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Comparisons among different plant breeding approaches applied to red clover

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Summary – Six hundred and six healthy plants were randomly collected from the Sardinian red clover ecotype "Gioscari" and submitted to three different breeding programs: Mass Selection 2 (MS2), Half Sib Family Selection (HSFS) and Half Sib Progeny Test (HSPT). Two cycles of MS2 selection and 1 cycle of HSFS did not produce an increase in red clover Dry Matter Yield (DMY) even if high Selection Differentials were applied; on the contrary, 1 cycle of HSPT gave rise to a significant response. The results of the breeding activities were not evident for total DMY (i.e. including weeds), but just for red clover DMY. Therefore the selection gave rise to a material able to increase its percentage in the botanical composition of the forage with respect to the initial material and, by this way, capable of contrasting weed growth under dense stand condition. The results show that the choice of the breeding procedure has to be carefully considered at the beginning of a new breeding programme in a forage species such as red clover. A breeding approach based on progeny evaluation followed by the intercrossing under isolation of the selected mother plants such as HSPT, is likely to give better results, in the same amount of time, in comparison to less complex selection approaches such as MS2 and HSFS.

Key-words: breeding, mass selection, progeny test, family selection, red clover

Résumé *- Des plants sains de l'écotype sarde de trèfle violet "Gioscari" ont été soumis à 3 programmes de sélection: 2 cycles de sélection massale (MS2), une sélection sur familles de demi-frères (HSFS) et une sélection sur test de descendances de plantes demi-frères (HSPT). MS2 et HSFS n'ont pas permis d'obtenir un accroissement de la production de matière sèche du trèfle violet, même quand de fortes pressions de sélection sont appliquées. A l'inverse, un cycle de HSPT a donné une réponse significative. Les résultats des schémas de sélection ne sont pas concluants pour la matière sèche totale, mais le sont pour la matière sèche du trèfle violet. La sélection a donc permis d'obtenir un matériel dont la contribution à la composition botanique du fourrage a augmenté, et par conséquent un fourrage de meilleur qualité. Ces résultats montrent que les schémas de sélection doivent être choisis avec précaution, surtout lorsque l'on débute un nouveau programme sur une espèce comme le trèfle violet. Un programme de sélection basé sur une évaluation des descendances suivie par un inter-croisement des meilleures plantes mères en isolement pollinique telle que HSPT donne de bien meilleurs résultats dans le même laps de temps que des méthodes moins complexes telles que MS2 ou HSFS.*

Mots-clès: *sélection, trèfle violet, sélection massale, test sur descendances, sélection familiale*

Introduction

Red clover (*Trifolium pratense* L., 2n=2x=14) is a forage species which deserves a particular attention in Mediterranean regions such as Sardinia, especially on soils characterised by sub-acid pH, not adapted to alfalfa cultivation. Most of the red clover varieties available in the seed market are adapted to temperate environments and need large water supply to grow and persist during summer drought under Mediterranean conditions. The local red clover ecotype "Gioscari", isolated from natural pastures of the "Nurra" region in N.W. Sardinia, Italy (Bullitta P., personal communication), proved to be characterised by high winter growth rates, drought resistance and persistence (Roggero *et al.* 1990). The use of

adapted materials of red clover could effectively contribute to increase the water use efficiency in areas of limited water availability during the summer. Therefore a breeding programme was started in 1986 with the objectives of obtaining a broad based variety of red clover adapted to Mediterranean climates and of testing the efficiency of different breeding approaches.

Materials and methods

Taking into account the increase of forage production, the following three breeding approaches (schematically shown in figure 1) have been applied starting from the ecotype "Gioscari".

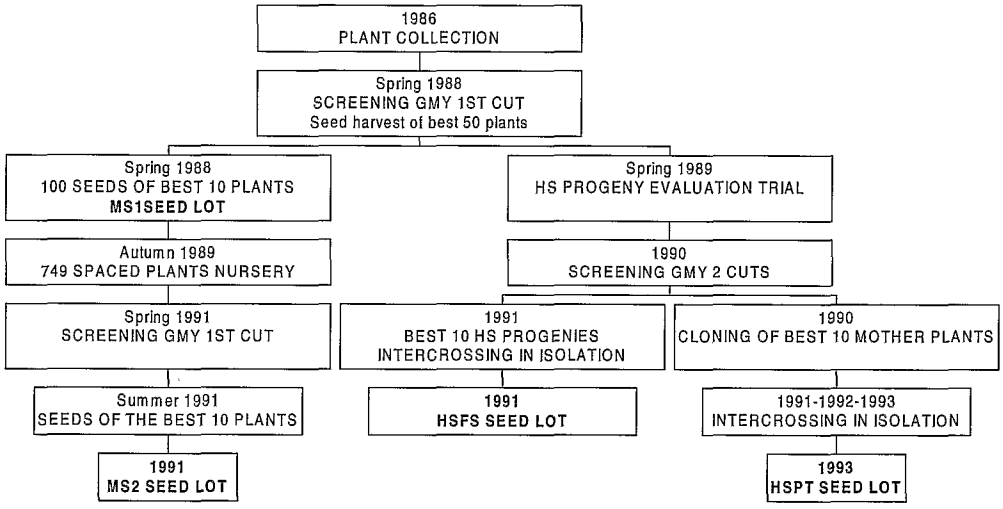

Figure 1 – Schematic map of the three breeding schemes.

