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METHODOLOGY

ASLncR: a novel computational tool for prediction of abiotic stress-responsive long non-coding RNAs in plants

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Abstract

Abiotic stresses are detrimental to plant growth and development and have a major negative impact on crop yields. A growing body of evidence indicates that a large number of long non-coding RNAs (lncRNAs) are key to many abiotic stress responses. Thus, identifying abiotic stress-responsive lncRNAs is essential in crop breeding programs in order to develop crop cultivars resistant to abiotic stresses. In this study, we have developed the first machine learning-based computational model for predicting abiotic stress-responsive lncRNAs. The lncRNA sequences which were responsive and non-responsive to abiotic stresses served as the two classes of the dataset for binary classification using the machine learning algorithms. The training dataset was created using 263 stress-responsive and 263 non-stress-responsive sequences, whereas the independent test set consists of 101 sequences from both classes. As the machine learning model can adopt only the numeric data, the Kmer features ranging from sizes 1 to 6 were utilized to represent lncRNAs in numeric form. To select important features, four different feature selection strategies were utilized. Among the seven learning algorithms, the support vector machine (SVM) achieved the highest cross-validation accuracy with the selected feature sets. The observed 5-fold cross-validation accuracy, AU-ROC, and AU-PRC were found to be 68.84, 72.78, and 75.86%, respectively. Furthermore, the robustness of the developed model (SVM with the selected feature) was evaluated using an independent test dataset, where the overall accuracy, AU-ROC, and AU-PRC were found to be 76.23, 87.71, and 88.49%, respectively. The developed computational approach was also implemented in an online prediction tool ASLncR accessible at https://iasri-sg.icar.gov.in/aslncr/. The proposed computational model and the developed prediction tool are believed to supplement the existing effort for the identification of abiotic stress-responsive lncRNAs in plants.

Keywords Machine learning · Abiotic stress · Long non-coding RNA · Computational biology

Introduction

Due to the growing world population, demand is going to be increased in global food consumption, and by 2050, that demand is expected to be doubled (Tilman et al. 2011). Abiotic stresses, on the other hand, present a substantial challenge to agriculture and the ecosystem due to changing climatic conditions, resulting in significant crop yield loss (Saeed et al., 2023; Wani et al., 2016). In order to adapt to challenging environmental conditions, plants modify the expression of several genes at the transcriptional, post-transcriptional, and epigenome levels in response to different abiotic stresses (Liu et al. 2022a; Choudhury et al. 2021; Zhu et al., 2022). The functional elucidation of many genes at the transcription, post-transcriptional, post-translational, and epigenetic levels has been significantly improved with the advancement in

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genome sequencing technology, especially next-generation sequencing (NGS) (Li et al. 2018). The NGS technologies have led to the identification of novel non-coding RNAs (ncRNAs) (Öztürk Gökçe et al., 2021; Bhogireddy et al., 2021; Yu et al. 2019) and their roles in the regulation of multiple biological processes, including plant response to various abiotic stresses (Yang et al. 2023;Yu et al. 2019).

The long non-coding RNAs (lncRNAs) are a group of ncRNAs which are more than 200 bp long and not be translated into a protein (Quan et al. 2015). Transcriptional, post-transcriptional, and epigenetic regulations of gene expression are three ways that lncRNA acts as a gene regulatory factor (Quan et al. 2015). The lncRNAs are reported to be important modulators of various biological processes (Mercer et al., 2009). Their involvement in controlling transcription through enhancers and providing regulatory binding sites has been well documented (Wang and Chekanova, 2017). These are also said to act as miRNA sponges, suppressing miRNA function by causing deflection to their potential target (Wang et al. 2010). The lncRNAs are also found in the nucleus, where they serve as major components of nuclear speckles (Hutchinson et al. 2007). In the cytoplasm, lncRNAs interact with a variety of RNA-binding proteins (RBPs) to monitor and control their regulatory dynamics (Glisovic et al. 2008).

Plant lncRNAs make up around 80% of all ncRNAs and are involved in a wide range of biological processes, including abiotic stress response (Wang et al. 2021). The first lncRNA reported in plants was ENOD40 in Soybean (Yang et al. 1993). Despite the fact that the plant genomes are more complicated than animal genomes, the number of experimentally identified lncRNAs in plants are much less than that reported for animals. Several lncRNAs that respond to abiotic stresses have been reported to be present in a wide range of plant species. Table 1 contains a list of recently identified lncRNAs reported to be involved in various abiotic stresses. Due to the discovery of abiotic stress-responsive lncRNAs and their target genes in a range of plant species, we now have a better understanding of the molecular mechanism underlying these stress adaptations. For example, in drought conditions of *Arabidopsis thaliana*, lncRNA lincRNA340 is induced to repress miR169, relieving nuclear factor Y (NF-Y) gene expression to improve stress tolerance (Qin et al. 2017). Further, lncRNA973 functions as a positive regulator of salt-responsive genes in ROS (reactive oxygen species), enhancing salinity tolerance in cotton (Zhang et al., 2019). Similarly, GhDNA1, which targets AAAG DNA double strands to regulate drought-responsive genes in trans, was discovered to be associated with drought tolerance in cotton (Tao et al., 2021). These findings support the idea that lncRNAs can be induced or suppressed in response to abiotic stress. Furthermore, these abiotic stress-responsive lncRNAs have been linked to phytohormone signal transduction, secondary metabolite biosynthesis, and sucrose metabolism pathways, each of which has been reportedly engaged in plant abiotic stress response (Ding et al. 2019; Yang et al., 2022; Lamin-Samu et al., 2022).

