Cold tolerance of subterranean clover in a continental-climate environment

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Cold tolerance of subterranean clover in a continental-climate environment

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Summary - Subterranean clover is a suitable species as cover crop in vineyards and orchards. This alternative use requires the development of varieties with specific features. A sufficient level of cold tolerance might be needed to extend the area of utilization of subterranean clover in environments with low winter temperatures. Two cycles of evaluation for cold tolerance were carried out under field conditions at Lodi, northern Italy, on 24 genotypes singled out from Sardinian native populations, and five commercial cultivars. The native strains were chosen as to represent sites of origin with contrasting altitude and, within these, genotypes with contrasting earliness. Wide variation among genotypes was found in both seasons for cold susceptibility and winter growth ability, which could be usefully exploited for breeding. Late genotypes from high-altitude environments were generally the best performing strains.

Key-words: cold tolerance, cover crop, winter growth, *Trifolium subterraneum* L

Résumé - *Le trèfle souterrain est une espèce convenable comme culture de couverture dans les vignobles et les vergers. Cette utilisation alternative demande le développement de variétés avec des caractéristiques spécifiques. Un niveau suffisant de tolérance au froid pourrait être nécessaire pour étendre la surface d'utilisation du trèfle dans des milieux à températures basses en hiver. Deux cycles d'évaluation pour la tolérance au froid ont été menés en plein champ à Lodi, en Italie du nord, sur 24 génotypes isolés de populations naturelles sardes, et cinq variétés commerciales. Les génotypes sardes avaient été choisis pour représenter des endroits d'origine à altitude contrastante et, à l'intérieur de ceux-ci, des génotypes à précocité contrastante. Au corse de deux saisons on a trouvé une grande variabilité parmi les génotypes pour la susceptibilité au froid et pour la capacité de croître en hiver. Les génotypes tardifs des endroits à haute altitude ont été généralement les meilleurs.*

Mots-clés: *croissance en hiver, culture de couverture, tolérance au froid,* Trifolium subterraneum *L.*

Introduction

Subterranean clover (*Trifolium subterraneum* L. *sensu lato*) is a species under widespread consideration in Italy for its possible use as technical cover crop in vineyards and orchards. Its cycle spanning from autumn to mid- or late-spring does not substantially interfere with that of the woody companion. Its morphology, especially the prostrate growth habit, provides soil protection from runoff erosion in the critical rainy period and maintains a remarkable ground sustenance. The vegetation residuals also carry out an important mulching effect in summer.

This alternative use may require the development of varieties with specific morpho-physiological features. Although subterranean clover is a species typical of Mediterranean environments (Morley, 1961), the current trend is to extend its area of cultivation as cover crop in sub-Mediterranean and, even, continental environments, with relatively low temperatures and limited solar radiation in winter, where vineyards and orchards are frequent in Italy. The success of this practice may be hindered by the possible cold susceptibility and the limited winter growth of the species which would render little effective the soil cover. The need arises therefore for a specific selection carried out under targeted conditions to identify germplasm suitable to novel environments of utilization. This study was undertaken to assess the variation of subterranean clover genotypes in the ability to grow and survive under cold-winter climatic conditions. The possible influences of the environment of origin and the length of the growing cycle of the genotypes (maturity grading) on the response to cold stress were also investigated.

Materials and methods

The trial was carried out in two seasons (1997-98 and 1998-99) under field conditions at Lodi, northern Italy (45°20' N, 9°15' E, 84 m a.s.l.), a location in the middle of the Po plain characterised by a continental-type climate. A set of 24 genotypes singled out from Sardinian native populations, and five commercial cultivars were evaluated. The native strains were chosen as to represent collection sites of contrasting altitude and, hence, minimum winter temperatures, and, within these sites, genotypes with contrasting flowering time. Four groups were then formed by combining altitude of origin and flowering time: high and late genotypes (HL), high and early genotypes (HE), low and late genotypes (LL), and low and early genotypes (LE), each represented by six entries. Flowering time, as number of days from sowing to first flowers, was previously recorded in two seasons of evaluation under Mediterranean climatic conditions (Piano, unpublished). Mean values of altitude of origin and flowering time were: 1020 m asl and 143.7 days for HL, 1034 m asl and 106.3 days for HE, 223 m asl and 143.7 days for LL, and 271 m asl and 105.7 days for LE, respectively.

