J. Agric. Sci. Mansoura Univ., 27(4): 2343 - 2359, 2002

HONEYCOMB SELECTION FOR HERB AND ALKALOID YIELD IMPROVEMENT IN EGEPTIAN HENBANE (*Hyoscyamus muticus,L.*)

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ABSTRACT

The effectiveness of the honeycomb selection method for herb and total Alkaloid content yields was evaluated in a population of *Hyoscyamus muticus,* L. (Egyptian henbane) grown at a plant spacing of 80x80 cm. Three cycles of selection were conducted using selection pressure of 14.3% and the traits of plant height, primary branching, fresh and dry herb yield and total alkaloid contents were evaluated. The plant were harvested two times. The mean single plant of the studied traits showed an increasing trend from the base population to the third cycle of selection in each harvest. The populations developed by honeycomb selection beside the base population were evaluated in yield test experiment using a randomized complete block design at high plant density (50 x 50 cm). The results of the yield test showed that honeycomb selection was effective in increasing the studied traits and the major constituents of alkaloid content; hyoscyamine and hyoscine. Improvement in each studied trait did not adversely affect any other studied trait. The estimates of realized heritabilities between successive cycles of selection showed an increasing trend from the base population to the third cycle of selection. In the 1st and 2nd harvests, the realized heritabilities ranged from 0.34 to 0.57 and from 0.47 to 0.53 for plant height, from 0.46 to 0.53 and from 0.45 to 0.58 for primary branching, from 0.40 to 0.57 and from 0.44 to 0.59 for fresh herb yield, from 0.42 to 0.58 and from 0.43 to 0.57 for dry herb yield and from 0.47 to 0.62 and from 0.47 to 0.59 for total alkaloid yield; respectively. The results indicated the presence of additive genetic variation for yield traits in the base population.

INTRODUCTION

Egyptian henbane (*Hyoscyamus muticus,* L., family *Solanaceae*) is an important perennial and medicinal plant in Egypt. It is grown as an annual in lower and upper Egypt and found in sandy and coastal regions (Kotb, 1987). The plant is coarse pubescent, clamy and ill-smelling. The lower leaves are alternate, ovate, toothed and petiolate, while the upper ones are ovate, entire and sessile. The flowers are white yellowish, short-pedicelled or sessile and in leafy spikes. Capsules are inclosed in the enlarging calyx contain small and brown or gray seeds. Sometimes the plant is used in some landscape purposes. The leaves and flowering tops are used in the form of tincture extract: in dried form, they constitute the drug Henbane Herba. However, since the main chemical constituent of the crude drug, viz, hyoscyamine also manifest hypnotic, mydriatic, sedative, antispasmodic and anticholinergic properties, it is widely employed in modern medicine as therapeutic agent in respiratory and intestingal disorders (Anon., 1982 and Kotb, 1987).

The wild plants of *H. muticus*, L. occurring in different regions in Egypt are regularly and indiscriminately collected by unscrupulous exploiters. Exploitation of wild resources may lead not only to the extinction of the species, but also to the deterioration of the drug quality due to heterogenous materials harvested (Sharma *et al.*, 1989). Besides, the cultivated *H. muticus*, L. suffers from low alkaloid contents and reduced quality of dry matter due to woody parts comparing with the wild plant (Kotb, 1987). Herb yield and alkaloid content are the main components in henbane crop. The alkaloid content is higher in the leaves and flowering tops than in the stems and roots. It varies with the agronomic practices and physiological stage of the plant, being highest at the beginning of the flowering in the early morning (Kotb, 1987).

The primary aim of almost every plant breeding program is to select genotypes with high yielding potential. Yield evaluation is carried out in advanced generations, where the range of the evaluated materials could be limited. Reliable evaluation of yield potential in the early generations helps to discard the low-yielding potential genotypes and detect those with high-yielding potential (Sneep, 1977). On the same line it is necessary to start selection in the F_2 generation, because in this generation the frequency of genotypes possessing a desirable allele on all segregating loci is high (Lungu *et al.*, 1987). This frequency declines in subsequent generations and delaying selection leads to loss of valuable genotypes (Sneep, 1977). The response to selection depends largely upon the presence of genetic variation in the population and the method used must be efficient in minimizing effects of variation in environmental conditions. Interplant competition and soil heterogeneity are two such environmental conditions which make single plant selection for yield ineffective with most selection methods as reported by Fasoulas (1973). He developed the socalled honeycomb selection which is partly based on the theory that single plant selection for yield can be effective, if interplant competition is eliminated. In honeycomb selection, single plants are space-planted and arranged in a hexagonal pattern of plant positions, like in a honeycomb, such that every plant is in the center of a hexagon. Single plant selection is conducted across a series of hexagons. The yield of the central plant in a hexagon is compared to the yields of its equidistantly spaced surrounding neighbours. A plant is retained only if it outyields each of its neighbours. Fasoulas (1973) stated that comparing the yield of a single plant to that of its equidistantly spaced neighbours in a small, and therefore, homogeneous area of land provides a better comparison of genetically determined yield potential among plants.

