Research Article

PLncWX: A Machine-Learning Algorithm for Plant lncRNA Identification Based on WOA-XGBoost

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Long noncoding RNAs (lncRNAs) are a class of RNAs longer than 200 nt and cannot encode the protein. Studies have shown that lncRNAs can regulate gene expression at the epigenetic, transcriptional, and posttranscriptional levels, which are not only closely related to the occurrence, development, and prevention of human diseases, but also can regulate plant flowering and participate in plant abiotic stress responses such as drought and salt. Therefore, how to accurately and efficiently identify lncRNAs is still an essential job of relevant researches. There have been a large number of identification tools based on machine-learning and deep learning algorithms, mostly using human and mouse gene sequences as training sets, seldom plants, and only using one or one class of feature selection methods after feature extraction. We developed an identification model containing dicot, monocot, algae, moss, and fern. After comparing 20 feature selection methods (seven filter and thirteen wrapper methods) combined with seven classifiers, respectively, considering the correlation between features and model redundancy at the same time, we found that the WOA-XGBoost-based model had better performance with 91.55%, 96.78%, and 91.68% of accuracy, AUC, and \( F_1 \) score. Meanwhile, the number of elements in the feature subset was reduced to 23, which effectively improved the prediction accuracy and modeling efficiency.

1. Introduction

Noncoding RNA (ncRNA) refers to a functional RNA molecule that cannot be translated into a protein, in which lncRNA is a class of ncRNA, longer than 200 nt previously considered "noise" and ignored. Until 1984, the study of lncRNAs had attracted increasing attention when Pachnis and his colleagues found the H19 gene in mice, which was the first eukaryotic lncRNA, and highly expressed during embryonic development [1]. The current researches on lncRNAs generally focus on lncRNA screening, identification, expression, and localization, so it is very necessary to accurately and efficiently screen out lncRNAs from mRNAs. There have been already several tools, which can be used to analyze the coding potential of transcript sequences. CPC [2], CNCI [3], PLEK [4], lncRScan-SVM [5], and CPC2 [6] classified the sequences using the support vector machine (SVM) algorithm, while CPAT utilized the logistic regression [7]. IncRNA-ID [8], PredLnc-GFStack [9], and CNIT [10] utilized ensemble learning algorithms such as random forest and XGBoost, whereas lncRNA-MFDL [11] and lncRNA-LSTM [12] identified the lncRNAs using deep learning.

Since lncRNAs participated in biological regulatory processes, such as transcriptional level regulation, epigenetic level regulation, and posttranscriptional level regulation, and associated with diseases [13–15], scholars at home and abroad mainly paid attention to lncRNAs of humans, mice, and other vertebrates, while the researches on plant lncRNAs were relatively few. However, many studies have shown that lncRNAs play a key role in the plant immune responses to biotic stress, such as regulating plant flowering, affecting male and female differentiation and pollen development [16], and participating in the plant responses to several abiotic stresses (e.g., drought, salt, and cold) [17, 18]. For the past few years, scholars have committed to use a variety of plants to build specialized plant lncRNA identification models. Urminder Singh et al. [19] developed consensus models for dicots and monocots with ten plant species (Amborella trichopoda, Arabidopsis thaliana, Chlamydomonas reinhardtii, Glycine max, Oryza sativa,
Physcomitrella patens, Selaginella moellendorffii, Solanum tuberosum, Vitis vinifera, and Zea mays). Caitlin M. A. Simopoulos et al. [20] used four different species (Homo sapiens, Arabidopsis thaliana, Mus musculus, and Oryza sativa) as negative training data sets. Siyu Han et al. [21] developed an integrated platform named LncFinder with the data sets of humans, mouse, wheat, zebra fish, and chicken. RNAplocn [22] used five plant species (Arabidopsis thaliana, Cucumis sativus, Glycine max, Populus trichocarpa, and Oryza sativa). Cagirici et al. [23] present a crop-specific, alignment-free coding potential prediction tool, LncMachine, using a set of publicly available lncRNA and mRNA sequences for 18 plant species. Shuwei Yin and his colleagues [24] downloaded the circRNAs and lncRNAs of Arabidopsis and maize from PlantcircBase and GreeNC, respectively, to test the universality of PCirc.