The experiment was laid out as a randomised complete block design with four replications, the genotypes belonging to the four groups and the commercial varieties being completely randomised within each block. In the first season, the genotypes were sown on October 6 in peat pots, and transplanted in the field on November 14 in rows of 15 plants, 15 cm apart on the row, with 20 cm between adjacent rows. In the second season, sowing and transplantation dates were much earlier, occurring on August 25 and October 13, respectively. In both seasons, each plot was visually rated at intervals of about. 14 days according to a score ranging from 1 (poor) to 5 (good), mainly based on the extent of symptoms attributable to cold/frost damage (e.g., leaf reddening, wilting and burning) but also considering the plant vigour. Scoring took place five times between January 9 and March 9 in the first season, and eight times between December 1 and March 15 in the second one. A mean score across the cold period was then computed for each season. On the two central plants per row, the number of fully expanded, healthy leaves was counted (and then averaged per plant) five times in the first season, at intervals of ca. 14 days between January 9 and March 9, and four times in the second one, at intervals of one month between December 15 and March 15. In both seasons, flowering time was recorded as number of days from March 1 to the appearance of the first inflorescences per plot, and the final survival was computed at the end of March as percent of transplanted plants.

For each season, an analysis of variance (ANOVA) was run for all the traits, holding the fixed factors 'group' (also including the set of commercial varieties) and 'genotype within group', and the random factor 'block'.

Results and discussion

The mean value of the daily minimum temperature from November through March was 1.6°C in the first season and 0.4°C in the second one. The number of frost days (≤ 0°C) was higher in the second season than in the first one (72 vs. 41), while the absolute minimum temperature was substantially similar, being –6°C in 1997-98 and –7°C in 1998-99. The coldest stretch in the first season was between January 20 and February 10 (18 frost days), while in the second one the whole month of December (23 frost days) and the span between January 20 and February 20 (25 frost days) were the coldest periods.

In both seasons, variation among genotypes was significant ($P \leq 0.001$) for the mean visual score across the cold period and for flowering time, and not significant for the percent of final plant survival (Table1).

Table 1. Results of the analysis of variance of genotypes and group mean values for three characters recorded in two seasons of evaluation.

	First season (1997-98)[†]			Second season (1998-99)[†]		
	(1)	(2)	(3)	(1)	(2)	(3)
F test significance of genotype means[‡]	***	ns	***	***	ns	***
Genotype range	1.6-4.0	61.7-86.7	23.7-54.0	1.4-3.5	83.3-98.3	35.2-60.0
F test significance of group means[‡]	***	ns	***	***	*	***
Group[§]:						
HL	3.4 a	80.6 a	43.8 a	2.7 a	95.3 a	51.5 a
HE	2.7 c	78.3 a	29.9 c	1.9 c	90.2 bc	40.5 c
LL	2.6 cd	78.9 a	40.1 b	2.4 b	91.9 abc	48.8 b
LE	3.0 b	75.0 a	28.3 c	2.3 b	88.3 c	38.3 d
CCs	2.4 d	72.3 a	40.5 b	2.4 b	93.7 ab	47.9 b

[†] (1): Mean visual score across the cold period (1 poor – 5 good); (2): Final plant survival (%); (3): Flowering time (dd. from March 1).

[‡] ns, *, ***: not significant and significant at $P \leq 0.05$ and $P \leq 0.001$, respectively.

[§] HL: high-altitude, late-flowering genotypes; HE: high-altitude, early-flowering genotypes; LL: low-altitude, late-flowering genotypes; LE: low-altitude, early-flowering genotypes; CCs: commercial cultivars. Group means followed by the same letter are not different at $P \leq 0.05$ according to Duncan's multiple range test.

On the whole, the plant mortality was lower than expected, indicating an intrinsic cold tolerance of the species in spite of its Mediterranean origin. The lower final plant survival in the first season relative to the second one (on average, 76.9% vs. 91.8%), in spite of the somewhat milder conditions, was probably related to greater vulnerability of the seedlings which were at a younger stage when the stressful conditions occurred, due to the later dates of sowing and transplanting. As indicated by the values of the visual score, both in 1997-98 and 1998-99 there was a wide range of genotypic response to the growing conditions (Table 1), and some genotypes were identified with consistently good behaviour in the two seasons. The range of flowering time among genotypes, which exceeded 50 days under Mediterranean conditions, was reduced to 25-30 days under continental conditions, confirming an already observed trend of shortening of the range when moving to colder conditions (Piano, 1987), which can be explained in terms of temperature interaction effects on the physiology of flowering (Evans, 1959).

