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Breeding of lucerne for higher symbiotic nitrogen fixation and testing in hydroponics with limited mineral nitrogen

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Summary

Two experimental lucerne synthetic populations were developed for higher symbiotic nitrogen fixation. The first one (ZE-N2-I) was developed in five cycles of recurrent phenotypic selection for indirect (root system size, number of nodules, dry herbage weight) and direct selection criteria (nitrogenase activity measured by acetylene reduction) from two varieties: "Palava" and "Zuzana". The second one consisted of 22 plants chosen from progenies of 13 selected clones. Both populations were tested in 17 cuts by a glasshouse experiment in hydroponics with limited levels of mineral nitrogen for three years (1987—1989). The ZE-N2-I gave a significantly higher dry herbage yield (increase of 13 %), significantly lower concentration of crude protein (CP) and significantly higher yield of CP (of 7 %) than the average of the parental varieties. The ZE-N2-II proved a significantly lower concentration of CP and nitrates in herbage than both control varieties. The yield of dry herbage and CP were similar to those of control varieties. Field experiments have been evaluated.

Key-words: *Medicago sativa,* breeding, symbiotic nitrogen fixation, yield, nitrates.

Züchtung von Luzerne auf höhere symbiotische Stickstoffixierung und Prüfung in Hydroponik mit begrenztem mineralischem Stickstoffangebot

Zusammenfassung

Zwei experimentelle synthetische Populationen von Luzerne mit höherer symbiotischer Stickstoffixierung wurden gezüchtet. Die erste Population (ZE-N2-I) entstand durch fünf Zyklen rekurrenter phänotypischer Selektion aus den Sorten „Palava" und „Zuzana". Selektiert wurde nach der Wurzelsystemgröße, Zahl der Knöllchen und oberirdischen Trockenmasse bzw. nach Nitrogenaseaktivität (bewertet anhand der Acetylenreduktion). Die zweite Population (ZE-N2-II) bestand aus 22 Pflanzen, die aus insgesamt 13 Nachkommenschaften von selektionierten Klonen stammten. Beide Populationen wurden in einem Glashausexperiment in Quarzsandkultur bei begrenztem Stickstoffangebot bei 17 Schnitten über drei Jahre (1987 bis 1989) getestet. ZE-N2-I brachte signifikant höhere oberirdische Trockenmasseerträge (um 13 %), einen signifikant niedrigeren Roh-

proteingehalt und signifikant höheren Rohproteinertrag (um 7 %) im Vergleich zum Durchschnitt der Elternsorten. ZE-N2-IIer brachte einen niedrigeren Rohprotein- und Nitratgehalt als beide Kontrollsorten. Der Trockenmasse- und Rohproteinertrag war den Kontrollsorten ähnlich.

Schlüsselworte: *Medicago sativa*, Züchtung, Stickstoffixierung, Ertrag, Nitrate.

1. Introduction

About 100 kg of mineral nitrogen per ha of arable land are used in Czechoslovakia every year. The average forage yield of lucerne is about 10 t of dry matter per hectare; a 16 to 18-%-level contains about 1600 to 1800 kg of crude protein, i.e. about 250 to 290 kg of nitrogen in harvested forage. From the ecological and economical point of view it is necessary to stress the fact that the crop should fix the amount through symbiotic fixation. But the relatively high mineral fertilization caused lowering of the process. It has been found that nodulation was limited by high rates of mineral nitrogen (Trimble et al. 1984). Nitrate caused quick senescence of nodules (Becana et al. 1985), but the lucerne plants can utilize nitrates and improve CO_2 assimilation without inhibiting N_2 fixation when plants are under conditions in which light is not saturating (Herrera et al. 1987). The real respiratory cost for nitrogen fixation was 4 mg C/mg N fixed (Warembourg and Roumet 1989). The breeding of lucerne for higher dinitrogen fixation is therefore important in regions with lower insolation since very high levels of nitrate have been found (5 to 9 g KNO_3 in 1000 g of dry matter) and have increased in Czechoslovakia recently (Matějček and Polopdrudská 1985). Similar problems are with the drinking water which contains mostly more than 50 mg of nitrates per 1000 g.

Breeding of synthetic populations of lucerne for higher dinitrogen fixation and the first results of evaluation are presented in this paper.

2. Materials and methods

The breeding began in 1983 at the Plant Breeding Station Želešice/Brno, using US publications (Viands et al. 1981, and others). Plants of varieties "Palava" and "Zuzana" were grown hydroponically in washed quartz sand with mineral nutrition without nitrogen. The substrate was inoculated by Rhizobium meliloti. The selection was done in three cycles in 1983, 1984 and 1985. The following traits were used as indirect selection criteria: root system size, using its electrical capacitance (Chloupek 1977), dry matter of herbage and number and volume of nodules (Chloupek et al. 1989).

The number of nodules per plant increased in the three cycles from 10 to 15 to 16 in "Palava" and to 20 in "Zuzana". Twenty to thirty plants (i.e. 2 to 3%) were selected in each cycle. Selection according to nitrogenase activity (using acetylene reduction) was used in the fourth and fifth cycles. A special inoculum consisting of nine strains of *Rhizobium meliloti* (four strains from lucerne stands — varieties "Isis" and "Palava" grown at the plant breeding station, two strains from *Melilotus albus*, two ineffective strains by Dr. Vance, USA, and one very effective strain isolated by Dr. Marečková, Prague) was used in the both cycles. The best twenty plants were vegetatively propagated and used as parental clones of the experimental population ZE-N2-I.

