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Genetic analysis of biochemical traits in F³ populations of rapeseed (*Brassica napus* **L***.***)**

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Abstract

The appropriate information on the biochemical characteristics of the plant is important for its usefulness in human health. The genetic basis for different brassica genotypes in terms of biochemical characteristics is important for the planning of effective breeding strategies. The present research was conducted to determine heritability, genetic advance and coefficient of correlation for biochemical traits in rapeseed. Breeding material comprising 10 parental lines and their 21 F³ populations was evaluated in the 2013-2014 at The University of Agriculture of Peshawar-Pakistan in a randomized complete block design with three replicates. For all studied traits, substantial differences were found among genotypes, parents, and F_3 populations. Similarly, significant differences for all the studied traits with the exception of protein and glucosinolates have been recorded for parent vs. F_3 populations. In most F_3 populations, the heritability estimates associated with the maximum genetic advance for oil content, protein content, linolenic acid, erucic acid, and oleic acid were low or moderate. In comparison, moderate to high heritability coupled with maximum genetic advance, have been observed for glucosinolates for most F_3 cross combinations. These findings show that selection in the early generation may be helpful in improving these characters. Oil content showed a negative and highly significant correlation with glucosinolate (r_p =-0.50) and linolenic acid (r_p =-0.57), so priority should be given to these traits to improve oil content. CA2 x DH5, DH7, and CA2 x DH7 performed better for oil content, protein content, and oleic acid, respectively, while DH2, DH5, and CA2 x DH8 showed better results for glucosinolates, linolenic acid, and erucic acid, respectively. These genotypes could be further utilized in rapeseed breeding programs.

Keywords: Brassica, Genetic variability, Genetic advance, Heritability, Oil content

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Introduction

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Pakistan has faced a severe shortage of edible oil in recent years due to the scarcity of varieties with higher seed yield and seed oil content. Pakistan is only capable of fulfilling 25% of its edible oil requirements, and the remaining imports are costly. In order to meet the demand and needs of Pakistan's

potential oil consumption, high-quality oilseed must be one of the main objectives of brassica breeding (Shengwu et al*.*, 2003). Both conventional and genetic engineering methods are used to improve the quality characteristics of Brassica. In 2012-13, 2.149 million tons of edible oil worth Rs. 270 billion (US\$ 2.7 billion) was imported (FBS, 2012-13). The import bill increases every day due to rapid population growth. The increase in population growth at an unprecedented rate and people's standard of living makes Pakistan a critically developing country for the development of consumable oil.

From a health perspective, brassica oil is considered beneficial. It contains nutritionally suitable linoleic acid and oleic acid, whose thermostability is ideal for cooking oil. (Vles, 1989). High oleic acid tastes better and can lead to good health too. This fatty acid's oxidative stability makes it also ideal for some industrial applications (Scarth and Mcvetty, 1999). High erucic acid oil has anti-nutritional effects, but ideal for particular industrial applications such as polyethylene and sticker anti-blocking agents, and anticorrosive materials in the metal sheets industries (Vles, 1989; Lühs and Friedt, 1994). In recent decades, brassica oilseeds have been a source of edible oil and a source of biofuels and industrial feed as well. Such genera are regaining interest in cosmetics, emollients for lubricants as well as adhesive and biodegradable plastic products (Mckeon et al*.*, 2016). Erucic acid therapy (X-ALD) has also been discovered, a genetic disorder that affects the nervous system and is associated with the accumulation of very-long-chain fatty acids (Moser et al*.*, 2007; Rizzo et al*.*, 1989). Thus, the fatty acid compositions of rape oils were tailored to different objectives by the conventional and molecular breeding (Mcvetty and Scarth, 2002). The development of biodiesel has provided new opportunities and has changed the course of usage and use of rapeseeds. Besides, the growing emphasis on renewable energy, chemical feedstocks, industrial oils, and the ever-rising bioeconomy would give industrial Brassica oils a significant growth potential.

