# Variability in Oil Content among Heads and Seeds within Heads of Sunflowers (*Helianthus annuus* L.)[1]

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## ABSTRACT

Oil contents were determined by nuclear magnetic resonance on four samples, each consisting of six seeds, from each of three ring positions (outer, middle, and inner) for 10 heads from each of two open-pollinated varieties and two inbred lines of sunflowers. The largest source of variability for three of the entries was due to differences among heads. Variability due to seed position in the head was the largest for the fourth entry. When averaged over all entries, % oil was the highest for seeds samples from the middle ring position. In studies in which oil content is determined on single seeds or small samples, error variation can be reduced by sampling from either of the outer two ring positions, rather than sampling from the inner position or at random.

## INTRODUCTION

Selection for oil content in oilseed crops has been facilitated greatly by NMR spectroscopy. NMR analysis is rapid, accurate, and nondestructive of seeds and allows for oil determinations on small samples, including single seeds. Selection is most effective if environmental effects that influence oil content are recognized. When selection is based upon individual plants or individual seeds within a plant, effects of major concern are those which contribute to between- and within-plant variability. The relative importance of variation among ears and among kernels within ears has been reported for corn (1,2) and among plants, pods, and seeds within pods for soybeans (3). The purpose of this study was to investigate the magnitude of variability among heads and among seeds within heads of sunflowers.

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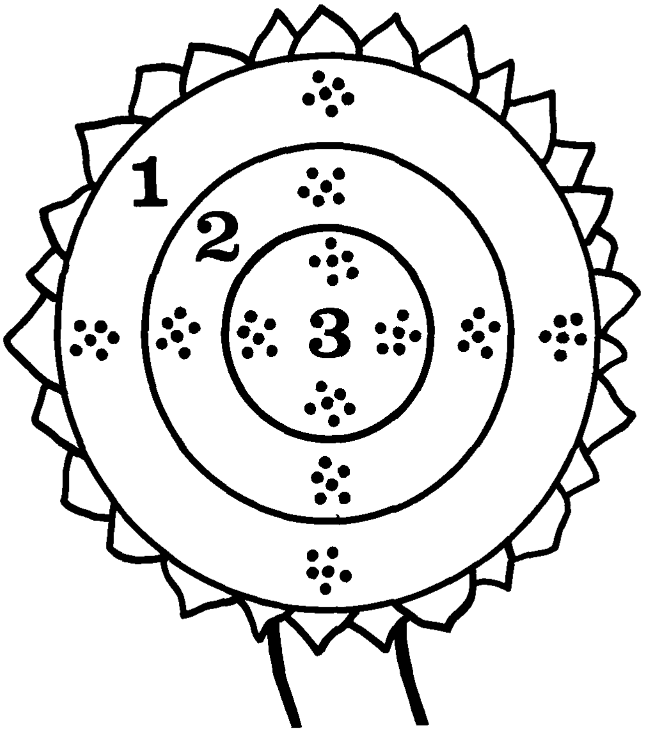


FIG. 1. Diagram of sunflower head showing the three concentric, ring-shaped zones from which six-seed samples were taken.

## MATERIALS AND METHODS

Ten mature sunflower heads were selected at random from commercial fields of each of two open-pollinated varieties, Krasnodarets and Luch, and two inbred lines, CM303 and CM323. Each head was divided into three concentric, ring-shaped zones, and six adjoining seeds (botanically achenes) were removed from within each zone at the 3, 6, 9, and 12 o'clock positions (Fig. 1). The

TABLE I

Model Analysis of Variance Used for Oil Content and Seed Wt of Four Sunflower Varieties and Inbred Lines

