OIL AND PROTEIN ACCUMULATION IN SUNFLOWER SEEDS

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The breeder should know not only how selection is developed but also what exactly to select and according to what combination of characters. And in order to choose the proper trend he has to know the prospects of breeding for certain quantitative characters. By determining the genetic correlations between characters we may obtain some information about the correlative effect of selection while the direct reaction to selection may be estimated by the heritability coefficient. But such empirical forecasts are not certain on account of the big errors found in these parameters as well as a result of the genetic changes undergone by the populations in the selection process. Neither do the methods applied in biometric genetics afford any information on the limits of selection in what concerns quantitative characters.

Prospective forecasts and choice of breeding trend as well as establishment of the way in which the breeding material shall be tested should be therefore based on the physiological processes of character formation in plant ontogeny. If these assumptions are erroneous, the breeding will not succeed for it has been performed following a wrong course. Such an error was made in his days by the famous breeder N. A. Uspenski. Shortly after the first variety of sunflower with higher oil content in the seeds appeared, this breeder studied the phisiological causes of oil content variation in order to foresee sunflower breeding prospects in this direction. He discovered a negative correlation between fat and protein content in seed and herefrom he drew the conclusion that breeding for high oil content has no perspectives, because this would lead to the creation of pathological sunflower forms which, on account of a weak root system would not properly absorb nitrogen from the soil. However, V. S. Pustovoit went on breeding sunflower for oil and developed varieties exceeding the initial types by 1.5 times in oil accumulation and holding however the same viability. And the mentioned correlation was not suppressed — it remains compulsory both for the new and for the old varieties.

The opinion still largely prevails that this correlation is due to the antagonism between fat and protein synthesis, consequent to their competition for acetyl-CoA, wich is formed after carbohydrate glycolysis and the pyruvat oxidative decarboxylation. It is moreover assumed that photosynthesis limits the accumulation of the reserve substances in sunflower seeds and thus oil accumulation can be intensified only by weakening the protein biosynthesis.

The results of trials with different sunflower varieties in different plant populations (table 1) proves that this viewpoint is wrong. The increase of oil yield as a result of breeding did not lead to an absolute protein decrease in kernel and protein percentage in the kernels of

Table 1

Oil and protein content in kernel yields of sunflower varieties with high and low oil content, at different plant populations

Sunflower variety	Weight (in a completely dry matter) q/ha					Content (% in completely dry kernels)		Nitrogen amount extracted kg/ha
	Achenes	Kernels	of which			Protein	Fat	
			Fat	Protein (N×6,25)	Total substances without fat			
				20,000 pl/ha				
Peredovik	27.7	21.7±1.3	13.1	5.6±0.3	8.6	25.9±1.0	60.3	151
Kruglik A—41	26.9	16.8±1.5	9.1	5.7±0.6	7.7	34.0±0.9	54.1	151
				40,000 pl/ha				
Peredovik	31.5	24.6±1.0	15.4	5.6±0.3	9.2	22.8±1.2	62.6	152
Kruglik A—41	30.5	19.6±1.6	11.1	5.8±0.4	8.5	29.9±1.6	56.8	162

sunflower varieties with high oil content decreased only on account of a high accumulation of fat and other nitrogen-free substances and not because of plant not absorbing the same amount of nitrogen. Similar correlations are observed when comparing the indices not only in different varieties but also in different genotypes of any variety-population, only if the same nitrogen nutrition is provided. Therefore Uspenski would have drawn a correct conclusion on the interdependent character of fat and protein accumulation had he considered the estimates of their absolute content in kernel yield and not of their relative content. The erroneous acknowledgement of these indices becomes quite clear when comparing the difference in total content of fatless substances in kernels. The amount of these substances increases as a result of breeding, although their relative content decreases exactly in the same percentage as increases oil content.

N. A. Uspenski's error must be mentioned even in our days because the erroneous notions on which it is based, regarding sunflower metabolic change as a consequence of breeding, are even now widely spread in literature; this leads to improper recommendations in breeding as well as to wrong appreciation of their future results. Such errors are even now existing when discussing sunflower breeding ways after the maximum oil content was reached as well as when discussing the protein content increase in the seeds.