The studies cited above indicate that lncRNAs may be exploited as genetic targets to develop crop cultivars that are resistant to abiotic stresses. However, the lncRNAs are needed to be identified first before using them as genetic targets. To date, techniques such as serial expression of gene expression (SAGE), the expressed sequence tag (EST), whole-genome tiling arrays, lncRNA microarray, RNA capture sequencing (RNA CaptureSeq), and RNA-sequencing (RNA-seq) have all been employed to identify abiotic stress-related lncRNAs. However, the wet-lab experiments consume a lot of resources (Lee and Kikyo 2012). Furthermore, the advanced sequencing techniques are species-specific. Thus, there is a need to develop a computational method for predicting abiotic stress-responsive lncRNAs using lncRNA sequence data. In other words, the development of machine learning-based computational methods may be a better alternative for predicting lncRNAs associated with abiotic stress. Considering the above facts, the present study is devoted to develop the first machine learning-based computational model for predicting abiotic stress-responsive lncRNAs using sequence-derived features. The proposed approach is expected to supplement wet-lab methods and other sequencing techniques for identifying abiotic stress-responsive lncRNAs in plants.

Materials and methods

Collection of abiotic stress-responsive lncRNA sequence data

The PncStress database (Wu et al., 2020) is the most recent source for abiotic stress-responsive lncRNAs. It contains experimentally validated ncRNA sequences linked to a variety of abiotic and biotic stresses. With 114 species responding to 48 abiotic and 91 biotic stresses, PncStress now has 4227 entries, including 2523 miRNAs, 444 lncRNAs, and 52 circRNAs validated by different experimental methods. The PncStress database (Wu et al., 2020) was accessed on July 30, 2022, in order to retrieve lncRNA sequences relevant to abiotic stresses. A total of 444 abiotic stress-responsive lncRNA sequences, representing 27 different abiotic stress categories, were obtained from 24 plant species.

Construction of positive and negative dataset

The abiotic stress-responsive lncRNA sequences obtained from the PncStress database were used to construct the positive set. On the other hand, 238,226 lncRNA sequences

Table 1 Representative lncRNAs found to be involved in plants responding to different abiotic stresses

Abiotic stress	Species	LncRNAs	References
Drought/dehydration	*Glycine max*	lncRNA77580	Chen et al., 2023
	Oryza sativa L.	TCONS_00021861; MSTRG.5679.8; MSTRG.19712.1; MSTRG.37152.2	Chen et al., 2021a; Yang et al., 2022
	Solanum lycopersicum	SlNCED1; SlAOC; SlLOX5; SlCWINV3-like;SlAgpL1; TomadPgps; SlMS1	Lamin-Samu et al., 2022
	Arachis hypogaea L.	MSTRG.70535.2; MSTRG.86570.2; MSTRG.86570.1; MSTRG.100618.1; MSTRG.81214.2; MSTRG.30931.1	Ren et al., 2022
	Solanum tuberosum L.	StFLORE	Ramírez Gonzales et al., 2021
	Gossypium hirsutum	GhDAN1	Tao et al., 2021
	Brassica napus L.	XLOC_052298; XLOC_094954; XLOC_012868	Tan et al., 2020
	Zea mays	TCONS_00043110; TCONS_00077962; TCONS_00084669; TCONS-00105920; TCONS-00166326; TCONS-00060596; TCONS-00149876; TCONS-00177501	Yu et al., 2020
Cold/heat stress	*Arabidopsis thaliana*	CIL1; XLOC_006026	Liu et al., 2022b; Rutley et al., 2021
	Oryza sativa L.	TCONS_00092993; TCONS_00043075; TCONS_00100154	Zhang et al., 2022
	Manihot esculenta Crantz	CRIR1; ncP12248	Li et al., 2022a; Suksamran et al., 2020
	Triticum aestivum L.	VAS	Xu et al., 2021
	Gossypium hirsutum	XH123	Cao et al., 2021
	Cucumis sativus L.	TCONS_00031790; TCONS_00014332; TCONS_00014717; TCONS_00005674	He et al., 2020
Salt/salinity	*Medicago truncatula*	MtCIR1	Tian et al., 2023
	Glycine max	lncRNA77580; Gmax_MSTRG.35921.1; max_MSTRG.18616.1	Li et al., 2022b; Chen et al., 2023
	Populus Trichocarpa	Ptlinc-NAC72	Ye et al., 2022
	Zea mays	MSTRG.8888.1	Liu et al., 2022a
	Gossypium hirsutum	lncRNA354; lncRNA973	Zhang et al., 2021; Zhang et al., 2019
Heavy metal stress	*Populus L.*	PMAT	Chen et al., 2022
	Populus tomentosa	MSTRG.22608.1; MSTRG.5634.1	Quan et al., 2021
	Betula platyphylla	LncRNA2705.1; LncRNA11415.1	Wen et al., 2020
	Oryza sativa L	XLOC_086307; XLOC_058523; XLOC_104363; XLOC_059778; XLOC_122123; XLOC_125848; XLOC_098316	Chen et al., 2018

retrieved from the PLncDB V2.0 database (accessed on August 05, 2022) (Jin et al., 2021) were used to construct the negative set. To prevent homologous bias in the prediction accuracy, the homology reduction at 50% sequence identity was applied to both positive and negative datasets using the CD-HIT method (Huang et al., 2010). After the redundancy sequences were removed, the positive and negative sets produced 364 and 97,654 lncRNA sequences, respectively. To avoid prediction bias toward the non-abiotic stress class having a larger number of sequences, a balanced dataset with an equal number of abiotic stress and non-abiotic stress-responsive lncRNA sequences was taken into consideration. In other words, 364 non-abiotic stress sequences were chosen at random from the pool of 97,654 sequences to prepare a balanced training dataset that comprises an equal number of sequences from both classes. Out of the 364 sequences in each class, 101 lncRNA sequences were kept aside to prepare the independent dataset. The remaining 263 stress-responsive lncRNAs and 263 non-stress-responsive lncRNAs were used as positive and negative sets for the training dataset.

