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Molecular cytogenetical and biochemical studies on some *Lupinus* species

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Abstract

Background: Lupins are cultivated as human consumption grains and forage legumes. The chromosomes of lupins are too small to be karyotyped by conventional techniques, because they reveal a general lack of distinctive cytological features. In the current study, Fluorescence in situ Hybridization (FISH) was used to locate 5S and 45S ribosomal gene sites on the chromosomes of *Lupinus albus* ssp *albus*, *Lupinus albus* ssp *graecus*, *Lupnus termis* (all with $2n = 50$), and *Lupinus polyphyllus* lindl var. polyphyllus ($2n = 48$), FISH together with seed storage protein electrophoretic patterns were used to find out the relationship among these species.

Results: The double-target FISH on the chromosomes of the studied species with rDNA probes revealed that the two types of rRNA genes are located on different chromosomes. The detected loci of rRNA genes partially reflected the taxonomical similarity among the two *Lupinus albus* subspecies and *L. termis*. *Lupinus polyphyllus* lindl var. polyphyllus was exception by having unique large chromosome mostly is covered by one signal of 45S rDNA, whereas its homologous chromosome seems to be normal-sized and have the other 45S rDNA locus. The similarity matrix among the *Lupinus* species as computed according to Jaccard's Coefficient from the SDS-PAGE, showed that *L. albus* ssp. *Albus* and *L. albus* ssp. *Graecus* are the most similar species (~ 97%), and then comes *L. termis*, and *L. polyphyllus* lindl var. polyphylus has been placed in separate clade and still the most related species to it among the studied species is *L. termis* (~ 70%).

Conclusion: It could be postulated from FISH and seed storage protein electrophoretic patterns that the relationships among the studied species is as follows, *Lupinus albus* ssp *albus*, is the most related species to *Lupinus albus* ssp *graecus* then comes *Lupnus termis* and *Lupinus polyphyllus* lindl var. polyphyllus at a distal position.

Keywords: *Lupinus*, 5S and 45S rRNA genes, FISH, Jumping satellite, Seed storage protein electrophoresis

Background

Leguminous plants are considered as essential protein supplements and other nutritious substances for animal and human in addition to fat content in the seed. Lupin is one of those crops that has biochemical and physiological traits for their ability to synthesize huge amount of protein, fats and other useful ingredients (Duranti and Morazzoni 2011; Lucas et al. 2015; Zhong et al. 2020). The number of *Lupinus* species is unclear, it ranges from 100 to more than 800 species. The distribution of lupins concentrated in two main regions: Mediterranean-African and American, the genus distributed also from the ocean level up to higher than 4500 m above the sea level. Twelve species of lupins are recorded in Africa and Mediterranean regions (Drummond et al. 2012; Mousavi-Derazmahalleh et al. 2018).

The commonly cultivated lupins species in several countries are *Lupinus albus* L., *L. angustifolius* L., *L. cosentinii* Guss, *L. luteus* L., *L. pilosus* Murr and *L. atlaticus* Gladstones. In Georgia *L. albus* was registered under the name hancholy, and in Egypt *L. albus* was described by Linnaeus as *L. termis*. In Palestine and ancient Egypt, *L. albus* was called thrums. Ecotypes of *L. albus* in Ethiopia, Sudan and Egypt genotypes are early maturity,

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tolerant to drought and many have resistance to fusarium (Christiansen et al. 2000; Dwiviedi et al. 2006; Atnaf et al. 2017).

So far, not many chromosomal researches have been done on *Lupinus*, despite the phylogenetic trees which have built up by using the recent genomic and transcriptomic data (Mahé et al2011; Drummond et al. 2012; Cannon et al. 2015; Susek et al. 2017). Some primary genetic investigates have been done to measure the genome size to be 0.97–2.44 pg/2C DNA (Naganowska et al. 2003b; Susek et al. 2016). Other investigations estimated the chromosome numbers to be from $2n=32$ to $2n=52$, and the basic chromosome numbers to be $x=5$–7, 9, or 13; therefore, Lupin species are supposed to be of polyploid origin, having interspecific variation (Conterato and Schifino-Wittmann 2006; Naganowska et al. 2006; Susek et al. 2019). Their small-sized chromosomes are difficult to be karyotyped by conventional techniques, because they exhibit a general lack of distinguishing cytological features (Naganowska and Zielinska 2002).

There are mainly two types of ribosomal RNA genes in eukaryotes, 5S and 45S (18S, 5.8S and 26S/28S), which are organized in tandem arrays in thousands of copies at one or more chromosomal loci. They involve in ribosome production, and their regions are essential elements of chromosomes of all cell types. The ribosomal RNA (rRNA) gene loci were the first chromosome markers used for cytotaxonomic and karyotyping purposes analysis especially in species with many small and equal chromosome sizes, since they could be easily visualized aby double FISH with heterologous ribosomal probes, thus allowed the physical localizations of both rDNA genes in a single metaphase preparation (Fransz et al. 1998; Pita et al. 2014).