A comparison of the four groups of native strains indicated that under the given climatic conditions late genotypes from high altitude (HL) were the best performing, considering both mean score and survival, over all the other groups (Table 1). Genotypes from high-altitude environments, which can represent an obvious target germplasm to select varieties for cold environments, performed differently according to their maturity class. Indeed, HE genotypes did not perform better than HL genotypes, indicating that earliness did not provide better winter growth while probably determining greater cold susceptibility, as evidenced by the lower survival in the second season. Also, lateness *per se*, which is often seen as a feature associated with cold tolerance, resulted in a different response to cold stress according to the environment of origin of the strains, LL genotypes being always less performant than HL genotypes (Table 1). The already mentioned trend towards a shortening of the flowering range was also exemplified by the the reduction to only ca. 11 days of the average differences between early and late strains within each group of altitude relative to the ca. 37 days recorded under Mediterranean conditions.

The vegetative activity across the cold period, which obviously relates to the ground cover capacity of the species as a cover crop in non-Mediterranean environments, reached in both years the lowest level in February, following the coldest stretch (Figure 1). A sudden and outstanding recovery took place as soon as the temperatures raised in early March. Differences among genotype groups for leaf number dynamics were generally consistent with those for the visual score, HL genotypes being significantly ($P \leq 0.05$) best performing, particularly at the most critical dates. From a breeding point of view, the most relevant finding was however the remarkable variation of winter growth among individual genotypes, the best strain showing a 2-fold and a 4-fold number of leaves relative to the worst one in the most stressful period of February in the first and second season, respectively (Figure 1).

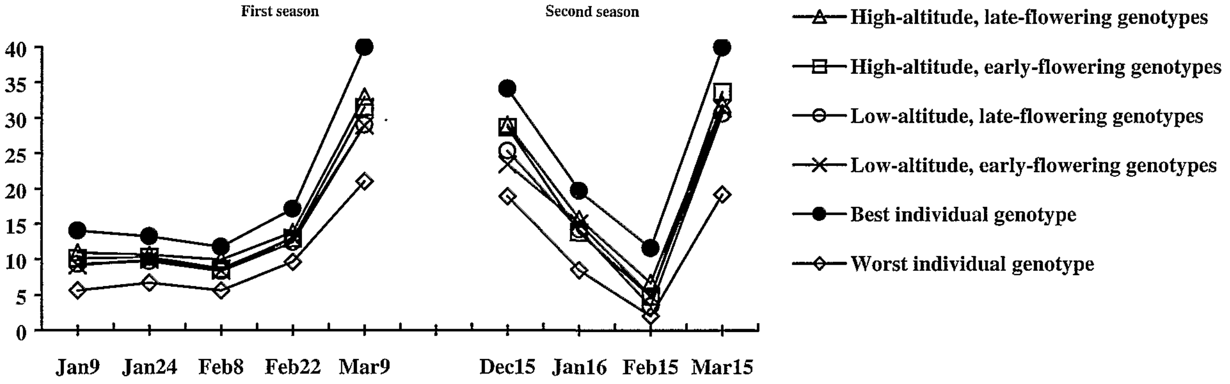

Figure 1. Number of fully developed, healthy leaves per plant of subterranean clover at different dates along the cold period in two seasons of evaluation, averaged over groups of genotypes.

Conclusions

The present results highlight the relevance of an appropriate choice of the germplasm resources, giving adequate consideration to the environments of origin, to select cover crop varieties for continental conditions. Germplasm from high-altitude sites appear the most suitable. The choice of early variants in this germplasm, as a possible selection criterion to enhance winter growth ability, does not seem to be a rewarding option on the basis of this study. The great variation found among genotypes for both stress tolerance and cold-season growing ability is a prerequisite to enable the selection of adapted varieties. As indicated by the lower mortality level in the second, colder season, an early crop establishment or regeneration is likely to play a relevant role in plant survival throughout winter.

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References

Evans, L.T. (1959). Flower initiation in *Trifolium subterraneum* L. I. Analysis of the partial processes involved. *Aust. J. Agric. Res.*, 10, 1-16.

Morley, F.H.W. (1961). Subterranean clover. *Adv. Agron.*, 13: 57-123.

Piano, E. (1987). Aspects of the variability of subterranean clover populations and their implications in breeding. The environmental influence on flowering time. In: *Proc. Eucarpia Fodder Crops Section Meet.*, Lusignan, France, 45-52.