Distribution of Heterochromatic Variability in Several Genotypes of Lens culinaris Medik (ssp Microsperma and Macrosperma)

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Abstract: The distribution of constitutive heterochromatin (highly repeat DNA sequences rich in CG bases) in ten genotypes of cultivated lentil (Lens culinaris sMedik) (2n = 2x = 14) was studied by “C-banding” technique marking. Comparative intra- and inter-genotype analysis of the cultivated lentil genome showed that genotypes are subdivided to three groups, according to the characterization and distribution of C bands (numbers, intensity and location of the bands) : the genotypes (Dahra, Nil45, Idlep2, Idlep3, and Balkan755) reveal an important and different marking (heterochromatin overload). The genotypes (Syria229, Metropole and Flip 90-31) are moderately rich in heterochromatin, while the genotypes (Idlep1 and Redjas) are poor. All these variations indicate the presence of an intra- and inter-genotype polymorphism with a percentage equal to 59.1%. We have also observed a remarkable structural difference within chromosome pairs 1 and 5 of the Dahra genotype. This means that these chromosomes are labeled with two types of heterochromatin (constitutive and optional). Note also, the presence of satellites and chromosomes B of types (euchromatic and heterochromatic). The results obtained confirm the existence of relation between the heterochromatin overload and the adaptation of the plant to the unfavorable conditions of the environment. The advantage of group 1 genotypes is that: they are well suited to adverse environmental conditions and have good productive potential. In selection, it is preferable to use them as genitors (especially Dahra) in crosses.

Keywords: Marker chromosomes, C-banding, Satellites, B chromosomes, Polymorphisms

1. Introduction

Lentil (Lens culinaris Medik) is an annual food legume, autogamous and diploid with 2n = 14 chromosomes. This species is classified into two sub-species according to the size and color of the seed: ssp macrosperma and ssp microsperma (Brink and Belay, 2006; Cokkizgin and Shitya, 2013). The lentil is probably the first legume domesticated by humans (Rasheed et al., 2010). Since its domestication, the lentil is grown in rotation with cereals and used for human nutrition through the richness of its protein seeds and other micronutrients (Bhatty, 1998).

In Algeria, the lens occupies 1.5% of the areas reserved for food legumes (Ait Abellah, 2011). It is cultivated in two main regions of Algeria: Mila (2124 ha) and Constantine (1091 ha).

Several cytogenetic studies are carried out on the number of chromosomes, the karyotypes of the Lens culinaris (Sharma and Mukhopadahaya, 1963, Kumar and Sinha 1992, Chafique and Altaf 1994, Gaffarzadeh-Namazi et al., 2007, Hammouda and Khalfallah, 2015). However, we are still far from an unambiguous identification of each pair of chromosomes and there is still disagreement about the exact karyotype. To establish an accurate karyotype becomes important for plant breeding programs providing assignment of genetic link groups to the chromosome and integration of physical and genetic recombination maps. The development and application of the differential staining technique (C-banding), demonstrating the constitutive heterochromatin (Arrighi and Hsu, 1971) resulted in numerous detectable markers for karyotype analysis.

This paper is part of a cytogenetic study of the cultivated lentil. We were interested in analyzing heterochromatin distribution and variability in genome of ten genotypes (local and introduced) (2n = 14), unveiled by the C-banding technique, in order to highlight:

- Detection of regions rich in heterochromatin (non-coding DNA sequence rich in CG base).
- Organization of the constitutive heterochromatin by the evaluation of its rate in the genome, its relation with B chromosomes and its role.
- Chromosomes of genome identification
- Structural comparative analysis of genotypes.
- Finally, to verify the hypothesis of an existing relationship between the heterochromatin richness of the vegetal species with B chromosomes, and their adaptation to unfavorable climatic conditions.

2. Materials and Methods

Plant Materials constitute of ten genotypes (Figure 1) belonging to a cultivated species: Lens culinaris Medik (2n = 2x = 14). The origins and sources of genotypes are described in Table 1:
Table 1: Genotypes Lists and their Origins of *Lens culinaris* Medik

