PHYSIOLOGY OF ACCUMULATION OF FATS AND PROTEINS IN HIGH OIL SUNFLOWER VARIETIES

By

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It is well known that Academician Pustovoit V.S. and his disciples, USSR, have markedly increased oil content in sunflower seeds. New high oil varieties have been developed with 53 to 55 percent oil versus 30 to 32 percent oil in old varieties.

It is of great theoretical and practical value to find out the reason of high level of oil accumulation in sunflower seeds from physiological standpoint.

At Plant Physiology Department at VNIIMK, Dyakov A.B., master of biological sciences, has conducted research work on the problem. The results obtained are given below.

Oil and protein are concentrated in kernels of sunflower seeds. Only very small amounts of lipids and nitrogenous components are contained in hull.

High negative correlation between oil and protein percentage in kernels was found long ago. In this connection a theory of antagonism between fat and protein synthesis in sunflower seeds appeared. Hence a conclusion is rather often made to avoid fertilizing of nitrogen on sunflowers. The schematic expression of the consideration is most distinctly shown by Konrad Mengel in fig. 1 (1965).

This theory may be true provided the intensity of oil formation is limited by amounts of assimilates, and provided the increase of nitrogen supply is not followed by intensive formation of the former. Competition between fat and protein synthesis from a common source of organic substances has been distinctly confirmed by tests with heterotrophic fat formation organisms. However, in treatments of nitrogenous fertilization on sunflowers or any other autotrophic oil crops, it has been found that decline of oil percentage at intensive N supply does not result in decrease of absolute amounts of fat. On the contrary, under such conditions absolute amount of fat increases; i.e., fat formation process is rather stimulated than inhibited (Table 1). Hence a conclusion may be drawn that there is no antagonism between the processes of fat and protein synthesis.

In the opinion of a number of authors, nitrogen promotes increase of oil yield as it is supposed that intensive N supply results in the increase of total amount of assimilates due to enlargement of leaf area and their higher productivity.

Table 1. EFFECT OF TIME OF NITROGEN APPLICATION ON SUNFLOWERSEED CROP

(1 g of nitrogen applied when packing pots; 3 gs of nitrogen applied as top dressing)

Top dressing at phases of	Yield in g/plant of			Oil content in kernels, %	Weight of dry leaves, g/plant
	Seeds	Fat	Nonfatty substances		
8 leaves	42.8	27.5±3.1	15.3±1.2	63.8±1.6	28.3
Appearance of bud	45.8	29.7±3.1	16.1±0.6	64.7±1.9	33.4
10 days after budding	41.1	26.2±1.5	14.9±0.8	64.4±1.6	29.1
Flowering	46.8	27.9±2.5	18.9±1.6	59.6±1.6	25.5
12 days after flowering	28.8	17.0±2.9	11.8±0.9	59.0±1.7	27.0
Control	23.1	15.8±1.6	7.3±0.5	68.3±1.0	20.0

The data given in Table 1 show that the nitrogen applied at different phases of plant growth, from 4 pairs of true leaves to flowering including, causes slight differences in absolute oil amount; in tests with ten replications the differences are not significant at the 5% level. Since by the commencement of flowering the sunflower leaves practically cease to grow, the increase of oil yield due to N application at this period cannot be attributed to enlargement of leaf area. Neither can the dressing applied at the time of anthesis effect the number of the florets per head.

Nitrogen applied after 12 days of florescene commencement did not increase oil yield significantly as compared with control, though intensive oil formation had not yet begun by the time. If the improved conditions of nitrogen supply had intensified oil formation due to direct effect of nitrogen upon intensity of photosynthesis or fat synthesis, the nitrogen