Molecular cytogenetic techniques like Fluorescence in situ hybridization (FISH) is one of the suitable tools for chromosome identification and analysis which has been applied to many plant genomes (Ali et al. 2005, 2009; Jiang and Gill 2006; Chiarin and Gauthier 2016; Ribeiro et al. 2016; She et al. 2017; Tan et al. 2017; Setiawan et al. 2018; Jiang 2019). FISH using BAC clones depending on the genome mapping was very effective tool to elucidate the comparative cytogenetics map in Lupin (Naganowska and Zielinska 2002, 2004; Hajdera et al. 2003; Wyrwa et al. 2016; Susek et al. 2019). rRNA genes have been used as FISH probes in a number of lupin species (Hajdera et al. 2003; Kong et al. 2009).

Sodium Dodecyl Sulfate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) is a useful tool to investigate the protein pattens which could be used as an informative marker in the plant genetic diversity investigations. Two classes of plant storage proteins are known, vegetative storage proteins (a group of proteins which accumulate in stems, tubers, and leaves), and seed storage proteins (a group of proteins which highly accumulated during the seed development). SDS-PAGE by using seed storage proteins is considered a reliable tool, because such proteins are extremely independent from the environmental changes. Therefore, their electrophonic patterns could be used as a capable tool for species and cultivars identification (Ahmad and McNeil 1996; Sammour et al. 2007; Vivodík et al. 2016; Špalekova and Galova 2018). However, only a few studies indicated that cultivar identification was not possible with the SDS-PAGE method (De Vries 1996).

The current study focused on using FISH to find out the numbers and chromosomal positions of 5S and 45S rRNA genes, and seed storage protein patterns by SDS-PAGE as genetic markers for *Lupinus* species identification. In our study, we used two *L. albus* subspecies (*L. albus* ssp *albus*, *L. albus* ssp *graecus*), in addition to *L. termis* and *L. polyphyllus* lindl var. polyphyllus.

Methods

Plant material

The seeds of the Lupin species were obtained from the germplasm collection of the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. Two *Lupinus albus* subspecies *L. albus* ssp *albus* (accession no. LUP: 203), *L. albus* ssp *graecus* (accession no. LUP: 512) have ($2n=50$), and *L. polyphyllus* lindl var. polyphyllus (accession no. LUP: 88), with $2n=48$ chromosomes, whereas *L. termis* L. ($2n=50$) was obtained from Egyptian germplasm (store).

Chromosome preparation

Chromosomes were prepared from the root tips of the germinated seeds and FISH experiments were done according to Fransz et al. (1998) with minor modifications. Seeds were sown on two layers of moistened filter paper in a Petri dish and kept in the dark at 25 °C for two days or more. The young germinated root tips were cut and treated with 0.02% aqueous 8-hydroxyquinoline for 3 h at 15 °C and then washed three times with sterile water before fixation in freshly prepared Chloroform–ethanol–acetic acid (6:3:1) then in acetic acid–ethanol (1:3) and stored in 70% ethanol.

Fluorescence in situ hybridization

The *A. thaliana* BAC clone T15P10 (AF167571) bearing the 45S rDNA sequence was labeled with digoxigenin by nick translation, and the 5S rDNA probe was amplified from genomic DNA of *A. thaliana* and labeled with biotin by PCR with primers specific for the coding region (Gottlob-McHugh et al. 1990). Digoxigenin-labeled probes were detected by mouse anti-digoxigenin (Jackson

Immune Research Laboratories, United Kingdom) and goat anti-mouse antibodies conjugated with Alexa 488 (Molecular Probes, USA). The biotinylated 5S rDNA was detected by avidin ~ Texas Red (Vector Laboratories, USA) and amplified by biotinylated goat anti-avidin (Vector Laboratories, USA) and avidin ~ Texas Red. The chromosomes were counterstained with DAPI (2 µg/ml). The images were captured with a Zeiss Axioplan 2 epifluorescence microscope equipped with a Spot 2e CCD camera. Images were pseudo-colored and merged using Adobe Photoshop CS software (Adobe).

SDS-protein electrophoresis

SDS-PAGE was performed according to the method of Laemmli (1970), as modified by Studier (1973); 0.2 g of seed samples were ground from the examined *Lupinus* species (*Lupinus albus* ssp *albus*, *Lupinus albus* ssp *graecus*, *Lupnus termis* and *Lupinus polyphyllus* lindl var. polyphyllus), then mixed with 1 ml of extraction buffer (2X) in Eppendorf tube and left in refrigerator over-night, then vortexed for 15 s and centrifuged at 12,000 rpm at 4 °C for 20 min. The supernatants containing total seed storage proteins were transferred to new Eppendorf tubes and kept in deep-freeze until use for electrophoretic analysis. Extracted protein samples and protein marker (BLUltra Prestained Protein Ladder, GeneDirex, USA) were loaded on 10% Protein separating gel for running. Protein fractionations were performed exclusively on vertical slab gel (19.8 cm × 26.8 cm × 0.2 cm) using the electrophoresis apparatus manufactured by Claver UK.

