Discussion

*Sesamum indicum* L. (family Pedaliaceae) is perhaps the oldest annual oilseed crop known to man. On the basis of its ancient history of cultivation, availability of diversity of forms of cultivated varieties and occurrence of the wild sesame, *Sesamum indicum* var. malabaricum, India is considered to be the basic center of origin of the crop (Brar and Ahuja, 1979). Sesame seed oil is valued for its high quality and stability. It is used as salad and cooking oil as well as in the manufacture of margarine, vanaspati, soap, paints and insecticides.

Previous study showed high heritability for plant height, number of branches, number of capsule/plant, 1000 seeds weight and seed yield/plant (Mohanty and Singh 1965; Sawant 1971; Salazar 1975; Chaudhari *et al.* 1977 and Gupta and Gupta 1977). Heritability was also found to be high for flowering (Shukla and Verma 1976) but low for flowering duration (Mohanty and Singh 1965). Dominant gene effects were regarded important in the expression of number of branches, number of capsules/main stem and number of capsules/plant, while both additive and dominant gene effects played important role in days to flowering and seed yield/plant (Dixit 1976). Chaudhari *et al.* (1977) and Yermanos and Kotecha (1978) considered additive gene effect to be more important in the inheritance of plant height, capsule/plant, days to flowering, number of branches/plant and seed yield/plant.

The present study reveals that additive component was important for plant height, number of leaves/plant, number of primary branches/plant, number of capsule/plant, number of seeds/capsule, number of loculi/capsule, capsule length, capsule width, number of capsule/axil, germination time, days to the first flowering, days to the first capsule, seeds weight, total lipid/oil and percentage of total lipid/oil characters.

5.1 F-test and DMRT:

Randomized block design layout was followed to compare the mean of all agromorphological parameters under study of 15 different germplasms of the *Sesamum* species. After analyzing the experimental data using analysis of variance significant differences among the mean were tested by Duncan’s Multiple Range Test which revealed significant differences with respect to the economic characters (here, oil content) and other associated quantitative characters (such as plant height, number of leaves/plant, capsule length, capsule width etc.) among 15 different germplasms in *Sesamum* species.
5.2 Estimates of genetic parameters and character association in 15 different germplasms of Sesamum:

The genetic variability for all the characters was also evidenced from the phenotypic and genotypic coefficients of variation (GCV and PCV). The highest GCV value was recorded for total lipid/oil obtained (mg) (25.897 %) followed by percentage of total lipid/oil (w/w) (25.893 %), number of leaves/plant (17.772 %), number of capsule/plant (16.481 %) and number of capsule/axil (15.199 %). On the other hand the PCV value was highest for number of capsule/axil (34.525 %), number of capsule/plant (27.015 %), total lipid/oil obtained (mg) (25.897 %), percentage of total lipid/oil (w/w) (25.893 %), number of primary branches/plant (24.868 %) and number of leaves/plant (20.984 %). The ECV value was highest for the characters like number of capsule/axil (37.723 %), number of primary branches/plant (23.490 %), number of capsule/plant (21.405 %) and number of loculi/capsule (18.717 %) respectively. The magnitude of GCV and PCV being same shows that there was least influence of environment in expression of the traits under study. Here, the examples of cases have been found perfectly in total lipid/oil obtained (mg) and percentage of total lipid/oil (w/w). In general, for all the 15 characters, PCV values are slightly higher than the corresponding GCV values, so we can conclude that environment play very little role for expression of the traits for example capsule/axil, germination time. Variability due to genetic component indicated that there was adequate scope of selection for improvement of certain traits.

Highest heritability (broad sense) was recorded for the characters like total lipid/oil obtained (mg) and percentage of total lipid/oil (w/w) (100 %) but moderately high for the characters like number of seeds/capsule (79 %), plant height (cm) (73 %), number of leaves/plant (71 %), capsule width (cm) (66 %) and capsule length (cm) (53 %). In some characters the gene expression was noticed in GCV, PCV and heritability was high for example total lipid/oil obtained, percentage of total lipid/oil (w/w), number of leaves/plant. It seemed that the expression of genes was not much modified or influenced by environment.

