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ORIGINAL ARTICLE

Evaluation of genetic diversity in some promising varieties of lentil using karyological characters and protein profiling

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Abstract Somatic chromosome study from root tip cells using the squash technique of the cytological method and seed protein profile of 5 varieties of *Lens culinaris* (Lentil) through SDS–PAGE were investigated. Karyotype analysis showed gross uniformity in morphology. Somatic chromosome number $2n = 14$ is constant for all the varieties. Chromosomes are mostly long to medium in length with secondary constrictions in one pair of chromosome. Primary constrictions in chromosome ranged from nearly median to nearly submedian in most of the cases. Notwithstanding the gross homogeneity, karyotype analysis revealed minute differences in details. Each lentil variety is thus characterized by its own karyotype, serving as one of the identifying criteria. The seed protein profile revealed that varieties are very close to each other with respect to similarity index that ranged from 0.594 to 0.690. With regard to seed protein banding patterns, slight polymorphism (14.285%) indicating low genetic diversity has been identified among the 5 varieties. A dendrogram indicates one variety is plesiomorphic and rest varieties are apomorphic. All the experimental varieties of lentil studied here show low genetic diversity due to their similar genetic base, indigenous genetic resources and conservative nature of the seed protein.

1. Introduction

Lentil (*Lens culinaris* Medik.) belonging to the family Fabaceae is considered as one of the ancient, domesticated, economically important winter legume crop agriculturally cultivated worldwide as human food [32]. The seeds of this plant are commonly used as edible pulse. Lentils are valued for their high protein content (as much as 30%) and good source of vitamins and other important nutrients.

Seed protein profiles obtained by gel electrophoresis have been successfully used not only to resolve taxonomic and evolutionary problems of several crop species but also to distinguish cultivars of a particular crop species [26,11,22]. In particular, seed protein profiles produced by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) have been successfully used for the identification or discrimination of various crop species, even at the varietal levels [18,39,20,42,8,40,44,2,35,43]. The technique is economical,

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simple, and rapid and generally free from environmental effects compared with the traditional morphological and other descriptive criteria derived from field trials [9,14,30,31,44]. Moreover, it is reported that SDS–PAGE of seed proteins is an extensively used technique for describing and assessing the seed protein diversity of many crop germplasm [12,21] and is potentially a useful identifier and descriptor for the purpose of seed identification and Plant Variety Rights [13,30,10].

Knowledge of cytological and molecular relationships between plant species is very useful in planning effective breeding strategies designed to transfer desirable genes or gene clusters from one species into another, thereby producing fruitful genomic reconstructions and disease free plants. Determination of genetic diversity of any given crop species is a suitable precursor for improvement of the crop because it generates baseline data to guide selection of parental lines and design of a breeding scheme. It is a valuable technique to get knowledge closeness between investigated genera (i.e., through similarity index) [23]. The aim of the present study was to find out genetic diversity of 5 different varieties of lentil using cytological characters and protein profiling.

2. Materials and methods

The seeds of 5 different varieties of lentil namely WBL 81 (Suvendu), WBL 256 (Ranjan), WBL 58 (Subrata), WBL 77 (Moitree) and B 77 (Asha) were officially procured from Pulses and Oilseeds Research Station, Berhampore, West Bengal, India. Characteristics of lentil varieties (Fig. 1) used in this work are presented in Table 1.

2.1. Karyological analysis

2.1.1. Study of somatic chromosome

Somatic chromosomes were studied from root-tip cells. Fresh healthy roots (November and December months are suitable for seed germination within a day), showing peak mitotic activity from 11 AM to 12.00 Noon, were collected and washed in distilled water. For scattering and clarification of chromosome morphology, pretreatment of root tips with mixture of saturated solution of pDB and aesculine for 3–3.15 h at 12–14 °C was found to be very effective for different varieties of lentil. The root tips putting in pre-treating solution were initially chilled at 0–5 °C for 4–6 min and then kept at 12–14 °C. For the sake of comparative karyological analysis, the same pre-treatment chemical was used for all the varieties. Root-tips were then carefully washed in distilled water and transferred to a suitable fixative such as, glacial acetic acid and ethanol (1:3) and kept overnight. The materials were then kept in 45% acetic acid for 3–5 min, subsequently warm over a flame in 2% acetic-orcein:HCl (1 N) mixture (9:1) for 3–4 s and finally kept for 2–3 h. Root-tips were squashed in 45% acetic acid for microscopic observation.

2.1.2. Karyomorphometrical analysis

The total length as well as the short arm length of all the chromosomes of the 5 varieties of lentil was measured accurately. In all the karyotypes, ratio of the short arm to the total length of the chromosome in percentage, F% (form percentage or centromeric percentage) was determined after Krikorian et al. [24].

The *centromeric index* (*F%*) i.e. the position of centromere of each chromosome was calculated using the following formula:

$$\text{Centromeric index (F\%)} = \frac{\text{Length of the short arm}}{\text{Whole length of the chromosome}} \times 100$$

Total centromeric index (*TF%*) was also determined in each taxa following Huziwara [19] by the formula:

$$\text{Total centromeric index (TF\%)} = \frac{\text{Sum of the short arm length}}{\text{Sum of total chromosome length}} \times 100$$

Disparity index (*DI%*) of chromosomes in a karyotype was calculated according to Mohanty et al. [28] by the formula:

$$\text{DI} = \frac{\text{Longest chromosome} - \text{shortest chromosome}}{\text{Longest chromosome} + \text{shortest chromosome}} \times 100$$

During the preparation of karyotypes at least 4–5 well spread metaphase plates were compared and analyzed. Photomicrographs were taken from the well spread preparations with the help of Olympus digital SLR camera and LM digital SLR adaptor fitted with Olympus CX 41 microscope.

2.2. Extraction of seed proteins

0.2 g dry seed of each variety was taken in pre-chilled pestle and mortar and homogenized in chilled 2 ml of 0.2 M phosphate buffer (pH-8.2). The extracts were centrifuged at 10,000 rpm for 15 min at 4 °C. The extracted crude proteins were recovered as supernatant which was used for protein profiling.

Protein concentration of extracts was measured immediately and directly from the supernatant by dye binding assay as described by Bradford [7]. A standard curve of absorbance at 595 nm versus 10–80 µg of BSA was also drawn and from this curve, the amount of protein in sample was calculated and finally expressed as mg per g of seed. Repetition of same experiment was done 3 times in order to check the reproducibility of the method.

2.3. SDS–PAGE

Just before starting electrophoresis process, supernatant was mixed (1:1) with 2X sample buffer [27] and heated in a 1.5 ml eppendorf tube in water bath at 85 °C for 3 min to denature the protein. After that, the protein samples were subjected to one dimensional SDS–PAGE in a gel slab of 1 mm thickness (4% stacking gel = 2.5 cm height and 10% resolving gel = 5.5 cm height). Total size of the gel was $8 \times 7.3\ cm^2$.

Electrophoresis was carried out in the discontinuous buffer system in a vertical electrophoresis apparatus (Bio-Tech India Pvt. Ltd) according to the method of Laemmli [27]. Using micro-pipette 20 µl protein samples were loaded to each well of the gel. In one well of the same gel, protein molecular weight marker (molecular weight range = 14–97 kDa) of Chrommas Biotech, India, was applied. 0.02% bromophenol blue (BPB) was added in the protein sample as tracking dye to see the movement of protein in the gel. The gel was run at 10 mA constant current mode. Then, the gel was stained for overnight in 0.025% Coomassie brilliant blue (CBB) R-250.