A study of gene action on erucic acid inheritance in selected geographical strains of oriental mustard (Brassica juncea, L. Cosson.)
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A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in GENETICS
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Abstract:
Erucic acid is a single unsaturated fatty acid with 22 carbon atoms. This has many potential commercial uses. It is found in varying quantities in different strains of Brassica juncea (L.) Cosson. This study was directed towards studying gene action relative to the inheritance of erucic acid content in the oil of B. juncea. General and specific combining abilities were to be estimated with a view to determining the future breeding program for increasing erucic acid content in commercial oriental mustard.

A modified method of micro-quantitative analysis was employed for oil extraction and esterification of seed oil. The gas liquid phase chromatograph provided the tool for the separation and quantitative study of fatty acid composition.

The effect of date of maturity on erucic acid content of seed oil was estimated from two years data. Results indicated that seed maturity was not a major factor in determining the erucic acid content of seed oil.

The effect of different climatic conditions on erucic acid content of seed oil was studied by analyzing one strain of B. juncea (oriental yellow mustard) grown at nine locations in 1960 and three Brassica accessions grown under seven different climatic conditions in 1962.

Results of analysis indicated that climatic differences influenced erucic acid content of the seed oil and that the effect was not uniform for different strains at different locations. The magnitude of the effect was not sufficiently great to interfere with the determination of major genetic differences.

Seventeen strains of Brassica differing in erucic acid content were selected for a study of erucic acid inheritance. Selfed and crossed seed from ten parental lines in three replications were analyzed for fatty acid composition. Erucic acid values were analyzed statistically for a study of gene action by correlation and regression and also by a method of analysis of variance suggested by Hayman (25). Results of statistical analysis indicated the presence of significant additive variance in these lines. Variance due to interaction was non-significant but there was evidence of the presence of dominance gene effects due to certain lines. Crosses involving one of ten lines exhibited large reciprocal differences indicating the presence of maternal effects. Further analysis by a method suggested by Sprague and Tatum (64) indicated that certain lines had high or low general combining ability and other lines combined high general and specific combining ability. Further testing would be necessary to determine the most effective parents and methods for a breeding program.

A STUDY OF GENE ACTION ON ERUCIC ACID INHERITANCE IN SELECTED GEOGRAPHICAL STRAINS OF ORIENTAL MUSTARD (*Brassica juncea*, / L./ Cosson.)

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A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

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ABSTRACT

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INTRODUCTION

Search for a new crop which could utilize acres not needed for food and fiber production is occupying the time of many researchers. This new crop must possess some unique quality with respect to either protein, starch or fat in order that it may be used as a source of raw material by industry. Chemical analysis in the past has indicated the presence of a typical fatty acid, erucic acid, in varying quantities, in the oils of seed of plants belonging to the family Cruciferae.

Erucic acid is a monoene of 22 carbons. The long unsaturated carbon chain makes it a potential raw material for conversion into different short chain carbon substances. At present it would seem that a fat source must contain more than 50 percent erucic acid for economic commercial extraction and utilization. Seed oil from crambe, which belongs to the same plant family Cruciferae, has been found to contain 45 to 55 percent erucic acid.

Strains of oriental mustard (Brassica juncea L.), which are commercially grown in the United States and are well adapted for high seed yields and high fat percentages, contain fats with low erucic acid content (from 20 to 25 percent). Oil from some of the related strains of B. juncea from the world collection have been found to contain erucic acid up to 50 percent. This source could possibly be used to transfer the gene or genes for erucic acid content to the commercial varieties of B. juncea.

Commercial requirements of the presence of at least 50 percent erucic acid presently favor the selection of crambe as a potential commercial crop even though it is relatively low yielding, both in respect to oil content and seed, and is not well adapted to dryland production.

At this point there would seem to be two alternatives, (1) accept crambe and work to improve yield, drouth resistance, and oil percentage and discard commercial varieties of B. juncea, which are known to possess these characters to a greater degree, or (2) locate additive genes for erucic acid in available strains of B. juncea and transfer them to adapted commercial types of B. juncea.

