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THE METHODOLOGY OF HONEYCOM MASS SELECTION IN YIELD IMPROVEMENT OF ALFALFA

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ABSTRACT

Honeycomb mass selection design have been used in a genetic broad based ecotype of Alfalfa to assess its efficiency in yield improvement through two cycles of selection. The selection intensities used were: 14.26%, 7.69% and 5.26% in the first and 14.26%, 7.69%, 5.26%, 2%, 3.22%, 2.77% in the second cycle of selection. According to the results obtained from field trials it was concluded that the application of this design was effective in improvement of yield by 13.5% in first cycle. In the second cycle only the first "selection" outyielded the initial population Heronia P2. The mean gain for two cycles of selection was 6.5%. The selection intensities when select from yield must be maintained in the borders 10-14%.

Key words: *Medicago sativa,* Honeycomb Design, selection intensity, yield improvement, breeding method.

INTRODUCTION

In a conventional plant breeding program, the opportunity for selection is limited not only by the parental genotypes and the size of the population grown in early generations but also by the ability of the plants to express their genotype to a degree distinguishable by the plant breeder(1).

The ineffectiveness of single plant selection for yield and yield components has been long recognized (2,3 4 1) and has been attributed to low heritability resulting from inability of a genotype to express itself sufficiently in the phenotype of one plant due to confounding effect of various macro-and micro-environmental factors (3,5). Of these factors, interplant, competition has been recognized (6,7) and wide plant spacing has been adopted to reduce its effect. Wide plant spacing involves a derivation from normal planting procedures and may introduce a new source of non-genetic variation, into the selection nursery due to larger nursery size and local micro-environmental differences. (7,8) Different methods have been proposed to reduce the negative effects of the interplant competition and the micro-environmental differences. Thus it was suggested the hill plots method (9).The line-test method (10), moving average method (11) and greed method (12). They have been adapted extensively in different plants by many researchers and criticized for their effectiveness.

Fasoulas (1973) identified interplant competition and soil heterogeneity as factors which make single plant selection for yield ineffective in most selection methods. He developed the so-called Honeycomb selection method which is based on the theory that single plant selection can be effective, if interplant competition is eliminated. If Honeycomb selection, single plant are spaced planted and arranged in a hexagonal pattern of plant positions, like in a honeycomb so that every plant is in the centre of a hexagon. Single plant selection is conducted across series of hexagons. The yield of central plant in a hexagon is compared to the yield of its equidistantly spaced surrounding neighbours. A plant is maintained only if it outyields each of its neighbours. Fasuolas (1973) stated that comparing the yield of single plant to that of its equidistantly spaced neighbours in a small and therefore homogenous area of land, provides a better comparison of genetically determined yield potential among plants.

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Mitchell *et al.*(1982) obtained significant response to selection for yield in tetraploid wheat (*Triticum turgidum* L.var.*durum*) using the Honeycomb selection method. Bos (1981) using this method obtained a significant response for plant height in ray (*Secale cereale* L.) but not so for yield. Kyriakou and Fasoulas (1985) were successful in demonstrating a significant response for yield in this crop. Almaliote *et al.*(1985) sing the Honeycomb mass selection method in the tomato (*Lycopersicum escelentum* Mill) obtained a significant response for yield and they concluded that this method can improve and maintain the cultivates of this crops.

This study was undertaken to evaluate the Honeycomb selection method developed by Fasoulas (1973) in mass selection for yield improvement in the population of alfalfa (*Medicago sativa*).

MATERIALS AND METHODS

One greek genetic broad based population of tetraploid Alfalfa (*Medicago sativa* L.) was selected for this study, namely Heronia, on the basis of its high yielding potential and its adaptation to the conditions prevailing in central and north-west of Greece.

Seeds from this population were sown in Jiffy pots with the intention of the preparation of the individual plants in the spring of 1985 in the greenhouse. Forty five days later these plants were transplanted in the field according to honeycomb design. The distance from plant to plant was 1 meter. In total 1300 plants were grown for the first cycle of selection.

All plants were tagged in the field . At the beginning of flowering, all the plants were recorded for the high of the stems, number of stems per plant, their injury by diseases and insects then they were cut individually and put in a paper bag together with their tags. When the green mass was dried in natural conditions every plant was weighted for its hay production.