<table>
<thead>
<tr>
<th>species</th>
<th>genotypes</th>
<th>G</th>
<th>origins</th>
<th>source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lens culinaris</em></td>
<td>Syrie229</td>
<td>F5</td>
<td>ICARDA</td>
<td>ITGC El Khroube</td>
</tr>
<tr>
<td>Microsperma</td>
<td>Dahra</td>
<td>F6</td>
<td>Local selection (Tieret)</td>
<td>ITGC Setif</td>
</tr>
<tr>
<td></td>
<td>clide1</td>
<td>F5</td>
<td>ICARDA</td>
<td>ITGC Mila</td>
</tr>
<tr>
<td></td>
<td>clide2</td>
<td>F6</td>
<td>ICARDA</td>
<td>CNCC El Khroube</td>
</tr>
<tr>
<td></td>
<td>clide3</td>
<td>F6</td>
<td>ICARDA</td>
<td>ITGC Setif</td>
</tr>
<tr>
<td>Macrosperma</td>
<td>Flip90-31</td>
<td>F6</td>
<td>ICARDA</td>
<td>ITGC El Khroube</td>
</tr>
<tr>
<td></td>
<td>Balkane775</td>
<td>/</td>
<td>ICARDA</td>
<td>CNCC El Khroube</td>
</tr>
<tr>
<td></td>
<td>Metropole</td>
<td>F5</td>
<td>Isolated in 1941, France</td>
<td>ITGC El Khroube</td>
</tr>
<tr>
<td></td>
<td>Nil45</td>
<td>/</td>
<td>ICARDA</td>
<td>CNCC El Khroube</td>
</tr>
<tr>
<td></td>
<td>Redjas</td>
<td>/</td>
<td>local Selection</td>
<td>ITGC Mila</td>
</tr>
</tbody>
</table>

**Figure 1:** The seeds of genotypes used in C-banding technique

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I.T.G.C: Technical Institute of Field Constantine, Algeria  
C.N.C.C: National Center of Control and Certification of seeds and plants (Constantine), Algeria  

The used C-banding technique is described by Gaffarzadeh-Namazi et al. (2007), on *Lens culinaris*, with modifications introduced in denaturation and renaturation of DNA steps (Hammouda, 2008).

**Preliminary steps**  
The seeds are set to germinate in Petri dishes on filter paper at room temperature. Root tips are treated with 8% hydroxyquinolene for 2-45h TO 3H30 and fixed in solution 3 ethanol/lacetic acid (3v/1v), and then squashed in 45% acetic acid. The coverslips are removed with liquid nitrogen (−196°C).

**C-banding**  
The best preparations have undergone the following steps: Hydrolysis; the slides were treated with 0.2 M HCl for 2 or 3 minutes at 60°C and rinsed in distiller water.  
Denaturation of DNA; the slides were submerged in barium hydroxide solution (50g/l) for 10 minutes at room temperature.  
Renaturation of DNA; the slides were incubated in a 2xSSC solution (0.3M Nacl and 0.03 M Na citrate), at pH 7 for 1h at 60°C.  
Staining; the preparations were stained with 4% Giemsa for 30 minutes in Sorensen phosphate buffer (pH 6.8).  
In experimental procedure, we have modified hydrolysis and coloration steps, by varying the concentrations of the solutions so as to have optimum band coloration.

**3. Results and Discussion**  
From obtained results, we have been able to identify the genome of the species *Lens culinaris* Medik (2n = 2x = 14). Indeed, the majority of chromosomes have dark heterochromatic bands (C-bands) (Figure 2, metaphase plates).  
Variations in chromosome forms are observed in genotypes, because the degree of condensation (or spiralization) is not the same for metaphase chromosomes (Figure 2).
The heterochromatin organization and distribution (corresponding to non-coding DNA sequences rich in CG bases) in all genotypes are analyzed and compared by the heterochromatic bands (Figure 2). This analysis revealed important variations in polymorphic bands. In fact, the zoning of chromosomes, in numbers and intensity of bands, differ, within one genotype and from one genotype to another (Figure 1, Figure 3).

We remind that, the C + bands exists in three forms; telomeric, centromeric and intercalary (Figure 2, Figure 3). The analysis by C-banding, of each chromosome showed a large heterogeneity in the heterochromatic bands distribution (Figure 3):

**Chromosome 1:**
This chromosome is marked by dark polymorphic C + bands observed in all genotypes with the exception of Redjas genotype. Also, we observed a pair of satellites on the short arms chromosome of Dahra and Balkan 755 genotypes.

**Chromosome 2:**
This chromosome revealed the maximum dark additional bands (or specific), of intercalary and telomeric types, with the exception of those of Idlep2 (short arm) and Balkan 755 (long arm). We also noted the presence of a pair of satellites marked on the short arms chromosome of Dahra and Syria 229.

**Chromosome 3:**
This chromosome is marked by dark, strongly colored intercalated bands, other fine, weakly colored, few centromeric and telomeric bands marked on this chromosome in all genotypes. Note also the presence of a pair of satellites near the centromere (short arm) of the genotype Flip90-31. Note here that only the Dahra genotype is characterized by the existence of a pair of secondary construction, located on the short arm.

**Chromosome 4:**
This chromosome shows a lot of extra intercalary bands, which are strongly colored and centrometal and telomere bands weakly colored, except for the Radjas genotype which is lacking shows a lot of extra intercalary bands, which are strongly colored and centrometal and telomere bands weakly colored, except for Syria 229.