The present series of experiments were designed to study the mode of gene action for erucic acid inheritance in strains of B. juncea of differing geographical origin.

A minimum of information on procedure was available at the time of initiation of this undertaking in 1960. It was necessary to first determine the effects of such factors as environment and maturity on erucic acid content and to develop a method for rapidly extracting and analyzing very small samples of oil for erucic acid content. These necessary experiments of a preliminary nature are made a part of this thesis.

The technique of diallel cross analysis was selected as the method to be employed in evaluating the mode of gene action.

General and specific combining ability of the different lines was

to be investigated in an effort to determine the feasibility and method of a future improvement program.

LITERATURE REVIEW

I. Fats, Fatty Acid Composition and Chemical Determination.

Fats obtained from seeds are in the form of triglyceride esters of fatty acids. Three molecules of the fatty acids are associated with one molecule of glycerol with ester bonds. These three fatty acids may be alike or different, as reported by Eckey (9), Markley (43), Jamieson (34) and Kirchenbauer (42). In general, fatty acids found in plants have an even number of carbon atoms and may be saturated or unsaturated.

The mechanism of fat synthesis in the plant is not known but it is thought that triglycerides, being insoluble, are actually synthesized in the parts where they are found.

Garner and Allard (15) noted that in peanut, soybean, cotton and safflower seed fat synthesis does not set in during the early stages of seed development but occurs about midway between blooming and final maturity of the seeds.

Mohamad and Sultan (47), working with B. campestris L., noted that rapid oil formation started 20 days after fertilization and continued for the next 20 days.

Jones (37) and Jones and Shaw (38) noted a similar phenomenon in Macadamia integrifolia, Maiden et. Betche. Very little oil formation in the beginning was followed by a period of rapid oil synthesis, followed again by a slower period of oil synthesis. A decrease in reducing sugars was associated with the increase in oil content.

Eckey (9) quoted several workers who studied oil formation in

walnuts, almonds and linseed. These workers found that a decrease in carbohydrate content occurred concomitant with a rapid increase in oil content.

Based on these findings, as well as those of Thor and Smith (quoted by Eckey) with pecan, it was concluded that the amount of carbohydrate involved was too small to account for the appreciable amount of fat formed. Eckey (9) suggested that much of the oil formed in the seed is from materials translocated from other parts of the plant. Eckey (9) and Kirchenbauer (42) suggested that probably the carbohydrates synthesized by the plant as a result of photosynthesis are translocated to the area of synthesis where the carbohydrates are converted to fat.

Esau (10) mentions development of a thick integument layer in the Cruciferae which consists of outer and inner integument each consisting of several cell layers. The outer integument serves as a protective covering whereas the inner integument serves as an area of starch storage during seed development. Hokansson (20, 21) traced the development of seed in B. oleracea and B. rapa. From his studies he concluded that embryo development takes place in eight stages. During the beginning stage, the endosperm starts growth immediately after triple-fusion and continues growth for 14 days. The endosperm surrounds the young embryo for a short period. Fourteen days after fertilization the endosperm starts diminishing and by 21 days the sporophyte tissues (integuments) as well as the endosperm disappears. The embryo grows and by 28 days the cotyledons are fully developed. The cotyledons are the fat storage tissues of the Brassica seed.

He further suggested the indication of three time periods in post fertilization development:

(1) The first period of about ten days during which an increase of sporophyte tissue, especially the inner integument, takes place. This is the location of starch storage.

(2) The second period of vigorous development of endosperm, accompanied by a diminishing of the inner integument.

(3) The third period of rapid growth of the embryo and development of cotyledons and formation of oil.

This suggests that starch is first stored in the integuments followed by its use in the manufacture of endosperm tissues. The endosperm supplies the nutrients for the growing embryo and development of the cotyledons.

Yermanos (72) suggested that fatty acid composition in oils from flax is controlled by the genotype of the seed. This would be expected on the basis of the evidence that fats are synthesized after fertilization and in tissues formed as a result of fertilization and subsequent embryonic development.

