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COMPARISON OF BREEDING METHODS FOR FORAGE YIELD IN RED CLOVER

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Abstract

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Three methods of red clover (*Trifolium pratense* L.) breeding for forage yield in two harvest years on locations in Bredelokke (Denmark), Hladké Životice (Czech Republic) and Les Alleuds (France) were compared.
Three types of 46 candivars[1], developed by A) recurrent selection in subsequent generations (37 candivars, divided into early and late group), B) polycross progenies (4 candivars) and C) geno-phenotypic selection (5 candivars) were compared. The trials were sown in 2005 and cut three times in 2006 and 2007; their evaluation is based primarily on total yield of dry matter. The candivars developed by polycross and geno-phenotypic selections gave significantly higher yields than candivars from the recurrent selection. However, the candivars developed by the methods B and C did not differ significantly. The candivars developed by these progressive methods were suitable for higher yielding and drier environment in Hladké Životice (where was the highest yield level even if averaged annual precipitation were lower by 73 and 113 mm in comparison to other locations, respectively); here was average yield higher by 19 and 13% for B and C in comparison to A method. Highly significant interaction of the candivars with locations was found. It can be concluded that varieties specifically aimed to different locations by the methods B and C should be bred; also the parental entries should be selected there.

red clover, breeding methods, geno-phenotypic selection, half-sib families, polycross, interaction varieties x locations

Red clover is one of the most important forage legumes in central and northern Europe, Canada and the U.S.A., due to high yields of protein and of energy, due to nitrogen fixation, high seedling vigour, ease establishment, excellent soil improvement characteristics, rapid growth, high forage quality and reasonably high yield. It is cultivated in Europe already three or four centuries as recorded in Italy 1550, in England 1645 and in the United States in 1663 (TAYLOR and QUESENBERRY, 1996).

Yield, persistency, pests and diseases resistance are major breeding aims (ABBERTON and MARSHALL, 2005). The most important goal in red clover breeding is to improve persistence, not only in Central and Northern Europe (POKORNÝ *et al.*, 2003), but also in America (TAYLOR, 2008). Persistence is related to resistance to stem nematodes (*Ditylenchus dipsaci*), clover rot (*Sclerotinia trifoliorum*) and root rot caused by many fungi (POKORNÝ *et al.*, 2003) and associated with general ability and yield (TAYLOR, 2008). Persistence is largely governed by programmed senescence but can be influenced somewhat by breeding for general adaptation (TAYLOR, 2008). High persistence derived from *Mattenklee* was controlled by one QTL on linkage group 3, which explained 10% of the variation observed for persistence (KÖLLIKER *et al.*, 2006). Yield can be improved also by breeding for effective nodulation, which increased this trait by 63% (NUTMAN and RILEY, 1981). Not only nodulation rate but also nitrogenase activity was connected to yield (ŠIMON and JAKEŠOVÁ, 2006). Effective was also selection for increased concentration of iso-

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[1] Candivar – candidate for variety, or candidate for cultivar (Jensen, 1988)

flavones in leaves for pharmaceutical use. Two cycles of the selection increased it by 17% (RUMBALL *et al.,* 2005).

Random amplified polymorphic DNA (RAPD) in breeding populations and varieties from South America and Europe found a high level of polymorphism, the proportion of polymorphic loci was 74% (ULLOA *et al.,* 2003). A population derived from a non-certified seedlot displayed a higher proportion of polymorphic loci than its respective certified seedlot. An analysis of molecular variance (AMOVA) revealed that the largest proportion of variation (80%) resided at the within population level. Using RAPD and similarity values found rather high genetic variability among a group of 16 elite red clover parents (CAMPOS and ORTEGA-KLOSE, 2001). Lower levels of genetic variability were detected in a subset of parents selected for resistance to the stem nematode. This data demonstrate sufficient variability for all known and desired breeding aims.

Most methods used in red clover breeding were first elucidated for maize and utilized spaced plant field populations. Some form of progeny testing was necessary later. Populations had to be larger to avoid inbreeding depression. Since forage is marketed through livestock it added responsible evaluation to the complexity of cultivar testing (TAYLOR and QUESSENBERRY, 1996). Mostly used methods of red clover breeding is development of open pollinated populations by recurrent selection and in limited extend also development of synthetic populations (due to problems connected with their maintenance by vegetative propagation) (CHLOUPEK, 2008). This can be seen also from the number of responsible entries tested in this paper.