In general, the karyotype formula of *Lens culinaris* is defined as:

\[ n = x = 7 = 4m + 3s. \]

Phylogenetic relationships and chromosome evolution in the genus *Lens* can be studied if the chromosome of the cultured lentil can be distinguished using a well-described karyotype as a reference. In species with a highly symmetrical karyotype and a very similar chromosomal morphology, chromosome length (LT), short / long arm ratio, centromere position and satellite localization and secondary constructions may not be the Radjas genotype which is lacking. Also, there is a pair of satellites localized on the short arms of chromosomes 4, genotypes Syria 229 and Idlep 1.

**Chromosome 5:**
This chromosome reveals numerous additional strongly colored intercalary bands and fewer telomerers and centromere bands on both arms.

**Chromosome 6:**
This chromosome is marked by intermediate and telescopic bands and dark centromeric bands, with the exception of that of the Metropole and Redjas genotypes. Note also the
presence of a pair of satellites located on the short arms of chromosome 6 of Balkan755 and Syria 229 genotype.

**Chromosome 7:**

This chromosome reveals interstitial bands, weakly colored, with the exception of those of genotypes Dahra, Idlep3 and Flip 90-31.

The karyotypes of genotypes possessing respectively C bands:

54 (Dahra) - 50 (Nil45) - 46 (Idlep2) - 44(Idlep3) – 44 (Balkan755) - 42 (Syrie229) – 40 (Metropole) – 38 (Flip 90-31) – 33 (Idlep1) - 30 (Redjas). (Table 2).

We remind that karyotypes of the studied genotypes are symmetrical both in the form and size of the chromosomes and this means that they are primitive. These karyotypes constitutes four metacentric chromosome pairs (m) and three sub-metacentric pairs (sm) with the exception of those of the Metropolis and Nil45 genotypes which are characterized by the presence of five metacentric pairs and two sub-metacentric pairs (Hammouda and KHALFALAH, 2015).

**Phylogenetic relationships** and chromosome evolution in the genus Lens can be studied if the chromosome of the cultured lentil can be distinguished using a well-described karyotype as a reference. In species with a highly symmetrical karyotype and a very similar chromosomal morphology,

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chromosome length (LT), short / long arm ratio, centromere position and satellite localization and secondary constructions may not be sufficient, to determine and study chromosomal pairs. For this, a "C-banding" marking technique was used to differentiate each chromosome individually.

The comparison of studied genotypes, shows a large structural heterogeneity (constitutive heterochromatin) marked by thick, dark and fine C * bands (specific gray bands) on the chromosome zoning (Figure 3). Table (3) allows us to distinguish the differences and similarities between the studied characters in the genotypes

![chromosome]{image}

Figure 4: Detection of both types of heterochromatin: constitutive (homologous bands marked on short arms) and facultative (non-homologous bands detected on long arms) labeled on chromosomes 1 and 5 (metacentric and metacentric type) of genotype Dahra

A heterochromatic differentiation was revealed in chromosomes of the genome of the lentil. Chromosomal structural differences, which appear as specific C* bands (marked in gray) (Figure 3) in all chromosomes. And a detailed karyological analysis of chromosomes of the examined genotypes confirmed the presence of intra- and inter-genotype variability.

According to the table, the genotypes (Dahra, Nil45, Idlep2, Idlep3 and Balkan755) showed an important and distinct marking (heavy heterochromatin) compared to the others, these genotypes form the group1.

The genotypes (Syria229, Metropolis and Flip 90-31) are mainly rich in heterochromatin, therefore, they constitute the group2. While, genotypes (Idlep1 and Redjas) are poor. As a result, they consisted of third group (Figure 3, Table 3).

The observations demonstrated a remarkable structural difference (number, intensity and localization of bands) within chromosomes 1 and 5 of Dahra genotype. This means that chromosomes are marked by two types of heterochromatin (constitutive and facultative). It should be noted that constitutive heterochromatin is localized near the centromere and in telomeres or satellites which have the Nuclear Organizer Regions (NOR). The latter consists of highly DNA sequences repeated in the form of bands. While, facultative heterochromatin is rich in LINES sequences (Hammouda et al., 2017).

According to Gaffarzadeh et al. (2007), in the lentil, four chromosomal pairs of metacentric types (m) and three sub-metacentric pairs (sm) are detected, which is our case (Figure 1). Sharma & al. (1983) reported, the first time, that the ploidy level in L. culinaris was $2n = 4x = 28$.

Localization of the satellites

The localization and number of vital zones of chromosomes (satellites / secondary construction, centromere) are different from those observed by the authors (Sinha et Singh, 1982; Sindhu et al., 1984; Slinkard 1985; Raziuiddinet al., 1990), Ahmad et al. (1992) Galasso et al., 2001, Gaffarzadeh et al., 2007). The latter have been able to show a pair of satellite on chromosomes 2 and 4 (near of centromere of the long arm). While in our case, the satellites are localized on the markers chromosomes of genotypes (Dahra, Balkan755, Syria229; Flip90.31 and Idlep1) in a different position. Also we could identify a secondary construct marked on chromosome 3 (short arm) of Dahra genotype (Figure 3, Table 3). But, Shaﬁque et al. 1994 have reported the total absence of satellites in lentil.