The selection of the plants was based on the total hay production of three cuts made at the stage of first flowers according to the procedure which has been described by Fasoulas (1973). This was accomplished by moving hexagonal grids (Kyriakov and Fasoulas 1985) In order to use different selection intensities a slight modification was made in this procedure. This modification consists on the comparison of central plant of the hexagon with six, twelve and eighteen its equidistant neighbours. A plant was retained if it outyielded its neighbours. It was applied three selection intensities in the first cycle of selection and concretely 14.26%, 7.69% and 5.26%.

One plant was classified in the first group (selection intensity 14.28%) if it overyielded its six neighbours on the corners of first and second hexagons with the same centre and in the third group (selection intensity 5.26%) if it outyielded its eighteen neighbours on the corners of first, second and third hexagons with the same centre.

Selected plants were left on the field to develop and seed setting freely while undeserved plants were put away immediately.

At flowering time about ten racemes per plant, only for the third group of selected plants, were isolated and hand-pollinated without emasculation with a mixture of pollen collected from all the plants of this group.

The seed produced by selected plants was threshed and manipulated individually, then the same quantities of seeds from every plant were mixed accordingly to the group it they belong. Thus it were created three seed samples or strains which originated from three selected groups of plants by free pollination. These samples of seeds were saved with the intention of the establishment of comparison tests of productivity.

The hand pollinated racemes were collected individually before the total collection of the seeds produced by the third selection group. The threshing and the manipulation were the same as above mentioned and thus, it was created a sample of seeds which would be used for the creation of a new population in the next year.

Summer 1986 the sample of seeds produced by hand pollination of the plants of the third group, was used to create the second population upon which was applied another cycle of selection. The preparation of individual plants, the layout on the field and all other observation, and procedures were the same as/for the first cycle. The only exception was that selection intensities were stronger, and concretely; the first 14.28%, second 7.69%, third 5.28% fourth 4.22%, fifth 3.22%, creating thus six selected groups of plants.

The statistical analysis of the individual selected plants was made for the mean hay production, quadratic deviation from the mean, while for the selected groups of plants were computed same practical values of selection parameters as , the proportion of selected plants "q" %, selection Differential S gr; the truncated point upon which start the selection C6p expressed with unit of phenotypic deviation, and selection intensities "i" expressed also on the base of the units of phenotypic deviation 6p. These values are tested with the theoretical values according to Falconer (1981).

In September 1987 was established an experiment to test the success made by these two cycles of selection. The same experiment was established in March 1988. The experimental design was the randomized complete block with five repetitions. The analysis of the recorded data was made according to this design (Sokol and Rolf 1968) for the hay production and the statistical significance of the means was tested by the Dunnett's criteria.

RESULTS AND DISCUSSION

The application of selection according to Honeycomb methodology, where the central plant is compared with six other its equidistant neighbours in the corners of hexagon have resulted in creation of the three selected groups of plants in the first cycle and six groups in the second cycle. The classification of plants into the groups or "selections" was made according with how many "groups of neighbours" were outyielded by the central plant.

The Table 1 give the "selections" created in the first and second cycle (C1 and C2).

Table 1. The selections created in C_1 and C_2 with some theoritical and practical selection parameters

	Number of plants	q %	Mean weight plant (g)	Deviation 6_p	Some selection´s parameters				
					Theoretical			Practical value	
					C_{6p}	L_{6p}	S (g)	C_{6p}	L_{6p}
First cycle C_1									
Heronia (Pop1) P1	1320		182	66					
Heronia C_1-1-86	63	14.26	221	32	1.071	1.583	38	-0.8	0.6
" C_1-2-86	52	7.69	236	38	1.426	1.874	54	-0.5	0.8
" C_1-3-86	27	5.28	303	34	1.616	2.038	120	+0.4	0.8
Second cycle C_2									
Heronia P_2	1100		254	79					
C_2-1-87	58	14.26	312	73	1.071	1.583	70	-0.8	0.8
C_2-2-87	27	7.69	347	43	1.426	1.876	90	+0.4	1.1
C_2-3-87	19	5.28	363	51	1.616	2.038	108	0.5	1.4
C_2-4-87	4								
C_2-5-87	6	3.22	387	45	1.852	2.243	129	1.1	1.7
C_2-6-87	23	2.2	40	58	1.934	2.328	163	1.4	2.1

The distribution of the plants in the field according to the Honeycomb design has given the possibility on the individual plants to produce much more, and the differences between them were more distinctly in comparison of dense stands. The higher the selection intensity is, the higher is the selection differential "S" which represents the differences between the means of the "selections" and the population.