The success of any crop improvement depends on the existence of genetic diversity, heritability, correlation, as well as genetic gain in selection (Khan et al*.*, 2006). An essential aspect of plant breeding is to measure the fraction of the overall variability between plants in a population due to genetic differences. It is called heritability, an integral component of the breeder's equation, which predicts the predicted selection response. The higher the heritability of a character, the greater the expected genetic gain since artificial selection can be achieved more efficiently (Mazurkievicz et al*.*, 2019). Correlations are necessary to determine the degree to which various characters are linked (Belete, 2011). A useful tool for crop improvement would be plant traits with adequate variability, high heritability, and genetic advance. In light of the importance and scarcity of edible oil in the region, this study has been conducted to evaluate genetic diversity, heritability, genetic advance and correlation in parental and F_3 populations for important biochemical traits and to identify the best segregants for future brassica breeding programs.

Material and Methods

This experiment was performed at The University of Agriculture, Peshawar-Pakistan, during the 2013-14 crop season to study genetic diversity, heritability, genetic advance, and association of traits in F_3 populations of *Brassica napus*. The breeding material (Table-1) comprised of three lines (CA-2, CA-4, and CA-5 procured from Canada) and seven testers (DH-2, DH-3, DH-4, DH-5, DH-6, DH7, and DH-8; collected from China). From 2007-2010 this material was evaluated for its stability at Peshawar conditions. In 2010-11, F_1 hybrids were developed and evaluated during 2011-12, whereas F_2 populations were raised in 2012-13 at The University of Agriculture, Peshawar. All genotypes were planted in three replicates in a randomized complete block design. Standard agronomic and cultural practices were applied to the experiment throughout the growing season. Data from ten randomly selected plants were collected at the right time from three central rows for each genotype. At the biochemical laboratory, Nuclear Institute for Food and Agriculture (NIFA) Peshawar-Khyber Pakhtunkhwa-Pakistan, seed samples from each selected plant were examined for oil (%), protein (%), oleic acid (%), glucosinolates (μ Mg⁻¹), linolenic acid (%), and erucic acid (%).

Analysis of variance

The data were statistically analyzed according to the method suggested by (Singh and Chaudhary, 1979) for RCB design. Mean separation was performed following the LSD $_{(0.05)}$ test.

Parents	F₃ Populations				
CA2	$CA2 \times DH2$	$CA4 \times DH5$			
CA4	$CA2 \times DH3$	$CA4 \times DH6$			
CA5	$CA2 \times DH4$	$CA4 \times DH7$			
DH ₂	$CA2 \times DH5$	$CA4 \times DH8$			
DH ₃	$CA2 \times DH6$	$CA5 \times DH2$			
DH ₄	$CA2 \times DH7$	$CA5 \times DH3$			
DH ₅	$CA2 \times DH8$	$CAS \times DH4$			
DH ₆	$CA4 \times DH2$	$CAS \times DH5$			
DH7	$CA4 \times DH3$	$CAS \times DH6$			
DH ₈	$CA4 \times DH4$	$CAS \times DH7$			
		$CAS \times DH8$			

Table-1. List of genotypes used in the experiment

Heritability (broad sense)

The broad-sense heritability for different traits was determined by using the updated version of the formula suggested by Mahmud and Kramer 1951:

$$
h^{2}(b.s) = \frac{\text{VF}_{3} - \sqrt{\text{VP}_{1} \times \text{VP}_{2}}}{\text{VF}_{3}}
$$

Where, V_{F3} = variances of F_3 population for a specific trait, V_{P1} , and V_{P2} = variances of parent 1 and parent 2.

The heritability was categorized as low, moderate, and high.

 $0-30\% = Low$, 31-60% = Moderate, 61% and above $=$ High

The genetic advance was computed using the following formula (Panse and Sukhatme, 1954)**:**

$$
GA = k.\sqrt{\sigma^2 p} . h^2
$$

Where, GA = genetic advance, $K = 1.76$ at 10 % selection intensity, h^2 = heritability in fraction for a particular cross, $\sqrt{\sigma^2 p}$ = Phenotypic standard deviation.

The genetic advance was categorized as low, moderate, and high as given (Johnson et al*.*, 1955).