The images were captured by a digital camera (Sony, Japan) and transferred directly to the computer, then the protein bands were analyzed by Total Lab program to detect the molecular weight of each protein. The protein analysis data were imported in Past program (Paleontological statistics software package for education and data analysis, Hammer et al. 2001) to find the similarity matrix and dendrogram (UPGMA, using Jaccard's coefficient) which reflect the relationships among the studied species.

Results

Ribosomal genes exist in many copies per genome and expressed in each cell. The double-target FISH on metaphase chromosome preparations with rDNA probes revealed that the two types of rRNA genes are located on different chromosomes.

Figure 1 shows the obtained loci of 45S rRNA genes (green) and 5SrRNA genes (red) in *L. albus* ssp *albus*, *L. termis*, *L. albus* ssp *graecus*, and *L. polyphyllus* lindl var. polyphyllus (Fig. 1a–d), respectively, while the same figure (a1, b1, c1 and d1) show the stained chromosomes with DAPI for more chromosome resolution.

Two signals of 5S rRNA genes were observed in *L. albus* ssp *albus* and *L. termis,* while four signals were found in *L. albus* ssp *graecus*, and *L. polyphyllus* lindl var. polyphyllus. *L. albus* ssp *albus*, *L albus* ssp *graecus* and *L. termis* showed four signals of 45S rRNA genes, whereas in *L. polyphyllus* lindl var. polyphyllus two 45s rRNA signals were detected, one of them covered almost the whole chromosome. The detected 45S and 5S rRNA gene loci using double-FISH experiment on the metaphase chromosomes of the studied species is as follows:

In ***Lupinus albus ssp albus*** ($2n=50$), FISH exhibited two pairs of strong, large signals of 45S rDNA, one on the long arm of sub-telomeric chromosome, the other signals were extended to cover the major part of another chromosome, including its secondary constriction, whereas only one locus (two interstitial signals) of 5S rDNA was observed in this species (Fig. 1a).

Lupinus termis ($2n=50$) exhibited two pairs of 45S rDNA sites (two gene loci) in its genome, one locus was transcriptionally active and gave stretched signals, while the other locus was tiny on small chromosome. Only one terminal 5SrDNA locus was observed in this species (Fig. 1b).

Lupinus albus ssp graecus ($2n=50$) expressed two pairs of 45S rDNA loci, one almost covering the whole chromosome pair, and another of a small terminal locus, in addition to two terminal 5S rDNA loci, one medium size on one chromosome pair and the other appears as a minor locus on another small chromosome pair (Fig. 1c).

Lupinus polyphyllus lindl var. polyphyllus ($2n=48$) was an exception with having one large chromosome. This species showed only one locus of 45S rDNA, one site of this locus (gene) is covering the large chromosome, and the other site of the same gene on another shorter chromosome (homologous chromosome), this species showed two normal loci of 5S rDNA on the terminal part of two chromosome pairs (Fig. 1d).

Seeds storage protein patterns

SDS-electrophoretic patterns of total seed storage protein fractions were used to identify and find the phylogenetic relationships among the studied *Lupinus* species.

The distribution of electrophoretic banding patterns of total seed storage proteins in *Lupinus* species is shown in Table 1 and Fig. 2. There was a total number of 37 bands in the different *Lupinus* species with molecular weight ranged from 240 to 12 Kilo Dalton (KD). A maximum number of 34 bands were detected in *L. termis,* whereas the minimum number was 29 bands in *L. polyphyllus* lindl var. polyphyllus. In *L. albus* ssp. *graecus* 31 bands were found, while 30 bands were detected in *L. albus*