Honeycomb selection method has attracted considerable attention (Mitchell *et al.*, 1982 on durum wheat; Kyriakou and Fasoulas, 1985 on rye; Lungu *et al.*, 1987 on spring wheat; Robertson and Frey, 1987 on oat; Onenanyoli and Fasoulas, 1989 on maize and Kulkarni, 1990 and 1991on palmarosa grass and davana).

Studies on Egyptian henbane were restricted on histological, taxonomical and physiological studies, while breeding works were rare. The main objective of the present work was to study the possibility of increasing herb and alkaloid content yields in the cultivated Egyptian henbane using honeycomb selection method adopted by Fasoulas (1981).

MATERIALS AND METHODS

Field experiments were carried out at the Agricultural Experiment station, Faculty of Agriculture, University of Alexandria, during the seasons from 1997 to 2001.

Nonselected seeds of a local variety of Egyptian henbane (*Hyoscyamus muticus, L.*) obtained from the National Research Center, Cairo, Egypt, were soaked in water for 48h. On April 3, 1997 the soaked seeds were planted to raise the base population (C_o). The seeds were planted in a non-replicated honeycomb design (Fasoulas, 1981). A wide spacing of 80cm was used to enhance differentiation and gene fixation and avoid competition among different genotypes. Each hill was overplanted and latter thinned, at the fourth to fifth leaf stage, to one seedling per hill to give a plant density of 6562 plants per feddan. Special attention was given to weed and pest control, four irrigations were given during the whole crop season at 21 days intervals (Gad *et al.*, 1982) to remove moisture stress and fertilization was applied after 40 days from planting at a rate of 22 kg N, 13kg P and 13kg K per feddan (Gad *et al.*, 1982 and Sharma *et al.*, 1989). These agronomic practices were used to allow an unrestricted expression of single plant yields. On June 30, 1997 (After 88 days from planting), at flowering time (as alkaloid content is maximum), the first cycle of honeycomb selection, using the principle of the moving hexagonal grid, was applied to all plants (Fasoulas, 1981). Each grid contained seven plants for a selection pressure of 14.3%. A plant in the grid was selected if its herb yield was not less than that of any of its six neighbours. Also, a random sample of 300 plants was selected to represent the unselected population by honeycomb selection. The plants selected by honeycomb selection and those of the random sample were harvested (cut) for the first time at 10cm from the ground. The same rate of fertilizer mentioned before was applied and the irrigation was conducted two times. On August 9, 1997 (40 days from the first harvest), the same plants were harvested again at the flowering time with the same manner mentioned before. Equal quantity of seeds from each plant selected by honycomb selection was bulked to raise the next generation (C_1 population and resembles the offspring of selected plants). Also, equal quantity of seeds from each plant of the random sample was bulked to raise the offspring of unselected population. The sowing date of C_1 population was April 2, 1998 and harvesting dates were July 4, 1998 (For the First harvest, 94 days from sowing) and August 16, 1998 (for the second harvest, 43 days from the first one). The sowing date of C_2 population was March 31, 1999 and its harvesting dates were July 1, 1999 (First harvest, 92 days from sowing) and August 19, 1999 (Second harvest, 49 days from the first one). C_3 population was planted on April 3, 2000, harvested for the first time on July 3, 2000 (91 days from planting) and for the second time on August 16, 2000 (44 days from the first harvest). The planting, agronomic practices, honeycomb selection application, selection of random sample and harvesting for the populations from C_1 to C_3 were the same as mentioned in C_0 population and each population was grown from the seeds of plants selected from the preceding cycle. The total number of plants and the number and percentage of plants selected by honeycomb selection during the different cycles are illustrated in Table 1.

Table (1): Total number of plants and number and percentage of plants selected by honeycomb selection during the different cycles of selection

Cycle	Total number of plants	Number of selected plants	Percentage of selected plants
C_0	1054	66	6.3%
C_1	1338	80	5.9%
C_2	1004	75	7.41%
C_3	863	70	8.1%

A sample of seeds from each cycle was stored in the refrigerator till the seeds were used for yield test. The yield test was carried out on April 7, 2001, where C_0, C_1, C_2 and C_3 populations were evaluated. The experiment of yield test was conducted and the entries were arranged in a randomized complete block design of 4 replicates (Snedecor and Cochran, 1974). Each plot consisted of 4 rows, 5m long. The hills were spaced at the 50 x 50 cm spacing recommended for raising a commercial crop (Kotb, 1987), which gave a plant density of 16800 plants per feddan. Standard agronomic practices were followed in the test. At the flowering time, the two central rows of each plot were harvested two times. The first harvest was on July 8, 2001 (92 days from planting) and the second one was on August 20, 2001 (43 days from the first harvest).

During the different cycles of selection data recorded on the different groups of plants were (1) plant height (in cm) from the ground surface to the uppermost point of plant, (2) number of primary branches per plant, (3) fresh and dry weight (in g) per plant, where the plants were air dried to a constant weight and (4) total alkaloid content as a percentage of dry matter. For total alkaloid content determination, all different groups of plants (in each harvest) were harvested group wise, air dried, grinded using a small size sample mill and 3 representative samples of about 200g each from the total grinded herbage of each group were used for determining the alkaloid content as mentioned by Cromwell (1955).