Other studies (Abdo et al. 1994, Galasso et al., 2001, Kumar et al., 2001) have demonstrated a nucleolar organizing regions (N.O.R) on chromosome 4 by in situ hybridization, using the probe ( pTa71). According to the literature, satellites are still associated with nucleolar organizers (NORs) correspond to the heterochromatin containing DNA sequences (GAA)m (GAG)n of the satellites, carrying genes responsible for ribosomal ARN coding.

Jones (2008) described the entire satellite and secondary constriction as a "satellite region". The existence of satellite DNA (repetitive DNA) is considered as a genetic marker that may play a role in chromosome pairing during meiosis and protect terminal genes against the process gains and losses of chromosomal.

Table 3: Study of some characters in Lens culinaris

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Characters</th>
<th>Heterochromatin rate</th>
<th>Number and localization</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S / CS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Location</td>
<td></td>
</tr>
<tr>
<td>Dahra</td>
<td>70.3%</td>
<td>2 / 0</td>
<td>Telomere, centromere</td>
<td></td>
</tr>
<tr>
<td>Nil45</td>
<td>68%</td>
<td>0 / 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idlep2</td>
<td>65.3%</td>
<td>0 / 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idlep3</td>
<td>63.4%</td>
<td>0 / 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balkan755</td>
<td>62.4%</td>
<td>2 / 0</td>
<td>Telomere</td>
<td></td>
</tr>
<tr>
<td>Syria229</td>
<td>61%</td>
<td>3 / 0</td>
<td>Telomere</td>
<td></td>
</tr>
<tr>
<td>Metropolis</td>
<td>68%</td>
<td>0 / 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flip90.31</td>
<td>56.2%</td>
<td>1 / 0</td>
<td>Centromere</td>
<td></td>
</tr>
<tr>
<td>Idlep1</td>
<td>51%</td>
<td>1 / 0</td>
<td>Telomere</td>
<td></td>
</tr>
<tr>
<td>Redjas</td>
<td>39%</td>
<td>0 / 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All the variations revealed by C-banding were probably due to the amplification or reduction in quantity of highly DNA sequences repeated in these regions (Friebe and Gill 1994,Gill, 2009).

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Chromosomes B

Our results, in comparison with all the authors (cited above), showed the existence of supernumerary B chromosomes, observed in Dahra, Idlep3, Redjas (in number of 1), Flip 90-31 (in number of 2) genotypes (Figure 2, Table 3). According to the authors (Slebbin, 1974, Amirouche2007, Houben 2011, Hammouda and khalfallah, 2008, John et al., 2012, Hammouda et al., 2015, Hammouda and al. 2017), the apparition of these chromosomes B is a form of adaptation of the species in difficult environment conditions.

The existence of a correlation between their presence and the ecological distribution of populations, noted by John et al. (1982), showed that Myrmeleotettix maculatus populations exhibit several B chromosomes in dry and warm regions, whereas in wetter and colder climates these chromosomes are present in few numbers, or even absent. These authors conclude that B chromosomes play a role in the adaptation of organisms to environmental variations. According to Hammouda (2015, 2017), there is a positive correlation between the constitutive heterochromatin rate and the increase in the number of B chromosomes in triticales (8x and 6x).

Finally, we can deduce the presence of an intra and inter-genotype polymorphism, also the vital zones (satellites / secondary construction, centromere) marked on different chromosomes of Lens culinaris

4. Conclusion

Chromosomal analysis of Lens culinaris genotypes showed inter and intra-genotype structural variations. From the results obtained, the genotypes are subdivided into three groups based on characters studied were: (i) the rate heterochromatin content (non-coding DNA sequences rich in (GC)) (ii) the presence of satellites/ secondary constriction and (iii) the number of B chromosomes, characterizing Dahra, Nil45, Idlep2, Idlep3 and Balkan755 genotypes.

The presence of satellites is remarkable in Dahra, Syrie229,Balkan 775,Flip90-31 and Idlep1 genotypes.

We reported also B chromosomes (heterochromatic type) in the genotypes Dahra, Flip90-31 and Idlep3 and a euchromatic chromosome in the Redjas genotype. This heterogeneity observed in the cultivated lentil showed a very high level in polymorphism which is of major interest at the economic and agronomic scale, because this richness of heterochromatin and chromosome B is translated in the field by adaptation and resistance to factors. Adverse environmental conditions. In selection, it is preferable to use them as a parent in crosses.

References


