Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 12(1): 58-68, 2022
Journal of the Institute of Science and Technology, 12(1): 58-68, 2022
ISSN: 2146-0574, eISSN: 2536-4618
Bilgisayar Mühendisliği / Computer Engineering **DOI: 10.21597/jist.884156**
Araştırma Makalesi / *Research Article*
Geliş tarihi / Received: 21.02.2021 **Kabul tarihi / Accepted: 05.12.2021**

Atıf İçin: Emre İ, Tuncer T, Dogan S, Kursat M, Gedik O, Kıran Y, 2022. Yerel ikili örnek kullanarak üç *Lallemantia* Fisch. & C.A. Mey. türünün kromozom sınıflandırmasına farklı bir yaklaşım. Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 12 (1): 58-68.

To Cite: Emre İ, Tuncer T, Dogan S, Kursat M, Gedik O, Kıran Y, 2022. A different approach to the chromosome classification of three *Lallemantia* Fisch. & C.A. Mey. species by using local binary pattern (LBP). Journal of the Institute of Science and Technology, 12 (1): 58-68.

Yerel İkili Örnek Kullanarak Üç *Lallemantia* Fisch. & C.A. Mey. Türünün Kromozom Sınıflandırmasına Farklı Bir Yaklaşım

İrfan EMRE[1], Turker TUNCER[2], Sengul DOGAN[2], Murat KURŞAT[3], Osman GEDIK[4] and Yaşar KIRAN[5]

ÖZET: Geleneksel karyotip çalışmaları, bitki sistematiğinde türlerin konumlarını değerlendirmek için yaygın olarak kullanılmaktadır. Ancak çalışmalar bazen sistematik sorunları çözememektedir. Son yıllarda bilgisayar tabanlı sistemler taksonomik problemlerin çözümüne katkı sağlamada önem kazanmıştır. Bu çalışmanın amacı, mitozdaki kromozom görüntülerine dayalı yerel ikili örüntü doku operatörünü kullanarak üç *Lallemantia* türünü belirlemektir. Yerel ikili örüntü, yerel görüntü modellerini tanımlamak için en güçlü ve kolay uygulanabilir araçlardan biridir. Bu çalışmada mitozun metafaz evresindeki 641 hücrenin mikrofotoğrafları kullanılmıştır. Yerel ikili örüntü, ön işleme, özellik çıkarma, özellik seçme ve sınıflandırmayı içerir. Sınıflandırma aşamasında DT, LD, SVM, KNN, BT ve SNN kullanılmıştır. Bu çalışma, başarı oranı yüzde yüz olduğu için en iyi sınıflandırıcının SNN olduğunu buldu. Ayrıca, üç tür arasındaki benzerliği ölçmek için bir dendrogram oluşturulmuştur. Sonuç olarak, LBP, kromozom görüntülerini kullanarak bitkileri sınıflandırmak için bir araç olarak kabul edilebilir.

Anahtar Kelimeler: Bitki sınıflandırması, kromozom, görüntü işleme, *Lallemantia,* yerel ikili örüntü

A Different Approach To The Chromosome Classification of Three *Lallemantia* Fisch. & C.A. Mey. Species By Using Local Binary Pattern (LBP)

ABSTRACT: The traditional karyotype studies are widely used in plant systematics to evaluate the positions of species. However, studies sometimes can not solve systematic problems. In recent years, computer-based systems have gained importance in contributing to the solution of the taxonomical problems. The aim of this study was to identify three *Lallemantia* species by using the LBP (local binary pattern) texture operator based on chromosome images in mitosis. LBP is the one of the most powerful and easily applicable tool for identifying local image patterns. In this study, microphotographs of 641 cells in the metaphase stage of mitosis were used. The LBP involves preprocessing, feature extraction, feature selection, and classification. Decision tree (DT), linear discriminant (LD), support vector machine (SVM), K-nearest neighbor (KNN), bagged tree (BT) and ensemble subspace nearest neighbor (SNN) were used in the classification stage. This study found that the best acting classifier was SNN because achievement rate was one hundred percent. Also, a dendrogram was formed to measure the similarity among the three species. As a result, LBP can be accepted as a tool for classifying plants by using chromosome images.