A. Mass Selection 2 (MS2): with this scheme, individual plants are selected on the basis of their phenotypes and the seed is produced under open pollination (OP), which means that maternal plants are selected but pollen sources are coming from the whole population. Equal amounts of seed from each one of the selected plants are mixed to produce a selected seed lot. Repeated cycles of this selection procedure correspond to a Simple Recurrent Selection breeding approach;
B. Half Sib Family Selection (HSFS): with this procedure open pollinated seed is collected on phenotypically selected plants and maintained as distinct seed lots. All the obtained Half Sib progenies are evaluated and the best progenies are intercrossed under isolation. Equal amounts of seed coming from each one of the selected progenies are mixed to produce a selected seed lot;
C. Half Sib Progeny Test (HSPT): with this breeding approach, also known as Polycross Progeny Test, individual plants are selected on the basis of the performances of their Half Sib progenies. Once selected, the plants are intercrossed under isolation and the seed (equal amount of seed for each plant) is mixed to produce a new seed lot.

Further information on the above reported breeding schemes can be found e.g. in Tysdal & Kiesselbach (1944), Allard (1960), Penny *et al.* (1963) and Rowe & Hill (1985).

The three breeding methods are characterised by an increasing degree of difficulty from MS2 to HSFS and HSPT. We wanted to clarify if such an increasing difficulty could led to a clear superiority in the breeding responses or not.

In autumn 1986, 606 plants were randomly collected from a 4 years old meadow of "Gioscari" and transplanted as spaced plants (50x80 cm) in the experimental farm of the

Faculty of Agriculture of Sassari, at Ottava (N.W. Sardinia, 80 m a.s.l., 500 mm annual rainfall, calcareous sandy-loam soils).

All the transplanted plants have been checked for Green Matter Yield (GMY, g plant^{-1}) at the first cut of 1988 and, on the basis of the results, OP seed from the best 50 plants has been collected and stored as separated seed lots. In autumn 1989, 100 OP seeds for each one of the 10 best plants selected on the basis of the spaced plant trial established in 1986 were mixed and utilised to produce a new spaced plant population of 749 plants. These materials have been evaluated for GMY at the first cut of 1991. In the same year, seed was harvested from the 10 best plants of the population; equal amounts of seed for each plant were mixed giving rise to the final product of two cycles of MS2.

Part of the seed obtained in 1988 has been utilised in spring 1989 to establish an HS progenies evaluation trial (50 entries) along with a randomised block design with three replication and a plot size of 2 m^2. In 1990 data have been collected on GMY of 2 cuts (g m^{-2}) and, in 1991, the best 10 HS progenies have been intercrossed in isolation by means of micro hives of *Bombus terrestris*. The harvested seed gave rise to the HSFS seed lot.

In 1990, the mother plants of the best 10 HS have been cloned and the cuttings transplanted in pots and intercrossed under isolation in 1991. Seed production was not high enough, so, further cycles of intercrossing under isolation were carried out in 1992 and 1993, in order to get sufficient seed for the HSPT approach.

A trial (randomised complete block design with three replication and 2 m^2 per plot) was established in 1994 to compare the materials obtained with the three different selection methods, the original population "Gioscari" as a control and the variety "Valente", the reference suggested by the official national variety list (Sovrano Pangallo, 1974). During 1995-1997, Dry Matter Yield (DMY, kg m^{-2}) has been evaluated at six different cuts (2 cuts per year). At each cut, red clover green matter yield has been sorted by the weeds and separately dried.

Results and discussion

GMY of the initial population (first cut, 1988) ranged from less than 100 to more than 1,900 g plant^{-1} (Figure 2). The 10 plants selected in the 1st MS2 cycle showed an average GMY of 1,763 g plant^{-1}, which resulted in a Selection Differential (S) of 1,058 g plant^{-1}. The average GMY of the best 10 mother plants selected on the basis of the HS progeny test showed an S value of 801 g plant^{-1}.

The distribution of GMY at the first cut of 1991 relative to the population obtained by the 1st cycle of MS2 is reported in Figure 2. Average GMY was equal to 762 g plant^{-1} and, in relation to the average production of the 10 selected plants (2,094 g plant^{-1}), a S of 1,332 g plant^{-1} was applied in the 2nd cycle of MS2.