Dry matter yields are usually measured in forage-breeding programs and influenced by genotype, cultural and environmental factors (i.e. by years and locations). New candivars are compared with standard varieties in more environments, not only for yield, but also for distribution of dry matter production over the growing season (MILLER and HANNA, 1995).

Such papers are not frequent, TAYLOR and KENDALL (1965) and TAYLOR *et al.,* (1968) compared intra- and inter-polycross progenies of red clover. They suggested that red clover polycross progenies should be screened first by the separate progeny test, and then by mixed progeny test in order to evaluate the differences in competitive ability. MARTINELLO and IANNUCCI (1998) studied genetic variability of populations of berseem clover (*Trifolium alexandrinum* L.) which were naturally cross-pollinated by half-sib breeding method. It was found that the mean value of the traits (dry matter yield, stem density and seed yield) in half-sib progenies and in advanced populations were higher than the mean observed in the original populations and may indicate a significant genetic response achieved by half-sib mating. The evaluation of breeding methods is important, since the methods bring usually different results. For example, open-pollinated and self-pollinated progeny tests were correlated while correlation between polycross and selfed progenies not for dry matter yield in meadow bromegrass (DE ARANJO and COULMANN, 2002). The aim of this paper was comparison of three breeding methods of red clover as shown in yield of green and dry matter.

MATERIAL AND METHODS

The trials were sown on three locations, Bredelokke (Denmark), Hladké Životice (Czech Republic) and Les Alleuds (France) in 2005 and harvested three times in two following years (2006 and 2007). Characteristics of the locations are given in Tab. I. Fifty-six diploid entries were compared, including four standard varieties (*Milvus,* Switzerland, *Mistral,* France, *Rajah,* Denmark and *Suez,* Czech Republic), six varieties for mutual testing, 37 half-sib progenies (24 early, 13 late), 4 polycross and 5 geno-phenotype selection progenies.

I: *Main annual weather characteristics of three testing locations*

Location	**2006**		**2007**		**Long-term means***	
	Mean temperature (°C)	**Precipitation (mm)**	**Mean temperature (°C)**	**Precipitation (mm)**	**Mean temperature (°C)**	**Precipitation (mm)**
Bredelokke	9.5	649	9.7	775	8.0	589
Hladké Životice	8.8	625	9.8	653	7.9	642
Les Alleuds	no data	755	no data	749	no data	597

*Bredelokke (Zealand island) 1961–1990; Hladké Životice (North Moravia) 1951–2000; Les Alleuds (Maine-et-Loire) 1951–1980.

Recurrent selection in subsequent generations was used for breeding of the 37 candivars. The best plants were selected according to their phenotypes from segregating populations after open pollination and their seed was sown into half-sib families. Due to their evaluation in two generations were selected the best of them and after multiplication evaluated in our trials.

From a genetically broad gene-pool were selected clones to form four candivars, consisting of 5–8 clones. The clones were tested for general combining ability in more environments. Similar clones, in particular in regard to earliness, were crossed in space- and time-isolation and produced syn-0 generation. Seed from second synthetic generation (syn-2) was used for our trials. Some similarity of clones in flowering time was necessary for mutual crossing and for morphological uniformity of developed populations.

Five candivars were developed by the geno-phenotypic selection. It is combination of the polycross method (genotypic selection) and recurrent phenotypic selection (SANDHA and TWAMLEY, 1973). Polycross seed was produced as described above, but instead of selecting the superior parents based on progeny means, superior plants selected within superior progenies were crossed to form new populations.

Comparison of selection efficiency in genetically different populations is complicated by their different genetic back-ground; precise comparison should use the same starting material. We undergo the estimation due to relatively high number of compared candivars which could be therefore regarded for randomly selected.

Our experiments were evaluated in three environments, i.e. in three locations. Both experimental years cannot be regarded for different environments due to significant correlation (r = 0.76**) between the dry matter yields of the candivars in both years.

The analysis of variance and Fisher's least significant difference (LSD) were used for evaluation. The relationship and calculation of results were estimated by Agrobase 98 (Agromonix software, Inc., Canada).