Keywords: Chromosome, image processing, *Lallemantia,* local binary pattern, plant classification.

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INTRODUCTION

Plant identification is a vital process for scientists working in such fields as agronomy, natural products, and bio-conservation (Lee et al., 2017). They use identification keys including some morphological, anatomical, phytochemical, karyological, and molecular characters to describe species (Wang et al., 2014). However, these classical techniques require specialized botanists and are time consuming (Lee et al., 2017; Goyal & Kumar, 2018). Moreover, sometimes these techniques are insufficient for solving taxonomical problems. Therefore, the automatic identification steps based on image features has recently gained in importance (Yigit et al., 2019). Similarly, these systems use many characters that exists in flora including color and shape of leaf, flower, fruit, root and cell type, and tissue or genetic structures (Bhardwaj & Kaur, 2013).

Today, image processing and machine learning have become valuable alternative systems for classifying species among (Lukie et al., 2017). The aim of image processing is to manipulate pixels to obtain the best clarity in the images and to extract target zones by using an input image (Uchida, 2013). Local binary pattern (LBP) is one of the strongest and most popular tools for obtaining local image patterns (Kazak & Koç, 2016; Muthevi & Uppu, 2017; Tuncer and Aydemir, 2020). LBP, proposed by Ojala et al. (2000, 2002), is characterized as a gray scale texture developed from the main texture in local neighborhood pixels (Kaya et al., 2015). In LBP, which is based on the principle of determining a binary number obtained by matching the pixel with its eighth neighbor, 0 is preferred if the neighboring pixel is smaller than the center pixel, while 1 is preferred if it is larger than the center pixel (Zhao et al. 2012; Ozturk & Kurnaz, 2020).

The aim of present study is to classify three *Lallemantia* species grown in Turkey by using LBP based on metaphase chromosome images in mitosis. *Lallemantia* Fisch. & C.A. Mey., a small genus belong to Lamiaceae, is the genus for the case study; it includes five annual and perennial species distributed in the Himalayas, southwestern Asia, and Europe (Kamrani & Riahi, 2018). In Turkey, the genus is represented by three species including *Lallemantia peltata* (L.) Fisch. & Mey., *Lallemantia iberica* (Bieb.) Fisch. & Mey., and *Lallemantia canescens* (L.) Fisch. & Mey. (Davis, 1982; Guner & Aslan, 2012; Ozcan et al., 2014). *L. canescens* is perennial while the *L. peltata and L. iberica* species are annual plants (Edmondson, 1982). The *Lallemantia* species in Turkey have 2n=14 chromosomes (Ozcan et al., 2014). Detailed chromosome studies are extensively used in comparative cytotaxonomy (Martin et al., 2018). Some studies have used plant leaves by LBP to make a classification (Pahikkala et al., 2015; Grinblat et al., 2016; Saleem et al., 2019), but chromosome images-related studies have not been found. This study was the first to use LBP based on chromosome images to do plant classification, as far as we know. Also, this study was intended to contribute both to plant systematics and image processing studies.

MATERIALS AND METHODS

Dataset

Plant materials were collected from natural habitats, and samples were deposited at the Bitlis Eren University Herbarium (BEUH). A karyological analysis of three *Lallemantia* species was performed according to the method proposed by Elci (1982). Microphotographs of 641 cells in the metaphase stage of mitosis belong to each species were taken using an Olympus BX51 light microscope after the staining process was completed. This study demonstrated that three species of *Lallemantia* had 2n=14 chromosomes including median (m) and sub-median (sm) centromeres. The chromosome images of three *Lallemantia* species were then utilized as input for the proposed method. Attributes of the used dataset are given as follows. The used dataset consisted of three classes. There were 211, 216, and 214 images in the first, second, and third classes (first class, *L. canescens*; second class, *L. peltata*; third class, *L. iberica*), respectively. These images are RGB and JPG, and the size of the images was 3072 x 2304.

