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# Induced mutation analysis with biochemical and molecular characterization of high yielding lentil mutant lines

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ABSTRACT

Induced mutagenesis generates macromolecular variations which ultimately alters the bio-physiological and morphological nature of the crop genotypes. In the present study, molecular characterization of six high yielding lentil mutant lines, developed from hydrazine hydrates (HZ) and gamma rays mutagenesis, was carried out with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and random amplified polymorphic DNA (RAPD) markers. Activity of nitrate reductase (NR) and content of chlorophyll and carotenoid were found to be significantly high in the mutant lines. Protein and mineral (Fe, Zn & Cu) contents were also increased considerably in the mutant lines compared to their respective parent genotypes. SDS-PAGE profile of seed storage proteins showed 35 unique bands with 97.14% polymorphism. Genetic divergence analysis generated total 41 reproducible RAPD bands with average calculated polymorphic percentage of 63.06%. Among the primers, OPA-10 showed the highest polymorphism with significant PIC value. Genetic divergent analysis revealed that genome of cultivar DPL 62 mutated relatively more than the cultivar Pant L 406 due to the mutagen treatments, while DPL 62-B and Pant L406-A were the most divergent mutants induced in the present study. Biochemical and molecular profile of the induced mutant lines facilitates a basis for future conservation and utilization strategies to widen the genetic base of the current lentil breeding population.



## 1. Introduction

Agriculture is the backbone of Indian economy contributing 13.7% of its total GDP and employing around 55% of the total working population in India. Grain legumes are the major component of agricultural system, successfully boosting nutrition, income and environment around the world and therefore considered vital for achieving food and nutritional security for both poor producers and consumers, especially in India. India is the world's largest producer and consumer of pulses accounting about 35% of the world area, about 26% of the total production with a yield gap of about 18% and about 30% of the total consumption in the world [1]. Among the 11 primary pulses recognized by Food and Agriculture Organization (FAO), lentil (*Lens culinaris* Medik.) is one of the most commonly consumed pulses in India. Lentil is an extremely nutritious affordable grain legume with rich protein contents and high mineral density and thus playing a pivotal role in combating food insecurity and malnutrition in developing countries including India [2,3]. Also, its cultivation enhances the soil nutrient status by adding nitrogen, carbon and organic matter, while serving economic advantage to farmer's livelihood with high market returns. India is the second most producer of lentil after Canada [1] and produces about 21.90% of world production whereas yield of lentil in India is very low compare to world average [1], which indicates the lack of efficient agricultural practices and high yielding varieties. Assessment on annual growth rate of lentil cultivation in India between 2005 and 2014 showed a positive shift of 3.06% and 2.13% in total area harvested and total production respectively while a negative shift of 0.91% in yield. This showed that after tremendous scientific efforts, low yield potential remains the major constraints for achieving the desirable lentil production goal. Cubero [4] subdivided *Lens culinaris* Medik. on the basis of seed size into two races, namely *macrosperma* (large seeded) and *microsperma* (small seeded). The cultivated lentil and their wild relatives are predominantly self-fertilizing diploids having seven haploid chromosomes, with length ranged from 3.0 μ to 9.2 μ [5]. Being a self-pollinated crop with narrow genetic base, induction of genetic variability is prerequisite to initiate breeding programme in lentil.

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**Table 1**
Brief description of the mutants cultivated in $M_4$ generation of lentil.

| S. No. | Strain Codes | Origin/Pedigree | Remarks |
|---|---|---|---|
| | DPL 62-CONTROL | JLS X LG 171 | |
| 01 | DPL 62-A | 0.2% HZ | High yield |
| 02 | DPL 62-B | 100 Gy γ rays | High yield |
| 03 | DPL 62-C | 200 Gy γ rays | High yield |
| 04 | DPL 62-D | 200 Gy γ rays + 0.02% HZ | High yield |
| | Pant L 406-CONTROL | SELECTION P-495 | |
| 05 | Pant L 406-A | 0.2% HZ | High yield |
| 06 | Pant L 406-B | 300 Gy γ rays | High yield |

**Table 2**
List of primers used in the RAPD study.

| Sl. No. | Primer Code | Primer sequence 5′ to 3′ |
|---|---|---|
| 01 | OPA-06 | 5′-GGTCCCTGAC-3′ |
| 02 | OPA-07 | 5′-GAAACGGGTG-3′ |
| 03 | OPA-08 | 5′-GTGACGTAGG-3′ |
| 04 | OPA-09 | 5′-GGGTAACGCC-3′ |
| 05 | OPA-10 | 5′-GTGATCGCAG-3′ |
| 06 | OPA-11 | 5′-CAATCGCCGT-3′ |
| 07 | OPA-12 | 5′-TCGGCGATAG-3′ |
| 08 | OPA-13 | 5′-CAGCACCCAC-3′ |
| 09 | OPA-14 | 5′-TCTGTGCTGG-3′ |
| 10 | OPA-15 | 5′-TTCCGAACCC-3′ |
| 11 | OPX-01 | 5′-CTGGGCACGA-3′ |
| 12 | OPX-02 | 5′-TTCCGCCACC-3′ |
| 13 | OPX-03 | 5′-TGGCGCAGTG-3′ |
| 14 | OPX-04 | 5′-CCGCTACCGA-3′ |
| 15 | OPX-05 | 5′-CCTTTCCCTC-3′ |
| 16 | OPX-06 | 5′-ACGCCAGAGG-3′ |
| 17 | OPX-07 | 5′-GAGCGAGGCT-3′ |
| 18 | OPX-08 | 5′-CAGGGGTGGA-3′ |
| 19 | OPX-09 | 5′-GGTCTGGTTG-3′ |
| 20 | OPX-10 | CCCTAGACTG |