Among the parameters, the point of truncation upon which the selection is starting C 6 p, the selection intensity 6 p not only are in disagreement with the theoretical values, but for some "selections" differential result by low practical value of the point of truncation selections upon which the selection is starting according to the mass selection Honeycomb method. This phenomenon is the feature of this method because the central plant is tested against the mean of its surrounding neighbours and not against he population mean; giving so the possibility to select and maintain some individuals, which in can of using the directional truncated selection, would not be selected. (Kiriakov et Fasoulas 1985).

The effectiveness of Honeycomb design for selection of superior plants, is a mass selection program for the improvement of hay production in alfalfa was estimated by the comparison test of selections. In these experiments were tested three "selections" created at the first cycle and five "selections created a the second cycle of breeding. The data are given in the Table 2.

Table 2. Mean yield kg/dy of the “selections” and statistical significance of differences between observed means according to Dunnett (Sokal and Rolf 1969)

"Selection"	Mean yield	Differences		Significance	%	
		T1	T2		T1	T2
Heronia (Pop) T1	1461.97			c	100	
Heronia (selec) T2	1624.00	162.03		bc	111.1	100
Heronia C_1-1-86	1754.55	292.6	130.57	a	119.9	108
C_1-2-86	1630.4	168.4	6.4	b	111.52	100.4
C_1-3-86	1608.01	146.03	-16	bd	110	99.00
C_2-1-87	1720.66	259	97	a	117.8	106.0
C_2-2-87	1623.6	161.7	0	b	111.0	100
C_2-3-87	1597.84	135.87	-26.2	cde	109.2	98.4
C_2-5-87	1590.72	128.75	-33.3	de	108.8	97.9
C_2-6-87	1570.00	108.3	-53.7	e	107.3	96.7

1 dy is equal to one to tenth of the hectar

There were significant differences between the means of hay production of the selections made at the two cycles. This indicate the positive effect of the method in selection of the better individuals from a broad based population.

The genetic gain from the first cycle of selection (C1) for all the "selections" was 187 kg/dy or 13.5%. While the gain of the selections separately was 20% for the first 11% for the second 10% for the third selection intensities.

The gain for all the "selections" made in the second cycle of selection was the same as in the first, that is to say we lack to improve more the productive ability of the "selections". But we can note the fact that all selection intensities yield more than the initial population did, while they did outyield the parental population Heronia T2 (tester Two).

The mean profited gain for two cycles together was 6.7%. The results of this study are in agreement with the others found out by some researchers who worked this design in other crops. Robertson *et al* (1982) obtained significant response in increasing yield of 126 F 10 lines of Arley by 9.%. Mitchel et al (1982) also obtained a gain by 4% in three crosses of hard wheat. Lungu et al (1987) announce a gain by 14% using divergent selection for yield. Kontsiotov et al (1984) profited a gain by 22% applying two consecutive cycles mass selection combined with family evaluation. in clover (*Trifolium Alexandrinum*)

The progress of yielding ability resulting from "selections" come down when the selection intensities tend to be higher. The greatest gain of yielding ability resulted in low selection intensities. The "selections" Cl-1-86 and C2-I-87 which have been selected at low selection intensity are more productive by 17-19% than the other "selections" selected at higher intensities. This is in disagreement with what Fasoulas (1988) supported: "the higher the selection intensities, more productive the created selections".

This disagreement can be explained when we consider the effects of different factors, briefly described down.

The tetraploid nature of alfalfa is accompanied by "doublemeioses" conducting in cases to homogamy in the new selection derived from higher selection intensities. (Demarly 1963, Busbice 1970: Gallais 1981).

The number of selected plants per "selection" was low. May be the pollination by insects was not enough to provide a great degree of cross-pollinated seeds.

CONCLUSIONS

The application Honeycomb mass selection in the population Herona of alfalfa was effective in the identification and selection of superior individual plants.

The gain obtained at the first cycle was 13% for the first "selections" (selection intensity I = 13.26%) C1-I-86 was 20%. At second cycle the gain obtained was 7.5% only for the first selection C2-I-87.

The application of higher selection intensities conducted to lower yielding abilities of the selections. The effectiveness of selection intensity 14.26% was higher than others.

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