Our investigations proved the relative independence of oil and protein biosynthesis in sunflower kernels. Intensification of oil formation does not lead to protein accumulation decrease, because plant foliage possibilities are not by far completely employed in the formation of a usual seed yield. The level of fat and protein accumulation is determined by quite different causes. Data given in table 1 show that even on a rich chernozem the increase of the soil volume per plant twice as compared to optimum, at the expense of the crop stand, does not bring about a substantial decrease of the protein yield and of nitrogen extraction per hectare. This proves that in sunflower field crops each plant gets at least an amount of nitrogen twice more reduced than it could take up. Other experiments have proved that in order to supply the entire nitrogen requirement to sunflower plants, their number per hectare should be reduced to 5000, i.e. to increase by 8—10 times the volume of soil per plant as compared to optimum volume for crop productivity. Therefore the possible differences among genotypes cannot appear with respect to their nitrogen uptake capacty under usual field crop conditions. The plants assimilate as much nitrogen as it is contained in the soil afforded to them. Analyses on a vast experimental material also showed a surprisingly reduced genotype variability in the share of nitrogen admitted in the seeds, as compared to the entire amount absorbed by plants. Thus, the hereditary variability of protein percentage content in kernels with which the breeder deals is in general determined by the genotype differences according to productivity and not to nitrogen absorbtion from the soil and seed capacity for protein accumulation. This explains the cause of undesired correlative effects of selection for increasing the protein percentage in sunflower kernels i.e. a decrease in seed and oil yield as well as in oil content in the seeds without an increase of the nitrogen uptake degree and of the absolute protein content in kernel yield (table 2).

The weakening of oil or other nitrogen-free substance biosynthesis, although accompanied by an increase in protein percentage, cannot as a rule lead to an increase in protein production, because the amount of raw protein is calculated by nitrogen content and the plants cannot change the nitrogen-free substances into nitrogen. When estimating the breeding material by protein percentage, the most productive genotypes get lost. There is only one adequate way of breeding sunflower for protein: it consists in the selection at the beginning of the most productive genotypes holding a relatively reduced protein content,

Table 2

The productivity of sunflower genotypes isolated from variety-populations with high and low oil content, by their protein percentage in kernels. The averages by groups of indices are calculated in absolutely dry kernels. Experiment in pots

Groups of plants by protein % in kernels	Protein content (N × 6.25) in kernels		Nitrogen in above ground plant organs g/pl.	Kernel yield g/pl.	Oil content	
	%	g/pl.			%	g/pl.
			Kruglik A—41 variety			
High	16.3	3.60	1.09	22.1	63.6	14.1
Moderate	14.5	3.74	1.11	25.8	67.7	17.5
Reduced	13.4	3.75	1.13	28.0	68.9	19.3
			VNIIMK 8931 variety			
High	13.3	3.88	1.13	29.2	67.2	19.6
Moderate	12.1	3.87	1.15	32.0	68.8	22.0
Reduced	11.2	3.99	1.10	35.6	70.2	25.0

and only after their positive appraisal in big plots, the genotypes yielding heighest amount of protein per hectare, shall be detected ; this will be due to a reduction in seed yield losses, an enhancing of nitrogen flow from the vegetative organs and an increased response to nitrogen fertilizers. But there is no reason for developing parallelly to sunflower varieties for oil other varieties particularly rich in protein, as there is no antagonism in the biosynthesis of oil and protein in the seeds. Those genotypes should be detected that produce more oil and more protein per unit area.

When investigating the cause that determine the volume of oil production, it was found that in the seed filling stage there was no difference at the cell level between varieties with high and low oil content in what concerns oil formation rate. These diferences are noticed only at the whole plant level and they may be explained by the fact that the varieties rich in oil and developing under the same conditions form at the beginning of seed filling stage more cells in the cotyledons in which they deposit their fat supplies. This is linked to the more sudden cessation in growth of their vegetative organs during anthesis and to the intensive flow of nitrogen and other substances from these organs into the growing seeds where, during this time, the constitutional proteins of the cell protoplasm are synthesised ; it is also linked to the embryo mitoitic activity before the onset of seed filling.

