alison [6], defining it as Turkish clover having dense, compact, leafy clover with leaves resembling arrowleaf clover, and being tolerant to grazing and having also better natural reseeding than arrowleaf clover.

The vegetative parts of *T. vesiculosum* do not differ from those of *T. mutabile* in the case of vigorous plants and when grown in fertile soil. The latter species, more thermophilous and xerophilous, often appears with plants of very small size [3].

It has been well documented that the forage quality of clover is a function of the composition of the individual plant parts: each differing in quality and quantity as plants mature [7–9]. Cutting is the main agronomic factor that affects morphology and the expression of yield potential, and indirectly determines nutritive value. Optimizing the use of forages in livestock feeding requires an understanding not only of its dry matter production and plant composition but also of the changes in its nutritive value. Consequently, alternative grasslands are required and the *T. mutabile*, recently found in Italy, could be an interesting option for integration into productive systems in the Mediterranean climate zone.

Therefore, the present study described the main agronomic characteristics of different accessions of *T. mutabile* collected in south of Italy and evaluated the forage production, nitrogen fixation and the qualitative characteristics in two different phenological stages.

## 2. Materials and Methods

### 2.1. Experimental Field Trial

A field trial was carried out during the years 2015–2016 in Southern Italy at Gravina in Puglia, Bari (40°53′ N; 16°24′ E; 415 m above sea level), on a loamy soil, characterized as sub-alkaline, medium in total N (nitrogen) (1.22‰ Kjeldahl method), high in available P (phosphorus) (74 mg $kg^{-1}$; Olsen method), and exchangeable K (potassium) (362 mg $kg^{-1}$; $BaCl_2$; TEA method). The experimental site was characterized by a summer-dry climate with a total annual rainfall of 560 mm distributed from Autumn to Spring and a mean temperature of 16 °C. During the experimental period (from November 2015 to June 2016), the total rainfall was 336 mm, and temperatures did not show any significant variation from the average.

Seedbed preparation consisted of plowing at 40 cm during summer and dish-harrowing twice during autumn. Before sowing a total of 100 kg/ha of $P_2O_5$ as superphosphate was used. The previous crop was wheat (*Triticum durum*). Six different accessions of *T. mutabile* collected in Apulia Region of Southern Italy (Table 1) were seeded in 18 November 2015 at a seeding rate of 10 kg/ha seed and a row spacing of 20 cm, and ryegrass (*Lolium multiflorum* cv Trinova) was used as non-fixing reference crop at seeding rate of 40 kg/ha with a 20 cm row spacing.

**Table 1.** Collection sites of the different *T. mutabile* accessions.

| Accession Code | Collection Site | Coordinates |
|---|---|---|
| TMU01 | Restinco | 40.6422 N; 17.8657 E |
| TMU02 | San Donaci | 40.5462 N; 17.8747 E |
| TMU03 | San Pietro Vernotico | 40.5353 N; 18.0590 E |
| TMU04 | Specchia (A) | 39.9709 N; 18.2815 E |
| TMU05 | Specchia (B) | 39.9781 N; 18.1060 E |
| TMU06 | Spinazzola | 41.0072 N; 16.2658 E |

A randomized block experimental design was used with four replicates having a plot area of 8 $m^2$. The macroplot sizes were 2 × 4 m, whereas microplot sizes were 1 × 1 m; each microplot was located in the centre of individual macroplots. Both the *T. mutabile* and ryegrass crops were fertilized uniformly with the solution of 10 kg N $ha^{-1}$ $^{15}N$ $NH_4SO_4$ (10% $^{15}N$ atomic excess) (fertilizer dissolved in 1 L of distilled water). The macroplots also received 10 kg N $ha^{-1}$ of unlabelled $NH_4NO_3$.

Forage biomass yield was determined by harvesting the whole plot at two main phenological stages including the vegetative stage and (stem elongation) and the regrowth at the flowering stage (inflorescence emergence-main shoot). The flowering time of the six accessions was also evaluated.