Numeric feature generation

In this study, we generated Kmer features to transform each lncRNA sequence into a numeric feature vector. The Kmer features are represented as the occurrence frequencies of *K* neighboring nucleic acids (Lee et al. 2011), which has been successfully used in several computational studies including lncRNA prediction (Sun et al. 2013). The numeric value for the Kmer size k can be calculated as

$$f_k(t) = \frac{N_k(t)}{N - k + 1}, \tag{1}$$

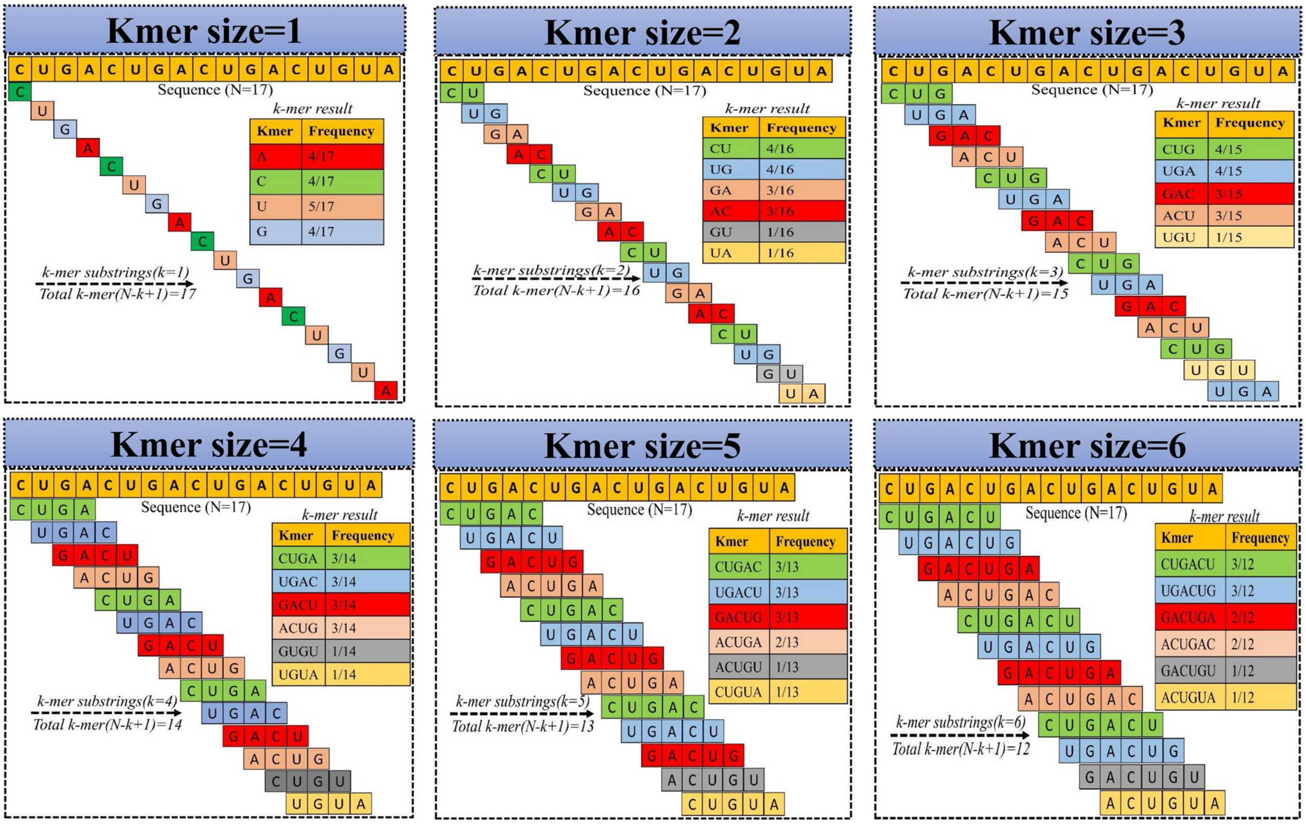
where $N_k(t)$ is the number of Kmer type t of size k, and N is the length of the nucleotide sequence. For example, for an RNA sequence 'CUGACUGACUGACUGUA', $f_1(C) = \frac{4}{17}$, $f_2(CU) = \frac{4}{16}$, $f_3(CUG) = \frac{4}{15}$, $f_4(CUGA) = \frac{3}{14}$, $f_5(CUGAC) = \frac{3}{13}$, and $f_6(CUGACU) = \frac{3}{12}$. A brief representation of the Kmer feature is shown in Fig. 1. The number of Kmer features of size k is 4^k. In this study, we have considered Kmer sizes 1 to 6 to generate the features for each sequence. Thus, for Kmer sizes 1, 2, 3, 4, 5, and 6, the number of features generated was 4, 16, 64, 256, 1024, and 4096, respectively. The Kmer sizes 1 to 6 were denoted as K_1, K_2, K_3, K_4, K_5, and K_6. In total, 5460 features were generated for each lncRNA sequence.

Prediction algorithms

Several bioinformatics fields have effectively applied machine learning techniques for prediction purposes (Guo et al. 2017, Pradhan et al. 2022, Abbas and EL-Manzalawy 2020, Pradhan et al. 2021). The support vector machine (SVM; Vapnik 1963), extreme gradient boosting (XGB; Chen and Guestrin 2016), random forest (RF; Breiman 2001), light-gradient boosting machine (LGBM; Ke et al. 2017), bagging (BAG; Breiman 1996), adaptive boosting (ADB; Freund and Schapire 1999), and gradient boosting decision trees (GBDT; Friedman 2001) were the seven machine learning techniques we used in this study. Table 2 lists the R-packages used to implement the learning models and the parameter settings for each learning model.