A Local Binary Pattern (LBP) -Based Multi-Level Learning Model

To distinguish three *Lallemantia* species by using metaphase chromosome images LBP-based multi-level learning model was proposed. The suggested method included preprocessing, feature extraction, feature selection, and classification levels. A block diagram of the suggested learning method is as Figure 1:

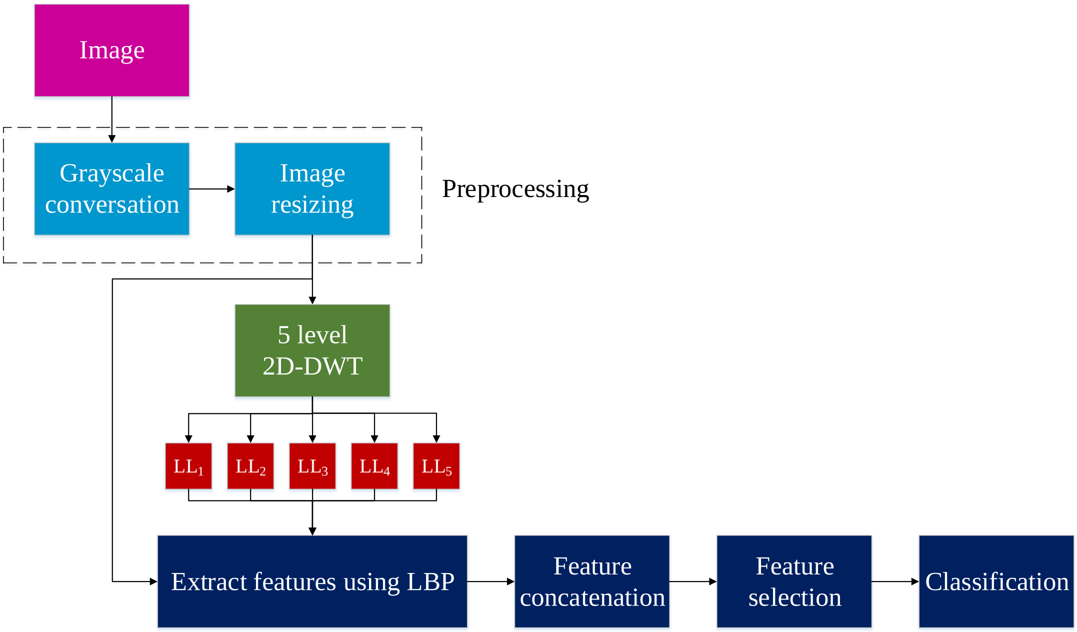

Figure 1. Schematically explanation of the proposed method.

A pictorial example of this method also is shown in Figure. 2.