G.A < 10%: Low, 10% < G.A < 20%: Moderate, G.A $> 20\%$: High

Phenotypic correlation

Phenotypic correlations (r_p) between two traits x and y were calculated as suggested by (Johnson et al*.*, 1955). The relationship between yield and its components was derived from the following formula:

$$
r_p = Cov_p(x, y) / \sqrt{V_p(x) V_p(y)}
$$

Where r_p is the phenotypic correlation coefficient, $Cov_p(x, y)$ is phenotypic covariance between (x) and (y), $V_p(x)$ is the phenotypic variance of characters (x) , and $V_p(y)$ is the phenotypic variance of characters (y).

Results

Oil content (%)

One of the ultimate goals of breeders is to increase oil content in Brassica. Its oil content determines the quality of oilseed Brassica. Analysis of variance showed highly significant genetic variability among genotypes, parents, F_3 population, and parents vs. F_3 (Table-2). Among all genotypes, mean values regarding oil percent ranged from 49.6 to 54.4%, with an overall mean of 52%. Among parents, the range was from 50.2 to 54.2% for DH8 and DH6, respectively. Among F_3 populations, oil percent varied from 49.6 to 54.4% for CA2 x DH2 and CA2 x DH5, respectively (Table-3). Among parents, for oil content, maximum variance (3.8) was observed in DH8, while minimum variance (0.5) was observed in DH4. Similarly, among F_3 populations, maximum variance (4.7) was observed in CA5 x DH8, whereas, minimum variance (2.5) was observed in CA2 x DH4 and CA4 x DH3. The majority of F_3 cross combinations showed moderate broad sense heritability. The highest heritability (0.68) coupled with high genetic advance (2.6) was estimated for CA5 x DH3, whereas, the lowest heritability (0.30) coupled with low genetic advance (1.1) was determined for CA2 x DH8 (Table-4). The phenotypic correlation coefficient for oil percent with protein content ($r_p = -0.86$), glucosinolate ($r_p = -0.50$), and linolenic acid $(r_p=0.57)$ was negative and highly significant. The rest of the traits showed no association with oil percent (Table-6).

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Table-2. Mean squares of parental genotypes and F³ populations for biochemical traits.

 $*$, $**$ = Significant at the 1% and 5% level of probability, respectively

Table-3. Means of parental genotypes and F³ populations of important biochemical traits.

Protein content (%)

Protein is the primary growth and development necessity for all living organisms. Highly significant differences were recorded among genotypes, parents, F_3 population, while parents vs. F_3 showed nonsignificant differences (Table-2). For all the genotypes, protein content varied from 15.6 to 20.3% for CA2 x DH5 and DH7, respectively, with a mean value of 17.8%. Among parents, its range was from 15.8 to 20.3% for DH2 and DH7, respectively. Among F³ populations, it varied from 15.6 to 20.1%. Maximum protein content (20.1%) was observed for CA5 x DH6, while the minimum value (15.6%) was calculated for CA2 x DH5 (Table-3). Variances for protein content ranged from 0.5 (DH3, DH5, DH7) to 2.9 (DH8) among parental lines. F_3 crosses varied from 1.3 to 4.1 for CA2 x DH7 and CA5 x DH3, respectively. Moderate heritability was observed for the majority of F_3 cross combinations. Broad sense heritability estimates for protein content ranged from 0.36 (moderate) to 0.63 (high) for CA4 x DH2 and CA5 x DH3, respectively. High genetic advance (2.2) was observed for CA5 x DH3, while low genetic advance (0.8) was calculated for CA2 x DH7. High heritability, coupled with high genetic advance for protein contents indicated effective selection for this trait due to additive gene action (Table-4). A positive and significant correlation was observed of protein content with glucosinolate $(r_p=0.66)$, linolenic acid $(r_p=0.47)$ and erucic acid $(r_p=0.40)$ (Table-6).

Glucosinolate (µMg-1)