Feature selection approach

By eliminating duplicate and irrelevant features, feature selection reduces the computational burden while increasing classification accuracy (Pradhan et al., 2022). The support vector machine recursive feature elimination (SVM-RFE; Guyon et al., 2002), random forest variable importance measure (RF-VIM; Daz-Uriarte and Alvarez de Andrés, 2006), XGB variable importance (XGB-VIM; Sandri and Zuccolotto, 2008), and LGBM variable importance measure (LGB-VIM; Ke et al., 2017) were used to select important and relevant features. According to past studies (Guyon et al., 2002; Pradhan et al., 2022), the top features in this study that led to a classifier with the best classification accuracy was



Kmer	Frequency
A	4/17
C	4/17
U	5/17
G	4/17

Kmer	Frequency
CU	4/16
UG	4/16
GA	3/16
AC	3/16
GU	1/16
UA	1/16

Kmer	Frequency
CUG	4/15
UGA	4/15
GAC	3/15
ACU	3/15
UGU	1/15

Kmer	Frequency
CUGA	3/14
UGAC	3/14
GACU	3/14
ACUG	3/14
GUGU	1/14
UGUA	1/14

Kmer	Frequency
CUGAC	3/13
UGACU	3/13
GACUG	3/13
ACUGA	2/13
ACUGU	1/13
CUGUA	1/13

Kmer	Frequency
CUGACU	3/12
UGACUG	3/12
GACUGA	2/12
ACUGAC	2/12
GACUGU	1/12
ACUGUA	1/12

Fig. 1 Pictorial representation of the computation of Kmer features of sizes 1 to 6

Table 2 Software used and parameter setting for different machine learning models used for prediction of abiotic stress-responsive lncRNAs

Method	Parameter	Software
Support vector machine (SVM)	Kernel = "Radial Basis Function", **γ** = 1/ #number of column, cost = 1	*e1071* R-package (Meyer et al. 2021)
Extreme gradient boosting (XGB)	max_depth = 3, **η**= 1, nrounds = 2, objective = "logistic"	*xgboost* R-package (Chen et al., 2021b)
Random forest (RF)	Ntree =1000, Mtry = sqrt (#number of column)	*randomForest* R-package (Liaw and Wiener 2002)
Light-gradient boosting machine (LGBM)	objective= 'binary', boosting="gbdt", learning_rate = 0.1 , num_leaves = 31, nrounds =1000	*lightgbm* R-package (Shi et al. 2022)
Gradient boosting decision tree (GBDT)	shrinkage =0.01, distribution = 'bernoulli', cv.folds=5, n.trees =1000, interaction.depth =6, n.minobsinnode =10	*gbm* R-package (Greenwell et al. 2022)
Adaptive boosting (ADB)	v = 5, mfinal = 1000	*adabag* R-package (Alfaro et al. 2013)
Bagging (BAG)	nbagg=25, method=c("standard")	*ipred* R-package (Peters et al. 2023)

chosen. The *sigFeature* R-package was used to implement the SVM-RFE technique (Das et al., 2020). The R-packages *randomForest* (Liaw and Wiener 2002), *xgboost* (Chen et al., 2021b), and *lightgbm* (Shi et al. 2022) were used to implement the RF-VIM, XGB-VIM, and LGB-VIM methods, respectively.

Cross-validation and performance metrics

A five-fold cross-validation approach was used to assess the performance of the prediction models. Both the positive and negative datasets were randomly separated into five subgroups of equal size to perform the five-fold cross-validation (Jiang and Wang, 2017). In each fold of the cross-validation, one randomly selected subset from each class served as the test set, while the remaining four subsets from both classes were pooled to serve as the training set. With distinct training and test sets for each fold, the experiment was carried out five times, and the accuracy over the five folds was recorded. The different steps involved to develop the proposed approach are shown in Fig. 2. The following metrics were used to evaluate the performance of the prediction models: sensitivity, specificity, accuracy, precision, area under receiver operating characteristic curve (AU-ROC; Fawcett, 2006), and area under precision recall curve (AU-PRC; Boyd et al., 2013). In the following formulae, TP and FP respectively represent the number correctly and wrongly predicted positive samples, whereas TN and FN respectively represent the number correctly and wrongly predicted negative samples.

$$\text{Sensitivity} = \frac{TP}{TP + FN} \tag{2}$$

$$\text{Specificity} = \frac{TN}{TN + FP} \tag{3}$$

$$\text{Accuracy} = \frac{1}{2}\left(\frac{TP}{TP + FN} + \frac{TN}{TN + FP}\right) \tag{4}$$

$$\text{Precision} = \frac{TP}{TP + FP} \tag{5}$$

$$AU - ROC = \int_0^1 \frac{TP}{P} d\left(\frac{FP}{N}\right) \tag{6}$$

$$AU - PRC = \int_0^1 \frac{TP}{TP + FP} d\left(\frac{TP}{P}\right) \tag{7}$$

Results

Performance analysis of MLAs with independent Kmer feature set

The performance of each machine learning method was evaluated independently with each Kmer feature set (K_1 to K_6). The highest sensitivity of 69.05% was achieved with LGBM for K_4, followed by the BAG (67.59%) with K_2 (Fig. 3). In comparison to the other combinations of Kmer size and learning algorithm, BAG also achieved the highest specificity (72.68%) with K_4. The BAG algorithm also achieved the highest precision of 68.10% for K_4 (Fig. 3). As far as overall accuracy is concerned, RF achieved the highest value of 61.79% with tri-nucleotide compositional features (K_3), followed by XGB (61.95%) and GBDT (61.21%) with dinucleotide (K_2) and tri-nucleotide (K_3) features, respectively (Fig. 3). With K_3, RF also achieved the highest AU-ROC (70.70%) and AU-PRC (70.69%). In comparison to the remaining learning algorithms, XGB with K_2 was found to produce higher AU-ROC (70.32%) and AU-PRC (69.51%) (Fig. 3). Because the