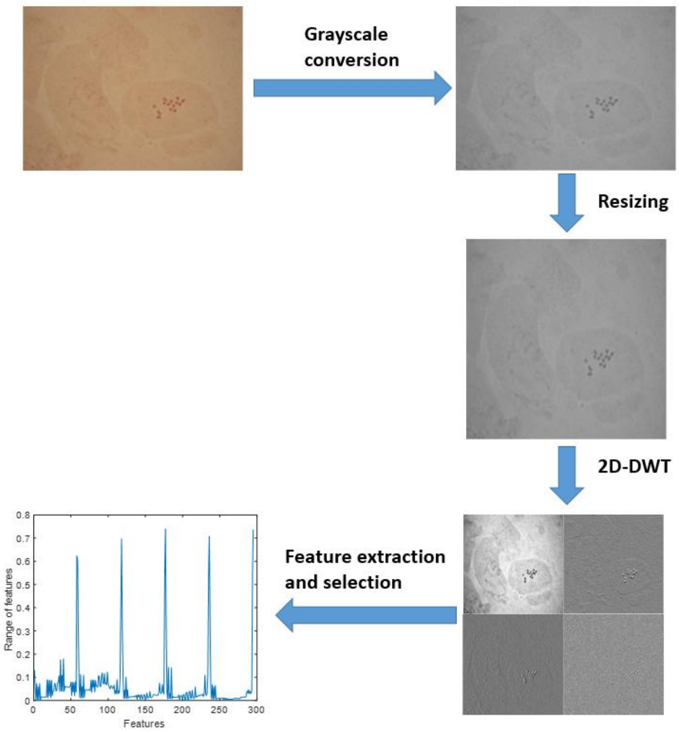

Figure 2. Pictorial example of the proposed 2D-DWT and LBP based biological image classification

Preprocessing

The first stage of the method was preprocessing. Three operations were applied to an image in preprocessing stage, gray-scale conversation, image resizing, and five leveled 2D-DWT.

Step 1: Convert RGB image to gray-scale image.

$$gray = R * 0.299 + G * 0.587 + B * 0.114 \quad (1)$$

where $gray$ was grayscale image and R, G and B are the red, green, and blue layers of the RGB image.

Step 2: Resize the grayscale image to a 512 x 512-sized image.

$$gray = imresize(gray, 512 \text{ x } 512) \quad (2)$$

where $imresize$ defines the resizing function.

Step 3: Apply a five-level 2D-DWT to the resized image and obtain LL_1, LL_2, LL_3, LL_4, and LL_5 sub-bands.

The equations of the five-level DWT method were as follows:

$$[LL_1, LH_1, HL_1, HH_1] = dwt2(gray, haar) \quad (3)$$
$$[LL_2, LH_2, HL_2, HH_2] = dwt2(LL_1, haar) \quad (4)$$
$$[LL_3, LH_3, HL_3, HH_3] = dwt2(LL_2, haar) \quad (5)$$
$$[LL_4, LH_4, HL_4, HH_4] = dwt2(LL_3, haar) \quad (6)$$
$$[LL_5, LH_5, HL_5, HH_5] = dwt2(LL_4, haar) \quad (7)$$

where $dwt2\ (.,.)$ 2D-DWT, LL_i, LH_i, HL_i, and HH_i are i^{th} level low-low, low-high, high-low, and high-high sub bands of the image, i was the number of levels, and $i = \{1,2, \ldots, 5\}$.

Feature Extraction

In this stage, a feature extraction process was performed by using the LBP method. The suggested method was multilevel feature extraction; therefore, the properties were subtracted from the resulting gray-level image and the LL_i sub bands. The LBP algorithm, the basic building block of the method, was presented in the following section.

Local Binary Pattern

LBP is a descriptor, and digital imagery is used to extract features from the digital images (Chang et al., 2013; Lu et al., 2013). This method was proposed by Ojala et al. as an image texture element and is used in many fields (Ojala et al., 2000, 2002). An LBP operator creates a medium for developing a number of descriptors such as dual cross pattern (DCP), local quadruple pattern (LQPAT), and local ternary pattern (LTP) (Liao et al., 2010; Ding et al., 2015; Chakraborty et al., 2017). These methods are called LBP-like descriptors (Berata & Pedrycz, 2013; El-Khadiri et al., 2018). The LBP operator extracts a feature using the basic neighborhood matrix of 3 x 3 dimensions. First, the image was divided into 3 x 3-dimension overlapping blocks, and the binary feature of every block was obtained from the signum function (Hong et al., 2014). The equations of the LBP were presented in Equation 1 and 2.

$$LBP(I_c) = \sum_{i=1}^{8} s(I_i - I_c) \text{x } 2^{i-1} \quad (8)$$

$$s(t) = \begin{cases} 1, t \geq 0 \\ 0, t < 0 \end{cases} \quad (9)$$

The nominal value given in the example in Figure 3 were obtained by using Equations 1 and 2.

