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Characterization of native populations of *Trifolium* spp.

R. Tuttobene, F. Gresta, O. Sortino, F. Frasca Polara, M. Dipasquale and V. Abbate
Department of Agronomy, Agrochemistry and Animal Production Sciences, University of Catania, Via Valdisavoia 5, 95123 Catania, Italy

SUMMARY – Annual legumes represent an important vegetative resource for pasture improvement. Twenty-eight ecotypes belonging to 11 species of *Trifolium* were collected in the Hyblean Plateau (south-east Sicily) from sea-level to 810 m. Plant height, maximum plant diameter, number of primary branches per plant, maximum branch length, leaf number, time to flowering, flowering length, seeds per pod, 1000 seeds weight and seed plant production were determined. Principal Component Analysis was performed on these characteristics. The altimetric level of collection sites did not influence morpho-phenological and productive traits. *T. pallidum* proves to be a valuable species as forage crop (in pure or mixed swards); *T. campestre* and *T. glomeratum* can very easily be harvested with conventional grain harvesters and may be an interesting species for pasture overseeding. *T. cherleri* and *T. tomentosum* may be improved for marginal pastures and to prevent soil erosion.

Keywords: *Trifolium*, variability, correlation, PC analysis.

RESUME *– "Caractérisation des populations autochtones de Trifolium spp.". Les écotypes de légumineuses annuelles représentent une importante ressource pour l'amélioration des pâturages. Une collecte de vingt-huit écotypes appartenant à 11 espèces de* Trifolium *a été effectuée dans le Haut Plateau des Iblés (Sud-Est de la Sicile) du niveau de la mer jusqu'à 810 m. La hauteur de la plante, le diamètre maximal de la plante, le nombre de branches par plante, la longueur de la branche maximale, le nombre de feuilles, l'époque et la durée de la floraison, le nombre de graines par cosse, le poids de 1000 graines et la production en graines de la plante ont été déterminés. Une Analyse en Composantes Principales a été exécutée sur ces caractéristiques. L'altimétrie des sites de collecte n'a pas influencé les caractéristiques morpho-phénologiques ainsi que les caractéristiques productives.* T. pallidum *se révèle comme une précieuse espèce fourragère (en culture pure ou associée) ;* T. campestre *et* T. glomeratum *peuvent être des espèces intéressantes à semer dans les pâturages,* T. cherleri *et* T. tomentosum *peuvent être améliorées pour les pâturages marginaux et pour prévenir l'érosion du sol.*

Mots-clés : Trifolium*, variabilité, corrélation, analyse en composantes principales.*

Introduction

Management to encourage the restoration and survival of species rich communities on agricultural land has become increasingly important as European agricultural policies are adapted to incorporate environmental objectives. Self-reseeding legumes (e.g. *Trifolium* spp., *Medicago* spp.) have been the primary components of Mediterranean grasslands for thousands of years.

In communally owned grasslands of the Mediterranean basin, modern surveys have confirmed that legumes are an important component of non-arable agriculture. In the Mediterranean basin, Porqueddu *et al*. (2000) recorded 33 herbaceous legumes in 1 ha survey of Sardinia, while Abbate *et al.* (2006) recorded 30 self-reseeding legumes in the Hyblean plateau of South East Sicily. The Hyblean plateau is characterised by intensive animal husbandry based on native pasture, a grassy surface in rotation with winter grain and forage crops, in which grasses (mainly consisting of annual species) grow spontaneously. Nowadays, native pastures have become rather deteriorated due to single crop grain cultivation and to the intensive grazing of livestock with a consequent reduction of pabular species. Naturally occurring ecotypes can perform as well or better than available cultivars in some environments, and therefore, the selection and evaluation of wild ecotypes is an important ongoing research programme (Sackville-Hamilton and Cresswell, 1999).

The genus *Trifolium* contains approximately 240 species (separated into eight sections), 110 of which have their centre of diversity in the Mediterranean Basin (Zohary and Heller, 1984). The objective of this research was to evaluate a collection of ecotypes belonging to 11 species of *Trifolium* using multivariate analysis with the practical purpose of establishing and directing a selection programme.

Materials and methods

Field investigation was conducted in 2006 - 2007 in a representative area of the Hyblean Plateau of Eastern Sicily (Italy). A collection of 28 ecotypes belonging to 11 species of *Trifolium*, collected in sites with altitude ranging from sea level to 810 m and classified in three level classes (A< 250, B= 250-500, C > 500 m), was evaluated in nursery rows. Each species was represented by 3 ecotypes, excepted *T. pallidum*, *T. spumosum* (2) and *T. angustifolium* (1). Individual seedlings of each population were grown in jiffy-pots; when the seedlings reached the fourth leaf they were transplanted in plots of 6.25 m^2. (2.5 x 2.5 m). In each plot, 50 plants were placed in five rows 0.50 m apart, spaced at 0.25 m within rows. The experiment was a randomized block with three replicates.