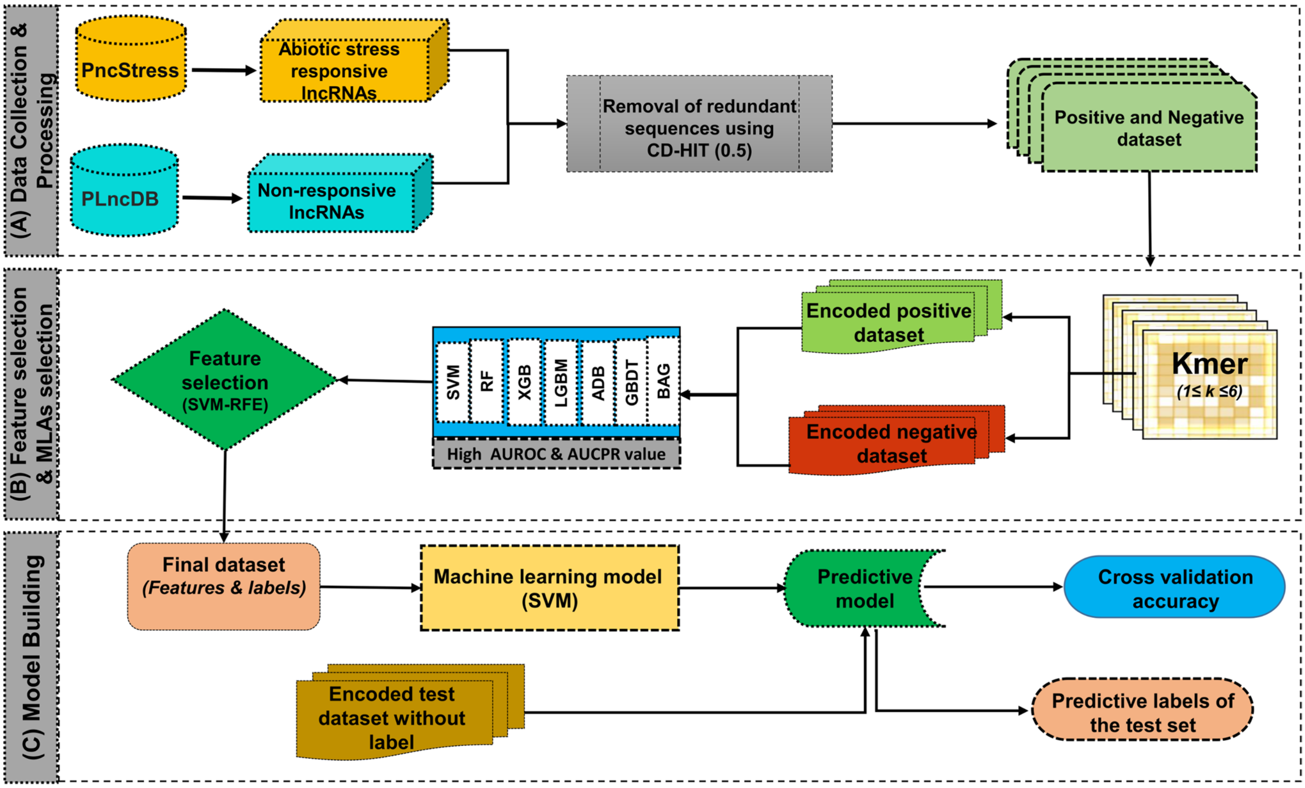
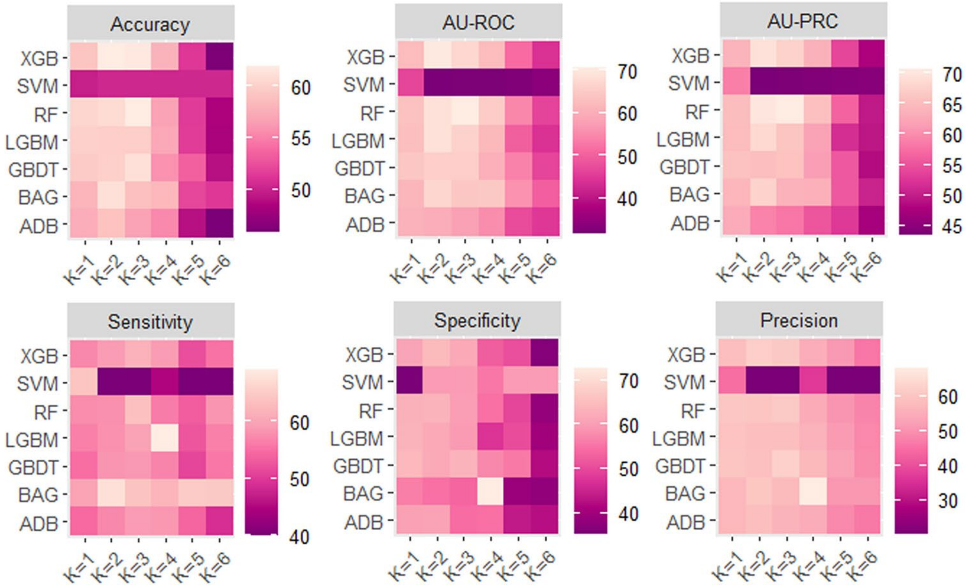

(A) Data Collection & Processing
PncStress
Abiotic stress responsive lncRNAs
PLncDB
Non-responsive lncRNAs
Removal of redundant sequences using CD-HIT (0.5)
Positive and Negative dataset
(B) Feature selection & MLAs selection
Kmer
(1≤ k ≤6)
Encoded positive dataset
Encoded negative dataset
SVM
RF
XGB
LGBM
ADB
GBDT
BAG
High AUROC & AUCPR value
Feature selection
(SVM-RFE)
(C) Model Building
Final dataset
(Features & labels)
Machine learning model (SVM)
Predictive model
Cross validation accuracy
Encoded test dataset without label
Predictive labels of the test set

Fig. 2 Illustration of the brief outline of the proposed computational approach. The diagram depicts the overall workflow of the entire computational strategies followed to develop the abiotic stress-responsive lncRNA prediction models. (**A**) Retrieval of experimentally validated abiotic responsive and non-responsive lncRNA sequences from the PncStress and PLncDB V2.0 database and processing of sequence data; (**B**) sequence-derived Kmer feature generation and selection of most important features and machine learning algorithm (MLA) based on AU-ROC and AU-PRC; (**C**) model building using machine learning technique and cross-validation with selected features and assessment of model in the independent test dataset

Fig. 3 Heat maps of the performance metrics for different machine learning algorithms with independent Kmer feature set



features generated with large Kmer sizes are sparse, the accuracy obtained with K_5 and K_6 may be worse than with K_1, K_2, K_3, and K_4, similar to the present study.