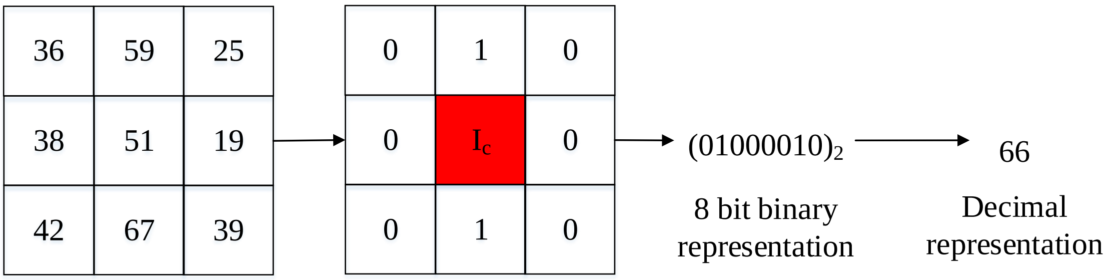

Figure 3. Schematic illustration of LBP

Using the steps illustrated in Figure 3, the decimal value of each block was obtained, and the LBP image was obtained by using these values. The histogram of the calculated LBP image was subtracted and used as the histogram property vector. The length of the histogram was $2^8 = 256$ because every block was encoding with eight bites.

The procedure of the LBP-based feature extraction function was given in Algorithm 1.

Algorithm 1. Feature generation procedure of the LBP.

Procedure: $LBPFeatExt(Im)$

Input: Input image (Im) with size of W x H
Output: LBP feature ($feat_{LBP}$) with size of 256.

$feat_{LBP} = zeros(1,256)$; //Assign LBP features to 0 because LBP is a histogram-based feature extraction method.
for $s = 1$ to $W - 2$ do
  for $d = 1$ to $H - 2$ do
    $window = Im(s{:}s+2, d{:}d+2)$; // Windows division.
    $counter = 1$;
    $LBP(s,d) = 0$; // Pixel of the LBP image defining.
    for $i = 1$ to 3 do
      for $j = 1$ to 3 do
        if $i \neq 2$ or $j \neq 2$ then
          $LBP(s,d) = LBP(s,d) + [window(i,j) \geq window(2,2)] * 2^{8-counter}$;
          $counter = counter + 1$;
        end if
      end for j
    end for i
    $feat_{LBP}(s,d) = feat_{LBP}(s,d) + 1$;
  end for d
end for **s**

By using the $LBPFeatExt$ procedure defined in Algorithm 1, features were generated. This process was mathematically defined below,

$$feat^1 = LBPFeatExt(gray) \quad (10)$$
$$feat^2 = LBPFeatExt(LL_1) \quad (11)$$
$$feat^3 = LBPFeatExt(LL_2) \quad (12)$$
$$feat^4 = LBPFeatExt(LL_3) \quad (13)$$
$$feat^5 = LBPFeatExt(LL_4) \quad (14)$$
$$feat^6 = LBPFeatExt(LL_5) \quad (15)$$

where $feat^i$ is i^{th} level feature with length of 256.

Feature Concatenation

Six feature vectors with 256 properties were obtained in the feature extraction stage. At this stage, a single final feature vector was calculated by combining the resulting feature vectors. The final feature vector contained 1536 features,

$$feat^{final} = feat^1|feat^2|feat^3|feat^4|feat^5|feat^6 \tag{16}$$

where | was the concatenation operator and $feat^{final}$ describes the final features.

Feature Selection

The ReliefF feature selection function is one of the frequently used feature selection and reduction functions in the literature. ReliefF is a function that calculates the weight using the distance metric (Robnik-Šikonja & Kononenka, 2003). ReliefF produces both positive and negative weights. Negative-weighted properties are called unnecessary properties. In this study, feature selection was performed by eliminating negative ReliefF weights.