RESULTS

Averaged yields of dry matter from both years of the candivars differentiated in regard to the breeding methods on three locations are given in Tab. II. Last two rows in the table show the breeding progress when all tested candivars were compared with the four standard varieties. The averaged higher yield amounted to 3.1%, the highest was on location with the highest yielding level (Hladké Životice, 9.3%), followed by second yielding location (Bredelokke, 3.3%) and no progress on the lowest yielding location (Les Alleuds, –4.1%). Within the four groups due to breeding methods differed significantly (P 0.01) candivars developed by recurrent selection in both groups of earliness. Candivars developed by other two methods did not differentiate significantly (P 0.05).

II: *Comparison of dry matter yields from two harvest years of different types of varieties on three locations (t.ha^{-1})*

	Bredelokke	**Les Alleuds**	**Hladké Životice**	**Average**
Half-sib progenies, late	25.42	22.38	27.43	25.08
Half-sib progenies, early	26.40	21.80	27.52	25.24
Progenies of polycrosses	26.25	24.73	31.70	27.56
Progenies of geno-phenotype selections	31.12	22.44	30.24	27.93
Average yield of all tested candivars	27.30	22.83	29.22	26.45
Average yield of four standard varieties	26.42	23.80	26.73	25.65

The variance analysis showed locations, candivars and their interaction as significant factors for green and dry matter yield. Most important were the locations, followed by the genotypes and the interaction as given for dry matter in Tab. III and can be seen also in Tab. II. The identified interaction among varieties and locations means necessity to breed different varieties for the different locations and/or climatic conditions or to find the rare populations with a low genotype x environment interaction which gives high yield in more environmental conditions. Such varieties can better respond to different conditions, better than perhaps adaptable varieties for such a broad range of environments and the different interactions.

The progenies of polycrosses were most yielding (119%, in comparison to averaged standards) on location with the general highest yield (Hladké Životice, 29.0 t.ha^{-1}). Progenies of geno-phenotype selections brought highest yield on the second fertile location (Bredelokke) as can be seen in Tab. II. Polycross method and geno-phenotype selection as progressive methods enabled highest yield in the driest location Hladké Životice (by 19 and 13% in comparison to standards). The difference in annual precipitation in both experimental years amounted to 73 and 113 mm (comparison of Hladké Životice with Les Alleuds and Bredelokke, respectively), as can be seen in Tab. I.

III: *Analysis of variance for dry matter yield harvested in 2006, 2007 and total*

Source of variation	**dF**	**MS**		
		2006	**2007**	**Total**
Blocs	1	1.80	0.02	2.22
Candivars	55	4.14 **	3.66 **	12.77 **
Locations	2	1 822.88 **	463.25 **	944.70 **
Candivars x Locations	110	2.15 **	2.37 **	6.83 **
Residual	167	1.22	0.65	2.59

dF = degree of Freedom, MS = mean square, *P 0.05; **P 0.01

The tables IV–VI show differences and their significance among different types of candivars due to different breeding methods for dry matter yield. It can be seen that candivars developed by polycross and geno-phenotypic selection gave higher yields than progenies of half-sib families (both groups of earliness). Significant differences (P 0.05) in dry matter yield in the first harvest year were found between polycrosses and geno-phenotypic progenies in relation to yield of half-sib progenies (both group). Highly significant differences (P 0.01) were found between progenies of geno-phenotypic selections and progenies of polycrosses in comparison to progenies of the recurrent selection in dry matter yield in the second harvest year, and in total dry matter yield. However, the first two methods did not differ significantly.

IV: *Comparison of the groups´ means, including least significant differences (LSD), for dry matter yield in the 1st harvest year 2006 ($t.ha^{-1}$)*

DM yield in 2006 (1st harvest year, $t.ha^{-1}$)	Mean yields	Half-sib progenies – late	Half-sib progenies – early	Polycross
Mean yields		**14.08**	**14.10**	**15.32**
Half-sib progenies – late	**14.08**			
Half-sib progenies – early	**14.10**	0.02		
Progenies of polycrosses	**15.32**	1.24*	1.22*	
Progenies of geno-phenotype selections	**15.14**	1.06*	1.04	0.18

*LSD (α 0.05) 1.05 $t.ha^{-1}$

V: *Comparison of the groups' means, including LSD, for dry matter in the 2nd harvest year 2007 ($t.ha^{-1}$)*