For the yield test experiment, the foregoing traits were recorded and 2 samples of 200g each from the total grinded herbage per harvest of each plot were used for determining the total alkaloid content. Alkaloid content was analysed by gravimetric method following Cromwell (1955) and its active constituents, i. e. tropane alkaloids, viz., hyoscyamine and hyoscine were determined by standard Gas Liquid Chromatography (GLC). The GLC was conducted on Varian VISTA-6000GC fitted with FID detector, 2m x 3mm SS column packed with 10% OV-101 on 80/100 Mesh Chromosorb WHP nitrogen inlet pressure of 19.5 psi and the column temperature programed from 150 °C to 275 °C @ 10 °C per min. (holding initially for 4 min.) for hyoscyamine (r.t. 15.1 min.) and hyoscine (r.t. 16.2 min.) contents. Retention time was compared with the authentic/standard samples of the drug. The fresh and dry weight values calculated from each plot were converted to ton per feddan and the yield values of total alkaloid content, hyoscyamine and hyoscine per plot were converted to kilogram per feddan.

Standard analysis of variance was carried out for the traits of the yield test experiment. Differences between means were tested by LSD at 0.05 and 0.01 levels of probability. For all percentages, angular transformation was settled and the statistical analysis was carried out using values resulting in form transformation. Realized heritability values for each trait were calculated as the ratio of selection response: selection differential (Falconer, 1981) for each cycle of selection using the formula employed by Newell and Eberhart (1961) and Vogel *et al.*, (1981).

$$h^2 = 2\left[\left(\overline{X}_{05} - \overline{X}_{op}\right)/\overline{X}_{op}\right]/\left[\left(\overline{X}_{ps} - \overline{X}_{p}\right)/\overline{X}_{p}\right],$$

where, $\overline{X}_{05}$ = mean of the offspring of plants selected by honeycomb selection, $\overline{X}_{0p}$ = mean of the offspring of unselected population (offspring of random sample), $\overline{X}_{ps}$ = mean of plants selected by honeycomb selection and $\overline{X}_{p}$ = mean of unselected population (random sample).

RESULTS AND DISCUSSION

The mean single plant of any trait (at a plant spacing of 80 cm) shown an increasing trend from C_0 to C_3 (Tables 2 and 3). As these populations were grown during different years, valid comparisons are possible from data of yield test experiment where the populations were grown during the same year (Kulkarni, 1991). It was clear in each harvest that the largest improvement (as the difference between any cycle and the preceding cycle) was obtained in the C_1 population for each of the plant height and primary branching, in the C_2 population for each of the fresh and dry weights and in the C_3 population for the total alkaloid content. The speed of improvement was trait depending.

The estimates of realized heritabilities between successive cycles of selection showed in both harvests that the realized heritabilities were low in the first cycle of selection for all traits then gradually increased in the following cycles (Tables 2 and 3). In the first and second harvests, the realized heritability ranged from 0.34 to 0.57 and from 0.47 to 0.53 for the plant height; from 0.46 to 0.53 and from 0.45 to 0.58 for the primary branching; from 0.40 to 0.57 and from 0.44 to 0.59 for the fresh biomass yield; from 0.42to 0.58 and from 0.43 to 0.57 for dry biomass yield or from 0.47 to 0.62 and from 0.47 to 0.59 for the total alkaloid yield; respectively. These results are in agreement with those reported by Kulkarni (1991) on davana. Heritability estimates for a trait are not absolute but vary according to the method used for calculation, the differences between seasonal conditions which the progeny was established under it and those of the parents and germplasm used to obtain the estimates (Vogel *et al.*, 1981).

Table (2): Mean values of plant height (cm), primary branches, fresh and dry weight (g) and total alkaloids (% of dry weight) and realized heritability values (h^2) as established in successive cycles of honeycomb selection in *Hyoscyamus muticus, L.* Data of the first harvest for plants grown at a plant spacing of 80 x 80 cm.

Cycles and groups	Plant height (cm)		Primary branches		Fresh weight (g)		Dry weight (g)		% Total alkaloids	
	Mean	h^2	Mean	h^2	Mean	h^2	Mean	h^2	Mean	h^2
C_0										
All plants [v)]	57.5		3.1		164.8		26.5		0.42	
H- plants [x)]	77.3		4.1		250.9		40.1		0.48	
R- plants [z)]	55.4		3.0		159.1		25.6		0.37	
		0.34		0.46		0.40		0.42		0.47
C_1										
All plants [w)]	74.5		3.8		255.1		40.8		0.46	
H- plants [x)]	85.5		4.7		306.9		49.4		0.53	
RC_0- plants [y)]	69.8		3.5		229.0		36.4		0.43	
R- plants [z)]	69.7		3.3		248.6		39.5		0.45	
		0.54		0.51		0.53		0.54		0.47
C_2										
All plants [w)]	80.1		4.1		368.9		59.0		0.50	
H- plants [x)]	93.3		4.9		433.8		68.5		0.63	
RC_1- plants [y)]	75.4		3.7		347.3		55.2		0.48	
R- plants [z)]	76.0		3.8		352.9		56.0		0.48	
		0.57		0.53		0.57		0.58		0.62
C_3										
All plants [w)]	92.5		4.2		413.3		67.0		0.56	
H- plants [x)]	100.7		5.4		460.7		73.3		0.73	
RC_2- plants [y)]	86.8		3.9		387.8		62.9		0.51	
R- plants [z)]	89.2		4.1		394.5		65.5		0.55	

v) Plants of the base population.
w) Offspring of plants selected by honeycomb from preceding cycle.
x) Plants selected by honeycomb selection.
y) Offspring of plants selected randomly from preceding cycle.
z) Plants selected randomly.