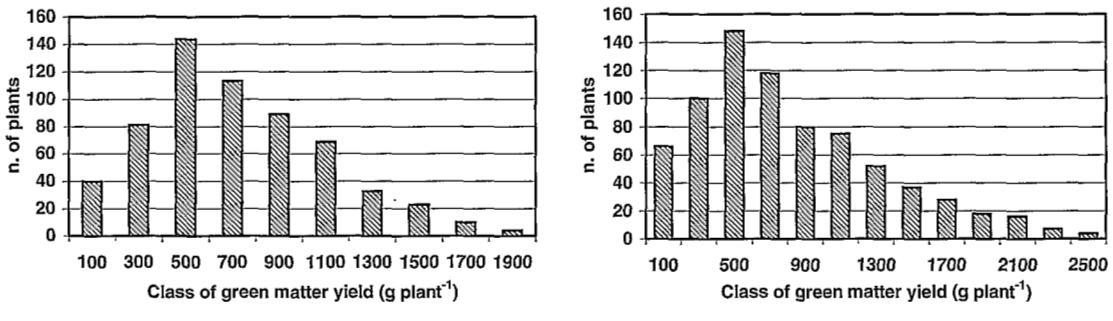

Figure 2 – *Left:* GMY (g plant^{-1}) of the initial population (606 spaced plants); *right*: GMY of the 749 spaced plants derived by the 1st cycle of MS2.

Analysis of variance (ANOVA) of the 1990 DMY data (sum of 2 cuts) of the HS progeny test showed that the source of variation "progenies" was highly significant (F=24.1** with 49 df for progenies and 98 df for error). On the basis of these results we concluded that a wide variability for the trait was present so that the selection of the best 10 HS was consistent.

The results of ANOVA relative to the final comparison among the materials derived from the three different breeding approaches, the ecotype "Gioscari" and the variety "Valente" are reported in table 1. Results relative to total (including weeds) and red clover DMY are reported separately. While the source of variation "Entries" was not significant when total DMY is considered, the same source of variation is significant for red clover DMY.

Table 1. ANOVA results relative to the final comparison trial.

Sources of variation	df	Total DMY		Red clover DMY	
		MS	F	MS	F
Total	14	613		852	
Blocks	2	195		10060	
Entries	4	321	<1 ns	1701	4,37*
Error	8	863		389	

* = Significant at $P<0.05$ level.

The 5 treatments showed similar total DMY, ranging from 3.06 to 3.34 kg m^{-2} (HSFS and "Valente", respectively) (table 2). When red clover DMY is considered, a higher variability among materials was clearly evident, with DMY ranging from 1.87 to 2.24 kg m^{-2} (MS2 and "Valente", respectively).

In particular, HSPT (2.03 kg m^{-2}) appeared to be characterised by a red clover DMY significantly higher than the initial material "Gioscari" (1.62 kg m^{-2}) while it was not significantly different from "Valente".

Table 2. DMY (sum of 6 cuts, kg m^{-2}).

Materials	Total	Red clover
MS2	3.19	1.87 abc*
HSFS	3.06	1.77 bc
HSPT	3.17	2.03 ab
Gioscari	3.22	1.62 c
Valente	3.34	2.24 a
$LSD_{0.05}$	-	0.37
CV	3%	10%

* = Numbers followed by the same letter are not significantly different at $P<0.05$ level.

When the [red clover DMY/total DMY] ratio is considered, all the selected materials showed a higher percentage of red clover forage in respect to "Gioscari" (59% for MS2, 58% for HSHS, 64% for HSPT and 50% for "Gioscari", respectively). Furthermore, the percentage of HSPT was very close to that of "Valente" (67%).

Conclusions

Two cycles of MS2 selection and one cycle of HSFS applied to the Sardinian ecotype "Gioscari" did not produce a significant increase in red clover DMY even if high S levels were applied. On the contrary, one cycle of HSPT gave rise to a significant increase, slightly higher than 25%. It is interesting to note that the result of the breeding activities was not evident for total DMY but just for red clover DMY. Therefore the selection gave rise to a plant material characterised by higher interspecific competition ability and capable of better performance with regard to the initial material under dense stand conditions. In this respect, also MS2 and HSFS appeared to be able to increase the [red clover DMY/total DMY] ratio. The analysis of DMY of each cut (data not reported) shows an increase with time of red clover percentage in the forage, so that the selected material appeared to be also characterised by a better survival under competitive conditions, a very important characteristic for this forage species. Furthermore, the results show that the choice of the breeding procedure has to be carefully considered at the beginning of a new breeding programme in a forage species such as red clover. A breeding approach based on progeny evaluation followed by the

intercrossing under isolation of the selected mother plants such as HSPT is more likely to give better results in comparison to less complex selection approaches such as MS2 and HSFS.

Acknowledgements

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