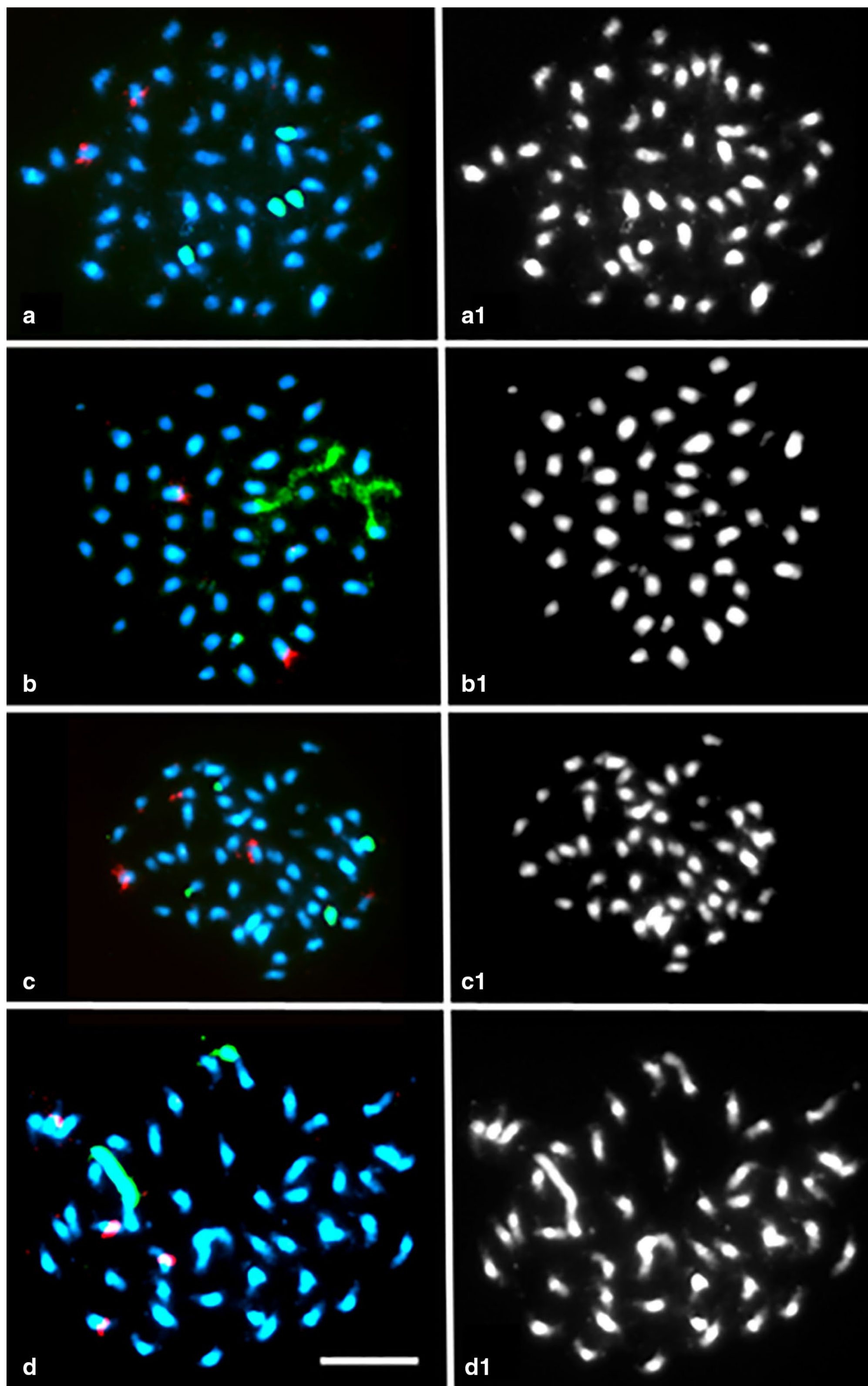

Fig. 1 Mitotic metaphase chromosomes of four *Lupinus* species after FISH with rDNA probes (blue color images), 45S rDNA probe was detected by FITC (green signals) and 5S rDNA probe by Texas red (red signals). The chromosomes were counterstained with DAPI (white images). **a**, **a1** *Lupinus albus* ssp *albus*; **b**, **b1** *Lupinus termis*; **c**, **c1** *Lupinus albus* ssp *graecus*; **d**, **d1** *Lupinus polyphyllus* lindl var. polyphyllus. Bar = 5.0 µm

Table 1 Approximate molecular weight and intensity of total seed storage protein bands in *Lupinus* species

Band No	MW (KD)	*Lupinus albus* ssp. *albus*	*Lupinus albus* ssp. *graecus*	*Lupinus polyphyllus*	*Lupinus termis*
1	240	–	–	+	+
2	210	–	–	+	+
3	180	–	–	+	–
4	160	+	+	+	+
5	140	+	+	+	+
6	120	+	+	+	+
7	109	+	+	–	+
8	103	+	+	+	+
9	96	+	+	–	+
10	89	+	+	++	+
11	73	+	+	+	+
12	70	+	+	++	+
13	65	+++	+++	+++	+++
14	64	+++	+++	+++	+++
15	63	+++	+++	–	+++
16	62	+	+	+	+++
17	60	–	–	++	+
18	54	++	++	+	++
19	48	+++	+++	++	+++
20	44	+++	+++	+	+++
21	42	+	+	+	+
22	38	++	++	++	++
23	36	+	+	–	+
24	34	+	+	+	+
25	32	++	++	–	++
26	31	+	+	+	+
27	30	–	–	–	+
28	29	+	+	+	+
29	28	+	+	–	+
30	26	+	+	+	+
31	25	–	–	+	+
32	23	+	+	+	–
33	20	+	+	+	–
34	19	+	+	–	+
35	17	++	++	++	++
36	15	+	+	+	+
37	12	–	+	+	+
Total	30	31	29	34	