Erucic acid is a characteristic fatty acid component of Cruciferae oil and constitutes a major portion of the fatty acids in the oils of the genus Brassica.

It is monoethenoid acid, also termed as monoene, and has 22 carbons. A monoethenoid acid contains two less hydrogen atoms than the corresponding saturated acid. The empirical formula of erucic acid is $C_{22}H_{42}O_2$ $\left[CH_3(CH_2)_7CH{=}CH(CH_2)_{11}\text{-}COOH\right]$. According to Markley (43) its molecular

weight is 338.5, neutralizing value 165.7, and iodine value 75.0. To date it has been found in only two plant families, the Cruciferae and Tropacolaceae.

Seeds from plants of Brassica species, viz. B. nigra (L.) Koch, B. campestris, B. juncea and B. napus L., have been analyzed for fatty acid composition at various times by different workers. Eckey (9) reported analysis of several samples of B. napus with an observed erucic acid content of 45 to 55 percent.

Hilditch (29, 30) reports 50 percent erucic acid in samples of rape seed and 50 percent in black mustard. Mehlenbacher (45) reports 45 to 54 percent erucic acid content in oils of B. napus, 40.6 percent in B. nigra and 38.7 percent in B. campestris. Mikolajizak et al. (46) have reported erucic acid content in oils from different sources of Brassica as follows: 31 percent in B. campestris, 43 percent in B. nigra, 36 percent in B. oleracea, 37 to 42 percent in B. carinata Braun., 22 percent in B. juncea and 44 to 50 percent in B. napus. Steffanson (66) has isolated one variety of rape (B. napus), completely devoid of erucic acid in the seed oil. Chemical analysis of seed oils from different strains of B. nigra, B. campestris and B. juncea performed by the Montana Agricultural Experiment Station indicated differences in erucic acid content among these species and among strains within species.[1/]

[1/] Quarterly Report Nos. 1-12: Investigation on fatty acid composition of the oil from Brassica and related genera of mustard seed. Prepared by Dr. K. Goering.

Craig and Wetter (3), and Craig (4) studied the influence of environment on fatty acid composition of rape seed. Their studies indicated that soil and moisture differences significantly influence erucic acid content.

Harvey (23) studied the inheritance of erucic acid content in B. napus L. In his studies he used one variety of rape which was free of erucic acid to cross with another having a high percentage of erucic acid. He determined gene action by studying F_2 and F_3 progenies. From his studies he concluded that erucic acid content was controlled by two additive genes and that each gene had an equal effect. He found no evidence of dominance or maternal effects.

Soxlet's method of oil extraction is commonly used for quantitative determination of total oil percentage. This method is quite accurate for quantitative determinations but it takes approximately 18 hours for complete extraction, and a minimum of ten grams of seed. This method is not readily adapted to the extraction of oils from smaller quantities of seed for qualitative purposes. Mehlenbacher (45) suggested grinding seed in a Waring blender and extraction of oil from the crushed seed by ethyl ether or other suitable solvent. This method requires a relatively large sample for grinding in the blender. The "Swedish method" suggested by Tröeng (69) consists of grinding five g. of seed in a metallic tube by metallic balls in the presence of Skelly F solvent. A large number of samples can be handled by this method in a comparatively short time. Harvey (22) used a Carver laboratory press equipped with a plunger and

cylinder and pressure gauge to crush the seed at a constant pressure. The maximum pressure should not exceed 2500 pounds per square inch. Traces of oil present on the top of the press are washed with an excess of Skelly F into a beaker, filtered, and evaporated on a steam bath under vacuum. This method is rapid and makes possible extraction of oil from small samples.

The general process of trans-esterification for determination of fatty acid composition consists of refluxing one g. of oil with 100 ml. of methanol and 10 ml. of sodium methoxide for about six hours. This is followed by a second process of trans-esterification by treatment with diazomethane. The complete process requires eight to ten hours. The amount of methyl esters obtained is usually sufficient for several injections.