The second type of the breeding material originated from strains (half-sib progenies of selected clones). Ten plants of each of the fifteen strains were evalu-

ated after growing in the hydroponics. There were not any significant differences in nitrogenase activity among the strains and therefore individual phenotypic selection was used. The best 22 plants out of 13 strains were cloned and used as parental clones of the experimental population ZE-N2-II.

Both populations were tested in the syn-2 generation. An experiment was done under the same conditions where the breeding had been done (in washed quartz sand with hydroponics nutrition without nitrogen, with the special inoculum consisting of the nine Rhizobium strains, etc.). The sand contained about 15 mg of nitrates per kg of sand. The experiment was sown into a bench where the plants were spaced 10 × 5 cm and 75 plants of the four traits (two parental varieties and two experimental populations) were tested in three replications. The experiment was sown in 1987 and then root system size (in 13 cuts when the plants were about 20 cm high), dry herbage yield, concentration of CP in dry matter (%), yield of CP and concentration of nitrates in individual plants were evaluated in 17 cuts in 1987, 1988 and 1989.

The data were evaluated using the variance analysis and the average values were separated using Duncan's test.

3. Results

Variance analysis proved significant effects of cuts and populations in all of the traits (table 1). Interaction of cuts and populations was not significant.

Table 1

Variance analysis of yield, protein and nitrate concentrations in experimental populations and comparison with control varieties

	dF		[1]Root size	[2]Dry matter	[3]Protein content	[4]Yield of protein	[5]Nitrate content
Mean squares for							
cuts	16	(12)	1461.61^{++}	4.048^{++}	64.596^{++}	0.25125^{++}	0.04946^{++}
varieties	3	(3)	73.11^{++}	0.370^{++}	7.854^{++}	0.00628^{+}	0.00297^{++}
interaction	48	(36)	11.88	0.035	0.857	0.00041	0.00082
residuum	136	(104)	13.66	0.050	0.474	0.00203	0.00041
Separated means (Duncan's test)							
ZE-N2-I			31.11^{b}	1.500^{bc}	19.58^{a}	0.294^{b}	0.128^{b}
ZE-N2-II			30.48^{b}	1.409^{b}	20.18^{b}	0.284^{ab}	0.116^{a}
Zuzana			31.89^{b}	1.300^{a}	20.61^{c}	0.268^{a}	0.130^{b}
Palava			28.68^{a}	1.357^{ab}	20.53^{c}	0.279^{ab}	0.128^{b}
LSD (0.05)			1.74	0.092	0.28	0.018	0.008

Degrees of freedom in brackets are given for root system size because it was measured only in 13 regrowths,
[1] electric capacity of the root system (pF. 10^4),
[2] herbage dry matter (g/plant),
[3] concentration of crude protein (% of dry matter),
[4] yield of crude protein (g/plant),
[5] concentration of nitrate (% of dry matter),
a, b, c — number in columns followed by the same letter are not significantly different,
$^{+}$, $^{++}$ significant at P = 5 % and P = 1 %.

Root system size was greater in the new synthetic population "Zuzana" and in both experimental populations than in the "Palava" variety. The dry herbage yield was higher in both the experimental populations than in the original varieties. However, the CP concentration was lower, the yield of CP was at least in

one of the populations higher. Concentration of nitrate was significantly lower in one of the experimental populations.

4. Discussion

Inheritance of ineffective nodulation and non-nodulation traits in lucerne were published by PETERSON and BARNES (1981). Four out of five clones had a different basis for ineffective inheritance. The first three were conditioned by a single tetrasomically inherited recessive gene (in 1, in 2 or in 3), the nulliplex genotype in each instance producing ineffective nodules. The fourth was conditioned by two recessive genes (in 4 and in 5), the nulliplex condition at both loci being required for production of ineffective nodules. The non-nod trait was conditioned by two tetrasomically inherited recessive genes (nn 1 and nn 2).

Two cycles of simple recurrent mass selection increased top dry weight of 65 % in comparison with the original population (VIANDS and BARNES 1978). In our experiment the yield of the ZE-N2-I was 13 % higher than the average yield of both the parental varieties in the condition of limited mineral nitrogen nutrition. Total nitrogen uptake provided reliable indication of N_2 fixation (GRAHAM et al. 1988). In comparison between the ZE-N2-I with the average of both parental varieties the increase was 7 %. The greatest variability of nitrogen fixation indices was proved among plants within the cultivars. The variability between cultivars was not significant (HOFFMAN and MELTON 1981). Similar results have been found in the breeding of the experimental population ZE-N2-II.

The first lucerne variety, developed for higher dinitrogen fixation was Nitro (BARNES et al. 1988). It is a non-winter-hardy cultivar for use as a 1-year hay source and it can be used as a fall-down green manure crop, too. Its average is 140 kg $N \cdot ha^{-1}$ compared to 128 and 106 kg $N \cdot ha^{-1}$ for unselected nondormant and dormant cultivars, respectively. However, N available for plow-down was not greater in Nitro compared to control varieties in other experiment (PETERSON and HUSET 1988). Nitro contained higher levels of nitrogen in roots and crowns but root and crown yields were lower. In the experimental populations the root system sizes were comparable with parental varieties.

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