DM yield in 2007 (2nd harvest year, $t.ha^{-1}$)	Mean yields	Half-sib progenies – late	Half-sib progenies – early	Polycross
Mean yields		**11.00**	**11.14**	**12.24**
Half-sib progenies – late	**11.00**			
Half-sib progenies – early	**11.14**	0.14		
Progenies of polycrosses	**12.24**	1.24**	1.10**	
Progenies of geno-phenotype selection	**12.80**	1.80**	1.66**	0.56

**LSD (α 0.01) 1.10 $t.ha^{-1}$

VI: *Comparison of the groups' means, including LSD, for total dry matter yield ($t.ha^{-1}$)*

Total dry matter yield (t/ha)	Mean yields	Half-sib progenies – late	Half-sib progenies – early	Polycross
Mean yields		**25.08**	**25.24**	**27.56**
Half-sib progenies – late	**25.08**			
Half-sib progenies – early	**25.24**	0.16		
Polycross	**27.56**	2.48**	2.32**	
Geno-phenotype selection	**27.93**	2.85**	2.69**	0.37

**LSD (α 0.01) 2.18 $t.ha^{-1}$

No significant differences between the breeding methods for green matter yield of first harvest year (2006) and total green matter yield were identified. Candivars from geno-phenotypic selection showed higher (P 0.05) yield of green matter in the second harvest year when compared to the progenies of both half-sib families progenies.

DISCUSSION

The paper shows that breeding of synthetic populations (open pollinated progenies of only selected clones exhibiting in general combining ability as found in polycrosses in the second and following generations syn-2 till syn-4), and progenies of geno-phenotype selection had higher performance than progenies of half-sib family breeding. The difference can be explained by the open pollination also with unselected fathers in the progenies of half-sib families; both better methods select not only females but also males. Similar results were found in alfalfa (CHLOUPEK, 1986).

Polycross progenies in stand and spaced plants differed in their fertility. It was therefore recommended simultaneous use of dense stands in plots to select superior half-sib families and spaced-planted nursery to select superior plants within the selected families in *Lotus corniculatus* L. (SANDHA and TWAMLEY, 1973). CHLOUPEK (2008) described the method as an alternative to conventional breeding methods. The recurrent selection in subsequent generation was also used for maintenance of varieties as expensive and little effective method (CHLOUPEK, 1979).

This paper describes response of genetically very different candivars to very different environmental conditions. It was found that the progenies of polycrosses and of geno-phenotype selection had not only higher yield, but also better response to more fertile locations in comparison to both groups of half-sib progenies. In such a way was supported finding of CHLOUPEK and HRSTKOVÁ (2005) that more responsible crops showed quicker growth of yields in more countries. It could be explained by more detailed selection in this method; when the whole half-sib progenies were selected, then only the whole groups were selected. But the progenies of polycrosses and of geno-phenotype selection were developed by more detailed selection.

However, when necessary, also candivars selected in these trials can be used on all locations, since the interaction of the candivars with the locations showed lower mean square (Tab. III) than the candivars tested. Our finding in Bredelokke corresponds to results cited by CHLOUPEK (2008) with breeding of *Lotus corniculatus* L. where geno-phenotype selection was more successful than polycross.

We confirm results of COPE and TAYLOR (1985) indicating that adaptable varieties have good performance in all conditions but in comparison with varieties bred for particular conditions had only average performance. However, the locally adapted varieties have higher risk due to unusual weather, what is actual in this time.

This paper demonstrates problems to reach high adaptable variety of red clover, which is population variety, similarly as in the most small seed legumes and grasses. However, in this time are registered high adaptable varieties of following forage crops in many countries, e.g. *Amos* (*Trifolium pratense* L.) in 6 countries, *Rivendel* and *Klondike* (*Trifolium repens* L.) in 15 and 7 countries, *Hykor* (*xFestulolium*) in 6 countries, *Calibra* (tetraploid *Lolium perenne* L.) in 19 countries, *Conni* (turf *Poa pratensis* L.) in 19 countries.

Impact of modern genome approaches to breeding of clover, i.e. to breeding of population varieties published ROSS-IBARA *et al.*, (2007). Use of population genetic analyses to identify potentially adaptive genes and bioinformatics to connect selected genes to a phenotype promises study of adaptation and as mean to identify genes that contribute to agronomically important traits. Such an approach is more perspective than quantitative trait loci and linkage disequilibrium mapping.

SUMMARY

The aim of this paper was comparison of three breeding methods of red clover as shown in yield of green and dry matter. The evaluation of breeding methods is important, since the methods bring usually different results.