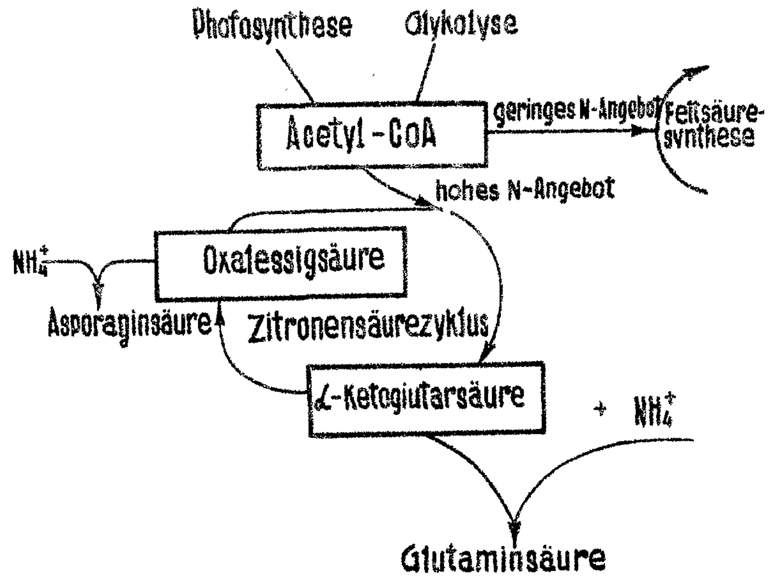

Figure 1. Relation between oil and protein synthesis in kernels of oil crops.

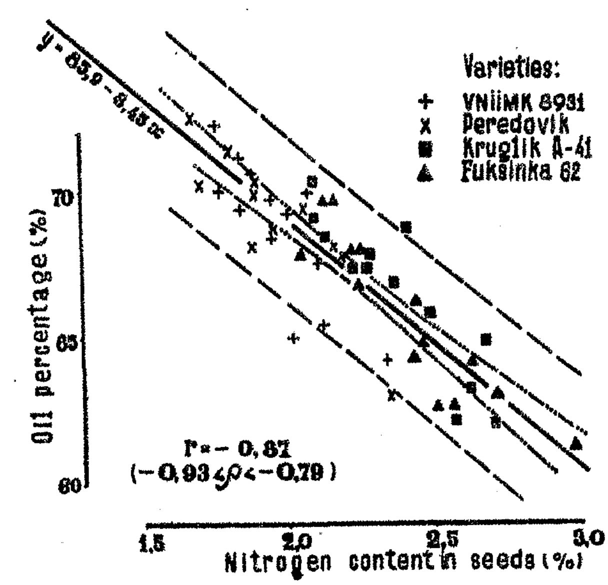

Figure 2. Regression of oil content on nitrogen content in sunflower kernels.

applied at that phase would have enhanced oil yield. Consequently, nitrogen effects oil yield by influencing some processes which precede the period of intensive oil formation but last over a short time. These processes are apparently responsible for intensity of fat synthesis as yield can be markedly influenced provided the limiting process is influenced.

Thus it was found that sunflowers respond qualitatively different to the conditions of nitrogen nutrition from flowering stage on to the beginning of oil formation in seeds. Special observations showed that at this period the growth of kernels tissue in kernels proceeds. It is completed in primary zones of sunflower head after 14 to 16 days of flowering. Total achenes (kernels) growth is evidently the process which attributes to the intensity of oil formation, whereas oil yield is directly proportional to amount of fat storage tissue.

It is known that at the initial stages of the growth of seed tissue constituent proteins are formed, and at the seed filling period reserve proteins are accumulated. The nitrogen seems to intensify fat synthesis provided it is utilized for the synthesis of constituent proteins. Significant increase of amount of nonfatty substances in kernels due to accumulation of crude protein in them, with nitrogen applied before the filling phase, did not cause lower oil yield as compared with control (Table 1). Consequently, synthesis of reserve proteins has no effect on the intensity of oil formation. However, increased amount of the proteins leads to the change of the ratio between oil and protein amounts in favour of the latter. As a result, oil percentage in kernels declines. Thus, the increase of oil yield is associated with the increased amount of constituent proteins in seeds, and the decrease of oil percentage is related to the enhancing of reserve protein accumulation in seeds.

Genetically conditioned differences in kernels oil precentage are closely negatively correlated with those in protein content (fig. 2, vegetative test, $r=-0.87$, confidence interval at the 5% level -0.95 -0.79). This fact was found long ago. According to the antagonism theory it was presumed that higher intensity of oil formation in high oil varieties was caused by less intensive protein synthesis, due to lower nitrogen uptake from soil as compared with low oil varieties. The hypothesis verified by a number of field and laboratory tests. The data presented in Table 2 show that this hypothesis is not true. Though, actually, relative nitrogen content in kernels of high oil varieties is markedly lower than that of low oil varieties, varietal differences on oil percentage, cannot be attributed to different N amount uptaken by the plant from soil. At full maturity no, less nitrogen is transported from soil to air parts of high oil varieties than to those of low oil varieties.