Twenty plants per plot were randomly selected from each of 28 populations. The selection of characteristics (descriptors) was made following the International Board for Plant Genetic Resources guidelines for *Trifolium repens* descriptors, but some additional descriptors were also taken into account. The following traits were measured: plant height (PH - cm), maximum plant diameter (MPD - cm), number of primary branch per plant (BN), maximum branch length (MBL - cm), leaves number (LN), time to flowering (FT - days from sowing date to 30% plant with inflorescences), flowering length (FL – days between the first and last dates of flowering), seeds per pod (SP - number), 1000 seeds weight (SW - g), seed plant production (PP- g). The relationships between the different traits and between them and altitude were quantified using Pearson correlation and Principal Component Analysis (PCA). PCA on standardised data was performed with Systat 12 (SYSTAT Software Inc.). Principal components with eigenvalues >1.0 were selected and correlation values >0.6 were considered as relevant for the PCA.

Results and discussion

Results of Pearson correlation analysis (data not reported) support the use of multivariate analysis. The 16% of the 55 correlation coefficients were statistically significant. The main positive correlation between the evaluated traits appeared as follows: plant height with plant diameter (0.68***), maximum branch length (0.793***) and flowering time (0.50**); branch number with leaves number (0.71***); seeds per pod with maximum branch length (0.38*) and seed plant production (0.49**). On the other hand, negative strong correlation appeared only between the time of flowering and flowering length (-0.86***). PCA showed four principal components accounting, as a whole, for 80.4% of the total data variability (Table 1).

Table 1. Variance of principal components (PC) and the cumulative contribution to the total variance

Value	PC 1	PC 2	PC 3	PC 4
Eigenvalue	3.90	2.01	1.81	1.12
% of Variance	35.49	18.30	16.42	10.20
Cumulative %	35.49	53.79	70.22	80.42

The first one, explaining 35.5% of the variation, is associated mainly with morphological traits (Maximum Branch Length, Maximum Plant Diameter and Plant Height) (Table 2); component two, responsible for 18.3% of the variation, is almost exclusively represented by leaf number and seed weight; component three contributed to 16.4% of variation and is associated with flowering parameters, receiving the highest positive loading from the variable time of flowering, whereas the highest negative loading coming from the variable flowering length; finally principal component four, responsible for 10.2% of variation, is associated mainly with seed production characteristics (seed per pod and seed plant production).

The graphic representation of the relationships among species, given on the basis of the evaluated traits, shows moderate amount of variability, except for some cases (Fig. 1). The two populations of *T. pallidum* clearly stand out for high PC1 values, corresponding mainly to high plants (50 cm in average compared to 18.4 cm general mean) with long branches (85.6 cm compared to 44.0 cm) (Table 3).

Moreover, *T. pallidum* presents a late flowering time. Another group that can be identified includes the three populations of *T. campestre* and one population of *T. glomeratum*, mainly characterized by several branches (142.5 in the average of four populations compared to 81.1) and consequently numerous leaves (1124 compared to 601.9), but a short *habitus*, as showed by MBL, MPD and PH traits. A third group is composed by the three ecotypes of *T. tomentosum* and one ecotype of *T. cherleri* that show less values of BN and LN compared to *T. campestre* and *T. glomeratum* and a more prostrate *habitus* compared to both previous groups.

Table 2. Results of the principal component analysis

Traits	Factor 1	Factor 2	Factor 3	Factor 4	Mean value	s.d.
Plant height (cm)	0.828*	-0.091	0.171	0.160	18.4	13.1
Maximum plant diameter (cm)	0.895*	0.021	0.217	0.144	77.3	25.2
Branch number (n)	-0.371	0.871	-0.065	0.008	81.1	33.5
Maximum branch length (cm)	0.971*	0.025	0.081	0.117	44.0	14.0
Leaf number (n)	0.198	0.892*	0.310	-0.058	601.9	318.5
Time to flowering (dd)	0.475	0.063	0.784*	-0.007	101.4	16.2
Flowering length (dd)	-0.234	-0.160	-0.822*	-0.137	48.4	16.3
Seeds per pod (n)	0.337	0.052	-0.374	0.803*	42.7	30.7
1000 seed weight (g)	-0.113	-0.626*	0.489	0.018	1.1	1.0
Seed production per plant (g)	0.104	-0.100	0.384	0.861*	11.7	8.3
Altimetry	0.037	-0.091	0.588	-0.024	--	--

*Correlation values > 0.6.