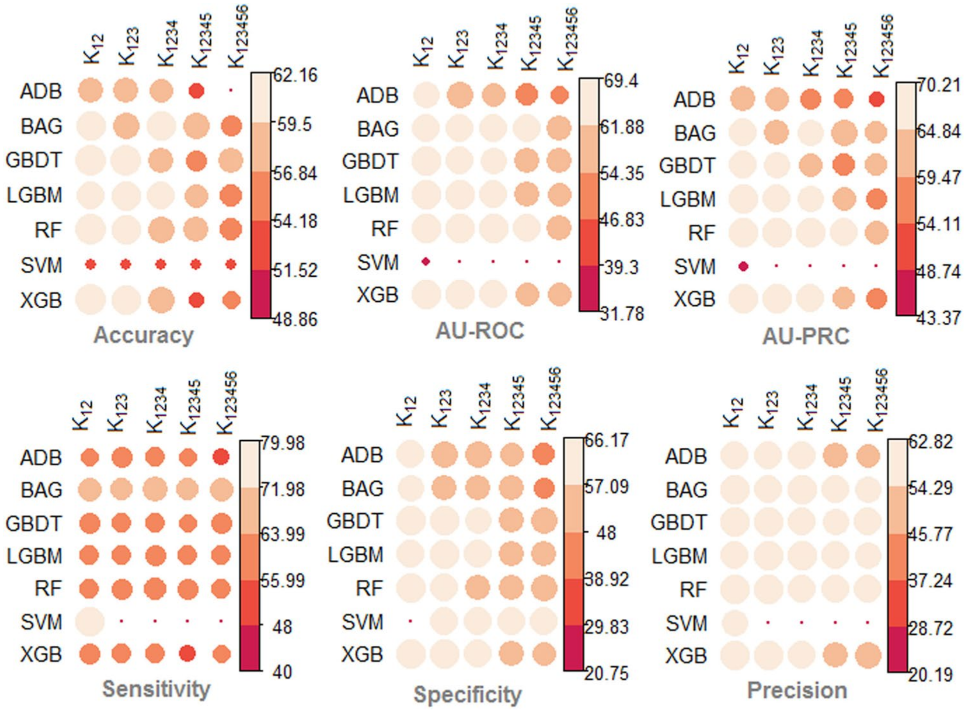
Performance analysis of MLAs with combined Kmer feature set

In addition to evaluating the accuracy of each Kmer feature set separately, the performance of machine learning algorithms was evaluated using combined Kmer feature sets such as K_{12} (K_1+K_2), K_{123} (K_1+K_2+K_3), K_{1234} (K_1+K_2+K_3+K_4), K_{12345} (K_1+K_2+K_3+K_4+K_5), and K_{123456} (K_1+K_2+K_3+K_4+K_5+K_6). The highest sensitivity (79.98%) was achieved by SVM with K_{12} features, whereas the BAG method achieved the highest sensitivity for the rest of the feature combinations (Fig. 4). The highest specificity (66.17%) and precision (62.82%) was achieved by GBDT with K_{123}, followed by RF (65.36%, 61.91%) with K_{12} features. When XGB was used, the highest accuracy was found to be 62.16% with K_{12} features, followed by GBDT (62.15%) with K_{123} and RF (62.14) with K_{12} features (Fig. 4). Barring a few exceptions, the accuracies were seen to be declining with an additional increase in the Kmer features (Fig. 4). The RF achieved the highest AU-ROC (69.4%) with K_{123}, followed by XGB (69.37%) with K_{12} features (Fig. 4). The highest AU-ROC with K_{123} features was seen to be less than that obtained with RF for K_3 (70.70%). When RF was employed as the classifier, K_{12} produced the highest AU-PRC (70.18%), which was also lower than the AU-PRC of RF achieved with K_3 (70.70%) (Fig. 4).

Performance analysis MLAs with selected Kmer features

In order to improve prediction accuracy further, four different feature selection procedures (SVM-RFE, RF-VIM, XGB-VIM, and LGB-VIM) were employed to identify relevant and non-redundant features. The features were ranked in order of relevance, with the first being the most significant and the final being the least important. The prediction accuracy of learning algorithms was further evaluated in terms of AU-PRC by adding 10 top features at a time (Fig. 5). The BAG method was observed to achieve the highest AU-PRC of 65.08% using the top 70 XGB-VIM features (Table 3). Similarly, BAG achieved the highest AU-PRC of 65.66% with 590 top-selected features of LGB-VIM. SVM was found to achieve the maximum accuracy (72.66%) among the considered models with 100 top features chosen by RF-VIM (Table 3). Furthermore, SVM was observed to achieve the highest AU-PRC of 76.16% using the top 530 SVM-RFE features (Table 3). The prediction accuracy of the learning algorithms was observed to be improved when compared to the performance with all 5460 features. The SVM was found to be the best performer, followed by the RF when the prediction was done using the selected features of SVM-RFE and RF-VIM (Fig. 5). The BAG method was found to be the better achiever when it came to prediction using the chosen features of XGB-VIM and LGB-VIM in comparison to the other methods (Fig. 5).