Table 2 YIELD OF HIGH- AND LOW OIL SUNFLOWER VARIETIES (Pots experiment)

Variety	Yield in g/plant			% oil in kernels	% nitrogen in kernels	N amount in air parts, g/plant
	Achenes	Kernels	Oil			
p						
VNIIMK 8931	43.0	32.3±2.5	22.3	68.9±1.5	1.95	1.13±0.04
Kruglik A-41	45.2	25.2±2.6	16.8	66.8±1.5	2.38	1.11±0.04
Peredovik	42.8	32.4±1.7	22.5	69.3±1.7	1.98	1.13±0.04
Fooksinka 62	44.3	26.4±2.5	17.4	66.0±1.6	2.41	1.11±0.05

To understand why N content in kernels of different varieties is different, it is necessary to consider their yielding capacity. The concept common in literature is that sunflower breeding results in increase of seed oil percentage only with almost no effect on seed crop. It is true with respect to achenes only. In fact, there is a nonsignificant difference in seed yield between high- and low oil varieties. However, basic amount of nitrogen is chiefly concentrated in kernels, and high oil varieties exceed low oil varieties much more in kernels yield than in oil percentage (Table 2). Therefore, high oil varieties, though with a lower relative protein content in kernels, give slightly higher protein yield and contain still higher absolute amount of fractions of nonfatty substances in kernels per unit of seeded area. It should be noted that absolute amount of protein in seeds depends mainly on available N amount in soil; heritable differences in relative protein content are linked with different kernels yield. Under equal conditions of plants nutrition in vegetative tests, high relationship between N percentage in kernels and kernels yield is exhibited not only between varieties but also between individual plants within varieties-populations (Fig. 3, r=0.87, -0.93 -0.78).

As higher kernels yield leads to lower N percentage, and reduced relative N content results in the increased oil percentage, there must be a positive correlation between genotypic variation on yielding capacity and oil percentage in kernels. Indeed, the higher is the yield of kernels, the higher is its oil percentage. The terms of nutrition being equal, this relationship is observed both in field and vegetative trials. Correlation coefficient ranges from 0.8 to 0.9 (Fig. 4, r=0.84 confidence interval at the 5% level $0.73 \leq r \leq 0.90$).

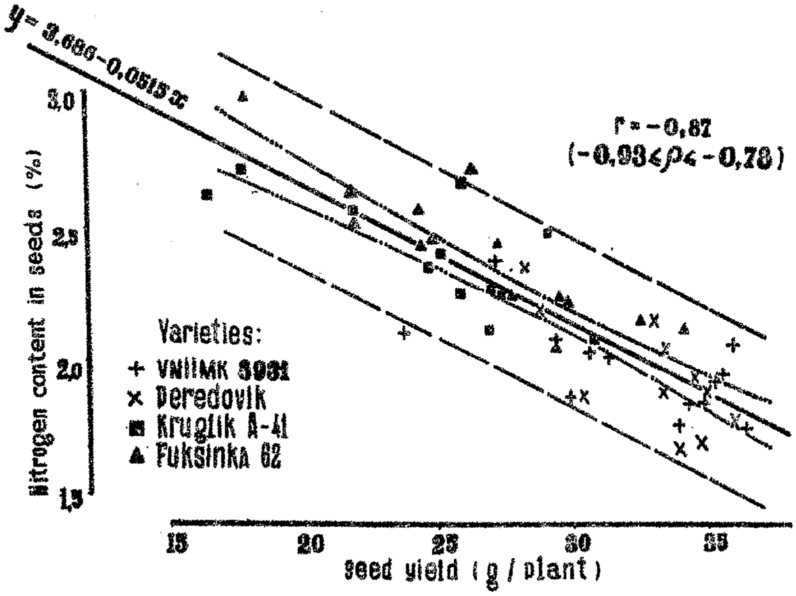

Figure 3. Regression of nitrogen content in sunflower kernels on yield of kernels.