Classification

In the classification stage, six classifiers were selected: decision tree (DT), linear discriminant (LD), support vector machine (SVM), K nearest neighbor (KNN), bagged tree (BT), and ensemble subspace nearest neighbor (SNN), respectively (Sun et al., 2007; Garcia-Pedarajas & Ortiz-Bayer, 2009; Nanni & Franco, 2011). These classifiers were run using MATLAB Classification Learner, and the default settings are used. Training and testing were performed using 10-fold cross validation. The main reason for using these classifiers was to show that the extracted features were distinctive because these classifiers are conventional classifiers.

RESULTS AND DISCUSSION

To obtain the results of the proposed LBP-DWT based method, six conventional classifiers were used, and the results obtained from these classifiers are presented in this section (Reyad et al., 2014). In addition, confusion matrices belonging to each classifier are given in order to clearly show the achievements obtained according to the classes. The most commonly used classification parameter in the literature is accuracy and the accuracy formula is Eq. 17 (Tuncer et al., 2019).

$$CA = \frac{ntp + ntn}{ntp + nfp + ntn + nfn} \tag{17}$$

CA: Classification accuracy
ntp: Number of true positives
ntn: Number of true negatives
nfp: Number of false positives
nfn: Number of false negatives
The calculated CA values are given in Table 1.

Table 1. Accuracy values of the proposed DWT-LBP based classification method according to used classifiers

	DT	LD	SVM	KNN	BT	SNN
Accuracy	81.0 %	96.9 %	99.5 %	%99.4	93.9 %	100.0 %

As shown in Table 1, the best performing classifier was SNN which achieved a classification rate of 100.0%. Among these classifiers, DT had the lowest performance rate with an 81.0% yield. The confusion matrices obtained according to classifiers are given in Figure 4.

Decision Tree

True Class \ Predicted class	1	2	3
1	172	24	15
2	22	175	19
3	17	25	172

Linear Discriminant

True Class \ Predicted class	1	2	3
1	208	1	2
2	2	207	7
3	3	5	206

Quadratic SVM

True Class \ Predicted class	1	2	3
1	210		1
2		215	1
3		1	213

Fine KNN

True Class \ Predicted class	1	2	3
1	210		1
2		215	1
3	1	1	212

Bagged Tree

True Class \ Predicted class	1	2	3
1	204	1	6
2	12	194	10
3	2	8	204

Ensemble Subspace KNN

True Class \ Predicted class	1	2	3
1	211		
2		216	
3			214

Figure 4. The confusion matrices obtained according to classifiers

Confusion matrices were used to show performance rates by class. The green cells represent correct predictive values while pink cells indicate wrong predictive values. The green cell values in each row and the sum of the values in that row show the performance rate for that class. For example, according to the confusion matrix of the BT, the success rates of the first, second, and third classes was calculated as $\frac{204}{211}$, $\frac{194}{216}$ and $\frac{204}{214}$. respectively.

The similarity of these specifies were calculated by using the extracted and selected features, and dendrogram graph was constructed. The dendrogram graph is shown in Figure 5 (Seo et al., 2003).