### 2.2. Nitrogen Fixation Analysis

Plant samples were oven-dried at about 70 °C for 48 h. The plant material was ground to pass through a 0.2 mm sieve. Total N and % $^{15}N$ atomic excess (a.e) of plant samples were analyzed at the Iso-Analytical Limited (Cheshire, UK) using elemental analysis isotope ratio mass spectrometry (EA-IRMS). The % $^{15}$N excess was calculated by the difference of the atomic % $^{15}$N in the plant material (*T. mutabile* and ryegrass) and that of the natural abundance in the atmosphere (0.3663%). The N fixation in *T. mutabile* was calculated using the $^{15}$N-isotope dilution method [10], using the equations (Equation (1)) mentioned below; and the amount of $N_2$ fixed was determined from the product % nitrogen derived from fixation (Ndfa) and N yield (as sum of the two cuts) for each replication, and the average was then calculated (Equation (2)):

$$\%\ \text{Ndfa} = 100 \times [(1 - \%\ ^{15}\text{N a.e } (T.\ mutabile) / \%\ ^{15}\text{N a.e (ryegrass)}] \quad (1)$$

$$\text{N}_2 \text{ fixed (kg ha}^{-1}) = [(\%\ \text{Ndfa} \times \text{total N in } T.\ mutabile \text{ (kg ha}^{-1})]/100 \quad (2)$$

## 2.3. Forage Chemical Analysis

Samples of forage were ground in a hammer mill with a 1-mm screen and analyzed in triplicate for dry matter (DM), ash, crude protein, (CP; Kjeldahl N × 6.25), crude fiber and crude fat (ether extract with previous hydrolysis) according to the procedures described by the AOAC (Association of Official Analytical Chemists) [11]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. [12], and were corrected for residual acid insoluble ash. Acid detergent lignin (ADL) was determined by the method of Van Soest and Robertson [13]. Representative samples of oven-dried forage weighing 1 g were placed in a muffle furnace at 550 °C for 4 h for total ash determination. The ash was wet with sulfuric and perchloric acids and diluted with distilled water [11]. Hemicellulose and cellulose also were estimated as NDF: (ash free)-ADF (ash free) and ADF (ash free)-ADL (ash free), respectively. All results were expressed on DM basis.

Digestibility of dry matter (DDM), dry matter intake (DMI), relative feed value (RFV), total digestible nutrients (TDN), net energy-lactation (NEl), net energy-maintenance (NEm) and net energy-gain (NEg) were calculated according to the following equations adapted from common formulas for forages [14]:

DDM % = 88.9 − (0.779 × ADF %);
DMI % = 120/NDF;
RFV = (DDM % × DMI %)/1.29;
TDN % = 96.35 − (ADF % × 1.15) (adapted equation from Lucerne formulas);
NEl: Mcal/1b = (TDN % × 0.01114) − 0.054;
NEm: Mcal/1b = (TDN % × 0.01318) − 0.132;
NEg: Mcal/1b = (TDN % × 0.01318) − 0.459.

## 2.4. Statistical Analysis

Data were analyzed by analysis of variance (ANOVA) using CoStat, version 1.03, Software (CoHort Software Incorporation, Monterey, CA, USA). The statistical analysis was applied to data following the one-way ANOVA design. Differences between means for significant effects were detected using Student-Newman-Keuls (SNK) test for biomass production and nitrogen fixation data. Unless otherwise stated, significance was declared at $p < 0.05$.

# 3. Results and Discussion

Unfortunately, previous studies evaluating the productive and nutritional characteristics including the nitrogen fixation have not yet been reported in *T. mutabile* forage. Thus, cross-referencing in the discussion of the findings in this study will be based on available results from other winter clover species under Mediterranean environmental conditions.