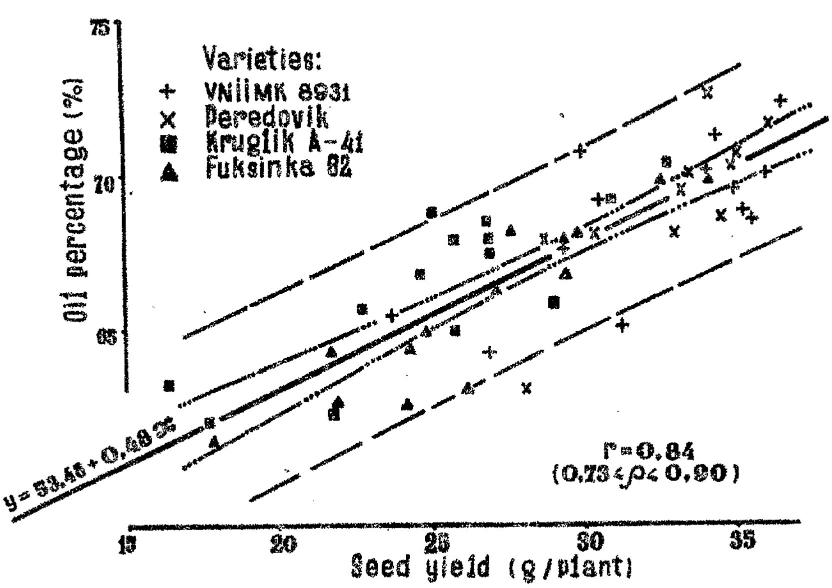

Figure 4. Regression of oil content on yield of sunflower kernels.

Oil yield depends on only two indices: yield and oil percentage of kernels. As inheritably conditioned variations on oil percent are mainly caused by differences in cropping power, oil yield practically wholly depends on kernel yield. Correlation coefficients of data of both vegetative and field trials with equal terms of nutrition amount to 0.99 and higher value (Fig. 5, r = 0.990, 0.983 0.994). The relationship is so close that it has become possible for a plant breeder dealing with yield from a plot, not from individual plants, to almost faultlessly choose superior entries and varieties, without oil content determination. Since dependence of oil yield (y) on seed yield (x), oil percentage being equal (a'), may be expressed with the equation:

$$y = a'x, \qquad (1)$$

and dependence of oil percentage on seed yield, environmental conditions being equal, can be described by the formula

$$a' = a+bx, \qquad (2)$$

it is possible to incorporate equation (2) into equation (1) as follows:

$$y = ax+bx^2, \qquad (3)$$

This well corresponds to the empiric data and to the condition that regression line should cross origin, as there cannot be any oil yield without seed yield, or any seed yield without lipids.

Equation (3) testifies to the fact that sunflower breeding is physiologically a breeding for yielding capacity as well as wheat or corn breeding. In the two last cases, the increase of yielding capacity through breeding also results in the decline of relative protein content due to reserve material, and in higher starch content. The deviation of regression line from lines of equal oil percentage is conditioned by relative depletion of protein in seeds (as it is shown above) as crop increases.

According to published information, the crossing of high oil sunflower biotypes with high yielding ones makes it feasible to develop forms superior in oil yield as compared with parents. This standpoint is derived from assumption that yielding capacity and oil percentage of kernels are inherited independently on each other. The suggestion proved to be incorrect as evidenced by the data above. Sunflowers cannot have special genes of oil percentage and protein content; seed oil percentage cannot be inherited independent on kernels yield, and these characters cannot be combined at hybridization. While breeding for higher oil yield per hectare yield of kernels and their oil percentage increase in certain rather limited proportion, a proportion in which these characters vary in individual plants within each variety-population (Fig. 4).

Thus, while estimating breeding material for kernels yield it is possible to carry out breeding program for higher oil yield too. Significant increase of kernels yield is followed by increased oil percentage as under equal terms of nutrition relative nitrogen content in kernels is reduced. When selecting for oil percentage, high yielding forms can also be released if oil percentage was higher due to the increase of seed yield. But if oil percentage is enhanced as a result of lower N uptake from soil or N transportation from vegetative organs into seeds, oil yield cannot be increased.