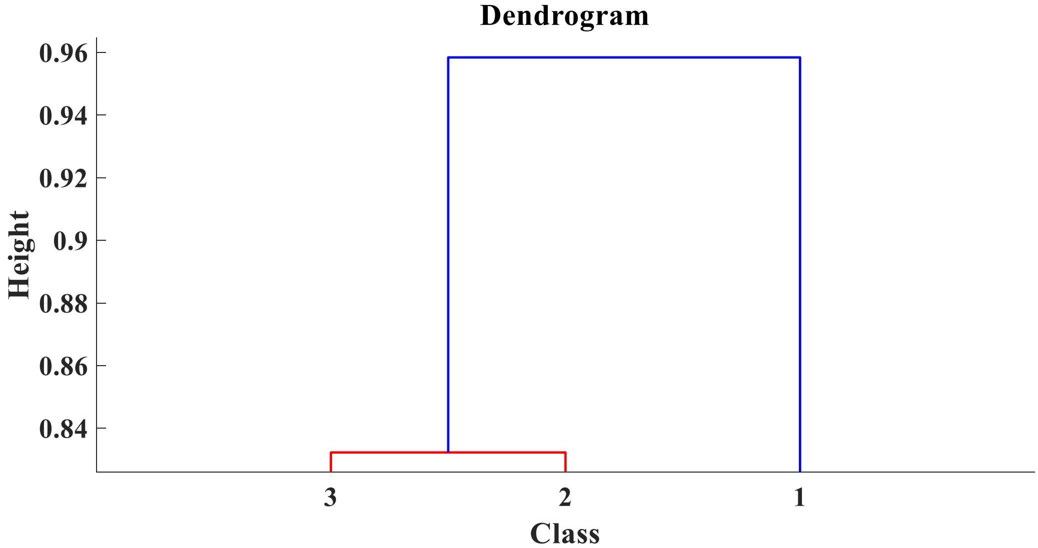

Figure 5. Constructed dendogram of studied *Lallemantia* species by using the extracted and selected features (1: *L. canescens*; 2: *L. peltata* and 3: *L. iberica*)

Morphologically, it has been reported that *L. iberica* is very similar to *L. canescens* because they have an analogous wide bracteole and a dentate calyx and exceed the corolla tube format (Kamrani & Riahi, 2018). *L. canescens* diverges only from *L. iberica* by being perennial (Edmondson, 1982). However, *L. peltata* and *L. iberica* are the annual species with a represented close relationship, and they clustered together, but *L. canescens* placed at a distance in the dendogram in this study (Figure 4). These results agree with some studies in the literature (Edmondson, 1982; Ozcan et al., 2014; Dolatyari & Kamrani, 2015). Ozcan et al. (2015) showed that *L. peltata* had a more symmetrical karyotype than *L. canescens*, and they indicated that *L. canescens* had a slightly longer total chromosome length than the annual species. Also, Dolatyari & Kamrani (2015) indicated that the total karyotype length of *L. canescens* was slightly lower than that of some annual species, but they found that *Lallemantia* is a karyologically stable genus. Similarly, Dinc et al. (2009) found that the pollen characteristics of the annual species (*L. iberica* and *L. peltata*) were similar. Nonetheless, they determined that the annual *L. iberica* and perennial *L. canescens* have similar nutlet characteristics which are most important morphological feature in the *Lallemantia* in determining the interspecific classification (Dinc et al., 2009). Moreover, results from Koohder et al. (2018) showed that *L. canescens* and *L. iberica* clustered in close proximity while *L. peltata* placed a distinct distance from them according to the pollen morphological analysis. Likewise, Kamrani & Riahi (2018) found that *L. canescens* and *L. iberica* represented a close relationship based on molecular data.

CONCLUSION

In this study, an LBP based classification method was presented. The aim of the proposed LBP-based multilevel classification method was to create an automated recognition of the *Lallemantia* species by using chromosome images. LBP was utilized as feature extractor, and it generated features from each layer. Then, the generated features were concatenated, and these concatenated features were reduced by using ReliefF. In the classification phase, six conventional classifiers were used to obtain a comprehensive benchmark. According to results, the SNN classifier achieved 100% classification accuracy. In conclusion, the advantages of the method are as follows:

- A high accuracy rate was obtained in biological data by using artificial intelligence.
- By using the proposed method based on this data set, the human factor was eliminated for classification of plant species.
- The proposed method had a high performance level because 100% CA was obtained.
- A quick and simple method was been proposed in this study.
- DWT and LBP methods were shown to be effective methods for solving this problem.
- In this study, both classification and clustering were best performed.
- This study showed that *L. iberica* and *L. peltata* are annual plants different from *L. canescens* which is a perennial plant.

Conflicts of Interest

The authors declare that no conflicts of interest exist.

Author's Contributions

The authors declare that they have contributed to the article.

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