The researchers mostly constructed models directly after extracting only a few features. When the number of extracted features was larger, containing more k-mers, they tended to select features before model construction. For example, Negri et al. [22] extracted 5468 features and selected 16 features with WrapperSubsetEval, InfoGainAttribute, and GainRatioAttributeEval methods before constructing the RNAplocn to prevent the model from overfitting. Here, we built a model specifically used to identify plant lncRNAs. Firstly, we extracted 89 features with Python and two online software packages about five different kinds of plant species (Arabidopsis thaliana, Brachypodium distachyon, Chlamydomonas reinhardtii, Physcomitrella patens, and Selaginella moellendorffii), especially including the algae, moss, and fern. Secondly, we chose the better-performing methods by comparing the accuracy of twenty feature selection methods containing seven filter and thirteen wrapper methods in three single classifiers. Finally, we combined these feature selection methods, respectively, with the meta-learner XGBoost to construct the model. Moreover, we found that the performance of the WOA-XGBoost-based recognition model was the best by comparing it with several selection methods and other classification algorithms.

2. Materials and Methods

2.1. Data Description. The identification of lncRNAs was actually a binary classification problem, and we defined lncRNAs as positive and mRNAs as negative. According to the way of survival, plants can be divided into algae, lichen, fungi, moss, fern, and seed (gymnosperm and angiosperm).

To establish a plant lncRNA identification model with strong generalization ability, we used five representative plant species: Arabidopsis thaliana (dicotyledon), Brachypodium distachyon (monocotyledon), Chlamydomonas reinhardtii (algae), Physcomitrella patens (moss), and Selaginella moellendorffii (fern), hereinafter referred to as AT, BD, CR, PP, and SM. The positive sample data (lncRNAs) were obtained from CANTATAdb 2.0 (https://yeti.amu.edu.pl/ CANTATA/), which was an online database of 39 species of plants such as Arabidopsis thaliana, Zea mays, Oryza, and three algae [25]. The negative data were downloaded from RefSeq (https://www.ncbi.nlm.nih.gov/refseq/), including nonredundant gene and protein sequences with biological significance provided by the National Center for Biotechnology Information (NCBI), in which we can screen for gene sequences by species, molecular types, source databases, sequence length range, and so on.

As shown in Figure 1, it was the frequency distribution histogram of the sequence length of lncRNAs and mRNAs in five different plants, where all sequence lengths of lncRNAs were below 10,000 nt, and over 98.5% mRNA sequence lengths of five plants were between 200 nt and 10,000 nt (see Table 1). Then, we removed gene sequences below 200 nt and above 10,000 nt. At this time, the positive data were still far smaller than the negative, which was a typical unbalanced dataset with a significant impact on the performance of the model to some extent, and therefore, we aligned the positive sample with the negative sample data size by stratified sampling.

The conventional stratified sampling was stratified by equal spacing of sequence length, followed by random sampling, so we took 2000 nt as one unit, and the results are shown in Figure 2, which indicated that the distribution of mRNA sequence lengths of the five plants was uneven. Consequently, we sorted the sequence lengths from small to large, divided them into ten parts, and randomly selected 120 samples from each part. Therefore, 1200 positive and 1200 negative samples for each plant were randomly chosen, and the sizes of original data and used data are shown in Table 2.

2.2. Feature Extraction. Prior to the model construction, we extracted 89 features including sequence features (sequence length, GC content, LORF length and coverage, k-mers) and structural features (RNA secondary structure), where the LORF length was defined as the longest open reading frame of the three forward frames, starting from a start codon (ATG) and ending with a stop codon (TAG, TAA, or TGA), extracted by the online software ORFfinder. The LORF coverage represented the ratio of LORF length to sequence length [26]. In terms of features like k-mers, we considered k = 1, 2, and 3, a total of 84 features. While the sequence features of the lncRNAs represented the surface content of the nucleotide sequence, the secondary structural characteristics might imply some important functional information [21]. We measured the RNA secondary structural feature using the minimum free energy (MFE) of the sequence extracted by the online software RNAfold. As shown in Figure 3, the lncRNAs of all five plants had higher MFE than mRNAs.

