6. SUMMARY

Lentil (*Lens culinaris* Medik) is one of the oldest protein rich food legume crop believed to be indigenous to south western Asia and the Mediterranean region. The Indian subcontinent is the largest lentil producing region in the world. Lentil has the potentiality to cover the risk of dryland agriculture. It helps to improve soil health and environment by fixing the atmospheric nitrogen in association with *Rhizobium* species and increase the fertility status of soil.

Genetic maps are valuable tool for geneticist and plant breeders dealing with crop improvement. A well defined linkage map also provides useful information for detection of analogous genes and linkage groups in related genera. It can also be useful in tagging the other genes such as disease resistance if they are associated with morphological markers. For example, tight linkage of a marker to a gene conferring disease resistance can be exploited for indirect selection of resistant plants in segregating population instead of testing for the resistance itself. Selection in the segregating population can be based on determination of the marker genotype.

Identification of morphological markers in sufficient numbers is prerequisite for development of comprehensive linkage map. However, it is important to note that in spite of great importance of this pulse crop, no concerted efforts were made in respect of identification and genetic
analysis of morphological markers. Keeping in view the above considerations, the present investigation entitled “Inheritance and linkage analysis of morphological markers in lentil (Lens culinaris Medik)” was initiated to worked out the mode of inheritance of identified morphological markers, and to establish the linkage between them and finally development of linkage map.

To initiate the investigation nineteen accessions, indigenous as well as exotic, were obtained from Division of Genetics, IARI, New Delhi and were used in 26 crosses. In each cross combination 2-9 morphological markers were present.

The crossing programme was undertaken during rabi season of 2000-2001 at Research Farm of Kisan Post Graduate College, Simbhaoli, Ghaziabad, Uttar Pradesh. The F₁s were grown in summer (May-September, 2001) at Summer Nursery Dalang Maidan, Lahaul Valley, Himachal Pradesh. The F₁ seeds as well as F₂ seeds were examined for cotyledon colour and F₃ seeds were grouped on the basis of cotyledon colour to raise the F₄ population.

The F₄ seeds with their respective F₂ parents and parents were space planted. The row to row and plant to plant distance was maintained at 40 cm and 20 cm, respectively. All the recommended cultural practices were adopted to ensure a healthy crop.

Twenty six morphological markers were used in the present study to determine their mode of inheritance and to detect linkage between them and to develop a genetic linkage map.

The trait wise summary of experimental findings are presented below.
6.1 Inheritance studies

6.1.1 F₂ population of twelve crosses comprising 4653 plants were studied to work out the inheritance of pigmented leaf (Bl) and found that pigmented leaf is monogenically dominant over non pigmented leaf.

6.1.2 Analysis of 2700 F₂ plants derived from 7 crosses demonstrated monogenic dominance of dark green leaves over light green leaves. The Dgl gene symbol has been proposed for this trait. Recessive condition dgl dgl produced light green foliage.

6.1.3 Study of 14 crosses having 5142 F₂ plants, revealed the monogenic dominance of plant pubescence (Pub) over non pubescence (pub).

6.1.4 The expression of incomplete dominance was observed for broad leaves size (Blf) character after analysis of 3860 F₂ plants derived from 10 crosses. This trait is governed by single gene and the heterozygous condition (Blf blf) produce intermediate size of leaves and the narrow leaves are produced under homozygous recessive state (blf blf).

6.1.5 Study on the inheritance of leaf shape demonstrated that oval leaf shape (Ol) is monogenically dominant over acute leaf shape (ol).

6.1.6 Non significant $\chi^2$ value was found when 5704 F₂ plant from 14 cross segregated in 4253 (tendrilled) : 1451 (tendrilless) revealing that the tendril formation is controlled by single dominant gene (Tnl) and tendrilless phenotype produces in recessive homozygous state (tnl tnl).
6.1.7 F$_2$ segregation of 1268 plants involving four crosses revealed the incomplete dominant of large stipule size controlled by a single gene. The gene symbol $Lst$ was proposed for the first time for this trait. The heterozygous ($Lst \, lsl$) and homozygous ($ls$ $ls$) conditions produces intermediate and small stipule size respectively.

6.1.8 Study on inheritance of stem pigmentation in 6450 plants derived from 16 crosses indicated the monogenic dominance of pigmented stem ($Gs$) over non pigmented stem ($gs$).

6.1.9 F$_2$ segregation for growth habit on the basis of 1163 plants from three crosses indicated that the spreading growth habit was controlled by single dominant gene. Gene symbol $Err$ was chosen for this trait in this study. The recessive condition ($erf$) produced erect growth habit.

6.1.10 The F$_2$ segregation of 672 plants from three crosses segregated in 505 (normal): 167 (globe) indicating dominance of normal plant phenotype ($Glo$) over globe plant phenotype ($glo$).