Three methods of red clover (*Trifolium pratense* L.) breeding for forage yield in two harvest years on locations in Bredelokke (Denmark), Hladké Životice (Czech Republic) and Les Alleuds (France) were compared. Three types of 46 candivars, developed by A) recurrent selection in subsequent generations (37 candivars, divided into early and late group), B) polycross progenies (4 candivars) and C) geno-phenotypic selection (5 candivars) were compared. The trials were sown in 2005 and harvested three times in two following years (2006 and 2007). Fifty-six diploid entries were compared, including four standard varieties (*Milvus,* Switzerland, *Mistral,* France, *Rajah,* Denmark and *Suez,* Czech Republic), six varieties for mutual testing and 46 candivars. Recurrent selection in subsequent generations was used for breeding of the 37 candivars. The best plants were selected according to their phenotypes from segregating populations after open pollination and their seed was sown into half-sib families. Four candivars were developed by the polycross method from clones with superior general combining ability (GCA). Seed from every second polycross progeny (syn-2) was used for the trials. This method is suitable for red clover breeding since the selected clones may be vegetatively propagated by

crown buds. Polycross progenies were tested in several locations as recommended e.g. by TAYLOR and QUESENBERRY (1996). Five candivars were developed by the geno-phenotypic selection. It is combination of the polycross method (genotypic selection) and recurrent phenotypic selection (SANDHA and TWAMLEY, 1973). Polycross seed was produced as described above, but instead of selecting the superior parents based on progeny means, superior plants selected within superior progenies were crossed to form new populations. Our experiments were evaluated in three environments, i.e. in three locations. Both experimental years cannot be regarded for different environments due to significant correlation (r = 0.76**) between the dry matter yields of the candivars in both years. The analysis of variance and Fisher's least significant difference (LSD) were used for evaluation. The relationship and calculation of results were estimated by Agrobase 98 (Agromonix software, Inc., Canada).
The candivars developed by polycross and geno-phenotypic selections gave significantly higher yields than candivars from the recurrent selection. However, the candivars developed by the methods B and C did not differ significantly. The candivars developed by these progressive methods were suitable for higher yielding and drier environment in Hladké Životice (where was the highest yield level even if averaged annual precipitation were lower by 73 and 113 mm in comparison to other locations, respectively); here was average yield higher by 19 and 13% for B and C in comparison to A method. Highly significant interaction of the candivars with locations was found. It can be concluded that varieties specifically aimed to different locations by the methods B and C should be bred; also the parental entries should be selected there.

SOUHRN

Srovnání metod šlechtění jetele lučního podle výnosu píce

Tři metody šlechtění jetele lučního (*Trifolium pratense* L.) byly porovnávány na výnos píce ve dvou sklizňových letech na třech lokalitách v Bredelokke (Dánsko), Hladké Životice (Česká republika) a Les Alleuds (Francie). Byly srovnávány tři typy 46 novošlechtění, které byly získány A) rekurentní selekcí v následných generacích (37 novošlechtění, rozdělených na rané a pozdní), B) polycrossem (čtyři novošlechtění) a C) geno-fenotypovou selekcí (pět novošlechtění). Pokusy byly založeny v roce 2005 a v každém sklizňovém roce (2006 a 2007) byly provedeny tři seče. Pokusy byly hodnoceny na základě celkového výnosu suché píce. Novošlechtění šlechtěná pomocí polycrossu a geno-fenotypové selekce měla významně vyšší výnos než novošlechtění pocházející z rekurentní selekce. Novošlechtění šlechtěné pomocí metody B a C se mezi sebou významně nelišila. Novošlechtění šlechtěná pomocí těchto metod byla vhodnější pro výnosnější a sušší prostředí v Hladkých Životicích (kde byl nejvyšší výnos, i když průměrné roční srážky byly nižší v porovnání s ostatními lokalitami (o 73 mm a 113 mm); zde byl průměrný výnos vyšší o 19 % a 13 % pro B a C metody v porovnání s A metodou. Byly zjištěny významné interakce mezi novošlechtěními a lokalitami. Závěrem lze konstatovat, že odrůdy určené pro určitá prostředí by měly být šlechtěny pomocí metod B a C; a také výchozí rodiče by měli zde být vybíráni.

jetel luční, metody šlechtění, geno-fenotypová selekce, kmeny, polycross, interakce odrůdy x lokality

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