ssp. *albus.* The examined *Lupinus* species shared twenty major bands (monomorphic bands) at molecular weights of 140, 120, 103, 89, 73, 70, 65, 64, 62, 54, 48, 44, 42, 38, 34, 31, 29, 26, 17 and 15 KD. The UPGMA method was used to calculate the similarity coefficient among the studied species as shown in Table 2 and their average was used as an approximate threshold value for recognizing groups of species in dendrogram (Fig. 3). The description of bands in each species was as follows.

Lupinus albus ssp. *albus*

This species exhibits a total number of 30 bands, among them 5 bands were dark, 3 moderate and the rest were faint. The largest-size band was observed at molecular weight of 160 KD and the smallest band was detected at molecular weight of 15 KD. It shared with *L. albus* ssp. *graecus* and *L. termis* in the absence of 4 bands (240, 210, 60 and 25 KD), and exhibited one unique negative band at 12 KD.

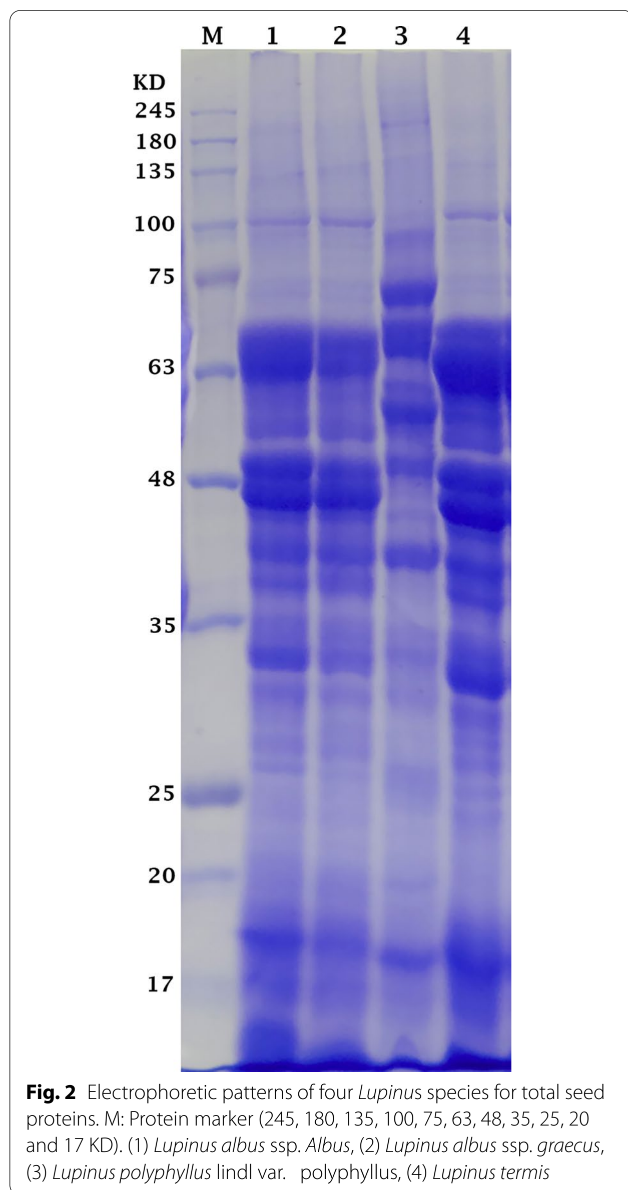

Fig. 2 Electrophoretic patterns of four *Lupinus* species for total seed proteins. M: Protein marker (245, 180, 135, 100, 75, 63, 48, 35, 25, 20 and 17 KD). (1) *Lupinus albus* ssp. *Albus*, (2) *Lupinus albus* ssp. *graecus*, (3) *Lupinus polyphyllus* lindl var. polyphyllus, (4) *Lupinus termis*

Lupinus albus ssp. *graecus*

A total number of 31 bands was detected in this species, among them 5 bands were dark, 4 bands were moderate and the rest were faint. The highest molecular weight band was at 160 KD, whereas the lowest one was at 12 KD. As mentioned above this species shared with *L. albus* ssp. *albus* and *L. termis* in the absence of 4 bands, it shared *L. termis* in the absence of one band at 180 KD.

Lupinus polyphyllus lindl var. polyphyllus

This species exhibited the lowest number of bands (29 bands) among the examined species. The largest size band was at molecular weight of 240 KD and the smallest band was at 12 KD. Among the 29 bands, 2 bands were dark, 5 moderate and the rest were faint. This species was characterized by the present of unique band at 180 KD and the absence of 7 bands at 109, 96, 63, 36, 32, 28 and 19 KD.