6.1.11 The observations on $F_1$ and $F_2$ population revealed the dominance of normal stem ($Fa$) over fasciated stem ($fa$). This trait is governed by single gene. Fasciated stem characterized by ($fa$ $fa$) the homozygous recessive state.

6.1.12 The inheritance of dwarf mutant was analysed in two crosses having 847 $F_2$ plants. The results indicated the monogenic dominance of normal phenotype ($D$) over dwarf phenotype. The homozygous recessive condition of this gene ($d \, d$) produces
dwarf phenotype. The inheritance for dwarfness has not been studied earlier.

6.1.13 Purple flower colour was found monogenically dominant over white flower colour when 1844 F$_2$ plants from five crosses gave non-significant $\chi^2$ values. Gene symbol $P$ was used for purple flower colour and homozygous recessive state $p$ $p$ was responsible for white flower colour.

6.1.14 Monocentric dominance of three flowers per peduncle ($Fm$) was observed over one flower per peduncle ($f_{m}$), in eight crosses having 2981 F$_2$ plants.

6.1.15 F$_2$ segregation of 1128 plants derived from three crosses demonstrated incomplete dominance of pod size. $Lpd$ gene symbol was used for this trait, $Lpd$ $lpd$ produced intermediate pod size while, the small pod size is produced by homozygous recessive condition $lpd$ $lpd$.

6.1.16 Pigmented pod trait was found to be controlled by single dominant gene ($Rdp$) over non-pigmented pod ($rdp$ $rdp$). The conclusion was based on 3007 F$_2$ plants generated from seven crosses.

6.1.17 Analysis of segregation for peduncle length in 381 F$_2$ plants of a cross (MC 6 x Sehore 74-3) revealed that the peduncle length was controlled by a single dominant gene. As this traits was studied for the first time gene symbol Pdl was proposed for long peduncle. The small peduncle was produced in homozygous recessive pdl pdl state.
6.1.18 The mottling pattern on testa was examined in 1272 F₂ plants over five crosses and the single gene dominance of mottling pattern over non mottling pattern was observed. Gene symbol \( M_{at} \) has been chosen for this trait in this study.

6.1.19 Inheritance for trait testa colour is described in following two sub-headings:

6.1.19.1 Four crosses were analysed for segregation of brown testa colour in F₂ and found that brown testa colour was monogenically dominant over tan testa. The gene symbol \( B_{rt} \) was used for brown testa while tan testa is expressed by \( b_{rt} b_{rt} \) the homozygous condition.

6.1.19.2 The inheritance of black testa was examined in 2109 F₂ plants from seven crosses indicating the monogenic dominance of black testa over non black testa. The F₂ plants produced varying proportion of seeds with either black or non black testa. The production of two types of seeds by about 50 per cent of F₂ plants, when approximately 25 per cent of the F₂ plants produced uniform black seeds and 25 per cent of F₂ plants produces uniform non black seeds. Most likely it is the case of differential penetrance and/or expressivity of the gene in the heterozygous conditions, a kind of dosage effect.
6.1.20  Inheritance for five cotyledon colours i.e., orange, yellow, brown, light green, and dark green was studied in following ten combinations:

6.1.20.1  A set of eight crosses was examined to find out the inheritance pattern of orange and yellow cotyledon colour in 11541 F$_2$ plants. The non significant $\chi^2$ value indicated the monogenic dominance of orange cotyledon colour ($D_gYD$) over yellow ($D_gYYbb$).

6.1.20.2  Orange cotyledon colour was found monogenically dominant over brown cotyledon colour ($D_gyyB^+$) when 1866 F$_2$ seeds derived from two crosses segregated in 3:1 ratio with non significant $\chi^2$ value.

6.1.20.3  Two crosses involving orange and dark green cotyledon parents revealed the monogenic dominance of orange cotyledon colour over dark green.

6.1.20.4  Two crosses were analysed for segregation pattern of orange and light green cotyledons in F$_2$ population. Four classes of cotyledon colours were obtained viz. 9 orange : 3 yellow : 3 brown : 1 light green with non significant $\chi^2$ value indicating involvement of two independent dominant genes controlling the cotyledon colour in lentil.

6.1.20.5  Another independent digenic segregation pattern was observed between yellow and brown cotyledon colours when 2783 F$_2$ seeds obtained from two
crosses segregated in 1556 (orange) : 531 (yellow) :
514 (brown) : 182 dark green, a good fit in 9:3:3:1 ratio.

6.1.20.6 In two crosses involving the parents having yellow and dark green cotyledon colour, F₁ population segregated in three classes orange, yellow and dark green in 9:3:4 ratio indicating the epistatic mode of digenic inheritance.