High heritability coupled with high genetic advance was also observed. Highest genetic advancement was obtained for the characters like total lipid/oil obtained (mg) (53.35 %), percentage of total lipid (w/w) (53.34 %), number of leaves/plant (31.01), number of capsule/plant (20.71 %) and number of seeds/capsule (14.63 %).
The result of present study also exhibited high heritability coupled with high GA for some characters like total lipid/oil obtained (mg), percentage of total lipid (w/w), number of leaves/plant. These traits should be considered for effective selection as most likely these traits are governed by additive genes effects. The characters like plant height (cm), capsule width (cm) showed high heritability with medium GA, indicating the influence of environment over the characters. Finally these results concluded that the characters are simply inherited and chances of selection for further improvement are more.

5.3 Correlation study and Path analysis of the traits:

Path analysis were performed to estimate the direct and indirect effects of different attributing casual characters which are important agronomically to select suitable plant materials for generating plants with higher oil yield. Path analysis results were discussed only considering lighter direct and indirect effects. The direct effect of any particular traits (variable) on the dependent variable i.e. percentage of total lipid/oil, in path analysis has been observed in accordance with the correlation of the other 14 traits to the trait percentage of total lipid/oil content. This means that when the correlation study was taken into account, it was found that one particular character which correlates positively with percentage of total lipid/oil also has direct effect on percentage of total lipid/oil during path analysis. But it was also found that whereas a particular trait correlates negatively with the percentage of total lipid/oil, it exercises direct positive effect on percentage of total lipid/oil (dependent variable) in path analysis and vice versa.

When the direct effect of causal character or variable on the response variable is almost equal to that of the correlation coefficient between them, then the direct selection of the causal character is useful and the correlation coefficients presents the true picture of the association between the response variable/character and the independent causal character.

When the direct effect is negligible or negative but the correlation coefficient between the causal variable/character is positive and significant, then indirect effects are the cause of such correlation coefficient. In this situation it is better to consider the other causal variables/characters rather than this variable/character.

When the direct effect is high and positive but the correlation coefficient is negative and negligible, then the direct effects are the cause of manifestation of such correlation. In this
case it is better to restrict the undesirable characters during selection based on the study of the individual path coefficients of these characters in likewise manner.

When both the direct effect and the correlation coefficient are negligible or negative then one should discard such characters/variables during selection.

However, detail effects can easily be extracted from respective path results displayed in the table 3.7). Path analysis results revealed that number of leaves/plant(0.00025), germination time (0.00003), days of first flowering (0.00011), 100 seeds weight(0.00008), and total lipid/oil obtained (0.99992) have high positive primary effect on percentage of total lipid/oil. On the other hand component characters like plant height (-0.00027), number of primary branches/plant (-0.00010), number of capsule/plant (-0.00010), number of seeds/capsules (-0.00002), number of loculi/capsule (-0.00003), capsule length (-0.00002), capsule width (-0.00014), and days to the first capsule (-0.00011) have negative primary effect on oil content. Capsule/axil (0.00000) has no effect on percentage of total lipid/oil.

It can be said in other way that the negative correlation and positive direct effect are mainly attributed by the indirect effect of the character via other characters/variables.

Thus from the analysis of path coefficient it is clear that the causal characters/variables are efficient to explain the variation in response characters/variable i.e. percentage of total lipid/oil content.