Lupinus termis

A total number of 34 bands were detected in this species, out of them 6 bands were dark, 4 bands were moderate and the rest were faint. The highest molecular weight band was at 240 KD, whereas the lowest one was at 12 KD. It was characterized by the presence of one unique band at 30 KD, and absence of two bands at 20 and 23 KD.

Discussion

The *Lupinus* species are characterized by a variation in their chromosome number (from $n=16$ to $n=26$) and the DNA content per haploid cell was found to be small (Weeden et al. 2000; Hajdera et al. 2003; Bennett and Leitch 2005; Naganowska et al. 2006). Compared to the other plant species, cytological work in genus *Lupinus* is quite limited because of the high number of chromosomes which ranges from $2n=32$ to 52, and small size of chromosomes, which made them difficult to be separated on the slide; subsequently, the karyotyping of the chromosomes by conventional techniques is complex, because they lack the general features, like centromere or secondary constriction positions. In addition, similarity in chromosome shape and size obstacles the chromosome pairs to be simply well-identified at the cytological level, which hampered the identification of individual species via chromosome size or arm ratios; therefore, the

Table 2 Similarity matrix among studied *Lupinus* species as computed according to Jaccard's coefficient as revealed by protein markers

L. albus ssp. *albus*	1			
L. albus ssp. *graecus*	**0.968**	1		
L. polyphyllus lindl var. *polyphylus*	0.639	0.667	1	
L. termis	**0.778**	**0.805**	0.703	1
	L. albus ssp. *albus*	*L. albus* ssp. *graecus*	*L. polyphyllus lindl* var. *polyphylus*	*L. termis*