| Source of variation | df[a] | Expected mean squares |
|---|---|---|
| | | Individual analysis |
| Heads | 9 | $\sigma^2_{s(hp)} + sp\sigma^2_h$ |
| Positions | 2 | $\sigma^2_{s(hp)} + s\sigma^2_{hp} + sh\theta^2_p$ |
| Heads x positions | 18 | $\sigma^2_{s(hp)} + s\sigma^2_{hp}$ |
| Samples in heads x positions | 90 | $\sigma^2_{s(hp)}$ |
| | | Combined analysis |
| Varieties | 3 | $\sigma^2_{s(hp)(v)} + sp\sigma^2_{h(v)} + shp\theta^2_v$ |
| Heads in varieties | 36 | $\sigma^2_{s(hp)(v)} + sp\sigma^2_{h(v)}$ |
| Positions | 2 | $\sigma^2_{s(hp)(v)} + s\sigma^2_{hp(v)} + shv\theta^2_p$ |
| Varieties x positions | 6 | $\sigma^2_{s(hp)(v)} + s\sigma^2_{hp(v)} + sh\theta^2_{vp}$ |
| Heads in varieties x positions | 72 | $\sigma^2_{s(hp)(v)} + s\sigma^2_{hp(v)}$ |
| Samples in positions x heads in varieties | 360 | $\sigma^2_{s(hp)(v)}$ |

[a]df = degrees of freedom.

[b]$\theta$ = component due to fixed variable, v(4) = varieties, h(10) = heads/variety, p(3) = seed positions/head, and s(4) = samples/position.

TABLE II

Estimates of Variance Components for Oil Content and Seed Wt. from Combined Analysis of Four Sunflower Varieties and Inbred Lines

| Source of variation | Oil content | Seed weight |
|---|---|---|
| Varieties | 38.0[a] | 0.00627[a] |
| Heads in varieties | 19.7[a] | 0.00484[a] |
| Positions | 15.2[a] | 0.00532[a] |
| Varieties x positions | 14.8 | 0.00277[a] |
| Heads in varieties x positions | 13.9[a] | 0.00218[a] |
| Samples in positions x heads in varieties | 7.2 | 0.00150 |

[a]Mean squares were significant at 1% level.

TABLE III

Estimates of Variance Components for Oil Content of Four Sunflower Varieties and Inbred Lines

| Source of variation | CM303 | CM323 | Krasnodarets | Luch |
|---|---|---|---|---|
| Heads | 6.4[a] | 20.5[a] | 40.1[a] | 11.7[a] |
| Positions | 5.9 | 10.8 | 24.5[b] | 22.8[b] |
| Heads x positions | 6.0 | 9.8[a] | 20.3[a] | 19.6[a] |
| Samples in heads x positions | 5.3 | 5.9 | 8.6 | 8.9 |

[a]Mean squares were significant at 1% level.
[b]Mean squares were significant at 5% level.

moisture content of the seeds was reduced to less than 3% by drying at 130 C for 1 hr. Each sample of six seeds was weighed to the nearest milligram, and oil determinations were made on a Newport NMR Analyzer (4).

A preliminary analysis of the data indicated that the differences due to clock positions of samples were not significant. Subsequently, a mixed-model analysis of variance was used, with varieties or inbred lines and ring positions as fixed variables and heads and samples as random variables. Analyses were obtained for each variety or inbred line and for the combined data for all of the varieties and lines. The degrees of freedom and expected mean squares for the mixed model analysis of variance for the individual and combined analyses are shown in Table I. Estimates of variance components and F tests were determined from appropriate mean squares. The term "variety" used in the tables includes inbred lines.

## RESULTS AND DISCUSSION

Highly significant differences existed for oil content and seed wt among varieties, heads within varieties, and ring positions (Table II). A nonsignificant variety x position interaction for oil content indicated that position effects were similar for all varieties. As indicated by a significant heads within variety x position interaction, position effects for both oil content and seed wt were different for heads within a variety. The component of variation due to varieties was the largest for both oil content and seed wt.

Estimates of the variance components for oil content of individual varieties or inbred lines (Table III) indicated that the variation due to heads was the largest source of variation in the sampling scheme for three of the four entries. The exception was the variety Luch, in which variation due to positions was the largest. These data suggest the importance of sampling procedures that result in determination of oil content for a particular variety or inbred on a large enough number of heads so that differences between plants and positions on the plant will average out. Adequate sampling would be particularly desirable for determining oil content of an open-pollinated variety, which, in addition to environmental variation, contains considerable variation due to genotype.

