

## EFFECT OF SOWING DATE ON THE PERFORMANCE OF SUNFLOWER FAMILIES UNDER BRITISH CONDITIONS

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### SUMMARY

Sixty-six families of sunflower were investigated for their genetic response under two different sowing dates at the School of Biological Sciences, University of Birmingham, England. The results showed significant differences for flowering time, height at flowering and area of head set with seed in both sowings. However, more significant genetic variations were detected in normal sowing compared with late sowing.

Significant effect of genotype x sowing interaction on the family performance suggested that a good agreement exists between family means across sowing for all the traits. A low magnitude of correlations, however, indicated that low genetic variation exists among families.

Negative correlations between H6 and flowering time revealed that early flowering and short plants flower earlier than slow growing plants. Critical association between flowering time and *Sclerotinia* infection, an epidemic disease of sunflower in EC countries, further evidence that early flowering and short plants are attacked more than tall and late flowering families. Thus, normal sowing during the first fortnight of May is the optimum time for obtaining good yield because it attained maturity before the onset of severe wet weather in September.

**Key words:** Genetic variation, intra-class correlations, late sowing, normal sowing, sunflower (*Helianthus annuus* L.).

### INTRODUCTION

One of the major problems that breeders face concerns the difference in the performance of breeding material under experimental and commercial conditions. At the early stages of a breeding programme the material is assessed under low density because of the scarcity of seed for particular families and the number of families being generally high. The shortage of seed also restricts replication and

usually small single row plots 1 or 2 metre long are grown for  $F_3$  and  $F_4$  families. At  $F_2$  level, only individual randomisation is practised because each plant is a separate genotype which has no replicates unless propagated vegetatively.

At later stages of a breeding programme, the above constraints do not apply, because the number of genotypes to be assessed is reduced to only a few most desirable ones and generally there is no limitation of seed. By this stage the families become more homozygous/homogenous also. Consequently their performance is more prone to intra-genotypic competition because they generally compete intensely because they have nearly identical requirements.

Another factor which makes the experimental and commercial performances of genotypes differ is the vast difference between agronomic practices. In early stages, experiments are generally started in the glasshouse or other protected areas where the material is less exposed to diseases and environmental stress such as frost attack, water stress and competition against weeds. Controlled temperatures also make the seed germination more even and consequently the experiments is given the best possible start. Commercial growing, on the other hand, involves direct sowing in the field where the seed may be sown at variable depth/density and also faces the prospect of slow germination and retarded initial growth due to low soil temperature and variable moisture content within the field site.

Sunflower breeding follows exactly the same pattern as above. The genetic/breeding work that has been carried out at Birmingham has employed sowing experiments in the glasshouse which were later transplanted in the field. Generally these experiments were sown in late May and the material was then ready to be transplanted outside during the first week of June. Further, because all the plants were measured individually, most experiments used either individual plant randomisation or small single plots of 5 plants usually at a low density of around 60,000 plants/ha.

Contrary to these situations commercial sunflower is sown around 1st May in the UK at a density of around 120,000 plant/ha. The crop is sown directly in the field and given an early start so that it can be harvested at the beginning of September, before the onset of autumn. A late sown corn is assumed to lose out at the end of season because wet and cold conditions expose the ripening seed to *Botrytis*/*Sclerotinia* attack.

In addition to the breeding work we have also been investigating the effects of factors like plot randomisation and high and low density on the performance of sunflower families. Holtom *et al.*, (1992) compared  $F_6$  families of sunflower under small plot and individual plant randomisation and showed that the trial design had a marginal effect on the performance and ranking of the families. The overall means of the families differed significantly for only two traits (out of 12), namely, height at flowering and seed set. Plot design showed poorer performance in both cases. Similarly, within-plot variation was also found to be significantly lower than within-family variation for the area of seed set and head angle but no differences were detected

for genetic component of variation or heritability or genetic correlation between characters.