5.4 Genetic divergence:

A meaningful classification of experimental materials depending upon the different characters help to distinguish genetically close and divergent genotypes which is a prerequisite for any genetical study. The 15 different germplasms of Sesamum collected from 4 different geographical regions of West Bengal could be grouped into five clusters. The cluster number 1 included 2 entries, cluster number 2 includes 3, cluster number 3 includes 3, cluster number 4 includes 3 and cluster number 5 includes 4 germplasms which reflecting narrow genetic diversity among them. The narrow genetic diversity may be attributed to similarity in the base material from which they have evolved. It is interesting to note that the original area of cultivation of the germplasms with in a cluster is localized with in a particular pocket of zone for example, in cluster number 2, which includes 3 germplasms (V01, V03, and V13) in which V01 and V03 from Murshidabad district of north zone. It is true for other germplasms also.
Average intra and inter cluster $D^2$ values among 15 germplasms revealed that cluster number 1 had the minimum intra cluster $D^2$ value (18.482) indicating that germplasms with in this cluster were similar, while cluster number 3 showed maximum intra cluster $D^2$ value (933.722) indicating existence of diverse genotypes that fell in this cluster.

The inter cluster $D^2$ value ranged from 366.469 to 1493.346. Minimum inter cluster $D^2$ value was obtained between cluster number 4 and cluster number 2 indicating close relationship among the germplasms included in these clusters. Maximum inter cluster $D^2$ value was observed between cluster number 4 and cluster number 1 (1493.346) followed by cluster number 4 and cluster number 3 (1219.754), cluster number 2 and cluster number 1 (1209.493), cluster number 5 and cluster number 4 (1114.921), cluster number 3 and cluster number 2 (974.724) revealing that germplasms included in these clusters are genetically diverse and may give rise to high heterotic response.

5.5 Cluster Mean:

The cluster mean of each 15 characters is presented in table 3.10. It is evident from the table that the cluster number 1 was characterized or noted for highest plant height, number of leaves/plant and number of capsule/axil. Cluster number 2 was distinguished for number of primary branches/plant, number of capsule/plant and percentage of total lipid/oil (w/w). Cluster number 3 was marked for its number of loculi/capsule and capsule width. Cluster number 4 had higher days to the first flowering, days to the first capsule, 100 seeds weight and total lipid/oil obtained. Finally cluster number 5 was distinguished for highest number of seeds/capsule, capsule length, capsule/axil and germination time.

There for hybridization among members of the distant cluster is suggested to get desirable progenies.

5.6 Major Fatty acids composition:

From the table 3.11, it reveals that percentages of total lipid/oil contents (1 gm. of dry seeds material) have been assessed with particular reference to fatty acid composition of 15 germplasms collected from different districts of West Bengal. From the result it is clear that there is a wide difference in total lipid/oil content (mg.) and also % of total lipid/oil (w/w). The highest % of total lipid/oil content was recorded in the germplasms V-JTS-8 of North Zone followed by V-B-67, V-SWB-32-10-1, South 24 Parganas-1, East Midnapore-2, V-SWB-18, Hooghly, East Midnapore-1, Rama, South 24 Parganas-2, West Midnapore,
Burdwan, Seklar, Howrah, V-8-Sheera respectively. These results correspond with the statement of Lew and Tung (1992) about the existence of natural variability in the total lipid/oil content among the 15 germplasms of Sesame.

Attempts are also to be made to prepare methyl esters of fatty acids following standard protocol for determination of fatty acid composition of different germplasms of Sesame using GLC technique. The table 3.12 depicts in principle there are 6 components exist in the fatty acid composition in the Sesame seed germplasms, these are palmitic acid, stearic acid, oleic acid, linolic acid, \(\gamma\)-linolenic acid, \(\alpha\)-linolenic acid. It is clearer from the table 3.12, that the \(\omega\)- values represent the methyl end chain from the center of the double bond furthest remove from the carboxyl end. It was also interest to note all the 15 germplasms showed wide difference in their palmitic acid content ranges from 11.4-15.5, stearic acid content 4.4-5.7 \%, oleic acid content 39.3-44.3 \%, linolic acid content 14.7-41.3\%, \(\gamma\)-linolenic acid content 0.03 to .1 \% and \(\alpha\)-linolenic acid 0.2 to 0.8 \%. This results confirm the conclusion of WILLIAMS and SOBERING (1992), VELASCO and BECKER (1998), VELASCO et al (1999a) that GLC analysis may facilitate effective selection of oil seed Sesame germplasms with desired content of fatty acid in oil to other significance parameters of quality Mik et al (2003). GLC analysis also allows the processing selected samples immediately after harvest and characterize those samples with other feature with quality criteria. The influence of harvest time may decrease the prediction accurately specially when the course of whether is significantly different from the year when the samples were collected from the field for assessment.