Table (3): Mean values of plant height (cm), primary branches, fresh and dry weight (g) and total alkaloids (% of dry weight) and realized heritability values (h^2) as established in successive cycles of honeycomb selection in *Hyoscyamus muticus, L.* Data of the second harvest for plants grown at a plant spacing of 80 x 80 cm.

Cycles and Groups	Plant height (cm)		Primary branches		Fresh weight (g)		Dry weight (g)		% Total alkaloids	
	Mean	h^2	Mean	h^2	Mean	h^2	Mean	h^2	Mean	h^2
C_0										
All plants v)	60.3		3.5		172.8		27.6		0.44	
H- plants x)	79.4		4.2		256.6		41.1		0.49	
R- plants z)	57.2		3.4		167.1		27.1		0.38	
		0.47		0.45		0.44		0.43		0.47
C_1										
All plants w)	77.3		4.0		258.4		41.3		0.47	
H- plants x)	92.2		4.9		315.2		50.4		0.54	
RC_0- plants y)	69.9		3.8		230.9		37.2		0.44	
R- plants z)	71.5		3.6		250.5		39.7		0.43	
		0.50		0.54		0.53		0.53		0.49
C_2										
All plants w)	86.2		4.5		378.3		60.3		0.51	
H- plants x)	99.1		5.4		446.7		72.2		0.65	
RC_1- plants y)	80.3		4.1		354.0		56.2.		0.48	
R- plants z)	80.8		4.1		360.4		57.1		0.49	
		0.53		0.58		0.59		0.57		0.59
C_3										
All plants w)	95.6		4.7		427.2		68.4		0.57	
H- plants x)	112.2		5.6		472.0		76.5		0.76	
RC_2- plants y)	90.2		4.3		398.9		63.6		0.52	
R- plants z)	94.3		4.2		394.1		61.5		0.52	

v) Plants of the base population.
w) Offspring of plants selected by honeycomb from preceding cycle.
x) Plants selected by honeycomb selection.
y) Offspring of plants selected randomly from preceding cycle.
z) Plants selected randomly.

A system of selection which increases the frequency of desirable genes is expected to reduce the frequency of less productive plants and to increase population yield. Data tabulated in Tables 2 and 3 show this trend. Honeycomb selection based on plant height (fresh herb yield) of widely spaced individual plants was effective in increasing herb and alkaloid content yields of *H. muticus, L.* Similar results have been reported for grain yield in winter rye (Kyriakou and Fasoulas, 1985); in spring wheat (Lungu *et al.*, 1987); in maize (Onenanyoli and Fasoulas, 1989) and for herb yield in palmarosa grass and davana (Kulkarni, 1990 and 1991; respectively). According to Fasoulas (1981); Kyriakou and Fasoulas (1985); Onenanyoli and Fasoulas (1989) and Kulkarni (1991) optimal growing conditions and absence of competition in the selection field maximize genotype expression and differentiation. This was reflected in an increase in herb yield and total alkaloid contents and in moderately high estimates of realized heritability (Tables 2 and 3). These results indicated the presence of considerable additive genetic variation in the base population (Onenanyoli and Fasoulas, 1989 and Kulkarni, 1991), which may be due to the fact that the population was not previously subjected to artificial selection (Kulakarni, 1991).

The results of the yield test are shown in Tables from 4 to 9. With respect to the plant height, the differences among the different populations were highly significant in both harvests. The highest mean values were recorded in the C_3 population which significantly differed from other populations at 0.05 and 0.01 levels of probability (Table 4). The base population (C_0) had significantly the lowest mean values at both probability levels of 0.05 and 0.01 for the two harvests. In general, the mean of plant height showed an increasing trend from C_0 to C_3. These increments were 29.5% to 60.2% and 29.5% to 60.2% over C_0 in the first and second harvests; respectively (Table 4). These rustles are supported with those mentioned by Onenanyoli and Fasoulas (1989) on maize and Kulkarni (1991) on davana. On the other hand, Kulkarni (1990) reported that the plant height of palmarosa grass did not change by honeycomb selection. In the same field, Gad *et al.*, (1982) found that mass selection was not effective in increasing the plant height of henbane.

Table (4): Means and relative (%of C_0) values of plant height (cm) and primary branches of each of the two harvests of *Hyoscyamus muticus, L.* populations developed by honeycomb selection. The plant material was grown at a plant spacing of 50 x 50 cm.