Toms & Pooni (1994) obtained similar results for comparisons between experimental and commercial densities and between single row and two row plots. Little difference could be found between the two types of plot or of the density, especially when comparisons were made within the same sowing, suggesting that sowing differences were perhaps more important than those of plot size or density. The present investigation, therefore, was conducted specially to study the impact of sowing time on the performance of sunflower families, measured as their overall stand at the various stages of growth, and their comparative performances for various characters.

## MATERIALS AND METHODS

In 1992, Pooni, Virk & Hussain conducted a sunflower trial in which they raised 66 families derived from an 11 x 11 half diallel set of crosses (Pooni, Virk & Hussain, 1994). This diallel was based on 12 restorer lines that were previously selected by Virk & Pooni (1994) for their good characteristics in respect to maturity and yielding ability. The open pollinated seed of these 66 families were collected at the end of the season and used in all kinds of assessment like seed weight, seed size and oil content. These 66 open pollinated families formed the experimental material for the present study.

Two plots of each family were sown in two sowings, the first on 11th May, 1993 (normal sowing) and the second on 3rd June, 1993 (late sowing). The material was sown directly in the field at a row to row distance of 75 cm and 15 cm between hills within rows. The plots consisted of a single row of 5 plants and both plots and plants within plots were randomised using EXPLAN computer programme. In each hill, 2-4 seeds were thinned to one dibbler. The sowing depth was approximately 2.5-3 cm and the seedlings were thinned to one per hill at the 4-leaf stage. The experiment was scored for the following characters on individual plant basis.

Results of the sowings were compared at several distinct levels using appropriate statistical procedures. All analyses were carried out on the University of Birmingham Computer using various statistical packages as SAS and Minitab PC Version 9.2.

## RESULTS

### **Comparison of overall means**

The overall performances of families in two sowings were compared using Student's 't' test (see Snedecor & Cochran, 1969, for procedure). Comparison of the overall means (Table 1) reveals that most of the critical differences between sowings

Symbol	Description
Height (H6)	Distance in cm from the base to the growing point of the plant after 6 weeks of planting in the field.
Flowering time (FT)	Number of days from sowing to the first anthesis.
Height at Flowering (HFT)	Distance (cm) from the base to the top of the apical bud at the time of flowering.
Head Diameter (HD)	Distance across the apical head at its widest point measured in mm at maturity.
Seed set (SS)	Visual measure of the proportion of the apical head with filled seed scored on a 0-10 scale where: 0=no seed set and 10= complete seed set.
<i>Sclerotinia</i> infection (Si)	Visual score (0 to 10) of the infection on the apical head where: 0=no infection and 10=complete head infected.
Area of Head set (AHS)	Area of head set with seed estimated as: $\pi \left( \frac{HD}{2} \right)^2 \left( \frac{SS}{100} \right)$

are restricted to early measurements. The largest values of *t* are obtained for H6 and FT both of which are developmental characters. Clearly, the normal sowing grew slowly and consequently took seven days more to flower than the late sowing.

In fact, plants in the normal sowing remained shorter than those in the late sowing through their life. They are, on average, 10 cm shorter at the time of flowering. As there is a very high correlation between HFT and final height of sunflower at the end of the season (Virk & Pooni, 1994), one can assume that this difference of 10 cm will persist up to the end of the season. Other differences worth noting are those for head diameter and seed set. There is significantly more seed set in the normal sowing but the head size is marginally smaller. Consequently no critical difference is observed between the means of the two sowings for most seed traits.

Table 1: Comparison of the overall means in the normal and late sowings

Trait	Normal sowing		Late sowing		Diff.	't' value
	Mean	Se	Mean	Se		
H6	22.81	0.35	30.44	0.58	-7.63	7.91***
FT	119.53	0.25	112.23	0.26	7.30	10.22***
HFT	148.01	1.36	158.32	0.81	-10.31	6.99***
HD	131.92	2.15	133.72	2.02	-1.80	0.88 ns
SS	7.42	0.10	6.89	0.11	0.53	2.01 *
Si	3.30	0.21	3.15	0.25	0.15	0.22 ns
AHS	12.09	0.45	11.68	0.43	0.42	0.46 ns

ns=non-significant; \*=*P*<0.05; \*\*=*P*<0.01; \*\*\*=*P*<0.001

#### Effect on variances: ANOVA

Any agronomic factor such as sowing time can affect variation among families at two levels. It can influence the expression of variation between families thus making the between-families component heterogeneous. Equally, variation between plants may also be affected and this can influence the extent of heritable variation in two

sowings. The ANOVA of plots/families and individuals within plots follows a characteristic hierarchical pattern (Snedecor & Cochran, 1969) in which three distinct sources can be identified. These sources correspond to:

- (i) Between families,
- (ii) Between plots within families, and
- (iii) Within plots.

The mean squares corresponding to these three items were obtained from the ANOVA carried out by general linear model fitting, because of the unequal plot size caused by plant losses, presented in Table 2 & 3 for the normal and late sowing, respectively.

Table 2: Hierarchical analysis of variance for the normal sowing

Trait	Between families		Between plots/families		Within plots/fam	
	df	MS	df	MS	df	MS
H6	58	95.15*	41	56.12***	350	24.97
FT	57	142.69***	41	29.55 ns	329	28.47
HFT	57	2198.25***	41	801.33**	329	379.25
HD	54	2074.44*	36	1772.95 ns	225	1458.58
SS	53	4.71**	36	3.30 ns	218	2.84
Si	57	31.05**	39	22.82 ns	293	17.56
AHS	53	95.83*	36	78.28 ns	218	64.55

see Table1 for probability levels

Table 3: Hierarchical analysis of variance for the late sowing

Trait	Between families		Between plots/families		Within plots/fam	
	df	MS	df	MS	df	MS
H6	65	229.94 ns	56	193.49***	450	23.87
FT	64	122.51***	52	38.16 ns	341	31.49
HFT	64	1962.84***	52	397.02 ns	341	300.66
HD	62	1954.28 ns	41	1708.49 ns	249	1438.21
SS	62	6.64 ns	41	5.81 ns	245	3.98
Si	62	23.43 ns	43	23.91*	268	15.04
AHS	62	95.21*	41	87.11 ns	245	64.11

see Table1 for probability levels

One of the main features of ANOVA in these tables is that plot effects are significant mainly for the height measurements. This indicated that micro environmental variation affects the height much more than the other traits. It is also apparent from these analyses that families show more significant differences in normal compared with late sowing. Significant differences between families are observed for all traits except H6 in normal sowing while such differences are detected only for FT and HFT in the late sowing.

A combined ANOVA of the sowings, given in Table 4, shows that the 'between-families' component is highly significant for all the traits. This suggests that the differences between families were perhaps not detected in normal sowing due to smaller family size, which is plausible because variation between families must have been reduced by selection for early flowering and high yield. The non-significance of families x sowings interactions for all except 2 characters further shows that the families do not respond differently to sowing date in any critical manner.

The effects of sowing date on the within-plot variances were tested using Bartlett's test of homogeneity (Snedecor & Cochran, 1969). A significant difference between variances would show that the plants in each sowing show a markedly different response to sowing time as the two sowings were located adjacent to each other and therefore could not be affected much by differences in the soil type or agronomic practices. Bartlett's  $\chi^2(1)$  values comparing within-plot variances of various characters between sowings (Table 5) show that the within-plot variances are statistically the same for 5 out of 7 characters and the differences are restricted to HFT and SS. Further the variance in early sowing is smaller for SS and larger for HFT showing that neither sowing was more homogeneous than the other.