Glucosinolates are undesirable elements of *Brassica napus* meal and cause various defects in animals. Its low level is desirable. A significant amount of genetic variability was observed among genotypes, parents, F_3 population, while parents vs. F_3 showed nonsignificant differences (Table-2). Mean values for genotypes ranged from 58.2 to $105.9 \mu mg^{-1}$ for DH2 and DH7, respectively, with an overall mean of 74.5 µmg-1 . For parents, maximum glucosinolate (105.9 μ mg⁻¹) was observed for DH7. For F₃ cross combinations, CA2×DH4 had a minimum value of 59.9 μ mg^{-1,} while CA4×DH7had a maximum value of 94 µmg⁻¹ (Table-3). Glucosinolate ranged from 3.4 to 10.1 for DH5 and DH3, respectively. For F³ populations, minimum variance (14.1) was observed for CA4 x DH5, and maximum variance (30) was calculated for CA5 x DH4. Moderate (0.51) to high (0.78) broad-sense heritability was observed for glucosinolate. The highest value (0.78) was observed

for CA2 x DH2, while the lowest value (0.51) was observed for CA5 x DH6. The majority of values for heritability was found in the high range. The genetic advance was maximum (7.0) for CA5 x DH4 and minimum (3.7) for CA2 x DH4 and CA5 x DH6 (Table-4). The correlation coefficient for glucosinolate revealed a positive and significant relationship with erucic acid $(r_p=0.47)$, while it had a negative and significant correlation with oleic acid $(r_p = -0.55)$ (Table-6).

Linolenic acid (%)

Like glucosinolates, linolenic acid is also undesirable unsaturated fatty acid. In brassica oil, its low level is desirable because it's high-level interrupts storage and frying. Results revealed highly significant differences among genotypes, parents, F3 populations, and parents vs. F_3 (Table-2). Among all genotypes, linolenic acid ranged from 7.7 to 11.3%, with a mean value of 9.9%. For parents, its range varied from 7.7 to 11% for DH5 and DH7, respectively. Similarly, for F_3 cross combinations, varied from 8.9 to 11.3% for CA2 x DH5 and CA5 x DH6, respectively. Data regarding variances of linolenic acid, among parents ranged from 0.2 to 0.6, while among F_3 populations, it ranged from 0.4 to 1.6. Among F_3 crosses, minimum variation (0.4) was observed for CA4 x DH3 and CA5 x DH3, whereas maximum variation (1.6) was observed for CA5 x DH4. Most of the crosses showed moderate heritability. However, it ranged from low (0.19, CA4 x DH3) to high $(0.65, CA5 x)$ DH4). The genetic advance was maximum (1.4) for CA5 x DH4, while minimum (0.2) for CA4 x DH3 cross combinations (Table-5). The correlation findings showed no association of linolenic acid with other traits.

Erucic acid (%)

Erucic acid is also one of the most undesirable components of *Brassica napus* oil. Like glucosinolates and linolenic acid, its low level in oil is also desirable. Analysis of variance showed significant differences among genotypes, parents, F₃ populations, and parents vs. F_3 (Table-2). For all genotypes, erucic acid ranged from 39.0 to 57.2%, with a mean value of 48.6%. Among parents, its values ranged from 41.5 to 57.2% for CA2 and DH7, respectively. Similarly, for F_3 populations, the maximum value (56.6%) was observed for CA4 x DH7, while the minimum value (39.0%) was observed for CA2 x DH8 (Table-3). Variances for erucic acid ranged from 4.1 (DH4) to 11.9 (CA2) among parental

lines, while for F_3 populations, its range was 9.3 (CA4) x DH4) to 27.2 (CA5 x DH5). Broad sense heritability for erucic acid ranged from 0.22 (CA4 x DH4) to 0.68 (CA5 x DH5). The genetic advance was minimum (1.2) for CA4 x DH4, while the maximum (6.2) was recorded for CA5 x DH5 cross combination (Table-5). The relationship of erucic acid to oleic acid was negative and highly significant (rp=-0.77) (Table-6).