Harvey (22) used two ml. of two percent acetyl-chloride in methanol for trans-esterification of oils from single seeds. He found refluxing for 30 minutes to an hour was sufficient for complete esterification. Acetyl-chloride serves as a catalyst in this method as contrasted to sodium methoxide in the method described above. The amount of methanol is dependent on the amount of oil. If proper care is taken, sufficient esters are obtained from a few seed for injection into the gas chromatograph.

Gas liquid phase chromatography is similar to other systems of chromatography. Gas liquid phase chromatography also utilizes a system of two phases, one stationary phase which serves as the adsorbing or

partitioning agent and another, moving phase, which serves as a means of transport of material for separation. In gas liquid phase chromatography, the stationary phase is a liquid which is generally distributed over an inert solid support in order to give it a large surface area for exchange, (41). The moving phase is a gas.

Under difinitely set operational conditions, the retention value or retention time which is characteristic of a certain component is recorded on a graph.

Gas liquid phase chromatography has been successfully used for the analysis of fatty acid composition of seed oils from Brassica by Harvey (22, 23), Mikolajizak, et al. (46) and is also used in the analytical work at the Montana Agricultural Experiment Station.

II. Brassica juncea and its Relation to Other Species of the Genus Brassica.

The nomenclature of forms within the genus Brassica has been quite confusing in the past owing to the morphological similarities within the species and also due to some cross compatability among some species. Sinsakaia (63) tried to classify the species of Brassica according to chromosome number but still there remained some confusion in the B. campestris group as well as the B. napus group. Pearson (57) stated that all members of the ten chromosome group were closely related and should be classified as one species. His classification was based on chromosome number. Due to previous incorrect cytological investigations, he grouped B. juncea and B. napus as one species although he recognized

morphological differences in these two forms and noted that they did not intercross easily.

Moringa (48, 49, 50), after a series of cytological, morphological, and interhybridization studies, subdivided the genus into six main groupings. He indicated that the three forms with the lowest number of chromosomes possess the basic genomes, i.e. B. campestris (n = 10) - genome A; B. nigra (n = 8) - genome B; and B. oleracea L. (n = 9) - genome C; whereas, the other three forms B. juncea (n = 18); B. napus (n = 19); and B. carinata (n = 17) are amphidiploids having the genome constitution AB, AC, and BC, respectively. Work of U (70), Sikka (61, 62), Sun (67, 68) and Crane (5) gave further evidence to Moringa's groupings.

A wide variety of forms differing in general morphology are present in almost all the species of the genus Brassica. Given on the following page are certain distinguishing characteristics which have been suggested by Moringa (50), Sabnis (60), Sun (67, 68) and Musil (52) as useful key characters for differentiating species.

	Brassica species					
	campestris	nigra	juncea	carinata	napus	oleracea
Leaf characters:						
clasping	yes	no	no	no	no	no
leathery	no	no	no	yes	yes	yes
leaf shape	wide at base	short ovate	long	-	-	-
hairs	few marginal	sparingly and stiff	few marginal	glaucus	few marginal	glaucus
color	bright green	light green	bright green	blue green	blue green	blue green
Pod characters:						
length, mm.	30	11	29	-	48	-
beak length	long	short	medium	short	-	-
shape	flattened	angular	slightly flattened	flattened	-	-
position relative to raceme	-	appressed	spreading	appressed	appressed	-

Moringa (50) and U (70) proposed a possible explanation for the amphidiploid origin of B. juncea, B. napus and B. carinata. They suggested that interspecies hybridization in nature followed by doubling resulted in the formation of the amphidiploid. In crosses between B. campestris and B. oleracea, U (70) obtained a very low seed set but among hybrids there was one amphidiploid (2n = 38) which was phenotypically similar to B. napus.

Subsequently, the statement of Moringa (50) and U (70) regarding the amphidiploid origin of B. napus, B. juncea and B. carinata has been verified by many workers.

Howard (32) obtained napus forms as a result of crossing autotetraploid forms of B. chinensis L. and B. oleracea.

Frandsen (14) also obtained B. pseudo-napus by crossing colchicine induced tetraploid forms of B. campestris and B. oleracea. This pseudo-napus had fairly regular meiosis and crossed freely with natural occurring forms. Similarly, B. pseudo-napus has been obtained by Olsson (54, 56) using different forms from the campestris and oleracea group. Synthesis of winter-hardy rape by crossing tetraploid forms of B. campestris and B. oleracea has been accomplished.