6.1.20.7 The F₂ segregation of yellow and light green cotyledon colour was examined in two crosses with 3434 seeds indicating the monogenic dominance of yellow cotyledon (Dg Y bb) colour over light green cotyledon colour (Dg yy bb).

6.1.20.8 Two crosses involving the parents with brown and dark green cotyledon colour were examined and interactive digenic mode of inheritance was found as F₂ segregation was 9 orange : 3 brown : 4 dark green.

6.1.20.9 In a cross between brown and light green cotyledon parents, the F₂ population segregated into 3 brown : 1 light green cotyledons revealing monogenic dominance of brown (Dg yy B⁺) over light green cotyledon (Dg⁻ yy bb).

6.1.20.10 In a cross between light green cotyledon and dark green cotyledon parents, the F₁ seeds were with
orange cotyledon colour. The F₂ population comprising 1792 seeds segregated in 749 (orange) : 249 (yellow) : 255 (brown) : 87 light green and 452 dark green cotyledons which is a good fit in trigenic ratio (27.9 9.3 16) of Mendelian inheritance.

6.2 Linkage studies

In the present investigation 154 gene pairs were analysed to work out the linkage relationship among them. Only 17 pairs of genes were found linked and remaining 137 gene pairs segregated independently. The summary of linkage studies of these gene pairs are presented below in four linkage groups.

6.2.1 Joint segregation analysis for gene pairs Bl-Gs, Bl-Ert, Bl-Rdp, Gs-Ert and Gs-Rdp indicated presence of linkage relationship in these gene pairs with map distances 25.952, 25.695, 31.067, 28.534 and 35.684 Kosambi unit, respectively. It is evident from the observations that these four genes are located in same linkage group.
6.2.2 The gene pair Dgl-Pub founds to be linked in four crosses as \( \chi^2 \) values were significant. The recombination fraction was estimated as 25.166±1.195 per cent and map distance was 27.721 Kosambi Unit

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\begin{array}{c}
\text{Dgl} \quad \rightarrow 27.721 \quad \leftrightarrow \quad \text{Pub}
\end{array}
\]

6.2.3 The joint F₁ segregation was analysed for gene pairs. Blf-OI, Blf-Lst, Blf-Glo, Blf-Lpd, OI-Lst, OI-Lpd, Lst-Glo and Lst-Lpd. The genes in these pairs were found to be linked with significant \( \chi^2 \) values. The map distances in Kosambi unit were 26.803 for Blf-OI, 63.717 for Blf-Lst, 46.324 for Blf-Glo, 39.204 for Blf-Lpd, 32.822 for OI-Lst, 30.989 for OI-Lpd, 25.682 for Lst-Glo and 28.682 for Lst-Lpd. These five genes are located in same linkage group.

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\begin{array}{ccccccc}
\text{Blf} & \rightarrow & 46.324 & \leftrightarrow & 32.822 & \rightarrow & 26.802 \leftrightarrow 30.989 \leftrightarrow 25.682 \\
\text{OI} & \rightarrow & 39.204 & \leftrightarrow & 28.682 & \rightarrow & 63.717
\end{array}
\]
6.2.4 The linkage analysis was worked out for gene pairs P-Mot, P-Brt and Mot-Brt and linkage was detected in these gene pairs due to significant \( \chi^2 \) values. The map distances in Kosambi unit these gene pairs were calculated as 33.267 for P-Mot, 29.159 for P-Brt and 25.952 and for Mot-Brt.

Out of twenty six conspicuously identified morphological markers, four characters i.e. stipule size (large vs small), dwarf growth habit (normal x dwarf), peduncle length (long vs short) and cotyledon colour, orange vs brown, yellow vs brown, yellow vs light green, dark green vs yellow, dark green vs brown and brown vs light green, were studied for the first time for their inheritance. It is also for the first time that all five cotyledon colours (orange, yellow, brown, light green and dark green) were studied together.

Out of 154 gene pairs that were analysed for linkage relationship the seventeen gene pairs were found linked and remaining 137 showed independent assortment. In this study 33 gene pairs were studied for the first time.

Four linkage groups are thus identified in this investigation viz.. Pub-Dgl, P-Brt-Mot, Gs-Bl-Ert-Rdp and Blf-Ol-Lpd-Glo-Lst. The fourth linkage group has been intensively investigated for the first time and thus it becomes possible to prepare a comprehensive linkage map.
The present study on inheritance of morphological markers/traits will enhance the understanding of breeders about lentil genetics, which can help in manifestation of breeding programmes to improve the productivity, adaptability and plant type of this crop.

The establishment of four linkage groups in the present investigation will be helpful for geneticists, biotechnologist and for lentil breeders in planning the meaning lentil breeding programme. Hopefully its ultimate benefits will reach to the farmers of lentil growing regions particularly in rainfed and limited irrigation of our country and the world.