Genotypes	Oil percent		Protein content		Glucosinolate				
	Var.	h ²	GA	Var.	h ²	GA	Var.	h ²	GA
CA2	1.8			1.1			4.3		
CA4	1.3			1.4			6.2		
CA5	1.7			0.8			8.3		
DH ₂	2.3			1.4			4.6		
DH ₃	0.7			0.5			$10.1\,$		
DH ₄	0.5			0.6			8.1		
DH ₅	2.0			0.5			3.4		
DH ₆	2.0			0.8			7.5		
DH7	2.3			0.5			5.0		
DH ₈	3.8			2.9			4.6		
$CA2\times D H2$	3.7	0.45	1.5	2.2	0.43	1.1	21.6	0.78	6.3
CA2×DH3	$2.6\,$	0.52	1.5	1.4	0.44	0.9	19.0	0.56	4.3
CA2×DH4	2.5	0.52	1.4	1.6	0.49	1.1	16.1	0.53	3.7
$CA2\times DH5$	3.6	0.43	1.4	1.5	0.44	0.9	16.5	0.74	5.3
$CA2\times DH6$	3.9	0.42	1.5	1.5	0.41	0.9	16.1	0.62	4.4
CA2×DH7	3.2	0.35	1.1	1.3	0.38	0.8	14.7	0.67	4.5
$CA2\times DH8$	4.1	0.30	1.1	3.7	0.46	1.6	17.4	0.66	4.9
$CA4\times DH2$	3.0	0.41	1.2	2.1	0.36	0.9	19.7	0.61	4.8
CA4×DH3	2.5	0.60	1.7	3.0	0.60	1.8	20.9	0.56	4.5
CA4×DH4	3.0	0.65	2.0	2.1	0.57	1.5	27.1	0.73	6.7
$CA4\times DH5$	2.9	0.47	1.4	1.6	0.49	1.1	14.1	0.58	3.9
CA4×DH6	2.8	0.41	1.2	1.9	0.49	1.2	17.9	0.62	4.6
$CA4\times DH7$	3.1	0.41	1.3	1.6	0.43	0.9	16.5	0.55	3.9
$CA4\times DH8$	3.9	0.36	1.3	3.6	0.41	1.4	19.9	0.72	5.6
$CA5\times D H2$	3.5	0.43	1.4	3.0	0.57	1.7	18.0	0.62	4.7
$CA5\times DH3$	4.5	0.68	2.6	4.1	0.63	$2.2\,$	24.4	0.62	5.4
$CA5\times DH4$	3.4	0.58	1.9	2.3	0.53	1.4	30.0	0.72	7.0
$CA5\times DH5$	4.6	0.55	2.1	2.2	0.60	1.6	15.2	0.59	4.1
$CA5\times DH6$	3.8	0.49	1.7	1.9	0.54	1.3	16.9	0.51	3.7
$CA5\times DH7$	3.1	0.38	1.2	1.6	0.57	1.3	23.3	0.71	6.1
$CA5\times DH8$	4.7	0.37	1.4	3.8	0.44	1.5	15.5	0.59	4.1

Table-4. Variances, heritability, and genetic advance for oil percent, protein content, and glucosinolate.

Oleic acid (%)

Oleic acid is one of the most desirable monounsaturated fatty acids, which is essential for oil stability. Oleic acid showed significant differences among genotypes, parents, F_3 populations, and parents vs. F3 populations. (Table-2). Among genotypes, oleic acid ranged from 48.6 to 58.0%, with an overall mean of 53.7%. Among parents, the maximum value (54.9%) was recorded for CA4, and a minimum (48.6%) was noted for DH7. For F₃ crosses, CA2 x DH7 attained a maximum value of 58.0%, while CA4 x DH7 attained minimum value (48.9%) for oleic acid (Table-3).

Variances for oleic acid ranged from 2.3 (DH7, DH8) to 7.1 (CA5) among parental genotypes, while its range was from 7.7 (CA5 x DH5) to 17.9 (CA4 x DH3) among F_3 cross combinations. Broad sense heritability ranged from moderate (0.33) to high (0.65) . CA4 x DH5 and CA4 x DH8 crosses had maximum heritability (0.65) , followed by CA4 x DH7 (0.64) . Most of the heritability values were in the moderate range for oleic acid. CA4 x DH3 showed high (4.6) genetic advance, and CA5 x DH5 showed low (1.6) genetic advance for oleic acid (Table-5). Oleic acid showed a negative and significant correlation with glucosinolates ($r_p = -0.55$), and erucic acid ($r_p = -0.77$) (Table-6).

among some prochemical trans in 13 populations.						
	OP	PC	GSL	LINO	ERU	OLE
OP	0	$-0.86**$	$-0.50**$ $-0.57**$		-0.12	0.06
PC		0	$0.66**$	$0.47**$	$0.40*$	-0.33
GSL			θ	0.28	$0.47**$	$-0.55**$
LINO				$\overline{0}$	-0.13	0.17
ERU						$-0.77**$
OLE						θ

Table-6. The phenotypic correlation coefficient among some biochemical traits in F3 populations.