The six investigated accessions of *T. mutabile* led to a average total dry matter biomass production of 6.6 t/ha as sum of the two cuts at vegetative and flowering growth stages; the TMU06 and TMU02 accessions produced the higher biomass (~8.0 t/ha), whereas the two accessions TMU04 and TMU05 resulted in the lowest dry matter yield (5.5 and 5.1 t/ha, respectively) (Table 2). These results are comparable to those reported by Ovalle et al. [15] in *T. vesiculosum* in a high rainfall areas of the Mediterranean climate zone of Chile and by Cazzato et al. [1] in different species of annual clovers in Southern Italy.

**Table 2.** Total aerial biomass yield, 1st cut biomass (% on total yield), flowering time, Ndfa % and fixed N of the different *T. mutabile* accessions.

| Accession Code | Total Biomass Yield (t/ha) | Biomass Yield at 1st Cut (% on Total Yield) | Flowering Time | Ndfa * (%) | $N_2$ Fixed (kg/ha) |
|---|---|---|---|---|---|
| TMU01 | 6.2 $^{bc}$ | 30.3 $^{a}$ | Early | 64.5 $^{cd}$ | 116.3 $^{b}$ |
| TMU02 | 8.0 $^{a}$ | 23.6 $^{b}$ | Medium-Late | 73.4 $^{ab}$ | 191.2 $^{a}$ |
| TMU03 | 6.9 $^{ab}$ | 15.0 $^{c}$ | Late | 76.8 $^{a}$ | 190.2 $^{a}$ |
| TMU04 | 5.5 $^{bc}$ | 23.6 $^{b}$ | Medium-Early | 58.2 $^{d}$ | 96.3 $^{b}$ |
| TMU05 | 5.1 $^{c}$ | 26.1 $^{ab}$ | Early | 67.1 $^{bc}$ | 111.4 $^{b}$ |
| TMU06 | 8.2 $^{a}$ | 21.6 $^{b}$ | Medium | 72.8 $^{ab}$ | 186.0 $^{a}$ |
| Mean | 6.6 | 23.4 | - | 68.8 | 148.6 |

* Ndfa: N derived from the atmosphere; Means within a column with no common letters (a–d) differ significantly ($p < 0.05$).

Considering the percentage of biomass yield at the first cut, it was found that the accessions produced 23.4% on average of the total biomass. In particular, the highest values were obtained by the early accessions (TMU01 and TMU05), conversely the lowest was detected in the late accession TMU03. Our findings confirm the data reported in the unique study conducted on *T. mutabile* in terms of regrowth capacity after forage cut at vegetative stage [6].

Based on flowering time, it was observed in our study a good variability for this parameter, with an average difference of ~20 days (from 150 to 170 days from emergence) between the earliest and latest accession (data not shown).

The Table 2 reports the values of N derived from air (Ndfa) and the $N_2$ fixed. The mean Ndfa was 68.8%, varying from 58.2% (TMU04) to 76.8% (TMU03). Regarding the $N_2$ fixed, the *T. mutabile* accessions showed an mean value of 148 kg/ha, with a significant variability among the six accessions (from 96 to 186 kg/ha). Our results on Ndfa and $N_2$ fixed are in agreement with those reported by Giambalvo et al. [16] in berseem clover (*T. alexandrinum*). Further, under similar environmental conditions, Cazzato et al. [1] found similar findings on Ndfa and $N_2$ fixed in many annual clovers (*T. incarnatum*, *T. resupinatum*, *T. squarrosum*, *T. alexandrinum* and *T. michelianum*).

The chemical composition of the different *T. mutabile* accessions at vegetative and flowering stages is reported in Table 3.

As expected, the forage cuts at the vegetative stage had a higher crude protein value (25.0%) compared to forage harvested at flowering stage (18.9%). A significant variation was observed among accessions varying from 22.8 to 29.4% of protein (first cut) and from 16.1 to 22.4% at second cut. Conversely, a higher fiber percentage was found at the second cut compared to the forage harvested at flowering stage (26.4 vs. 21.3%). Ross et al. [17] reported in berseem clover a crude protein declined between 31 to 18% DM according to the plant maturity.