Material tested	Plant height (cm)				Primary branches			
	First harvest		Second harvest		First harvest		Second harvest	
	Mean	% of C_0	Mean	% of C_0	Mean	% of C_0	Mean	% of C_0
C_0	46.8	100.0	48.5	100.0	2.5	100.0	2.9	100.0
C_1	60.6	129.5	62.8	129.5	3.1	124.0	3.4	117.2
C_2	65.1	139.1	70.1	144.5	3.4	136.0	3.7	126.6
C_3	75.2	160.7	77.7	160.2	3.5	140.0	3.8	131.0
$LSD_{0.05}$	4.02		4.82		0.66		0.46	
$LSD_{0.01}$	5.79		6.94		0.95		0.66	

Regarding the number of primary branches, the differences among the tested populations were significant for the first harvest and highly significant for the second one. The highest mean values were recorded in the C_3 population followed by C_2, C_1 and C_0; respectively, in both harvests (Table 4). For the first harvest, there was a significant difference between each of C_2 and C_3 on one side, and C_0 on the other side at 0.05 level of probability, while at 0.01 level the significant difference was noticed between C_3 and C_0 only. For the second harvest and at 0.05 level all mean values of C_1, C_2 and C_3 were significantly higher than that of C_0, while at 0.01 level C_2 and C_3 significantly overcame C_0 (Table 4). After 3 cycles of selection the increments in the primary branches ranged from 24% to 40% and from 17.2% to 31% over the check population (C_0) in the first and second harvests; respectively (Table 4). Gad *et al.* (1982) reported that mass selection was able to improve the branching in henbane.

In examining the foregoing results one should not loose sight of the objective of the present study which was to ascertain the effectiveness of honeycomb selection procedure for early selection. Viewed in this light the results are encouraging in that in all three cycles, the method used was effective in increasing the plant height and branching. Furthermore the results show that the effects of selection practiced on individually spaced plants at wide distances (80 x 80 cm) carry over when the progenies of these plants are grown in dense stands (50 x 50 cm). Bos (1981) studied the relative efficiency of honeycomb selection and other procedures of mass selection in winter rye. When the effect of density on the result of honeycomb selection was considered, he offered experimental support for selection without competition. Again, the response in this study indicates the presence of considerable additive genetic variance in the base population.

For the fresh herb yield in the test experiment, statistical analysis indicated that there were highly significant differences among the tested materials after each harvest. For both harvests, the fresh herb yield was increased from C_0 to C_3 and the highest mean values were recorded in C_3 followed by C_2, C_1 then C_0; respectively (Table 5). Each cycle significantly differed from the other ones at both levels of 0.05 and 0.01 of probability for each harvest. Fresh herb yield increases of 54.8% to 150.8% over C_0 were indicated for the first harvest, while in the second one these increases were 49.5% to 147.2% (Table 5), which agree with published findings reported by Kulkarni (1990) on palmarosa grass. When the results of the fresh herb yield were converted to ton per feddan, the total productivity per feddan after the two harvests reached about 7.0, 10 and 11.5 tons for the tested materials of C_1, C_2 and C_3; respectively, while it was 4.6 tons for the source material (Table 5). There were insignificant differences in moisture content in fresh herb of the 4 populations after both harvests. The mean moisture contents in the fresh herb were 83.9% and 84.1% in the first and second harvests; respectively. In general, these results are in agreement with those obtained in perennial forage grass breeding programmes involving selection of individual plants in spaced plant nurseries. In perennial reygrass, successful selection for yield under spaced plant conditions was not accompanied by comparable increases in yield under competitive conditions (Hayward and

Vivero, 1984). In bahiagrass, Burton (1974 and 1982) obtained significant but relatively lesser increases in yield under sward than under spaced plant conditions, after 4 and 6 cycles of recurrent restricted phenotypic selection.

Table (5): Means and relative (% of C_0) values of fresh herb yield per plant (g) and their values converted to fresh herb yield per feddan (t) for each of the two harvests and total yield per feddan (t) of *Hyoscyamus muticus, L.* populations developed by honeycomb selection. The plant material was grown at a plant spacing of 50 x 50 cm.

Material Tested	First harvest			Second harvest			Total Yield per feddan (t)
	Yield per plant		Yield per feddan (t)	Yield per plant		Yield per feddan (t)	
	Mean (g)	% of C_0		Mean (g)	% of C_0		
C_0	133.9	100.0	2.25	140.4	100.0	2.36	4.61
C_1	207.3	154.8	3.48	209.9	149.5	3.53	7.01
C_2	289.0	215.8	4.86	307.4	218.9	5.16	10.02
C_3	335.8	250.8	5.64	347.1	247.2	5.83	11.47
$LSD_{0.05}$		13.79				11.00	
$LSD_{0.01}$		19.81				15.81	

In respect of dry herb yield in the yield test experiment statistical analysis revealed that there were highly significant differences among the different populations after each harvest. Each of C_1, C_2 and C_3 populations was significantly superior in the yield of dry herb over the base population. In the two harvests, C_3 population had the highest mean values of the dry followed by C_2, C_1 and C_0 populations; respectively (Table 6). Considering the first harvest, there was a significant differences between any population and the other one at 0.05 level of probability, but this significance disappeared between C_2 and C_3 at the probability level of 0.01. The comparisons between the populations after the second harvest were similar at the two levels of 0.05 and 0.01 and similar to those of 0.01 after the first harvest. It was registered that C_1, C_2 and C_3 out-yielded C_0 after the first harvest by 54.0%, 116.3% and 149.8%; respectively, and after the second one by 50.0%, 118.3% and 147.8%; respectively. These results are in harmony with those reported on dry herb yield in palmarosa grass and davana (Kulkarni, 1990 and 1991; respectively). To estimate the productivity of feddan, data of plots were converted to ton per feddan. The total dry matter per feddan after two harvests will be 0.74, 1.13, 1.60 and 1.83 tons for C_0, C_1, C_2 and C_3; respectively (Table 6).