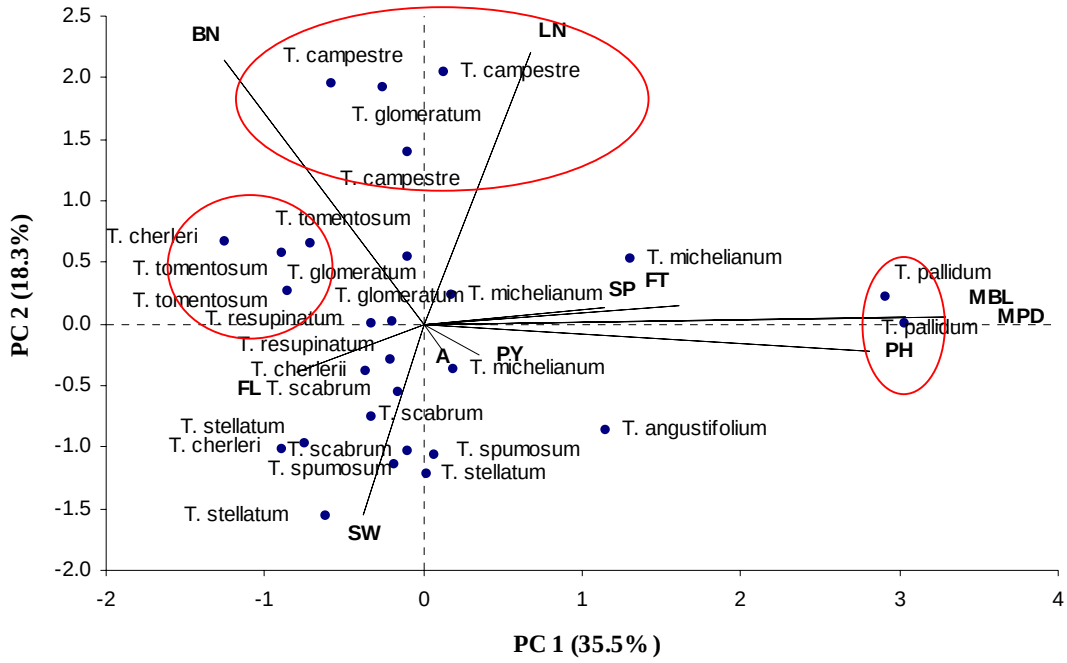

Fig. 1. Scatter plot grouping of *Trifolium* spp. based on PC1 and PC2 of the principal component analysis. The rays connecting the traits to the biplot origin are referred to as trait vectors. The vector length of a trait measures the magnitude of its effect (positive or negative). Almost all the vegetative traits (PH, PD, MBL, LN and BN) had relatively long vectors, suggesting that they could have relatively large weight. In contrast, most reproductive traits such as PP or SP and much more for the A parameter, had short vectors, suggesting that they had little association with descriptors selection.

Table 3. Main morphological and phenological traits + coefficient of variability

Species	PH	c.v.	MPD	c.v.	BN	c.v.	MBL	c.v.	LN	c.v.	FT	c.v.
T. angustifolium	50.0	--	63.3	--	42.3	--	55.8	--	285.0	--	124.2	--
T. campestre	18.6	41.5	66.8	5.7	146.3	9.3	40.4	14.5	1066.9	16.5	98.0	13.2
T. cherleri	9.4	30.3	61.4	5.6	86.0	32.8	34.9	10.2	652.1	56.3	110.5	5.6
T. glomeratum	11.0	22.2	78.6	14.9	97.4	37.4	42.5	7.8	794.1	44.3	113.5	19.2
T. pallidum	49.1	5.6	157.1	1.4	44.7	10.9	85.6	1.0	1019.5	7.8	132.1	5.4
T. michelianum	18.3	28.2	88.0	11.7	81.6	10.8	51.4	24.3	470.4	35.7	87.8	8.3
T. resupinatum	9.6	21.4	72.7	1.1	70.6	11.2	40.1	1.0	490.9	15.1	95.6	2.4
T. scabrum	9.3	24.5	67.2	0.9	53.1	12.5	38.3	3.4	307.1	14.5	90.7	9.7
T. spumosum	26.3	12.5	87.8	2.4	45.7	1.4	47.7	3.6	314.2	7.8	105.3	4.6
T. stellatum	19.3	79.9	70.8	5.0	68.7	17.5	37.6	7.7	294.3	34.9	96.3	6.4
T. tomentosum	12.9	18.8	55.7	9.2	102.6	13.6	31.1	8.9	721.6	13.8	85.8	16.3

The different growing *habitus* of the above mentioned *Trifolium* ecotypes, suggest their possible improvement for different uses: *T. pallidum* with a tall *habitus* and longer vegetative stage can be introduced in cultivation as forage crop (pure or mix sword); *T. campestre* and one ecotype of *T. glomeratum,* with a shorter *habitus* for overseeding in pasture; *T. tomentosum and* one ecotype of *T. cherleri* with a very short *habitus* and moderate BN in pasture of marginal lands and to prevent soil erosion.

T. cherleri and *T. glomeratum*, compared to the other species, showed a high variability within ecotypes, confirming what was observed by Bennett and Mathews (2003) for *T. glomeratum*. No interesting relationship appears between ecotypes and altitude. Almost all the remaining populations show negative values of PC1 and PC2, corresponding to a limited vegetative development and a shorter vegetative stage.

Conclusions

Results of PC analysis highlight moderate interspecific and intraspecific variability of the collected ecotypes. Altimetric level of collection sites did not influence morpho-phenologiocal and productive traits. *T. pallidum* stands out as a valuable new forage crop. In order to improve pasture of Hyblean plateau, *T. campestre* and *T. glomeratum can be used* in overseeding. *T. tomentosum* and *T. cherleri* may be interesting species in marginal pastures and to prevent soil erosion.

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