Bold numbers reflect the more related species

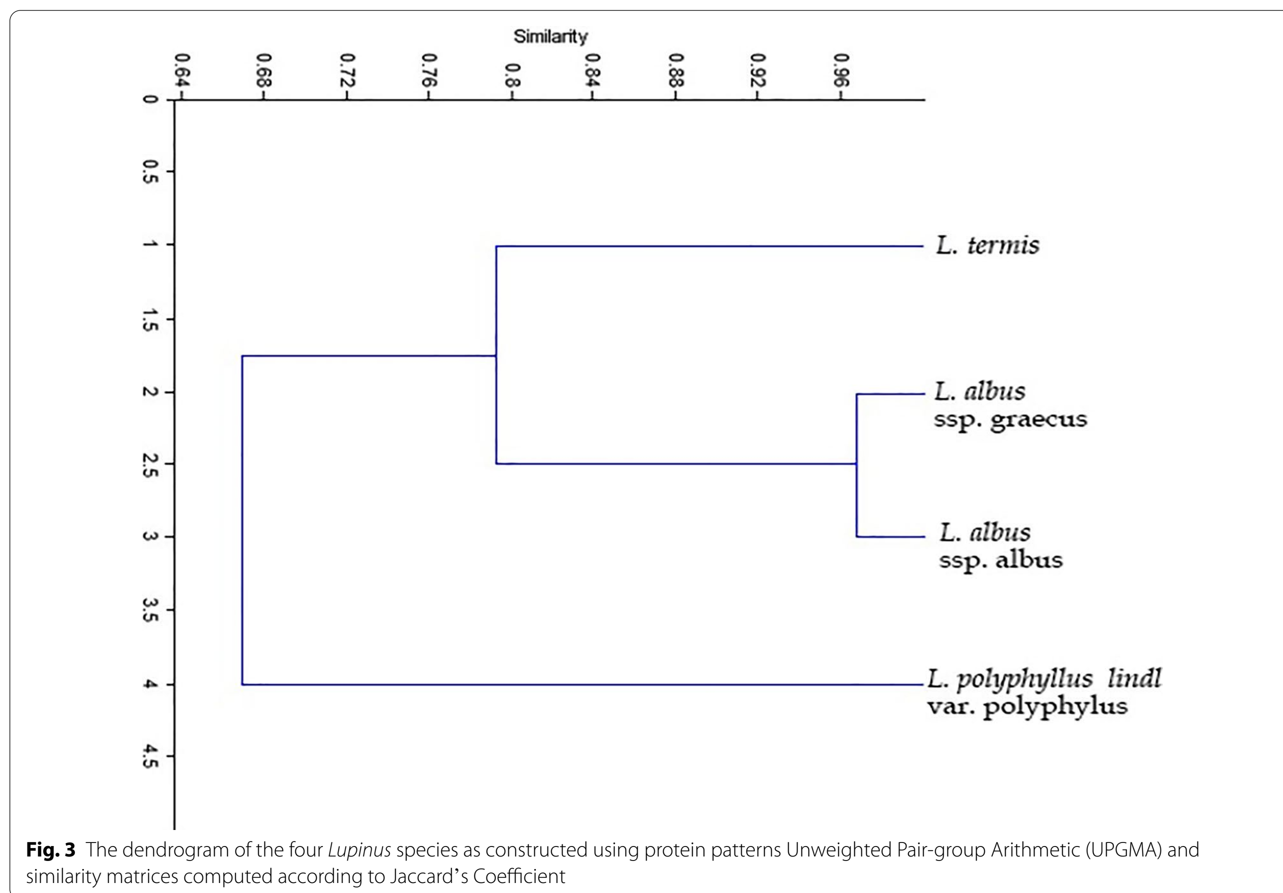

Fig. 3 The dendrogram of the four *Lupinus* species as constructed using protein patterns Unweighted Pair-group Arithmetic (UPGMA) and similarity matrices computed according to Jaccard's Coefficient

chromosome karyotyping in the species of this genus is very difficult. Nevertheless, chromosomes counting in lupins have been achieved for more than 60 years (Wolko et al. 2011).

Fluorescence in situ hybridization (FISH) is mainly based on hybridization of a molecular probe to its complementary sequence on chromosomes, and detecting the signals directly under a fluorescence microscope (Pita et al. 2014; Jiang 2019). The highly repetitive rRNA genes were suitable chromosomal markers to study the physical map of plant genomes using FISH. In lupins, most of FISH studies were carried out on *L. angustifolius* to locate rDNA loci (Kaczmarek et al. 2007, 2009), or for comparative genome mapping to assign many linkage groups to special chromosomes (Kasprzak et al. 2006; Lesniewska et al. 2011; Książkiewicz et al. 2013; Wyrwa et al. 2016; Susek et al. 2019). Double-target FISH was applied in other Lupin species to determine the localization of genomic rDNA in new and old world Lupin species, (Naganowska and Zielinska 2002, 2004; Hajdera et al. 2003; Naganowska et al. 2003a; Naganowska and Kaczmarek 2005; Kong et al. 2009).

It was observed in the previous studies that the positions of rRNA genes (5S and 45S rDNA as probes) were on separate chromosomal regions, thus allowing the differentiation of only up to five different pairs of chromosomes, no relationship between numbers of rRNA gene sites and number of chromosomes has been recorded. This is in agreement with the results in the current study, where we observed that the loci of 5S and 45S rDNA are on separate chromosomes in the investigated species, and the 45S rDNA signals are large and cover almost half of the chromosome, the stretched signals reflected the nucleolar activity in *L. albus* ssp *albus*, *L. albus* ssp *graecus*, and *L. termis*. In general, the large size of the signals and low number of rDNA loci, support the commonly known idea of ancient origin of polyploidy in *Lupinus*, where some rDNA sites might be lost, translocated or fused with other rDNA sequences during the course of evolution and the long diploidization process. The high rate and fixation of chromosomal rearrangements in Lupin genomes through the diploidization process, resulted in cytogenetically stable, functional diploids plants in the long history of lupins (Thomas et al. 1997; Weiss and Maluszynska 2000; Kroc et al. 2014;

Susek et al. 2019; Susek and Naganowska 2020). The same reduction in rDNA was observed in other polyploid plant species (Sakowicz and Olszewska 1997; Snowdon et al. 1997; Ali et al. 2005).

The DNA contents of three *Lupinus* sp. (*L. albus* ssp *albus*, *L. albus* ssp *graecus*, and *L. termis*) in the current investigation have been studied by Naganowska et al. (2003a), they found slight differences in measurements of the DNA content (C-value) in *L. albus*, *L. graecus* and *L termis* (1.16 ± 0.044, 1.13 ± 0.030 and 1.14 ± 0.032 (pg), respectively). Despite the similar chromosome number (2n = 50) in these three taxa, no previous investigations by FISH using 5S and 45S rDNA probes have been done on them.

This study was carried out to locate the loci of ribosomal genes on the metaphase chromosomes of four *Lupinus* species, the detected loci of rRNA genes in the current study partially reflected the similarity among them. FISH result revealed two of 45S rDNA in *L. albus* ssp *albus*, *L. albus* ssp *graecus*, and *L. termis* in which one of two loci of 45S rDNA was transcriptionally active and gave stretched intermediate signals. Concerning the loci of 5S rDNA, it was discovered one 5S rDNA in *L. albus* ssp *albus*, and one terminal 5SrDNA locus in *L. termis*, while *L. albus* ssp. *graecus* expressed two terminal 5S rDNA loci.