2.3. Feature Selection. According to the different combination methods with the learner, the feature selection methods could be divided into three categories: filter, wrapper, and embedded.

The filter feature selection methods are irrelevant to the learning algorithm that the feature selection is the preprocessing process of the latter, while the learning algorithm is the former’s verification process. The principle is to score each feature with specific evaluation criteria, to rank features
Figure 1: Sequence length frequency distribution of different plants. (a) *Arabidopsis thaliana* (AT). (b) *Brachypodium distachyon* (BD). (c) *Chlamydomonas reinhardtii* (CR). (d) *Physcomitrella patens* (PP). (e) *Selaginella moellendorffii* (SM). The horizontal axis is sequence length, and the longitudinal axis represents density.
according to the score, then to select the top \( k \) features as the subset of features (or to select the features whose values are larger than threshold as the feature subset), and finally to verify the quality of the subset by calculating the accuracy of machine-learning algorithms. Commonly used evaluation criteria include chi-square test and mutual information. Here, we discussed seven evaluation criteria including chi_square, \( f \_score \), gini_index, \( t \_score \), fisher_score, FCD, and FCQ for features screening [27,28].

...J_hewrapperfeatureselectionprocesscombineswiththelearningalgorithmtoencapsulatetheselectedlearnerintoablack box, evaluates the excellence of the selected features according to its prediction accuracy of machine learning, and adjusts the subset using the search strategy to finally obtain the approximate optimal subset. The wrapper feature selection methods generally include the sequence search and random search strategies. Sequence search can be divided into three categories: forward search, backward search, and bidirectional search, in which the sequential forward search (SFS) starts with an empty set, and the feature with the highest score is greedily added to the subset of selected features each time [29]. The intelligent optimization algorithms are random search strategies based on biological intelligence or physical phenomena, such as genetic algorithm and particle swarm optimization, which generally do not require the continuous type and convexity of objective functions and constraints, and also have strong adaptability to the data uncertainty in computation. We selected twelve intelligent optimization algorithms and one sequence search strategy-based algorithm as the wrapper feature selection methods, which are genetic algorithm [30], particle swarm optimization [31], differential evolution [32], cuckoo search...

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**Table 1:** Numeric description of mRNAs in the five plant data sets.

| Species                | Original | [0, 200 nt) | (10,000 nt, +∞) | [200 nt, 10,000 nt] |
|------------------------|----------|-------------|------------------|--------------------|
| *Arabidopsis thaliana* | 48,347   | 304         | 44               | 47,999 (99.28%)    |
| *Brachypodium distachyon* | 37,816   | 1           | 29               | 37,786 (99.92%)    |
| *Chlamydomonas reinhardtii* | 14,430   | 125         | 78               | 14,227 (98.59%)    |
| *Physcomitrella patens* | 47,897   | 0           | 175              | 47,722 (99.63%)    |
| *Selaginella moellendorfii* | 45,248   | 0           | 51               | 45,197 (99.89%)    |

*Figure 2:* Distribution of mRNA sequence lengths of the five different plants. (a) Number of mRNAs in each interval. (b) Corresponding frequency.

**Table 2:** Numeric description of the lncRNA and mRNA data sets used to build the model.

| Species                | lncRNA | mRNA   |
|------------------------|--------|--------|
|                        | Original data | Used data | Original data | Used data |
| *Arabidopsis thaliana* | 4373   | 1200   | 48,347       | 1200     |
| *Brachypodium distachyon* | 4945   | 1200   | 37,816       | 1200     |
| *Chlamydomonas reinhardtii* | 3425   | 1200   | 14,430       | 1200     |
| *Physcomitrella patens* | 1498   | 1200   | 47,897       | 1200     |
| *Selaginella moellendorfii* | 2267   | 1200   | 45,248       | 1200     |
algorithm [33], firefly algorithm [34], bat algorithm [35], flower pollination algorithm [36], grey wolf optimizer [37], sine-cosine algorithm [38], whale optimization algorithm [39], salp swarm algorithm [40], and Harris hawks optimization [41], hereinafter referred to as GA, PSO, DE, CS, FA, BA, FPA, GWO, SCA, WOA, SSA, and HHO.