Synthesis of pseudo-carinata has been achieved by Frandsen (14). The fertility of this amphidiploid was not very high but it had fairly regular meiosis and crossed freely with natural B. carinata.

B. juncea has been artificially synthesized by intercrossing different forms of B. nigra with different forms of B. campestris. Frandsen (13) synthesized B. juncea by crossing tetraploid forms of the two species, B. nigra and B. campestris. Howard (31, 32), Ramanujam and Srinivasachar (59), and Olsson (55) successfully isolated artificial B. juncea from hybrids between tetraploid forms of B. nigra and B. campestris. The pseudo-juncea resembled the natural B. juncea in morphological characters and intercrossed freely with the natural forms.

Dobzhansky (7) points out that different geographical races or

strains of allopatric organisms have originated as a result of environmental diversities and as a result of natural selection. These allopatric populations respond to environmental differences by genotypic differentiation. He defines geographical races as genetically distinct allopatric races. Diversities among allopatric organisms are much greater than among sympatric organisms. There is always the probability of finding diverse gene complexes in different geographical races or strains, which may be true in case of the strains under consideration.

Olsson (53), based on the result of the hybridization experiments between morphologically different forms of B. napus of diverse origin and on the result of cytological examinations, suggested that this species originated in different regions by intercrossing of B. campestris and B. oleracea on different occasions. B. juncea and B. carinata types may have originated in a similar manner. Gates (16) suggested that many new types have evolved in the genus Brassica at different times and places.

Allard (1) has discussed different types of incompatability mechanisms operating in self and cross fertilized plants which does not fit in the present situation. Pearson (57), Hokansson (20, 21) and Downey (8) found reciprocal differences in incompatability mechanisms. They report instances of interspecies hybridization of crosses involving diploid and tetraploid individuals. In general, they found better seed set when the individual strains with higher chromosome numbers were used as the female parent than in the reciprocal crosses. Müntzing (51)

suggests that disturbances in the hybrid seed development may be due to a change in the relationship between maternal tissues, endosperm tissues, and embryo tissues which normally have the respective chromosomal relationship of 2n : 3n : 2n. Hokansson (20) actually observed that quantities of endosperm and starch formed in reciprocal crosses of 2n x 4n types in barley were different. This could, in turn, have an influence on embryo development.

III. Estimation of Components of Variation in Quantitative Inheritance Studies.

Quantitative characters which exhibit continuous variations are, in most cases, controlled by a number of genes acting as a gene block. These genes may have an additive or complementary effect or the genes may be interacting to produce a phenotypic variation. It is not possible to separate the effects of individual genes in such cases, yet, measurement of the combined effect in appropriate materials may give a general idea of the genetic situation involved.

Another type of continuous variation may be found to be controlled by single genes. This type of continuous variation is due to different degrees of gene expression in different environments. Allard (1) believes that if environment is held constant for all the progenies of such monogenic hybrids, segregation into discrete classes should occur.

A study of continuous variation requires a statistical approach and no single method has been devised which will apply to all genetic situations. Different genetic models have been devised and methods

suggested to analyze the genetic situations characterized by a particular model. In all these situations, the model is usually much simpler than what may be the actual situation.

Fisher is considered to be the forerunner of modern biometrics. In his paper of 1918 (11) he presented a gene model which included dominance effects at a single locus. He stated that there may be a deviation from the simple additive effects between loci, similar to dominance at one locus, if more than one locus affected the genetic character. He called this deviation epistacy, and introduced the term dual epistacy to indicate the interaction of all possible pairs of loci affecting the gene character. In this paper he discussed the possibility of the study of the accumulated effect of additive and interacting factors. He suggested that the heritable variances observable among any group of organisms may be regarded as a sum total of the variance due to individual factors. The proportion of heritable variance may be easily estimated from covariance or the mean products of the measurements of related individuals so that without being able to recognize any single factor, we have a direct means of estimating their total contribution to heritable variance. This concept provided the basis for the development of techniques for measurements of heritability in animal breeding.