The forage fat content did not vary significantly considering both the accessions and the two cuts (1.75% on dry matter). The ash content decreased on average from 12.7 (vegetative stage) to 10.3% (flowering stage), whereas the N-free extract increased harvesting the forage at flowering (39.9%) compared to vegetative (48.2%) stage.

The fiber fractions of the different *T. mutabile* accessions harvested at vegetative and flowering growth stages are reported in Table 4. Based on the findings, it was observed that all the determined parameters increased significantly from vegetative to flowering growth stage; in particular, the mean NDF content varied from 42.4 to 49.1%, the ADF from 23.3 to 29.7%, ADL from 6.7 to 7.1%, cellulose from 16.8 to 22.7%. However, taking into account the single accessions, a high variation in the fiber fractions evaluated was observed. The results of our accessions of *T. mutabile* for NDF and ADF values are similar to the findings reported by Evans and Mills [18] in arrowleaf clover. Moreover, considering the NDF and ADF values, as proposed by the Hay Marketing Task Force of the American Forage and Grassland Council [19], the accessions of *T. mutabile* forage resulted of good quality when harvested at flowering stage and of "premium" quality when harvested at vegetative stage.

**Table 3.** Chemical composition of the different *T. mutabile* accessions at vegetative and flowering stages.

| Accession Code | Vegetative Stage | | | | |
|---|---|---|---|---|---|
| | **Crude Protein (%)** | **Crude Fiber (%)** | **Crude Fat (%)** | **Ash (%)** | **N-Free Extract (%)** |
| TMU01 | 29.4 (0.3) | 16.1 (0.8) | 1.6 (0.1) | 12.5 (0.6) | 40.3 (1.0) |
| TMU02 | 24.2 (0.1) | 20.9 (0.4) | 1.7 (0.1) | 12.5 (0.1) | 40.6 (0.9) |
| TMU03 | 24.1 (0.1) | 22.7 (0.1) | 1.9 (0.1) | 12.5 (0.4) | 38.7 (0.7) |
| TMU04 | 22.8 (0.1) | 23.1 (0.2) | 1.7 (0.1) | 12.7 (0.2) | 39.7 (0.2) |
| TMU05 | 23.1 (0.2) | 22.2 (0.6) | 1.9 (0.1) | 12.4 (0.4) | 38.5 (0.6) |
| TMU06 | 26.4 (0.1) | 22.4 (0.4) | 1.9 (0.1) | 13.7 (0.6) | 35.6 (1.2) |
| Mean | 25.0 | 21.3 | 1.8 | 12.7 | 38.9 |
| Significance level | $p < 0.01$ | $p < 0.01$ | ns | ns | $p < 0.01$ |
| | **Flowering Stage** | | | | |
| TMU01 | 22.4 (0.3) | 23.7 (0.2) | 1.7 (0.1) | 10.4 (0.2) | 41.8 (0.2) |
| TMU02 | 16.5 (0.1) | 24.1 (0.4) | 1.6 (0.1) | 9.6 (0.3) | 48.2 (0.8) |
| TMU03 | 17.7 (0.1) | 29.2 (0.3) | 1.6 (0.1) | 10.3 (0.3) | 41.3 (0.7) |
| TMU04 | 20.8 (0.1) | 26.6 (0.4) | 1.8 (0.1) | 11.7 (0.3) | 39.3 (0.1) |
| TMU05 | 19.4 (0.1) | 28.3 (0.4) | 1.9 (0.1) | 10.8 (0.2) | 39.6 (0.7) |
| TMU06 | 16.1 (0.1) | 26.2 (0.2) | 1.7 (0.1) | 9.1 (0.3) | 47.0 (0.2) |
| Mean | 18.9 | 26.4 | 1.7 | 10.3 | 42.8 |
| Significance level | $p < 0.01$ | $p < 0.01$ | ns | $p < 0.01$ | $p < 0.01$ |

Values in brackets indicate the standard error; ns: not significant.