Embedded methods embed feature selection into the construction of the model, such as Lasso and tree models, with higher accuracy and less computational complexity than filter and wrapper methods, yielding a subset of features when the classification algorithm training process ends. Since embedded methods based on the tree model are also classification models, we did not consider it here.

2.4. Model Construction. After the feature selection, we constructed the model with the meta-learner XGBoost. XGBoost is an abbreviation for extreme gradient boosting, an integrated machine-learning algorithm based on the decision tree, using a gradient boosting framework. The algorithm has a wide range of applications and can be adopted to solve regression, classification, ranking, and user custom prediction problems. XGBoost employs a gradient descent structure to improve the learning of weak learners (CART), implements the sequence tree building process using parallelization, and punishes more complex models via $L_1$ ridge $L_2$ regularization to prevent overfitting [42], while the algorithm carries built-in cross-validation methods in each iteration without assigning the exact number of boost iterations.

We obtained the best parameters of the model using the grid search technique to tune these parameters step by step including $n_{\text{estimators}}$, max_depth, min_child_weight, gamma, subsample, colsample_bytree, reg_alpha, reg_lambda, and learning_rate (see Table 3), and other hyperparameters were default. To evaluate the performance of the model, we followed the following five evaluation criteria:

\[
\begin{align*}
\text{Accuracy} & = \frac{\text{TP} + \text{TN}}{\text{TP} + \text{FP} + \text{TN} + \text{FN}}, \\
\text{Precision} & = \frac{\text{TP}}{\text{TP} + \text{FP}}, \\
\text{Recall} & = \frac{\text{TP}}{\text{TP} + \text{FN}}, \\
F_1 & = \frac{2\text{TP}}{2\text{TP} + \text{FN} + \text{FP}}.
\end{align*}
\]

and area under the receiver operating characteristic curve (AUC), where TP is the number of true positives (long noncoding transcripts correctly classified as IncRNAs), TN is the number of true negatives (coding transcripts correctly classified as mRNAs), FP is the number of false positives (coding transcripts incorrectly classified as IncRNAs), and FN is the number of false negatives (long noncoding transcripts incorrectly classified as mRNAs).

3. Results and Discussion

3.1. Comparison between the Feature Selection Methods. Several feature selection methods with better performance were selected by comparing the classification accuracy of 20 feature selection methods on each single classifier (K-nearest Neighbors, Naive Bayes, and Support Vector Machine, hereinafter referred to as KNN, GaussianNB, and SVM). For seven filter methods, we analyzed the variation tendency of the accuracy under different numbers of features and chose several filter feature selection methods that performed better in all three single classifiers.

Figures 4(a)–4(c) depict the changing characteristics of seven filter feature selection methods under the number of features in three single classifiers including KNN, GaussianNB, and SVM, respectively, and the results are shown in Supplementary Table S1. Figure 4(a) indicates that in KNN, when the number of features was $n \geq 1$, each of the seven filter feature selection methods had higher classification accuracy than using all features. When $n \geq 8$, the classification accuracy of all feature selection methods decreased with the number of features. Among them, the accuracy of the f_score, gini_index, and FCQ, respectively, achieved the highest (88.85% and 88.92%) at $n = 8$, and these two methods had similar accuracy rate and consistent change trend under different values of $n$. While the f_score, gini_index, and FCD have already reached the highest accuracy (91.21%, 91.21%, and 91.14%) at $n = 2$, chi_square had a 91.28% accuracy at $n = 8$, and the highest accuracy of FCD was 90.35% at $n = 3$. Therefore, in KNN, chi_square, f_score, gini_index, and FCD performed well.