Fig. 4 Heat maps of prediction accuracy for different shallow learning algorithms with the combining Kmer feature sets



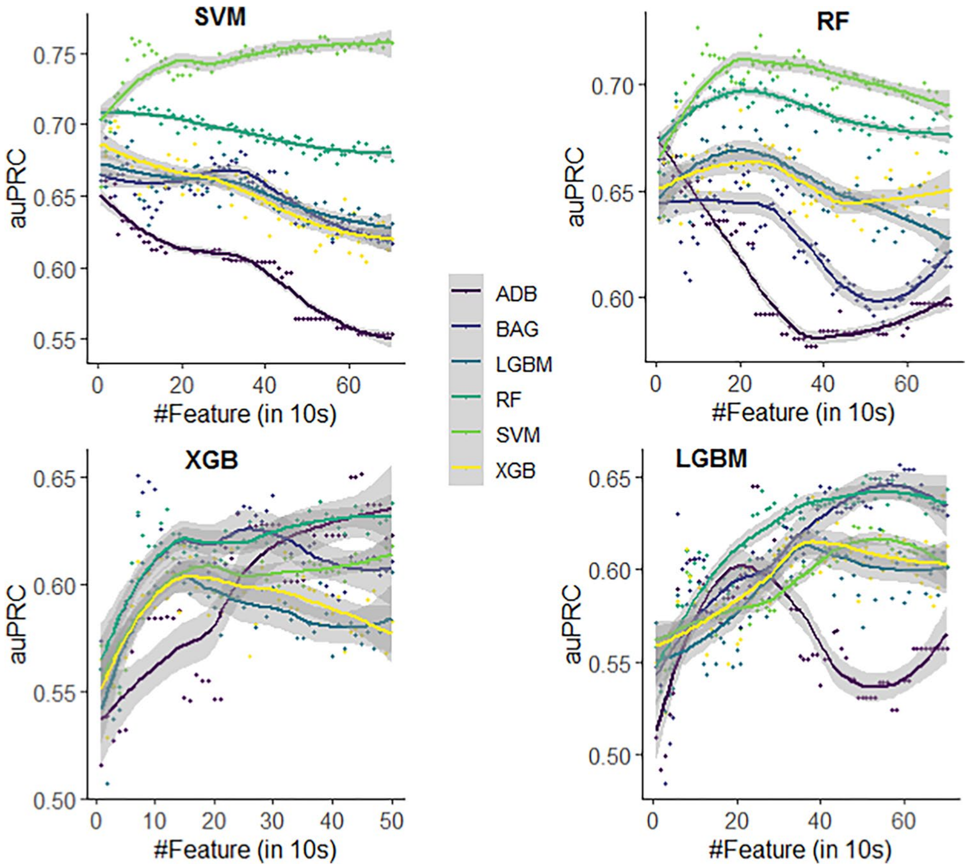


Fig. 5 Plot of the AU-PRC (auPRC) with the ranked features selected through four different feature selection methods

Table 3 Performance metrics of different machine learning methods using the selected features

Feature selection method	#Feature selected and metrics	ADB	BAG	LGBM	RF	SVM	XGB
LGB-VIM	#Feature	250	590	350	260	480	370
	AU-ROC	61.83	63.57	61.76	61.86	60.22	63.12
	AU-PRC	64.51	65.56	63.09	64.28	62.35	63.83
XGB-VIM	#Feature	430	70	140	70	170	230
	AU-ROC	60.55	60.31	61.80	70.61	60.53	60.41
	AU-PRC	64.96	65.08	62.32	63.35	61.92	61.60
RF-VIM	#Feature	10	20	50	250	100	240
	AU-ROC	66.54	65.45	66.40	68.44	69.01	67.64
	AU-PRC	67.51	66.28	69.21	70.11	72.66	67.86
SVM-RFE	#Feature	20	190	300	80	530	30
	AU-ROC	62.53	65.34	68.37	69.80	74.09	70.22
	AU-PRC	66.02	67.55	67.82	71.73	76.16	69.65

ADB, adaptive boosting; *BAG*, bagging; *LGBM*, light-gradient boosting machine; *RF*, random forest; *SVM*, support vector machine; *XGB*, extreme gradient boosting

Analysis of cross-validation and independent test set prediction

Since the SVM was found to achieve the highest accuracy with 530 top-selected features of SVM-RFE, the same combination was employed for cross-validation performance analysis. As far as cross-validation analysis is concerned, the sensitivity, specificity, overall accuracy, precision, AU-ROC, and AU-PRC were observed to be 73.03, 64.61, 68.84, 67.58, 73.98, and 75.54%, respectively (Table 4). The model trained with SVM using 530 selected features was also employed to predict the independent test set (101

Table 4 Performance metrics for the training and independent test datasets

Evaluation	Sensitivity	Specificity	Accuracy	Precision	AU-ROC	AU-PRC
Cross-validation	73.07	64.61	68.84	67.58	73.98	75.54
Independent dataset	91.08	61.38	76.23	70.22	87.71	88.49

The SVM with the 530 selected features was used for training and prediction

positive and 101 negative sequences). For the independent test set, the sensitivity, specificity, overall accuracy, precision, AU-ROC, and AU-PRC were found to be 91.08, 61.38, 76.23 and 70.22, 87.71, and 88.49%, respectively (Table 4). The higher degree of sequence similarity with the training dataset may be attributed to the higher accuracy of the independent test set when compared to the cross-validation accuracy.

Development of an online prediction tool

In order to predict the abiotic stress-responsive lncRNAs, we further developed an online prediction tool called ASLncR (https://iasri-sg.icar.gov.in/aslncr/). The front end of the server was designed using HTML, while its back end uses PHP to execute the developed in-house R-code. This server implemented the SVM model using the 530 chosen features. For prediction, the user has to either paste or upload the lncRNA sequences in FASTA format. The results are displayed in tabular format, where the probability of each lncRNA being associated with stress is provided.