L. polyphyllus lindl var. polyphyllus (2*n* = 48) was an exception by having large chromosome bearing one site of 45S rDNA covering most of the chromosome, whereas its homologous chromosome seems smaller in size and have the other 45S rDNA locus (gene). Therefore, it has been postulated the presence of satellite jumping from one of the chromosomes which bears the 45S rDNA to its homologous chromosome in this species. Such phenomenon is rare and has been observed by Sato (1981) and Sato et al. (1981) in their studies on *Allium cepa* with two chromosomes bear satellites, where it was found one of the homologues with a distinct satellite, while the other with a very weakly stained region at the end of the short arm. The observation of Schubert and Wobus (1985) by Ag-NOR patterns strongly supported that NORs can jump between terminal heterochromatin regions of different chromosomes of the parental species in *Allium cepa* and in their interspecific hybrids as well. In another cytological study by silver staining and C-banding (Georgiev and Topouzova 1998), it was recorded in the chromosome complement of *Allium cepa* bulbs the occurrence of a single, two, three and four chromosomes with satellites, the satellites were highly polymorphic in their staining intensity and size and they detected only on the subtelocentric and the shortest metacentric chromosomes. The authors concluded from their study that both deletions and translocation might have occurred which led to a variation in the number and size of the satellited chromosomes and consequently in the polymorphism of the subtelocentric and the metacentric chromosomes.

Seed storage protein gel electrophoresis in the different *Lupinus* have been Examined in several investigations (Tai and Bush 1997; El-Shazly et al. 2006; Marzouk and El-Darier 2008; Mahfouze et al. 2018). Among the *Lupinus* species, *L. albus* L. and *L. angustifolius* L. are the most intensively studied species by using seed protein electrophoresis (Yu et al. 1987; Melo et al. 1994; Magni et al. 2007; Foley et al. 2011; Islam et al. 2012; Czubinski and Feder 2019).

The total seed protein profiles have been used in the present investigation to elucidate the taxonomic relationships among *L. albus* ssp. *Albus*, *L. albus* ssp. *Graecus*, *L. termis* and *L. polyphyllus* lindl var. polyphyllus. In addition, to compare the *L. albus* ssp *albus* with its related relatives. As revealed by analysis of current study data depending on the variation in electrophoretic seed storage protein patterns, and with regard to their UPGMA tree building method (Table 2), it has been postulated that *L. albus* ssp. *Albus* and *L. albus* ssp. *Graecus* are the most similar species (~ 97%) which support their taxonomic criteria, and *L. termis* is more related to *L. albus* ssp. *Graecus* (~ 81%) than to *L. albus* ssp. *Albus* (~ 78%), and finally *L. polyphyllus* lindl var. polyphylus has been placed in separate clade and still the most related species to it among the studied species is *L. termis* (~ 70%).

Conclusion

Three of the analyzed *Lupinus* species in the current investigation (*L. albus* ssp *albus*, *L albus* ssp *graecus* and *L. termis*) are closely related at the taxonomical level. The observed number of rDNA loci by FISH partially reflected the relationship among them. Two signals of 5S rRNA genes were observed in *L. albus* ssp *albus* and *L. termis,* while four signals were found in *L. albus* ssp *graecus*, and *L. polyphyllus* lindl var. polyphyllus. *L. albus* ssp *albus*, *L albus* ssp *graecus* and *L. termis* showed four signals of 45S rRNA genes, while in *L. polyphyllus* lindl var. polyphyllus.one of the two detected 45s rRNA signals covered almost the whole chromosome.

The current studied postulated the satellite jumping in *L. polyphyllus* lindl var. polyphyllus (2*n* = 48), which was an exception by having a big single chromosome bearing one site of 45S rDNA covering it, whereas its homologous chromosome seems normal and have the other 45S rDNA locus. It has been assumed from the seed storage protein SDS-PAGE patterns that *L. albus* ssp. *Albus* and *L. albus* ssp. *Graecus* are the most similar species to each other, and *L. albus* ssp. *Graecus* is more related to *L. termis*, and finally *L. polyphyllus* lindl var. polyphyllus has been placed in separate clade and *L. termis* the most

related species to. It could be concluded that SDS-PAGE of seed storage proteins can reveal the similarity among the *Lupinus* species, which support their taxonomic criteria in an attempt to obtain information useful for breeders.

Abbreviations

FISH: Fluorescence in situ hybridization; SDS-PAGE: Sodium dodecyl sulfate-poly acrylamide gel electrophoretic; rDNA: Ribosomal DNA; rRNA: Ribosomal RNA; DAPI: 4′,6-Diamidin-2-phenylindol; Past: Paleontological statistics software package; (UPGMA): Unweighted pair group method with arithmetic; NORs: Nucleolar organizing regions.

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Authors' contributions

HA performed the FISH experiment part. SH performed the seed storage protein electrophoresis part. HA and SH wrote the manuscript, participated in the data discussion, data analyses, and drafting of the manuscript. Both authors have read and approved the final manuscript.

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Availability of data and materials

Authors declare that all generated and analyzed data are included in the article. All plant materials (three *Lupinus* species seeds) were identified and collected in Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. And one species *L. termes* is from Egypt germplasm (store).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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