If selection for oil content is based upon individual plants or individual seeds within a head, the question arises whether environmental variation might be reduced and selection effectiveness improved by sampling from a specific ring position of the head rather than by sampling at random from the entire head. Coefficients of variation for sampling from each ring position for each variety or inbred line (Table IV) were generally the least for sampling from either of the two outer ring positions. This suggests that if sampling involves a small number of seeds, as in this study, error variation may be reduced by sampling from either of the outer ring positions rather than from the inner ring position or at random. Selective sampling would be particularly important if oil content were largely under embryonic control, as in corn (5), and if selection on a single-seed basis were feasible. Pawlowski (6) reported that a strong maternal effect on oil content of seeds is present in sunflowers. Consequently selection of single $F_2$ seeds is not likely to be as effective as in corn. However, in this study, the higher variance in oil content among seeds within heads for the open-pollinated varieties than for the inbred lines (Table III) suggests that the genotype of the seed may have some influence on the oil content of the seed. Assuming that small geotypic differences do exist and can be detected, the variation among heads and among positions within a head should be considered in the sampling scheme.

The mean values for oil content and seed wt for seeds sampled from the three ring positions for each of the four entries and for the combined data are presented in Table V. Oil content of the seeds sampled from the middle ring position generally was the highest. Seeds from the outher ring position were next highest and those from the innermost position were the lowest. To ascertain whether

TABLE IV

Comparison of Coefficients of Variation Obtained from Analysis of Variance of Oil Content in Seeds Sampled from Three Positions in Head for Four Sunflower Varieties and Inbred Lines

| Variety or inbred | Combined analysis | Ring position[a] | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| CM303 | 5.2 | 4.9 | 5.0 | 5.6 |
| CM323 | 6.4 | 6.6 | 5.0 | 7.4 |
| Krasnodarets | 8.7 | 6.8 | 6.7 | 12.3 |
| Luch | 7.9 | 5.7 | 8.3 | 9.6 |

[a]1 = outer position of head, 2 = middle position of head, and 3 = inner position of head.

TABLE V

Mean Values for Oil Content and Seed Wt. of Seeds Sampled from Three Ring Positions of Head for Four Sunflower Varieties and Inbred Lines

| Variety or inbred | Seed position[a] | Oil content, % | Seed wt, g |
|---|---|---|---|
| | 1 | 44.0a | .338a |
| CM303 | 2 | 44.7a | .294b |
| | 3 | 44.8a | .229c |
| | 1 | 37.7a | .384a |
| CM323 | 2 | 39.8a | .416a |
| | 3 | 37.6a | .350a |
| | 1 | 35.0a | .353a |
| Krasnodarets | 2 | 35.4a | .340a |
| | 3 | 31.1b | .217b |
| | 1 | 38.8a | .380a |
| Luch | 2 | 39.0a | .354a |
| | 3 | 35.2b | .245b |
| | 1 | 38.9ab | .364a |
| Combined analysis | 2 | 39.7a | .351a |
| | 3 | 37.2b | .260b |

[a]1 = outer position of head, 2 = middle position of head, 3 = inner position of head. Values followed by the same letter for each variety in each column are not significantly different L.S.D. (5% level).

these differences were due to differences in hull percentages, rather than oil percentage of the embryos, oil content was determined on the dehulled seeds from three random heads for the entries CM323 and Krasnodarets. Oil contents of hulled and unhulled seeds (the latter in parentheses) for ring positions 1, 2, and 3 were 47.7 (38.3), 49.4 (39.9), and 41.5 (33.6) for CM323 and 47.3 (37.7), 46.9 (37.0), and 37.0 (26.8) for Krasnodarets. A high positive correlation (r = 0.93) for oil content between hulled and unhulled seeds and similar rankings of the three ring positions indicate that position effects were not due to differences in hull percentages.

Seed wts for three of the four entries were progressively less as sampling proceeded toward the center of the head (Table V). Simple correlation coefficients between oil percentages and seed wt of individual samples were 0.00, 0.38, 0.36, and 0.46 for CM303, CM323, Krasnodarets, and Luch, respectively. Values for the latter three entries were significant at the 1% level and indicate that the larger seeds were higher in oil percentage.

The results of this study indicate the bias that may be introduced in genetic and selection studies for oil content where single seeds or small samples are selected from a small number of heads or at random, instead of from a particular position within the head. Similar conclusions were reached for studies dealing with fatty acid composition (7). The effect of variability due to heads or due to sampling position within heads may be reduced by sampling larger numbers of heads and larger amounts of seed from individual heads. If it is desirable to sample single seeds or small samples or if necessary as a result of self-incompatibility or poor seed set for other reasons, error variation may be reduced by sampling seeds from the outer ring positions.

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