Performance analysis of ASLncR with experimentally validated dataset

To further confirm the efficiency of the developed tool ASLncR, lncRNA sequences for various abiotic stresses were manually collected from published literature (Jha et al. 2020; Urquiaga et al. 2020; Patra et al. 2023). For 9 different plant species, a total of 190 sequences were collected for the abiotic stresses cold, heat, light, salt, drought, flood, and others. We were left with 138 sequences for the evaluation using our model after eliminating the sequences that were present in the positive set of training and independent test dataset. The abiotic stress responsiveness of the sequences was predicted using the ASLncR server, and it was discovered that 81.88% (113 out of 138) of the sequences were correctly identified.

Discussion

Abiotic stresses brought about by climate change pose a serious challenge to crop production and productivity. Therefore, it is necessary to develop abiotic stress-tolerant crop cultivars to meet the food security demand. In the last decade, a considerable amount of research has focussed to understand the different regulatory roles of lncRNAs in plant response to abiotic stresses and their indispensable roles in environmental adaptation (Chen et al., 2023; Yang et al., 2022; Liu et al., 2022b; Zhang et al., 2022; Tian et al., 2023; Ye et al., 2022; Chen et al., 2022). To put it another way, lncRNAs are multifaceted regulatory components that are essential for controlling cellular stress in response to various abiotic stimuli. For instance, Eom et al. (2019) revealed that lncRNAs co-express with mRNA in tomatoes in response to drought stress. Network analysis of the interactions between lncRNA and miRNA in *Brassica juncea* reveals a target for regulating drought tolerance (Bhatia et al., 2020). In order to understand how plants respond to various environmental stresses, it is crucial to identify abiotic stress-responsive lncRNAs. However, due to intricate genomic architecture, wet-lab experiments for lncRNA identification are costly and time-consuming. Thus, we developed a machine learning-based computational model for predicting abiotic stress-responsive lncRNAs based on the sequence-derived features.

Though several tools are available for plant lncRNA prediction, no single tool is available for predicting abiotic stress-responsive lncRNAs. It has been shown that lncRNAs with related functions share comparable K-mer profiles (Kirk et al., 2018). Additionally, the K-mer features have been successfully utilized to establish relationships between sequence and function among lncRNAs (Kirk et al. 2018; Kirk et al. 2021). In order to capture the abundance of short motifs in an lncRNA, in the present study, the K-mer features were used to encode lncRNAs into numeric feature vectors. The Kmer features have also been successfully applied in other areas of bioinformatics such as sequence assembly (Li et al. 2010), metagenomics (Dubinkina et al. 2016), DNA barcoding (Meher et al. 2016), and lncRNA prediction (Sun et al. 2013). We considered Kmer sizes 1 to 6, where the accuracy obtained with individual Kmer features was found to be higher than the accuracy obtained by combining all 5460 Kmer features. Shorter K-mers are more common, and their relative frequencies are more strongly cross-correlated than for longer K-mers (Klapproth et al. 2021), which could be a probable reason for the low accuracy with higher K-mer features.

It was seen that while all the 5460 features were utilized, the prediction accuracy was low. Thus, in order to improve prediction accuracy, significant and non-redundant features were selected by employing feature selection methods. To choose important features, four distinct feature selection strategies, including SVM-RFE, RF-VIM, XGB-VIM, and LGB-VIM, were adopted. As compared to all the 5460 features, BAG achieved the highest accuracy with 70 and 590 features selected using XGB-VIM and LGB-VIM methods, respectively. Similarly, SVM achieved the highest accuracy with 100 and 530 features selected using RF-VIM and SVM-RFE methods, respectively. Compared to the other three approaches, SVM-RFE ranking features had greater accuracy. Furthermore, it was discovered that prediction with selected features improved the accuracy of learning algorithms. When using the 530 top-ranked features of SVM-RFE, SVM had the highest accuracy among the learning algorithms, despite being the least effective when the prediction was done with individual or combined Kmer features.

The robustness of the proposed approach was also assessed using an independent dataset. The higher accuracy with the independent dataset as compared to the cross-validation accuracy may be attributed to a higher degree of sequence similarity between the training and independent test dataset. For easy implementation of our computational approach to predict abiotic stress-responsive lncRNA, we have established an online prediction tool ASLncR. Furthermore, to check the effectiveness of ASLncR, 138 experimentally confirmed abiotic stress-related lncRNAs were revalidated. The accuracy obtained from the cross-validation, independent test set validation, and the revalidation of ASLncR supports the applicability of the proposed model for predicting abiotic stress-responsive lncRNA in a plant.

Conclusion

Intensifying evidence from various plant species signifies that lncRNAs play critical roles in abiotic stress responses. Compared to humans, the application of lncRNAs in plant breeding is still in its initial phases. Despite the fact that lncRNAs mediate plant regulation in response to abiotic stresses in many species, their potential as valuable genomic resources in plant molecular breeding or as indicators have yet to be confirmed. Studies of lncRNAs in a wider range of plant species will aid in understanding the evolution and diversity of their roles in environmental adaptation. Due to the dearth of wet-lab as well as computational approaches, potential applications of lncRNAs in plant abiotic stress are currently lacking. The present work provides one of the first computational methods, ASLncR (https://iasri-sg.icar.gov.in/aslncr/), for predicting lncRNAs that are responsive to abiotic stress. The ASLncR can be successfully employed for large-scale prediction of abiotic stress-responsive lncRNAs using only sequence information. The suggested strategy is expected to supplement the current experimental approaches for predicting abiotic stress-related lncRNAs, given the significance of lncRNAs in plant response to abiotic challenges.

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Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. All secondary data used in the study are available at https://iasri-sg.icar.gov.in/aslncr/dataset.php

Conflict of interest The authors declare no competing interests.

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