Mutation breeding is quick, cost effective, robust and proven method to accelerate the process of developing and selecting novel agronomic traits [6]. Mba et al. [7] and Mba [8] advocated the advantage of induced mutagenesis for unleashing new alleles of genes that control the traits desired for development of "smart" crop varieties. About 55 mutant legume varieties developed in India till 2017. Of the total mutant varieties, only 13 (0.40%) and 2 (0.60%) mutant varieties of lentil released in the world and in India, respectively (MVD-IAEA/FAO, 2017). This indicates the unexplored status of lentil genetic resources and huge potential for mutation breeding in lentil. An adequate amount of genetic variation in crops is must for employing selection [9], as the success of any crop improvement programme exclusively depends on selection [10]. Induction of mutation and selection of mutants are the two key components of mutation breeding. Mutagens directly or indirectly react to damage or modify almost all important structural and functional organic molecules, including proteins, lipids and nucleic acids and thereby differentially affect the morphology, anatomy, biochemistry and physiology of plants. Therefore, assessment on variations induced in the plant macromolecules is highly useful in mutation breeding to understand and select the most desirable mutant phenotypes.

For collection, conservation, utilization of plant genetic resources effectively, the knowledge of genetic diversity that exists naturally or induced is necessary in any crop improvement programme. In mutation breeding, accuracy and speed of the mutant selection depends on the correct estimation of the induced genetic divergence in the mutant lines using markers of different natures viz. morphological, physiological, biochemical and molecular. Lee [11] and Srivastava et al. [12] pointed out that the assessment of genetic diversity based on external features (phenotypes), which are in use for a long time in plant breeding, has limitations, since environmental factors coupled with developmental stages greatly influence the morphological characters of the plant. Therefore, molecular characterization of the induced mutants is decisive in mutation breeding. Although the morphological markers provides the conventional data for initial characterization of the mutant lines, but to target the polygenic traits like grain yield and nutrient contents, selection needs to be supplemented with biochemical and molecular markers for validating the induced genetic diversity observed with morphological and physiological markers, as these are least susceptible to the environmental flux.

Biochemical or protein markers are widely used in plant breeding to ascertain the crop genetic diversity [13–15], especially in pulses due to its abundance. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiling of seed storage proteins have been used by many workers for characterization and identification of genetic diversity in lentils [16–20,9]. Although, protein markers plays an important role in the study of crop diversity in the mutagenized population, but introduction of DNA markers in genetic diversity study revolutionized the marker based techniques, therefore, at present DNA are the most acceptable markers for confirming the induced divergence among the mutant lines. Also, variations in protein are the expression products of mutated genes at a given bio-physiological state of the cell, therefore, DNA markers reveal the more accurate state of induced changes in the genome of the crop. Kumar et al. [21] extensively elaborated that since most of the economic traits, especially yield, are complex, polygenic and influenced by environmental flux; therefore, in order to identify, fix, and select recombinants (mutants in the present case) with superior traits more accurately, there is a need to integrate biotechnological approaches such as marker assisted selection (MAS) in lentil breeding programme to mainstream new genetic variability in the cultivated gene pool. Molecular marker assisted selection has gained major attention in recent years due the availability of large number of DNA markers with better genomic coverage for analyzing the genetic distinctiveness of the crop genotypes [22]. Collard and Mackill [23] opined that "DNA markers have enormous potential to improve the efficiency and precision of conventional plant breeding via marker-assisted selection (MAS)". Random Amplified Polymorphic DNA (RAPD) [24], is an excellent DNA based marker approach for estimation of genetic diversity in pulses [25]. In RAPD, random primers were used in polymerase chain reaction (PCR) for amplification of target DNA [26] from different genotypes to evaluate the polymorphism information at the gene level. RAPD analysis needs relatively small amounts of DNA without prior sequence information [27], thus RAPD markers are generally preferred over other techniques in the construction of genetic maps, cultivar identification, and phylogenetic analysis [28]. Although there are many preliminary reports of mutagenic action of hydrazine hydrate and gamma rays on induction of variation in the plant phenotypes but molecular characterization of high yielding lentil mutants induced by them are meager. The main objective of the present study is to reveal the extent of genetic diversity induced in the NR activity, pigment (chlorophyll and carotenoids) contents, availability of seed protein and micronutrient, and macromolecules viz., protein and DNA assisted characterization of six high yielding lentil mutant lines.

## 2. Material and methods

### 2.1. Plant materials

6 mutant lines and 2 parent genotypes from ongoing lentil mutation breeding program were used in this study. The parent genotypes, cultivar DPL 62 (*macrosperma*) and cultivar Pant L 406 (*microsperma*), were received from the NBPGR, India.