**Table 4.** Fiber fractions of the different *T. mutabile* accessions at vegetative and flowering stages.

| Accession Code | Vegetative Stage | | | | |
|---|---|---|---|---|---|
| | **NDF (%)** | **ADF (%)** | **ADL (%)** | **Cellulose (%)** | **Hemicellulose (%)** |
| TMU01 | 48.27 (0.49) | 21.39 (0.52) | 7.07 (0.08) | 14.20 (0.48) | 27.17 (0.72) |
| TMU02 | 42.25 (0.30) | 23.34 (0.23) | 6.80 (0.09) | 17.14 (0.28) | 18.52 (0.14) |
| TMU03 | 39.68 (0.28) | 25.58 (0.11) | 6.16 (0.04) | 19.31 (0.04) | 14.35 (0.42) |
| TMU04 | 41.52 (0.23) | 22.60 (0.17) | 6.58 (0.08) | 16.46 (0.20) | 18.93 (0.41) |
| TMU05 | 40.88 (0.33) | 22.22 (0.25) | 7.19 (0.07) | 14.83 (0.11) | 18.78 (0.20) |
| TMU06 | 41.60 (0.13) | 24.78 (0.11) | 6.14 (0.09) | 18.79 (0.05) | 16.29 (0.31) |
| Mean | 42.42 | 23.31 | 6.70 | 16.84 | 19.00 |
| Significance level | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ |
| | **Flowering Stage** | | | | |
| TMU01 | 43.93 (0.28) | 24.14 (0.18) | 7.55 (0.19) | 17.49 (1.28) | 19.90 (0.21) |
| TMU02 | 50.03 (0.33) | 28.50 (0.30) | 7.18 (0.06) | 21.18 (0.22) | 22.01 (0.14) |
| TMU03 | 48.38 (0.26) | 31.86 (0.46) | 6.87 (0.02) | 24.98 (0.43) | 17.27 (0.04) |
| TMU04 | 52.46 (0.56) | 29.11 (0.15) | 6.99 (0.02) | 22.15 (0.16) | 23.77 (0.28) |
| TMU05 | 54.62 (0.42) | 34.37 (0.59) | 7.17 (0.19) | 27.23 (0.36) | 20.72 (0.64) |
| TMU06 | 45.46 (0.22) | 30.04 (0.09) | 6.99 (0.07) | 23.01 (0.11) | 15.58 (0.29) |
| Mean | 49.11 | 29.73 | 7.12 | 22.71 | 19.94 |
| Significance level | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ |

NDF, neutral-detergent fiber; ADF, acid-detergent fiber; ADL, acid-detergent lignin; Values in brackets indicate the standard error.

Significant differences were observed among the six accessions of *T. mutabile* in terms of DDM, DMI and RFV for animal feeding (Table 5). As a result of differences in ADF contents, DDM contents of the accessions were also different in the two harvest period. Because of NDF contents of the accessions were different, also the DMI varied significantly. As a result of differences determined in DDM and DMI values amongst *T. mutabile* accessions, the RFV values were also different amongst accessions.