Table 4: Combined analysis of sowings

Trait	Betw. families		Betw. sowings		Fam x Sowing		Plot/fam/sowing		Within plots	
	df	MS	MS (1 df)	df	MS	df	MS	df	MS	
H6	65	203.78***	15230.81***	58	112.81 ns	97	135.43***	800	24.35	
FT	65	223.31***	10632.25***	56	49.82***	93	34.36 ns	670	30.01	
HFT	65	3295.95***	24585.51***	56	668.36***	93	575.26***	670	339.25	
HD	64	2279.37***	1254.10 ns	52	1664.50 ns	77	1738.62 ns	474	1445.78	
SS	64	6.82***	45.11***	51	4.60 ns	77	4.64*	463	3.44	
AHS	64	119.63**	1.53 ns	51	71.58 ns	77	82.98 ns	463	64.32	

See table 1 for probability levels

Table 5: Test of heterogeneity of within-plot variances

Character	$\chi^2(1)$	Significance
H6	0.20	ns
FT	0.85	ns
HFT	4.49	*
HD	0.01	ns
SS	6.45	*
Si	1.66	ns
AHS	0.00	ns

See table 1 for significance level

### Components of variances

The main component of importance in the present context is  $\sigma_f^2$ , the between-families variance. Furthermore, its importance generally lies in estimating the heritability for each character. In the present case, however, it will be more appropriate

to estimate the intra-class correlation 't' (Falconer, 1989) which can be converted into heritability assuming that the families are a set of half sib families whose mother is known but the pollen parent(s) is not. The intra-class correlation was calculated for each sowing as follows:

$$t = \sigma_f^2 / (\sigma_f^2 + \sigma_p^2 + \sigma_w^2)$$

where:

$\sigma_f^2$  = between-families component,  $\sigma_p^2$  = between-plots component and  $\sigma_w^2$  = within-plot component. An equivalent estimate of the same statistic was obtained from the combined ANOVA of the two sowings as:

$$\frac{\sigma_f^2}{\sigma_f^2 + \sigma_{fs}^2 + \sigma_p^2 + \sigma_w^2}$$

where  $\sigma_{fs}^2$  represents interaction between families and sowings. Harmonic estimates of plot size and family size were used to calculate these  $\sigma_s^2$  from the MS given in Tables 2, 3 and 4. These estimates of t in Table 6 reveal that the extent of genetic variation observed in the experiment varies both across traits and across sowings.

Table 6: Intra-class correlations for the normal, late and combination of sowings

Character	Normal sowing	Late sowing	Combined
H6	0.14	-	0.06
FT	0.35	0.22	0.25
HFT	0.22	0.34	0.27
HD	0.06	-	0.05
SS	0.06	-	0.05
Si	0.07	-	-
AHS	0.04	0.05	0.08

ns genetic variation ns

Table 7: Correlation between family means ( $r_{\bar{x}}$ ) and rank correlation ( $r_{rank}$ ) for various traits

Character	$r_{\bar{x}}$	$r_{rank}$
H6	0.33	0.36
FT	0.55	0.45
HFT	0.63	0.53
HD	0.20#	0.27
SS	0.25	0.22#
Si	0.33	0.36
AHS	0.33	0.46

significant except when marked #

### Correlation between sowings

The impact of genotype x sowing interaction on family performance is further investigated by calculating Pearson's correlation based on the family means and correlation between their ranks (1=the lowest, 66=the highest) for each character



(see Steel and Torrie, 1980, for procedure). All correlations are significant and positive (Table 7), suggesting that a good agreement exists between the family means across sowings for all the traits (Table 7). This is further supported by the rank correlations which take similar values for all the characters. But the magnitude of correlations is mostly low, which indeed supports the low values of intra-class correlations listed in Table 6, *i.e.*, the genetic variation is low among the families.

### Correlations between characters

Characters often show correlated variation due either to genetic or environmental causes. Environmental correlations are caused when the same environmental factor affect more than one character simultaneously. This type of correlation is clearly observed among the individuals of inbred lines or of  $F_1$  hybrids. In other generations, such as  $F_2$  or back crosses, their correlations contain both genetic and environmental parts. Correlations between families, on the other hand, are due primarily to genetic causes particularly when family size is large and heritability is high (Falconer, 1989). In the present study, correlations between family means can be expected to differ in the two sowings particularly when the effect of sowing is large and it modifies the genetic relationship between traits.