In the present study, improvements in fresh and dry herb yield were associated with increases in plant height and number of primary branches, when the plants were grown wither at a wide space or at a narrow space (in test experiment).

The results of the test experiment indicate the efficiency of honeycomb selection based on the yields of widely spaced individual plants from the base population. Moderately high estimates of realized heritability and the herb yield which out-yielded the base population indicated the presence of additive genetic variation for yield in the source material. In addition the yield response in this study appeared after the first few cycles of selection. It is quite likely that, within the selected C_3 population, there is still sufficient additive genetic variation to permit further progress from selection in later cycles. However, as the population yield increases and all the desirable alleles are eventually fixed, the additive genetic variation will be reduced that the rate of gain from selection is expected to decrease. Such a situation has been mentioned by Moll and Hanson (1984). Nevertheless, it is also possible that crossing over in polygenic blocks can convert repulsion into coupling phase linkage and consequently release potential variability permitting continued response to selection (Onenayoli and Fasoulas, 1989).

Table (6): Means and relative (% of C_0) values of dry herb yield per plant (g) and their values converted to dry herb yield per feddan (t) for each of the two harvests and total yield per feddan (t) of *Hyoscyamus muticus, L.* populations developed by honeycomb selection. The plant material was grown at a plant spacing of 50 x 50 cm.

Material Tested	First harvest			Second harvest			Total Yield per feddan (t)
	Yield per plant		Yield per feddan (t)	Yield per plant		Yield per feddan (t)	
	Mean (g)	% of C_0		Mean (g)	% of C_0		
C_0	21.5	100.0	0.36	22.4	100.0	0.38	0.74
C_1	33.1	154.0	0.56	33.6	150.0	0.57	1.13
C_2	46.5	216.3	0.78	48.9	218.3	0.82	1.60
C_3	53.7	249.8	0.90	55.5	247.8	0.93	1.83
$LSD_{0.05}$	6.50			7.54			
$LSD_{0.01}$	9.34			10.84			

The results of the test experiment to assess advance through selection in the total alkaloid percent in dry herb, are presented in Table 7. Statistical analysis showed the presence of highly significant differences among the different populations after each harvest. The total alkaloid percent was increased from C_0 to C_3. Honeycomb selection resulted in significant increases in the total alkaloid content in C_1, C_2 and C_3 in dry herb of each harvest as compared with C_0 at 0.05 level of probability, whereas C_2 and C_3 significantly overcame C_0 at 0.01 level in each harvest (Table 7). The total alkaloid content in each of C_2 and C_3 after the first harvest was significantly higher than that of C_1, while after the second harvest this content of C_3 was significantly higher than that of C_1; these comparisons were registrated at 0.05 and 0.01 levels of probability (Table 7). Increases in total alkaloid contents in C_1, C_2 and C_3 were 16.7%, 41.7% and 52.8% over C_0; respectively, for the first harvest, while for the second one they were 18.4%, 31.6% and 47.4%; respectively. The suggestive values revealed that after two harvests of the tested inaterials the gained amounts of the total alkaloids from fedden will be 2.73, 4.88, 8.08 and 10.16 kilograms for C_0, C_1, C_2 and C_3; respectively (Table 7). Gad *et al.*, (1982) demonstrated that mass selection was effective in increasing total alkaloid content in henbane where the increase was proportional to selection intensity and estimate of heritability was low in magnitude (39%).

Table (7): Means and relative (% of C_0) values of total alkaloid contents in dry herb (%) and their values converted to total alkaloid yield per feddan (kg) for each of the two harvests and total alkaloid yield per feddan (kg) of *Hyoscyamus muticus, L.* populations developed by honeycomb selection. The plant material was grown at a plant spacing of 50 x 50 cm.

Material Tested	First harvest			Second harvest			Total Yield per feddan (kg)
	Yield per plant		Yield Per feddan (kg)	Yield per plant		Yield per feddan (kg)	
	Mean (%)	% of C_0		Mean (%)	% of C_0		
C_0	0.36 (3.44)	100.0	1.30	0.38 (3.53)	100.0	1.43	2.73
C_1	0.42 (3.72)	116.7	2.34	0.45 (3.85)	118.4	2.54	4.88
C_2	0.51 (4.09)	141.7	3.98	0.50 (4.05)	131.6	4.10	8.08
C_3	0.55 (4.25)	152.8	4.95	0.56 (4.29)	147.4	5.21	10.16
$LSD_{0.05}$	0.25			0.27			
$LSD_{0.01}$	0.35			0.39			

Parenthetical values are angles corresponding to the mean of percentages and were used in ANOVA and for comparison.

The significant actual advance from selection proved that improvement in yield of total alkaloid content can be achieved with safety and rapidly by honeycomb selection. Effective selection, however, depends on the presence of additive genetic variance in the base population and the realized gain from selection should be a good indication for the presence of this type of gene action (Allard, 1960 and Gad *et al.*, 1982).

As alkaloid content is higher in the flowering parts and leaves of *H. muticus, L.* (Kotb, 1987 and Sharma *et al.*, 1989), higher alkaloid contents in C_1, C_2 and C_3 over C_0 may have been partially due to an increase of herb yield (Sharma *et al.*, 1989).