**Table 5.** Digestibility dry matter (DDM), dry matter intake (DMI) contents, relative feed value (RFV), Total digestible nutrients (TDN), net energy lactation (NEl), net energy. Maintenance (NEm) and net energy gain (NEg) contents of the different *T. mutabile* accessions.

| Accession Code | Vegetative Stage | | | | | | |
|---|---|---|---|---|---|---|---|
| | **DDM** | **DMI** | **RFV** | **TDN** | **NEl** | **NEm** | **NEg** |
| TMU01 | 72.23 (0.40) | 2.49 (0.03) | 139.23 (0.62) | 71.75 (0.60) | 0.75 (0.01) | 0.82 (0.01) | 0.49 (0.01) |
| TMU02 | 70.72 (0.18) | 2.84 (0.02) | 155.73 (0.70) | 69.51 (0.27) | 0.72 (0.0) | 0.79 (0.01) | 0.46 (0.01) |
| TMU03 | 68.98 (0.09) | 3.03 (0.02) | 161.71 (1.35) | 66.94 (0.13) | 0.69 (0.0) | 0.75 (0.0) | 0.42 (0.0) |
| TMU04 | 71.30 (0.14) | 2.89 (0.02) | 159.72 (0.61) | 70.36 (0.20) | 0.73 (0.0) | 0.80 (0.01) | 0.47 (0.0) |
| TMU05 | 71.59 (0.20) | 2.94 (0.02) | 162.91 (1.77) | 70.79 (0.29) | 0.74 (0.01) | 0.80 (0.0) | 0.48 (0.01) |
| TMU06 | 69.60 (0.08) | 2.89 (0.01) | 155.64 (0.31) | 67.86 (0.12) | 0.70 (0.0) | 0.76 (0.0) | 0.44 (0.01) |
| Mean | 70.71 | 2.84 | 155.82 | 69.53 | 0.72 | 0.78 | 0.46 |
| Significance level | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ |
| | **Flowering Stage** | | | | | | |
| TMU01 | 70.09 (0.14) | 2.73 (0.02) | 148.43 (1.24) | 68.59 (0.21) | 0.71 (0.01) | 0.77 (0.01) | 0.44 (0.01) |
| TMU02 | 66.70 (0.23) | 2.39 (0.01) | 124.04 (0.38) | 63.58 (0.34) | 0.66 (0.0) | 0.71 (0.01) | 0.38 (0.01) |
| TMU03 | 64.08 (0.35) | 2.48 (0.01) | 123.23 (0.03) | 59.71 (0.52) | 0.61 (0.0) | 0.66 (0.01) | 0.33 (0.01) |
| TMU04 | 66.22 (0.11) | 2.28 (0.02) | 117.45 (1.03) | 62.87 (0.17) | 0.64 (0.0) | 0.69 (0.01) | 0.37 (0.01) |
| TMU05 | 62.13 (0.46) | 2.19 (0.01) | 105.83 (1.58) | 56.83 (0.67) | 0.58 (0.01) | 0.62 (0.01) | 0.29 (0.01) |
| TMU06 | 65.50 (0.06) | 2.64 (0.01) | 134.04 (0.78) | 61.81 (0.1) | 0.63 (0.01) | 0.68 (0.01) | 0.36 (0.01) |
| Mean | 65.82 | 2.46 | 125.52 | 62.23 | 0.64 | 0.69 | 0.36 |
| Significance level | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ |

Values in brackets indicate the standard error.

Differences in terms of TDN, NEl, NEm and NEg contents were obtained amongst accessions. The relative feed value (RFV) is a widely accepted forage quality index in the marketing of hays in the United States of America [20,21]. Based on our results on RFV, the forage obtained from the *T. mutabile* accessions can be classified as good-premium quality, resulting thus a valuable feeding resource for livestock species.

## 4. Conclusions

From our findings, significant differences were found among the accessions of *T. mutabile* in terms of biomass production, capacity of nitrogen fixation and forage quality characteristics. Besides the high forage yield, the collected accessions showed favourable values of production and quality, representing also valuable germplasm for further selection programs as well as the application for possible plant cultivar registration. Moreover, it is interesting to underline that *T. mutabile* may represent a valuable alternative to commonly cultivated annual clover species especially in Mediterranean areas, because of its longer vegetative cycle. However, further investigations are needed to assess the self-reseeding capacity of *T. mutabile* when utilized as pasture species.

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