Figure 4(b) indicates that, in Gaussian NB, all filter methods had higher classification accuracy than without feature selection. Thereinto, the methods of FCD, FCQ, gini_index, and chi_square had better performance (90.05%, 89.87%, 89.69%, and 89.03%) and achieved within $n = 7$. In Figure 4(c), t_score and fisher_score had the same tendency under different $n$ values, but within $n = 30$, no accuracy was larger than the one using all 89 features in SVM. The
### Table 3: Parameter setting by grid search for XGBoost.

| Parameter         | Range                     | Used | Description                                      |
|-------------------|---------------------------|------|--------------------------------------------------|
| max_depth         | [3, 4, 5, 6, 7, 8, 9, 10] | 3    | Maximum tree depth for base learners            |
| min_child_weight  | [1, 2, 3, 4, 5]           | 4    | Minimum sum of instance weight (hessian) needed in a child |
| gamma             | [0, 0.1, 0.2, 0.3, 0.4, 0.5] | 0.2  | Minimum loss reduction required to make a further partition on a leaf |
| subsample         | [0.6, 0.7, 0.8, 0.9, 1]   | 0.8  | Subsample ratio of the training instance         |
| colsample_bytree  | [0.6, 0.7, 0.8, 0.9, 1]   | 0.8  | Subsample ratio of columns when constructing each tree |
| reg_alpha         | [1e-5, 1e-4, 1e-3, 1e-2, 0.1, 1, 100] | 1e-05 | L₁ regularization term on weights |
| reg_lambda        | [0.05, 0.1, 1, 2, 3]      | 1    | L₂ regularization term on weights                |
| learning_rate     | [0.01, 0.05, 0.07, 0.1, 0.2] | 0.07 | Boosting learning rate                           |

#### Figure 4: Accuracy of different filter feature selection methods in three classification models.

- **(a)** KNN
- **(b)** GaussianNB
- **(c)** SVM

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The accuracy of different filter feature selection methods in three classification models is shown in Figure 4. Each line represents a different feature selection method: `chi_square`, `f_score`, `gini_index`, `t_score`, `fisher_score`, `FCD`, `FCQ`, and `All Features`. The x-axis represents the number of features, and the y-axis represents the accuracy.
classification accuracy of feature screening according to gini_index criteria has been floating around the classification accuracy for all features. All the accuracy of FCD was similar to FCQ, and the variation trend was consistent at the same time. In SVM, obtaining better performance was the FCD, FCQ, chi_square, and f_score, whose accuracy was 90.94%, 90.94%, 90.84%, and 90.83%, respectively.

In general, the filter feature selection methods with better performance in all three single classifiers were FCD and chi_square. However, the subset of features screening by the chi_square criterion contained both sequence length and LORF length, which had a high correlation coefficient of 0.84 (see Figure 5), easily resulting in model redundancy, so we chose FCD for the next experiment.

For the wrapper feature selection methods, we searched for each intelligent optimization algorithm twenty times to obtain different subsets of features and selected subsets of features with higher accuracy under an individual classifier. The number of elements in the feature subsets was specified for sequence forward search (SFS) from 1 to 30, and then, we chose the feature subset with higher accuracy on all single classifiers. The results are shown in Figure 6.

Figure 6 shows the classification accuracy of 20 feature selection methods on three individual classifiers, where on both Gaussian NB and SVM, the accuracy of wrapper feature selection methods was significantly higher than filter, but wrapper methods had higher computational complexity and were more time-consuming. Figure 6(a) illustrates seven filter feature selection methods showing that FCD had the best performance. Figure 6(b) shows the classification accuracy of the 13 wrapper methods, where methods with the highest accuracy, respectively, on these three classifiers are GWO, HHO, and WOA. Therefore, we used four feature selection methods (FCD, GWO, HHO, and WOA) combined with the ensemble learner XGBoost after tuning parameters to construct the model.

3.2. Performance Comparison with Other Classifiers. We analyzed seven classification algorithms (KNN, Naive Bayes, SVM, Decision Tree, Random Forest, AdaBoost, and XGBoost) and tuned parameters for each model using grid search techniques. We trained the models with fivefold cross-validation techniques, with the performance of each classification model shown in Figure 7.

As shown in Figure 7, XGBoost had the highest AUC value of the seven classifiers with better classification performance. It can be seen from Figure 4 that the accuracy of models after using a filter method was higher than that without feature selection, while from Figure 6, the overall accuracy of models with the wrapper feature selection methods was obviously higher than that of the filter. Therefore, the accuracy of the classifiers combined with a feature selection method was better than the one with all features. The performance of the four feature selection methods combined with seven classifiers is shown in Table 4.