Kulkarni (1990) reported that honeycomb-selected plants of palmarosa grass had higher oil content with moderately high heritability. The realized heritability values for the total alkaloid content in the present study were low in the first cycle of selection (0.47) then became moderately high through the successive cycles (0.62 and 0.59 for the first and second harvests; respectively). In another aromatic plant, davana, Kulkarni (1991) found that the effect of honeycomb selection for herb yield on oil content was encouraging and oil content increased from C_0 to C_2 and C_3 populations.

With respect to the major constituent of alkaloid content (hyoscyamine) in *H. muticus,* L., the results of the yield test experiment indicated that there were highly significant differences among the different populations considering the percentage of hyoscyamine in the dry herb for both harvests. There was an increasing trend in hyoscyamine percent from C_0 to C_3 (Table 8). The comparisons between the different populations after each harvest showed that honeycomb selection significantly increased hyoscyamine percentage in each of C_1, C_2 and C_3 comparing with C_0 at 0.05 level of probability and in each of C_2 and C_3 comparing with C_0 at 0.01 level of probability (Table 8). For the first harvest, hyoscyamine percentage in each of C_2 and C_3 was significantly higher than that in C_1 at the two levels of 0.05 and 0.01. For the second harvest, hyoscyamine percentage in C_3 was significantly higher than that in each of C_1 and C_2 at 0.05 level and higher than that in C_1 at 0.01 level (Table 8). C_1, C_2 and C_3 out-yielded C_0 by 18.2%, 40.9% and 54.5%; respectively, in the first harvest, while in the second one they registered increases of 17.4%, 30.4% and 52.2% over C_0; respectively (Table 8). Data in Table 8 show that the expected amount of hyoscyamine from feddan will be 1.66, 2.97, 4.88 and 6.33 kilograms for C_0, C_1, C_2 and C_3; respectively, after two harvests.

Table (8): Means and relative (% of C_0) values of hyoscyamine content in dry herb (%) and their values converted to hyoscyamine yield per feddan (kg) for each of the two harvests and total hyoscyamine yield per feddan (kg) of *Hyoscyamus muticus, L.* populations developed by honeycomb selection. The plant material was grown at a plant spacing of 50 x 50 cm.

Material Tested	First harvest			Second harvest			Total Yield per feddan (kg)
	Yield per plant		Yield per feddan (kg)	Yield per plant		Yield Per feddan (kg)	
	Mean (%)	% of C_0		Mean (%)	% of C_0		
C_0	0.22 (2.68)	100.0	0.79	0.23 (2.75)	100.0	0.87	1.66
C_1	0.26 (2.92)	118.2	1.45	0.27 (2.98)	117.4	1.52	2.97
C_2	0.31 (3.19)	140.9	2.42	0.30 (3.14)	130.4	2.46	4.88
C_3	0.34 (3.34)	154.5	3.07	0.35 (3.39)	152.2	3.26	6.33
$LSD_{0.05}$	0.18			0.21			
$LSD_{0.01}$	0.26			0.29			

Parenthetical values are angles corresponding to the mean of percentages and were used in ANOVA and for comparison.

Considering the percentage of hyoscine (the second important constituent of alkaloid content in *H. muticus*, L.) in dry herb, the results of the yield test experiment revealed that the differences among the tested populations were highly significant for the first harvest and significant for the second one. Hyoscine percentage increased from C_0 to C_3 (Table 9). After both harvests, hyoscine percentage in dry herb of C_2 and C_3 was significantly higher than that of C_0 at both levels of probability 0.05 and 0.01, also in the first harvest the dry herb of C_3 contained amount of hyoscine significantly higher than that detected in C_1 at 0.05 level of probability. All other comparisons were insignificant (Table 9). Hyoscine increased in C_1, C_2 and C_3 by 14.3%, 42.9% and 50.0% and by 20.0%, 33.3% and 40.0% over C_0 in the first and second harvests; respectively. The estimation of total hyoscine per feddan after two harvests were 1.07, 1.91, 3.20 and 3.83 kilograms for C_0, C_1, C_2 and C_3; respectively (Table 9).

Table (9): Means and relative (% of C_0) values of hyoscine content in dry herb (%) and their values converted to hyoscine yield per feddan (kg) for each of the two harvests and total hyoscine yield per feddan (kg) of *Hyoscyamus muticus, L.* populations developed by honeycomb selection. The plant material was grown at a plant spacing of 50 x 50 cm.

Material Tested	First harvest			Second harvest			Total Yield per feddan (kg)
	Yield per plant		Yield per feddan (kg)	Yield per plant		Yield per feddan (kg)	
	Mean (%)	% of C_0		Mean (%)	% of C_0		
C_0	0.14 (2.13)	100.0	0.51	0.15 (2.21)	100.0	0.56	1.07
C_1	0.16 (2.29)	114.3	0.89	0.18 (2.43)	120.0	1.02	1.91
C_2	0.20 (2.56)	142.9	1.56	0.20 (2.56)	133.3	1.64	3.20
C_3	0.21 (2.63)	150.0	1.88	0.21 (2.63)	140.0	1.95	3.83
$LSD_{0.05}$	0.28			0.23			
$LSD_{0.01}$	0.39			0.33			

Parenthetical values are angles corresponding to the mean of percentages and were used in ANOVA and for comparison.