Correlations between family means in Table 8 reveal that all characters are critically associated with each other except H6 & Si, HD & Si and SS & Si. Further, all significant correlations take positive sign except those between Si and other traits. It is further evident that *Sclerotinia* attack is highly influenced by the time of flowering and the height of the plants; *i.e.*, early flowering and short plants are attacked more often than tall and late flowering ones. Negative correlation between H6 and FT confirms the general rules of biology *i.e.*, fast growing plants flower earlier than the slow growing ones. The remaining correlations simply show that tall plants are also generally late flowering and have larger heads with more seed set.

Comparison of the sowings, on the other hand, reveals that the frequency of significant correlations does not differ between sowings; *i.e.*, 13 correlations are significant in normal sowing and 14 in late sowing. However, there are some subtle differences between the sowings in that H6 is more strongly correlated with other traits in the late sowing and the opposite is perhaps true for FT in the normal sowing.

When tested using the Z transformation (Fisher & Yates, 1963) most of the correlations differ significantly between sowings, except that of FT/HFT. When tested against a universal error of

$$\pm 0.18 = \sqrt{\frac{2}{(n-3)}}$$

the Z values (0.95 & 0.59) differ just at 5%. However, because it is only one significance out of 21, it falls well within the 5% margin of error.



Table 8: Correlation between characters in the normal (upper) and late (lower) sowings

Trait	FT	HFT	HD	SS	Si	AHS
<b>H6</b>	-0.21 ns	0.16 ns	0.45***	0.25 ns	0.16 ns	0.45***
	-0.34**	0.40***	0.41***	0.46***	0.03 ns	0.39***
<b>FT</b>		0.74***	0.37***	0.17 ns	-0.74***	0.36***
		0.53***	-0.4 ns	-0.03 ns	-0.62***	0.03 ns
<b>HFT</b>			0.49***	0.15 ns	-0.62***	0.47***
			0.27*	0.36**	-0.55***	0.31**
<b>HD</b>				0.60***	-0.22 ns	0.95***
				0.66***	-0.12 ns	0.96***
<b>SS</b>					-0.24 ns	0.71***
					-0.17 ns	0.74***
<b>Si</b>						-0.31*
						-0.21 ns

See table 1 for probability levels

Table 9: The minimum, maximum and range among the means for the normal and late sowings

Trait		H6	FT	HFT	HD	SS	Si	AHS
Minimum score	Normal	15.00	104.40	78.00	92.00	5.00	0.00	44.77
	Late	17.00	100.43	69.71	90.00	4.00	0.00	21.52
Maximum score	Normal	32.60	130.75	196.33	187.00	9.00	10.00	253.10
	Late	41.60	127.00	183.40	180.00	8.71	8.75	311.10
Range	Normal	17.60	26.35	118.33	95.00	4.00	10.00	208.95
	Late	24.60	26.57	113.69	90.00	4.71	8.75	261.61

## DISCUSSION

The significant differences that were observed between the overall means of the two sowings can arise due to several reasons. Although the two sowings were located side by side to minimise soil and other environmental differences, such effects, however, can never be ruled out entirely. Similarly inter-plant competition may also differ between sowings, especially when the plants of normal sowing will have plenty of time to grow while those of late sowing will have 23 days less to do so. In the present case, however, some of the differences must be attributed to the major effects of sowing date because they are too large to be accounted for by other minor differences associated with interaction and macro-environmental factors.

One of the most important differences is for flowering time (Table 1). Although the data give the impression that the late sowing flowered significantly earliest, which is of course true in terms of number of days, the normal sowing flowered during the first week of September while the late sowing flowered around 20th of

September. The late sowing is therefore exposed to a higher risk of bad weather at the end of season and therefore will fail more frequently than the early sowing.

Differences in the height of plants, on the other hand, reflect the genuine influence of improved weather later on in the season which makes plants vegetatively more vigorous and increases their growth rate.