According to the literature, the major compounds concerned of *Sesamum* germplasms are different. The observed differences may be due to the different environment and genetic factors, different chemotypes, and the nutritional status of the plants as well as other factors that can influence the oil composition.

**5.7 Characterization of germplasms based on molecular marker (RAPD):**

Accurate identification of germplasms of crop species and varieties by means of DNA finger prints is important particularly when new crop varieties are to be released, different accessions of wild species are to be characterized and purity of germplasms is to be determined. RAPD markers were utilized this study to evaluate the level of genetic variation among 15 germplasms of *Sesamum indicum* L. collected from different districts of West Bengal. We have chosen the RAPD technique as it does not require any previous knowledge
on DNA sequence of this species, is an inexpensive and easy to use for evaluating the degree of genetic diversity in Sesame and many other plant species (Bhat et al. 1999; Sangwan et al. 2001; Vieira et al. 2003; Ercan et al. 2004; Salazar et al. 2006; Kumar and Sharma 2009; Akbar et al. 2011).

10 RAPD primers detected sufficient genetic variation within 15 Sesame germplasms to allow for complete differentiation. According to Li and Midmore (1999) when the variation between genotype is high the use of few primers will be sufficient. For instance Ercan et al (2004) identified 38 accessions only by using 7 primers while 20 Sesame genotypes were determined with 10 primers by Akbar et al (2011). Similarly Millan et al (1996) reported a high level of genetic diversity among rose genotypes using nearly 10 RAPD primers. Schontz and Rether (1999) identified 37 lines of Foxtail millet employing just 4 RAPD primers.

A high level of genetic diversity was observed among the 15 germplasms of Sesame. Although Sesame is generally follow self-pollination but cross pollination have been reported between 5 and 60 % in this species (Yermanos 1998; Joshi 1961; Mazzani 1983; Brar and Ahuja 1979; Ashri 1989). Approximately 10 to 20 % of the genetic diversity among the population is due to out crossing could explicate the high genetic variability noticed in the present study. Our results are in conformity with the results of earlier workers based on RAPD and morpho-agronomic traits which have reported high genetic diversity in Sesame germplasms (Ashri 1998; Bhat et al. 1999; Ercan et al. 2004; Salazar et al. 2006; Pham et al. 2009; Akbar et al. 2011).