It was reported by Kotb (1987) and Sharma *et al.*, (1989) that the rate of hyoscyamine is higher than that of hyoscine in *H. muticus*, L. and this confirms the results of chemical analysis of the present work. Honeycomb selection for herb yield did not affect the constituents of the total alkaloid content in *H. muticus*, L. and this was in agreement with that obtained in other aromatic plants, where the major components of the oil of lemongrass (Kulkarni *et al.*, 1986);palmarosa (Kulkarni, 1990) and davana (Kulkarni, 1991) were not affected through selection for herb yield.

The present study suggests that increases in total alkaloid yield of *H. muticus*, L. can be made through honeycomb selection for herb yield. Simultaneous selection for both characters should also be possible as increases in herb yield and alkaloid content and quality were obtained without adversely affecting each other.

In conclusion, interplant competition may make single plant selection for yield ineffective irrespective the presence of the additive genetic variation in the population and the ability of selection experiment to identify phenotypically superior genotypes. This is because the genotypic expression and differentiation are reduced under competition besides the low yielding plants which have strong competing ability are selected which results in a negative response to selection.

Efficiency of honeycomb selection in each cycle depends on the magnitude of genetic variation and on the ability to identify phenotypically the few superior genotypes. In the present study identification of superior genotypes was possible through selection in the absence of competition. A very wide spacing (80 x 80 cm) was used to enhance differentiation and gene fixation overcome the disturbing effect of competition among genetically different plants in each cycle. Phenotypic identification of favourable genotypes was also possible by applying a very high selection pressure (14.3%). The high selection pressure results in the better response to a certain point by using moving hexagonal grid. Efficiency was also improved through application of high selection pressure through the progeny of plants selected by honeycomb selection. There was no sign of inbreeding depression. Besides the previously mentioned factors, there were some advantages such as: Soil heterogeneity was under control using the moving hexagonal grid for many times; selection at several sites in the field; optimal growing conditions were provided to minimize effects of environmental variation on individual plant yields and the test yield experiment was conducted under spacing (50 x 50 cm) recommended for raising a commercial crop. All these factors must have positively contributed together to the better selection response.

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J. Agric. Sci. Mansoura Univ., 27(4), April, 2002

الإنتخاب بإستخدام الشكل السداسى لتحسين إنتاج العشب والقلويدات فى السكران المصرى

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تم تقييم تأثير طريقة الإنتخاب بإستخدام الشكل السداسى على إنتاج العشب والمحتوى الكلى من القلويدات فى عشيرة من نبات السكران المصرى المزروعة على مسافات زراعة واسعة (٨٠ × ٨٠سم).

أجريت ثلاث دورات إنتخاب تحت ضغط إنتخابى مقداره ١٤,٣% وتم دراسة كل من: إرتفاع النبات – عدد الفروع الرئيسية – إنتاج كل من العشب الرطب والجاف – والمحتوى الكلى من القلويدات. وقد تم حش النباتات مرتين فى كل مرحلة من مراحل التجربة. هذا وقد حدثت زيادة فى متوسط الصفات المذكورة لكل نبات إبتداء من العشيرة الأصلية حتى دورة الإنتخاب الثالثة.

أجريت تجربة قطاعات عشوائية كاملة لإختبار إنتاجية النباتات المنتخبة وذلك تحت ظروف الكثافة النباتية العالية (مسافة زراعة ضيقة ٥٠ × ٥٠سم) أستخدم فيها العشائر الثلاث المنتخبة بالإضافة إلى العشيرة الأصلية. أوضحت نتائج تجربة إختبار الإنتاج هذه أن الإنتخاب بالشكل السداسي كان فعالاً فى تحسين الصفات المدروسة مع زيادة فى كمية المكونين الرئيسيين للمحتوى الكلى من القلويدات وهما الهيوسيامين والهيوسين، ولم يكن للتحسين الحادث فى كل صفة تأثيراً معاكساً على أى صفة أخرى تحت الدراسة.

قدرت قيم المكافئ الوراثى المحقق فى الدورات الإنتخابية المتتابعة وقد حدثت زيادة لهذه القيم فى الأجيال المتتابعة من الإنتخاب. ففى الحشة الأولى زادت هذه القيم من ٣٤% إلى ٥٧% ومن ٤٦% إلى ٥٣% ومن ٤٠% إلى ٥٧% ومن ٤٢% إلى ٥٨% ومن ٤٧% إلى ٦٢% وذلك بالنسبة لكل من إرتفاع النبات، التفريغ الرئيسى، الوزن الرطب للعشب، الوزن الجاف للعشب، المحتوى الكلى من القلويدات على الترتيب. أما فى الحشة الثانية فقد زادت هذه القيم من ٤٧% إلى ٥٣% ومن ٤٥% إلى ٥٨% ومن ٤٤% إلى ٥٩% ومن ٤٣% إلى ٥٧% ومن ٤٧% إلى ٥٩% وذلك بالترتيب المذكور. وقد دلت النتائج على وجود تباين وراثى إضافى لصفات الإنتاج فى العشيرة الأصلية.