In a subsequent paper Fisher et al. (12) discuss the effect of dominance and epistasis and describe what they call third degree statistics to measure the degree of dominance. F_2 and F_3 population data are required.

Fisher's suggestion was quite useful. Hull's (33) paper may be thought to be an extension of the work of Fisher. Hull (33), Griffing (17) and Kempthorne, et al. (40) used techniques of regression analysis for estimating heritability and to study inheritance of quantitative characters.

Similarly, Powers (58) studied the inheritance of quantitative characters by means of correlation and regression in tomatoes. Sprague and Tatum (64) evolved a method for evaluating general and specific combining ability for a set of single crosses in corn. Specific combining ability was used to measure heterosis effects.

Mather (44) developed statistical methods or formulas for partitioning components of variation under a given set of conditions. His genetic model is similar to that of Fisher (11). He proportioned the observed variations into three main components which he describes as:

E - Nonheritable - environmental

H - Genetic - unfixable (dominance or epistatic)

D - Genetic - fixable (additive)

Yates (71), about the same time, suggested a method of analysis of data from diallel crosses and also developed techniques of analysis of variance for testing reciprocal differences in diallel crosses.

Techniques developed by the above workers provided a foundation for the development of a much stronger analytical method for separation of components of variation in a given genetic environment. This analytical method is generally referred to as "Diallel Cross Analysis".

A diallel cross is taken to be a set of homozygous lines with a complete set of F_1 progeny including a total of n^2 individuals or families (depending on whether F_1 or later generations are employed).

The method of analysis and interpretation of results is dependent on the following conditions:

(1) Use of only one set of F_1 progenies or both reciprocal.

(2) Use of F_1 progenies (both reciprocals or single) plus the inbred parents.

(3) Nature of inbred parents, that is: (a) If they are random inbred parents from a random mating population, then genetic interpretation can be safely drawn with respect to the population as a whole. (b) If the inbred lines selected are non-random from a population, then the inferences drawn can be valid for only the particular population of inbreds.

(4) If F_2 and F_3 and backcross generations can be included in the study, extra information may be obtained, linkage may be determined and the number of alleles estimated, etc. Some loss in the precision of the experiment may occur due to inadequate population size.

Methods of analysis suggested by different workers from time to time may be categorized into three main approaches:

(1) Kempthorne (39) and Anderson et al. (2) defined a genetic environment characterized by a random mating population at equilibrium. If inbred lines of a diallel cross represent random samples from this population, then the genetic mechanism as studied from the analysis of

the diallel cross may be used to define the population as a whole. This approach is quite rigid. Kempthorne developed methods for estimating components of variation for inbreeding populations and also extended it to populations with partial inbreeding.

(2) Hayman (24, 25, 26, 27) and Jinks (35, 36) have proposed methods of analyses that are not as rigid regarding random selection of inbred lines. The inbred population may be selected for certain characters and accordingly they suggest methods to estimate components of variation under a given set of conditions. These components are descriptive of a population from which the selected lines might have been sampled. The components give an approximate description of the genetic situation in the given set of inbred lines.

In this kind of situation any particular line may be added or removed from the general diallel table without any effect on the final interpretation of the result, as stated by Dickinson and Jinks (6).

(3) The third approach to diallel cross analysis is that of Griffing (18, 19), who estimates the general and specific combining ability of the inbred lines and interprets the combining ability in terms of gene action. Griffing has suggested methods for determining the components of variance after calculating the combining ability.

These three approaches to diallel analysis are based on certain assumptions which characterize particular situations, discussed by each author. Methods have been extended further to apply to other situations. Studies of F_2 and F_3 or backcross generations have been used in the

diallel analysis to estimate parental homozygosity by Dickinson and Jinks (6) and for the study of linkage by Hayman (28).