The performance of the four different feature selection methods on seven classifiers is shown in Table 4. When the feature selection method was FCD, Random Forest and AdaBoost had the highest accuracy of 91.47% and the highest AUC is 96.38% on XGBoost, while the SVM had the highest F1_score of 91.56%. In the intelligent optimization algorithms such as WOA and HHO, the values of accuracy, AUC, and F1_score were taken on XGBoost at the maximum. Thereinto,
Figure 6: Accuracy of the 20 feature selection methods on the different classifiers. (a) Seven filter feature selection methods. (b) Thirteen wrapper feature selection methods.

Figure 7: ROC curves for the different classifiers. The AUC values for each model have been given.
values on the WOA were 91.55%, 96.78%, and 91.68%, while values on the HHO were 91.41%, 96.79%, and 91.48%. It can be seen that the WOA-XGBoost-based algorithm, with higher, AUC, and F1_score. Similarly, performed a little better than HHO-XGBoost and FCD. the intelligent optimization algorithm GWO achieved the highest accuracy, precision, AUC, and F1_score values on AdaBoost of 91.89%, 91.71%, 96.51%, and 91.90%, respectively. Although the GWO-AdaBoost-based algorithm performed better than the WOA-XGBoost-based algorithm, the features subset of the former contained sequence length and LORF length, which had a high correlation (see Figure 5) and would improve the redundancy of the model. Therefore, the performance based on the WOA-XGBoost algorithm was best among the twenty feature selection methods and seven classifiers. The identification model we developed was specifically for plants, using not only the dicot (Arabidopsis thaliana) but also adding the monocot (Brachypodium distachyon), algae (Chlamydomonas reinhardtii), moss (Physcomitrella patens), and fern (Selaginella moellendorffii) to enrich the diversity of the data sets. Moreover, the traditional recognition models generally either did not carry on feature selection containing a few features, directly using all the extracted features, which easily lead to overfitting of the model, or used some or some class of feature selection methods before building the model. While we compared seven filter and thirteen wrapper methods, the redundancy of the model was further considered. In short, 89 features were reduced to 23 features using the WOA-XGBoost algorithm. In future work, we plan to extract more features and use the identification algorithm to verify its performance in other plant data sets.

4. Conclusions

To establish a specialized plant IncRNA recognition model, we used five plant data sets of different species. Furthermore, after extracting 89 features, we compared the accuracy of 20 feature selection methods on three single classifiers, respectively, and then combined FCD, GWO, HHO, and WOA, respectively, with XGBoost while comparing the accuracy, precision, recall, AUC, and F1_score score of the other six models (parameters of all classification models were tuned). The results showed that the performance based on the WOA-XGBoost algorithm was best among the twenty feature selection methods and seven classifiers. The identification model we developed was specifically for plants, using not only the dicot (Arabidopsis thaliana) but also adding the monocot (Brachypodium distachyon), algae (Chlamydomonas reinhardtii), moss (Physcomitrella patens), and fern (Selaginella moellendorffii) to enrich the diversity of the data sets. Moreover, the traditional recognition models generally either did not carry on feature selection containing a few features, directly using all the extracted features, which easily lead to overfitting of the model, or used some or some class of feature selection methods before building the model. While we compared seven filter and thirteen wrapper methods, the redundancy of the model was further considered. In short, 89 features were reduced to 23 features using the WOA-XGBoost algorithm. In future work, we plan to extract more features and use the identification algorithm to verify its performance in other plant data sets.

Data Availability

The data used to support the findings of this study can be obtained free of charge from CANTATAdb 2.0 and RefSeq.
The positive sample data (IncRNAs) can be obtained from CANTATAdb 2.0: https://yeti.amu.edu.pl/CANTATA/, and the negative data (mRNAs) can be downloaded from RefSeq: https://www.ncbi.nlm.nih.gov/refseq/.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

Acknowledgments

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Supplementary Materials

Table S1: the accuracy of seven filter feature selection methods under the different numbers of features in three single classifiers including KNN, GaussianNB, and SVM, respectively. (Supplementary Materials)

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