Another important impact of sowing date has been on the expression of genetic variability. Less variation is expressed in the late sowing where the families have shown non-significant differences for 4 out of 7 traits scored. The between-plot and within-plot variances, on the other hand, were similar (Tables 2, 3 and 5) across the sowings suggesting that the experimental conditions did not differ greatly. These apparent differences that we have observed between the sowings are also reflected in the combined analysis where significant families x sowings interaction is detected for 2 traits. Furthermore, the intra-class correlations (Table 6) are much smaller for the combined analysis, even for those traits for which significant genetic effects were detected in both sowings. The sowings show no plausible differences for the other statistics such as family mean correlations and the range of extreme scores (Tables 7, 8 and 9). The correlations across sowings were generally low, mainly because the heritable variation is low in the material and secondly due to the families x sowings interaction which is detected for two characters (Table 4).

## CONCLUSIONS

The main conclusion of the present study is that only early sown sunflowers are likely to be commercially successful in this country. Although late sown crop catches up in growth it is unlikely to be successful because it will always suffer losses due to frost and bad weather during October. It also follows from this that early maturing varieties are a must for sunflower to be established as a minor crop in the UK. Any variety which matures after the second week of September, *i.e.*, takes more than 130 days from sowing to harvest, will be too risky to grow because its harvest will always be in doubt.

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## **EFFECTO DE LA FECHA DE SIEMBRA SOBRE EL COMPORTAMIENTO DE FAMILIAS DE GIRASOL EN CONDICIONES BRITANICAS**

### RESUMEN

Para investigar su respuesta genética bajo dos fechas de siembra 66 familias fueron evaluadas en la Escuela de Ciencias Biológicas, Universidad de Birmingham, Inglaterra. Los resultados mostraron diferencias significativas para fecha de floración, altura en floración y área del capítulo con semilla llena en ambas fechas de siembra. Sin embargo, la mayor variación genética significativa se detectó en siembras normales comparadas con siembras tardías.

Un efecto significativo de la interacción genotipo fecha de siembra sugiere que existe una buena relación entre la media de familias en las diferentes siembras para todos los caracteres. Una magnitud baja de las correlaciones, indicó sin embargo que existe una baja variación genética entre familias.

La correlación negativa entre H 6 y fecha de floración reveló que las plantas precoces y bajas florecieron más temprano. Una asociación crítica entre tiempo floración e infección de *Sclerotinia*, una enfermedad epidémica del girasol en los países de la Unión Europea (EC), evidenció una vez más que las plantas precoces y bajas son más atacadas que las familias altas y tardías. Por tanto, la siembra normal durante la primera quincena de mayo es la fecha más óptima para obtener buenos resultados ya que se espera la maduración antes del comienzo del clima húmedo y severo de septiembre.

## **EFFET DE LA DATE DE SEMIS SUR LA PERFORMANCE DE FAMILLES DE TOURNESOLS EN CONDITIONS BRITANNIQUES**

### RÉSUMÉ

On a étudié 66 familles de tournesol à l'Ecole des Sciences Biologiques, Université de Birmingham, Angleterre, pour leur réponse génétique à deux dates de semis différentes. Les résultats montrent des différences significatives pour la période de floraison, la taille à floraison et la surface du capitule portant des graines dans les deux dates de semis. Cependant, des effets génétiques plus significatifs ont été détectés dans le semis normal comparé au semis tardif.

Des effets d'interaction significatifs (génotype x semis) suggèrent qu'il existe une bonne concordance entre les moyennes des familles dans les différents semis, pour tous les caractères. Une faible valeur des corrélations indique, pourtant, qu'une variabilité génétique limitée existe entre les familles.

Des corrélations négatives entre H6 et la période de floraison révèlent que les plantes à floraison précoce et taille courte fleurissent avant les plantes à fort développement. L'association entre la période de floraison et l'infection par le *Sclerotinia*, maladie épidémique du tournesol dans les pays de la CE, renforce l'idée que les plantes à floraison précoce et courtes sont plus attaquées que les plantes des familles à taille élevée et tardives. Ainsi, le semis normal durant la première quinzaine de Mai correspond à la période optimale pour obtenir une bonne récolte car la maturité est atteinte avant l'arrivée des conditions très humides de Septembre.