MATERIALS AND METHODS

I. Collection of Plant Materials.

Seventeen geographical strains of B. juncea originally from ten different geographical regions were selected and obtained from different sources for the present study. Their place of origin, description of the type, and source of seed for this study are shown below:

	Identification	Place of origin	Seed source
1.	Fordhook Fancy	United States	W. Atlee Burpee & Co.
2.	Florida Broad Leaf	"	"
3.	Large Smooth Leaf	"	"
4.	Chinese Broad Leaf	"	R. H. Shumway, Seedman
5.	P.I. 173847	India	Regional Plant Introduction Station, Pullman, Washington
6.	P.I. 183920	"	"
7.	P.I. 175067	Nepal	"
8.	P.I. 207465	Afganistan	"
9.	P.I. 169077	Turkey	"
10.	P.I. 169085	"	"
11.	P.I. 179192	"	"
12.	P.I. 195552	Ethiopia	"
13.	Japan No. 91	China	Dr. Roebbelen, Goettingen, Germany, through Dr. J. R. Schaeffer
14.	Tibet No. 96	Tibet	"

Identification	Place of origin	Seed source
15. Montana Brown	Europe	Prof. R. F. Eslick, Original seed.
16. Oriental Yellow	Orient	" (60-9220-12)
17. (P.I. 173847 Sel.) B. juncea	India	" (60-8870)

These 17 strains were originally identified as B. juncea by the source from which the seeds were obtained. Some discrepancies have been noted. Percentage of oil, iodine number, and fatty acid composition in the seed of the 17 strains of Brassica selected for study of erucic acid content of the seed oil are presented in Table I.

II. Hybridization.

In the spring of 1961, nine seeds from each of the 17 strains were seeded in three replications in hills three feet apart. On emergence they were thinned to one plant per hill.

On initiation of flower buds, one inflorescence from each plant was bagged with kraft paper bags to insure self pollination. Care was taken to clip off the flowers which had opened prior to bagging.

In addition to selfing, every plant in the replicate was crossed to each other plant, i.e., all possible combinations, so that each plant served as a pollen donor for the rest and also received pollen from each of the other plants in the replicate. Summarily, a diallel series with reciprocals was established in triplicate.

Emasculations and pollinations were preferably done in the morning

Table I. Oil percentage in the seed and iodine number and fatty acid composition of the oil from 17 strains of *Brassica* selected for study of erucic acid content of the seed oil.

Identification	No. of samples averaged	Oil percentage	Iodine No.	Percentage of some of the fatty acids in oils of different strains of *Brassica*.					
				C_{16}	C_{18}	C_{20}	C_{22} Saturated	C_{22} Unsaturated	C_{24}
P.I. 169077	2	33.6	118.5	4.2	61.2	14.0	0.8	19.7	0.4
P.I. 169085	2	34.3	119.1	4.0	60.4	14.0	0.9	20.6	0.6
P.I. 179192	3	34.8	117.2	4.1	55.5	16.8	0.8	21.9	1.3
Brown Seeded *B. juncea*	2	35.8	106.6	3.2	50.6	11.8	0.8	23.5	0.4
Oriental Yellow	13	34.8	116.3	4.6	56.8	14.7	0.2	23.7	0.4
Montana Brown	2	34.1	121.2	4.0	56.7	15.1	0.4	24.0	0.5
Tibet No. 96	1	32.3	123.0	3.2	51.4	9.5	1.0	33.5	1.5
Japan No. 91	1	34.1	115.3	3.3	46.4	12.2	1.0	35.1	1.7
P.I. 183920	1	32.8	108.0	6.9	36.9	16.8	1.9	36.5	1.9
Chinese Broad Leaf	3	32.2	113.4	3.8	44.5	12.7	1.8	36.7	1.3
Florida Broad Leaf	2	32.1	112.7	3.6	46.8	11.2	0.6	36.8	0.7
Fordhook Fancy	2	32.6	111.5	3.2	42.9	11.6	0.6	40.8	0.8
Large Smooth Leaf	2	33.0	115.2	3.6	42.7	11.4	0.6	40.8	1.3
P.I. 195552	3	30.3	114.0	4.7	42.8	8.8	0.6	42.7	0.8
P.I. 207465	2	30.1	102.1	2.6	41.5	11.4	0.2	43.7	1.1
P.I. 173847	1	36.3	97.8	2.9	36.9	10.9	1.5	46.6	1.7
P.I. 175067	1	35.3	98.5	2.7	39.9	8.6	1.1	47.1	1.3

hours as usually it was difficult to find pollen in the afternoon. Occasionally extra flowers were bagged in the morning for pollen collection, if the pollination was to be done in the afternoon.