It should be pointed out that applying different methods would provide different results on the level of genetic diversity. Isshiki and Umezki (1997) reported little variation among 68 accessions of cultivated Sesame employing isozymes. Laurentin and Karvilsky (2006) applied amplified fragment length polymorphism (AFLP) to clarify genetic relationship among 32 Sesame accessions from the Venezuelan germplasms collections and reported a very low genetic relationship and diversity (0.14 to 0.21). Inter simple sequence repeat (ISSR) polymorphism was to determine the genetic relationship among 75 Sesame accessions of Korea and other countries showed a low level of polymorphism with this particular marker and genetic distances ranged from 0 to 0.255 (Kim et al. 2002). However, admonished that their method had low resolution.
In contrast, a very high level of genetic diversity among Sesame accessions by means of RAPD molecular markers has been reported by Bhat et al. 1999; Earcan et al. 2004 and Akbar et al. 2011. The present study detected a high level of polymorphism for Sesame between the different geographical areas of West Bengal. In our study a high level of polymorphism (94.42 %) obtained is comparable to the 86.75 % polymorphism noticed in a study of genetic diversity in Indian and exotic Sesame germplasms by Bhat et al. 1999 and also analogous to 82.99 % observed in a study of genetic diversity of Sesame of Vietnam and Cambodia (Pham et al. 2009). Salazar et al (2006) also observed 100 % of polymorphism in an analysis of genetic diversity of Sesame of Venezuela. Ercan et al (2004) also reported 78 % polymorphism in an analysis of genetic diversity in Turkish Sesame. Although a considerable level of genetic diversity was noticed among diverse Sesame germplasms collected from various geographical regions of West Bengal, it was found that some accessions situated geographically fur apart grouped together in the same cluster such as V1 and V6 (from North Zone) and V11 (from South Zone) grouped together in cluster I (Fig. 2.38). Similarly in the cluster II germplasms of North Zone (V3 and V5), East Zone (V8 and V10) and West Zone (V13 and V15) were clustered together. Furthermore, in the cluster III germplasms collected from different regions of West Bengal appeared in the identical group such as North Zone (V2, V4 and V7), South Zone (V12), East Zone (V9) and West Zone (V14). This could be an outcome of fairly large movement of West Bengal farmers to different regions (West Bengal) carrying Sesame seeds for cultivation into their new geographical locations. According to Bhat et al (1999) the cultivation practices and consumer preferences in respect to seed colour and seed texture and other quality attributes differ widely with the of cultivation. Stankiewicz et al (2001) pointed out that human factor could be responsible for lack of association between genetic and geographical detachment in some cases. Germplasms collected from the same zone were found to have a close genetic relationship for example cluster I included germplasms V1 and V6 from North Zone and V8, V10 from East Zone in cluster II. Interestingly, all the 3 clusters included germplasms collected from North Zone (Murshidabad district) which may be a consequence of largely substantial movement of farmers of Murshidabad district to different regions of West Bengal for collection of diverse germplasms of Sesame for cultivation.

Using the RAPD results attempts have been made to analyses the genetic diversity from the 4 different geographical zones of West Bengal. From the result it can be summarized that least gene diversity was among the germplasms collected from the South Zone or South 24
Parganas district and highest among germplasms of North Zone or Murshidabad district. The same order of genetic heterogeneity was discerned to Shannon’s information index (North Zone › West Zone › East Zone › South Zone) and the level of gene flow was 2.30. From the standpoint of population genetics, a value of gene flow (Nm) < 1 (less than 1 migrant/generation into a population) or equivalently a value of genetic differentiation (Gst) > 0.25 is generally regarded as the threshold quantity beyond which significant population differentiation occurs (Slatin et al. 1987). The high flow among the population detected in this study points towards the possibility of instances of single isolated populations possessing unique germplasms also found in other populations and which is in conformity with the studies of earlier workers Naik et al (2010).

Little studies have been carried out about the discriminatory power of RAPD primers in Sesame Salazer et al. 2006. But similar studies have been done in other crops such as Soyabean (Powell et al. 1996); Barley (Russel et al. 1997), Jatropha (Grativol et al. 2011) and Chickpea (Chowdhury et al. 2013). In this study we obtain a PIC of 0.40, higher than the 0.37 value reported by Salazer et al. 2006.

In conclusion, using as few as 10 primers, RAPD marker analysis revealed a high level of genetic diversity among Sesame germplasms collected from various geographical zones of West Bengal, India. Finally it can be surmountable that high level of diversity obtained by RAPD marker based analysis and thus this technique can be used for the selection of parents in Sesame breeding program.

The UPGMA cluster analysis and PCA showed a similar pattern in this study and similar relationship between both analyses have been reported by Ercan et al. 2004 and Salazer et al. 2006.