The above data directly relate to the estimation of prospects of sunflower breeding. Oil percentage of kernels cannot exceed 73 to 75% on dry seed basis. Physiologically, maximum oil percentage depends on limiting low protein content- under given terms of plant nutrition which hinders further breeding for higher yielding capacity. Therefore, it should be recommended to produce and breed sunflowers with higher rates of fertilizers. This will contribute to some increase of oil yield, however, abundant nitrogen supply will result in lower oil percentage as absolute and relative protein content in seeds are increased in this case. This will permit, however, to further continue breeding for yielding capacity. As a result of continuous breeding, oil accumulation is increased both absolutely and per unit of nitrogen absorbed which implies new higher oil percentage as well (in comparing varieties under identical conditions apart from higher level of nutrition).

Thus, increased intensity of oil formation in high oil varieties cannot be attributed to reduced intensity of protein synthesis. Literature offers two more theories accounting for varietal variations on seed oil accumulation. According to one of them, this phenomenon is conditioned by inheritable differences in photosynthesis mechanism which bring about non-identical amount of assimilates in different varieties. According to another theory, more oil is formed in high oil varieties because of higher activity of fat synthesizing enzymes in their cells. At Plant Physiology Department, VNIIMK, these theories have been substantiated to be erroneous. The results of one field trial may be summarized here. A close positive relationship has been found between dry weight of 100 seeds by the end of their growth and absolute oil amount in 100 seeds of the same plants by the end of oil formation period (Fig. 6, $r = 0.92$, 0.82   0.97). Before the period of seed filling content of reserve substances is not high. Therefore, by the end of the plant growth absolute content of constituent substances can be estimated by dry seeds weight. As no fat synthesis is possible without these substances in cells, and lipids are contained in any tissue of the plants, regression line is bound to cross the origin. As this relationship is not substantiated to be curvilinear, it can be expressed by the equation:

$$y = ax \qquad (4)$$

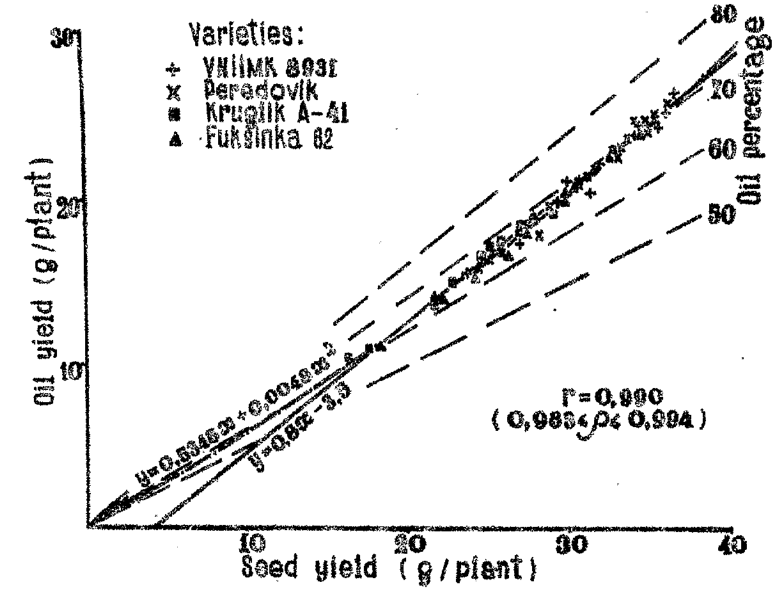

Figure 5. Regression of oil yield on yield of sunflower kernels.