In general, four buds were used for emasculation. They were emasculated about two days prior to opening and pollinated immediately. The pollinated flowers were bagged in kraft paper bags. Bags were secured by means of a support beside the plant. This helped prevent losses from wind. Plants were checked regularly for pod set. Bags were removed as soon as pods developed to minimize wind injury. Selfed and crossed seed was collected from each plant as the seed matured. At the onset of fall, all plants were harvested irrespective of maturity.

III. Effect of Immaturity on Erucic Acid Content.

Immaturity was anticipated as a possible complicating factor as some strains were quite late in flowering. Random eight-foot rows of oriental yellow mustard were harvested starting about a month before maturity. Subsequent harvests were made every four days until the final harvest. Seeds obtained after threshing were analyzed for fatty acid composition to determine the effect of premature harvest on erucic acid content.

On the various dates of harvest, pod samples were collected for moisture determinations. The percentage moisture in the seed is a relative measure of the degree of maturity. This experiment was conducted in the years 1961 and 1962. The correlation coefficient between percentage moisture and percentage erucic acid using the two years combined

data was calculated.

IV. Effect of Environment on Erucic Acid Content.

In trials conducted in 1960 and 1962, the effect of environment on percentage of erucic acid in B. juncea was studied. Four replication composited samples of oriental yellow mustard from each of the nine locations where mustard trials were conducted in 1960 were analyzed for erucic acid content. In the 1962 study, seeds of three Brassica ascessions grown at seven locations in four replications were analyzed for erucic acid content of the seed fat. Seed of replications one and two, and three and four were combined to provide two replications for analysis. A wider choice of species or strains within the genus Brassica permitted more reliable conclusions to be drawn with respect to the effect of environment on erucic acid content.

V. Chemical Analysis.

Based on a review of the literature, the fatty acid composition of the seed was considered to be controlled by the genotype of the seed rather than by the maternal plant. Seed obtained from the diallel cross constituted the F_1 population as far as the oil composition determinations were concerned. This seed and selfed seed from the parent plants were used for the determination of the oil composition.

The method of chemical analysis as suggested by Harvey (22) was followed with slight modifications. Initially, esters of a few samples were made using the method suggested by Harvey and also by the official method used for larger samples. Similarly, esters were injected in two-

foot and five-foot columns of the chromatograph to note any differences due to column length. The complete procedure for analysis, as used, is described below.

(1) Fat Extraction: For the chemical analysis, four seeds from each sample of selfed and crossed progenies were used for the determination. Seeds were cold pressed in a closed cylinder operated by hydraulic pressure. The instrument was provided with a pressure gauge so that care could be taken to maintain a pressure of less than 2500 psi. hydraulic pressure. At higher pressures the oil may undergo chemical changes due to the heat developed. The equipment used for this hydraulic press is a modification of the Carver Laboratory Press and was constructed for this study by using a hydraulic jack, a pressure gauge, a Carver cylinder and plunger, some channel iron and four long bolts. The instrument construction is illustrated in Figure 1. This apparatus cost only a fraction of the Carver Laboratory Press and proved satisfactory for these tests.

Oil obtained from the four seeds was not usually sufficient to overflow and remained on the plunger top with the meal. This meal and oil was washed from the cylinder and plunger with an excess of Skelly F into a 50 ml. beaker. Meal was separated from the oil-solvent solution by vacuum filtration using a porcelain vacuum filter. The oil-solvent solution was then transferred to a 25 ml. pear-shaped flask with ground glass neck and the solvent was removed by evaporation under vacuum using a rotary evaporator and hot water bath as illustrated in Figure 2.