As sunflower kernels are usually formed of fat and protein, their percentage content is determined by the ratio in which they accumulate in the kernels. As already mentioned, protein accumulation range is determined mainly by the available nitrogen content in the soil. The establishment of hereditary differences implies the supply of equal environment conditions and nitrogen nutrition. That is why high yielding and low yielding genotypes do not generally differ by protein production, and the variation in kernel production is mainly determined by the

uneven accumulation of oil. As a result, absolute and relative oil content increases and protein percentage decreases as kernel production is enhanced in the breeding process. The estimates of these two most important indices of sunflower yield vary in the breeding process in a ratio determined by objective laws and not according to breeder's desire. In whichever direction the selection may be performed — be it for oil content, oil production or kernel production — higher yielding genotypes with low protein percentage will be separated from sunflower populations.

From our findings it results that the joint variability of all these characters is controlled by the same genes. Thus oil production increases not because of gene combinations controlling oil percentage and yields, but because, alongside the intensification of the total growth of seed embryos, the total amount of depositing tissues also grows ; this in turn leads to the increase of kernel yield, more particularly as a result of the intensification of nitrogen-free substance accumulation of which oil holds the first place. All these phenomena are to the same extent suitable both to varieties and to sunflower hibrids between lines.

With respect to the slower rhythm of oil content increase in the seeds, the physiological limit of this index is presently of high practical importance. Some breeders think as follows about the matter : oil content cannot be raised to 100% because all the protein in the tissues cannot be superseded by oil, therefore sunflower varietal breeding for oil will soon reach a limit. And from the fact that hereditary changes have already decreased with regard to this indice, they draw the conclusion that this kind of breeding is already exhausted and that they therefore have to resort to hybridization between lines.

We should like to stress that the direction in which V. S. Pustovoit suggests to breed sunflower varieties seems the most adequate because by varietal improvement both percentage and oil yield per hectare increases (conversely to protein percentage and yield). But what speaks more in favour of this is the breeding for high oil yield per hectare ; in all the breeding process steps, beginning from elite plant rating by progeny, all the genotypes and families that do not exceed the control — as to oil yield per cropped unit area are discarded even if these families and genotypes are characterized by a high oil content.

The relative protein content is determined by the following formula :

$$Z = \frac{a}{x} \cdot 100 \qquad (1)$$

where Z is protein content in percentage ;
a — protein content q/ha ;
x — kernel yield q/ha. Herefrom the following equation may be used for calculating oil content :

$$Y = \frac{x - a}{x} \cdot 100 - V \qquad (2)$$

where Y is oil content in percentage ;
V — content of nonfat, nitrogen-free substances, % in dry weight.

If one starts with the notion that oil and protein vary on account of one the substances superseding the other, in the case of the same total production, that is if one accepts that x is constant and a variable, then those who develop special varieties with high protein content and consider as a limit of oil content in seeds that limit efficiently utilized in varietal sunflower breeding would be right. However, data presented here as well as other experimental material clearly showed, during sunflower genotype trials under the same conditions, that protein yield per hectare was incomparably more constant than kernel yields. For this reason the dependents (1) and (2) are hyperbolic : oil and protein percentage tend towards the limit, but theoretically they'll never reach their highest values, however high seed and oil yield may increase. This means that with respect to physiologic changes occurring in the plants, sunflower breeding stages differ only quantitatively and not qualitatively, both in the past and in the future. Therefore in the future the same main principles will be used in varietal improvement but changing correspondingly the relationships between characters when estimating the breeding material.

The main part, in the evolution process, is played by gene accumulation which operates additively and not by allele interaction. Therefore a cessation in population breeding might substantially hinder a prospective perfection of sunflower for, as N. I. Vavilov said : „Breeding is an evolution controlled by human will".