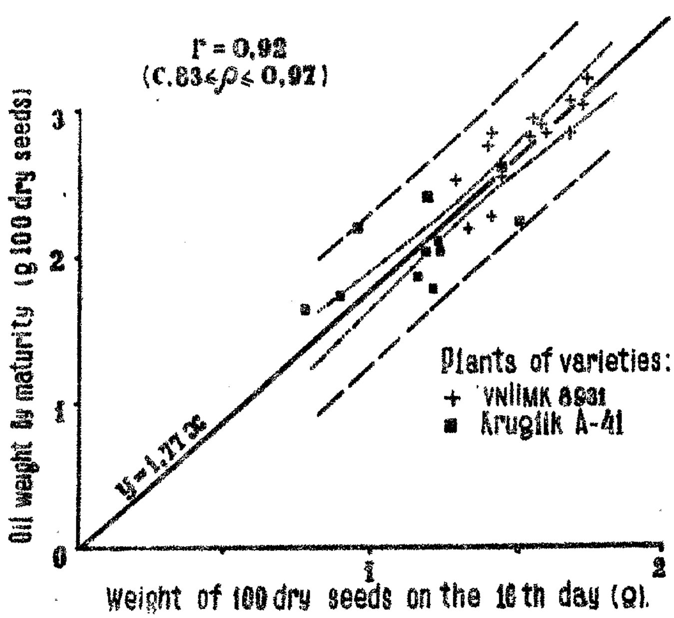

Figure 6. Dependence of oil yield in sunflower kernels on quantity of constitutional substances of kernels.

where y is oil weight by maturity period; x - dry seeds weight on the 16th day after commencement of flowering; a-coeffecient denoting oil amount synthesized through the filling period per unit of weight of constituent substances accumulated in seeds throughout their growth. The value of the coefficient in relation to y/x is different for high- and low oil varieties, and amounts to 1.77 in this experiment. Consequently, per unit of constituent substances identical amount of fat is accumulated in low- and high oil varieties. Therefore, inheritable variations on enzyme activity cannot be responsible for varietal variations on intensity of oil formation. As varietal variations on fat accumulation are quite evident, and by the time of maturity identical amount of oil is accumulated per unit of storage tissue, it means that varieties are differentiated by oil yield only because of dissimilar intensity of seed growth, the environmental conditions being equal. As a result, by period of filling nonidentical amount of fat storage tissue is formed. Thus, change of intensity of seed growth causes increase of oil yield not only through fertilizing but also through sunflower breeding.

No doubt, to synthesize more amount of oil high oil varieties should consume more assimilates accordingly. However, it has been proved that potentialities of assimilation apparatus are not wholly brought into action even in high oil varieties under normal field conditions. It is due to nonconformity between the ability of leaves to produce carbohydrates and that of seeds to consume them. Therefore, though varietal variations on intensity of photosynthesis have been found significant, they are an effect of different intensity of oil formation, and not a cause. Photosynthesis of high oil varieties is to a lesser extent inhibited by excess of assimilates as greater amount of storage tissue consumes more carbohydrates throughout oil formation what contributes to more intensive outflow of them from leaves as compared with low oil varieties.

It conforms well with the data above concerning exceedingly high dependence of oil yield on kernel yield. The relationship apparently indicates that inheritable variations on oil yield are conditioned by variations of intensity of total seeds growth. As plant breeder throughout the strain testing of crops endeavours to create similar environments for all the genotypes, while different genotypes produce dissimilar bulk of storage tissue, per unit of its amount there is synthesized nonidentical amount of protein with almost equal protein yield per unit of seeded area. In other words, differences (genotypycal) in oil yield are also attributed to intensity of seed growth. And protein yield is chiefly related to the terms of nitrogen nutrition of plants.

Intensive growth of seeds at the increased level of N supply is, in general, clear enough. However, from physiological standpoint it is more difficult to account for variations on intensity of seed growth conditioned by genetical factors. Direct determinations have shown that these variations are associated with differences in conditions of seed nutrition too. The fact is that under field conditions, seeds are supplied with nitrogen and phosphorus, for instance, almost wholly due to plastic substances mobilized from vegetative organs as by the commencement of flowering N and P uptake from soil has mainly ceased. It has been found that outflow of nitrogenous material from vegetative organs into growing seeds proceeds more intensively in plants of high oil varieties. Hence, more intensive seed growth of the latter results; higher amount of constituent proteins are formed, and less amount of reserve proteins respectively. All this causes varietal variations on oil yield and seed oil percentage.