 $**$ = highly significant, $*$ = significant and NS=nonsignificant, Note: OP- oil percent, PC- protein content, GSL- glucosinolates, LINO-linolenic acid, ERUerucic acid and OLE-oleic acid.

Discussion

Understanding the inheritance of genetic architecture of important biochemical traits is necessary to devise a systematic breeding program for the production of varieties with desirable biochemical characteristics. The existence of genetic variation, heritability, and association in any crop is deemed necessary for the selection of superior genotypes. The present study investigated the genetic variation, the association between the essential biochemical traits and heritability in a group of 31 rapeseed genotypes. The wide range of genetic variation estimated for all traits has shown that these traits can be used for the development of new rapeseed varieties. Previous researchers (Kumar et al*.*, 2018; Shaukat et al*.*, 2014; Iqbal et al*.*, 2014; Fayyaz et al*.*, 2014; Abideen et al*.*, 2013 and Ahmad et al*.*, 2013) also reported sufficient genetic variability among rapeseed genotypes. The present research demonstrates a broad range of germplasm variations for the studied traits. Character variations in any crop species are the raw material of a plant breeder, and the degree of variability in the population concerning the various characters is the success criteria of the plant breeder. In combination with high heritability estimates and high genetic advance, there are more possibilities for additive genes to influence the character and selection in the early generation is more effective. Most of the F_3 crosses revealed low to moderate broad-sense heritability for all the studied traits. Iqbal et al*.* (2019) also reported high broad-sense heritability with maximum genetic advance, thus indicating additive gene action, which is similar to the outcomes of the present experiment.

The characters showing a high heritability value are controlled by additive genes and can be used effectively for plant phenotypic selection. Kumar et al*.* (2018) also reported high heritability coupled with low genetic advance, which is almost similar to the findings of the present experiment. Ullah et al*.* (2017) studied advanced lines of Brassica and reported high broad-sense heritability for all biochemical traits. Similar results were reported by Chittora and Singh (2015), who found high heritability values for GSL content in inter-specific Brassica populations and *Brassica napus* genotypes, respectively. In contrast, Schierholt and Becker (2001) observed low heritability for GSL in winter oilseed rape. The difference in the results may be due to the difference in materials and environment as well as environmental influence on the studied traits. Some of the F³ populations revealed broad-sense heritability in low range for all the traits studied, hence indicating the influence of the environment, and thus selection may not be fruitful. Correlation studies are also of great importance to plant breeders for evaluating the traits linked to main breeding goals. Correlation analysis of important plant traits leads to a directional model for quality response. In the present study, negative and highly significant correlations of oil content with glucosinolates and linolenic acid showed that selection for important biochemical traits would be more effective. Therefore, in this and previous studies, different correlations of traits revealed that the studied materials are diverse, and knowledge extracted would help to develop various selection strategies. Ali et al*.* (2017), Ullah et al*.* (2017) and Ahmad et al*.* (2015) revealed a negative and significant correlation for all of the traits studied in this experiment.

Conclusion

A significant amount of genetic variability was observed among *Brassica napus* genotypes for the traits under investigation. Oil content, protein content, linolenic acid, erucic acid, and oleic acid manifested low to moderate broad-sense heritability associated with high genetic advance for most of the F_3 populations suggesting the possibility of improvement in new generations. Correlation analysis uncovered the presence of negative and highly significant oil content with glucosinolates and linolenic acid, so more importance should be given to these characters for indirect improvement of yield. CA2 x DH5, DH7, and CA2 x DH7 performed better for oil content, protein

content, and oleic acid, respectively, while DH2, DH5, and CA2 x DH8 showed better results for glucosinolates, linolenic acid, and erucic acid, respectively. These genotypes could be further utilized in rapeseed breeding programs.

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Contribution of Authors

Ahmad N: Wrote and revised the whole manuscript, including all the tables.

Raziuddin: Executed and planned the experiment Ahad F: Executed and planned the experiment Iqbal T: Data analysis, Review the manuscript Khan N: Help in recording the experimental data Nauman M: Help in recording the experimental data

Hameed F: Help in recording the experimental data