I cannot go into details about the findings, and confine myself to one of the experiments only which has been carried out to verify their adequacy with the reality. With that end in view, an attempt was made to experimentally decline seed yield and oil percentage of high oil variety VNIIMK 8931 down to the level of low oil variety Kruglik A-41. For this purpose it was necessary to decrease intensity of nitrogen inflow into growing seeds without changing its total rate. Therefore, nitrogen was applied to some pots after seed growth was completed, and not before flowering (Fig. 7, 1 g of nitrogen applied while paching pots, 3 gs of nitrogen applied at dressing). As a result of the fact that nitrogen applied as top dressing could not be utilized by the plants for growing seeds, both kernel yield and oil percentage decreased down to the level of low oil variety Kruglik A-41. It should be noted that these plants are plotted on the same regression line in the graph but in the zone of low oil varieties disposition. The plants to which nitrogen was applied after seed growth was completed could not be differentiated in oil yield from the plants of the same variety with no N dressing applied to it. (See diagram, lines of equal oil yield).

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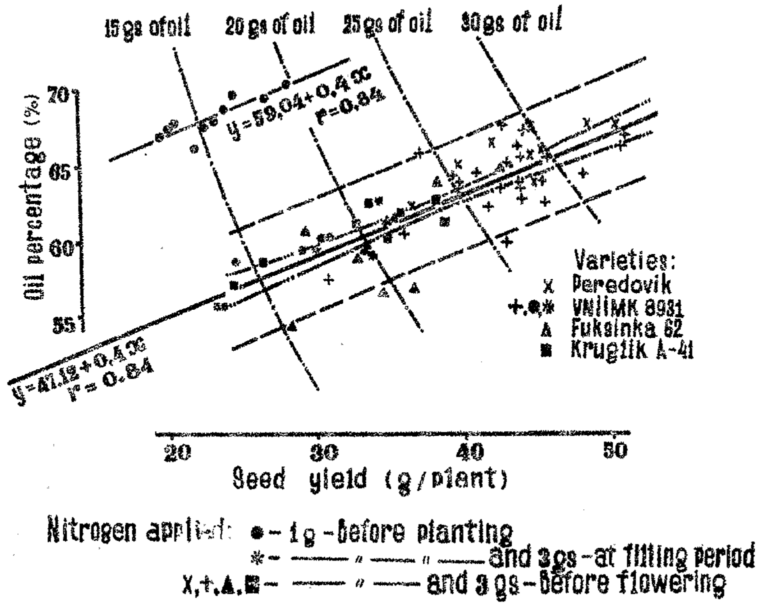

Figure 7. Experimental transformation of plants of high oil variety into low oil ones.

DISCUSSION

Orellana: What are the amino acids involved in this increase in protein content? Secondly, have you noticed any increase in susceptibility to disease as you increase protein content?

Panchenko: The total amount of nitrogen in the higher oil varieties does not decline. Our experience is that yield of protein in oil varieties goes up accordingly with the increase in high oil. The content of amino acids, in relative percentage, does not change as the amount of protein increases with the higher oil varieties. We have observed no differences in susceptibility to diseases as the amount of protein has increased.

Johnson: Have you found any differences with "time of application of fertilizer" with sunflowers?

Panchenko: Yes. The application of nitrogen increased the yield of achenes. It appears that we are now reaching the limit of oil percentage. We now must work on and introduce into our program of breeding high levels of fertilizer application - including nitrogen. Hopefully, this will result in still greater gains in percent of oil and percent of protein above the present levels in our breeding program.

Almgard: It appears to me that the fastest method of selection to make gains is to select just for oil. Is that true?

Panchenko: I indicated in my paper that, if you collect data on the total yield of achenes, this correlates rather closely with the amount of oil; yet, when you make individual selections, it is highly important to determine the amount of oil in individual selections. We have found that using either the yield of seed achenes or the percentage of oil to be about the same as far as making progress in the breeding program is concerned.

Almgard: Do you prefer one or the other?

Panchenko: As you know, we do a tremendous amount of oil determinations on thousands and thousands of individual plants in the breeding work. We, also, must be concerned about yield of achenes. The two measurements go together.

Knowles: As you increase kernel yield and oil content, is there any difference in the fatty acid composition?

DISCUSSION
(Continued)

Panchenko: Actually, Academician Pustovoit did about thirty years of work before we were concerned about the composition of fats. At that time, we found that the percentage of linoleic acid is higher in the high oil varieties. We find that in the earlier varieties that were produced, there was about fifty percent linoleic acid. In the present day improved varieties, there is about fifty-eight percent to sixty-five percent linoleic acid and with some decrease then in oleic acid in the newer varieties.

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