# NACSVM<sub>Pred</sub>: A MACHINE LEARNING APPROACH FOR PREDICTION OF NAC PROTEINS IN RICE USING SUPPORT VECTOR MACHINES

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#### ABSTRACT

NAC proteins are plant-specific transcriptional factors with diversified roles in various developmental processes and stress responses. Development of genome wide prediction tools for NAC proteins will substantially have an impact on rice gene annotation. NACSVM<sub>Pred</sub> is an effort in this direction for computational genome-scale prediction of NAC proteins in rice by integrating compositional and evolutionary information of proteins. Support vector machine (SVM)-based modules were first developed using traditional amino acid, dipeptide (i+1), tripeptide (i+2), four-parts composition and PSSM and an overall accuracy of 79%, 93%, 93%, 79% and 100% respectively was achieved. Further, two hybrid modules were developed based on amino acid, dipeptide and tripeptide composition, which achieved an overall accuracy of 83% and 79%. NACSVM<sub>Pred</sub> was also evaluated with PSI-BLAST, which resulted in a lower accuracy of 50%. The different statistical analyses carried out revealed that the proposed algorithm is useful for rice genome annotation, specifically predicting NAC proteins.

### **KEYWORDS**

SVM, NAC, RBF, PSSM

## **1. INTRODUCTION**

Rice (*Oryza sativa* L.) is an indispensable grain being the staple food for more than one-third of the world's population. Rice is truly a way of life for many societies who are dependent on ricebased production systems for their livelihood. Rice production is subjected to a number of stresses, both abiotic and biotic, and potential yields are seldom achieved because of the effect of these stresses. Abiotic stresses adversely affect the growth and productivity of rice-based farming systems. Present day challenges like global climate change, less water availability, urbanization etc. exacerbate these situations. These factors have lead to a mounting interest among researchers on investigations on tolerance to abiotic stress. Incorporation of stress tolerance into high-yielding rice varieties has proven to be a very effective approach to developing varieties that can cope with these extreme situations.

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The mechanism of molecular response of plants to abiotic stresses has been investigated by detailed studies of genes unregulated under the specific stress conditions. One class of important transcription factors induced during abiotic stress tolerance is NAC. NACs are genes encoding a polypeptide containing a plant-specific highly conserved N-terminal domain, NAC (for NAM, ATAF1, 2, and CUC2) and are represented by approximately 140 genes in rice [1]. These transcription factors regulate gene expression by binding to specific cis-acting promoter elements, thereby activating or repressing the transcriptional rates of their target genes [2, 3]. The identification and functional characterization of these transcription factors therefore assumes importance for the reconstruction of transcriptional regulatory networks.

In comparison to experimental methods, computational prediction techniques are fast and highly accurate for high-throughput analysis of large-scale genome sequences. Development of a fully automatic prediction system for NAC transcription factors in rice is a systematic attempt in this direction. In this work, a new prediction method NACSVM<sub>Pred</sub> has been developed, based on a powerful machine learning technique *viz.*, Support Vector Machine (SVM) for the prediction of NAC proteins in *indica* rice (*Oryza sativa* L. ssp. *indica*). This was developed using the evolutionary and compositional features of a protein sequence and the performance of the model was evaluated using cross-validation techniques. Finally, a web-based server was developed based on the best approach, based on accuracy, to provide service to the rice research community, where the users have the option to query their sequence/sequences for the prediction of stress-responsive NAC proteins in rice.

# 2. MATERIALS AND METHODS

## 2.1. Dataset

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An important concern during the development of a prediction method is the selection of a dataset. In the present study, the data set consisting of 95 NAC proteins of *indica* rice and another set of 95 NAC proteins from diverse plant families *viz.*, Arabidopsis (*Arabidopsis thaliana*), soybean (*Glycine max*), wheat (*Triticum aestivum*), poplar (*Populus trichocarpa*), maize (*Zea mays*) and cotton (*Gossypium hirsutum*) were taken from Uniprot Knowledgebase. 'All Plants' dataset was used to cross-check the performance of our species-specific classifier (indica rice) on some non-trained plants. The 95 NAC proteins of indica rice, of which some where uncharacterized proteins, were confirmed to be of NAC family through Prosite and Pfam databases. Out of 95 NAC proteins, 10 were randomly selected for the creation of test set and remaining 85 proteins were used for positive dataset / training set. Non-NAC proteins in test set and training set and these were used for independent dataset test as training set and test set were entirely different. Similar method was applied for all plants model also.

# 2.2. Support Vector Machine

Support vector machine (SVM), initially conceived by Cortes and Vapnik [4, 5], is conceptually simple, and easy to implement algorithm. It is defined as a computer algorithm that learns by example to assign labels to objects [6]. SVMs are becoming popular in a wide variety of biological applications like classifying objects as diverse as protein and DNA sequences, microarray expression profiles and mass spectra [7]. An SVM is a mathematical entity, an algorithm for maximizing a particular mathematical function with respect to a given collection of data. For constructing an optimal hyperplane, SVM employees an iterative training algorithm which is used to minimize an error function. In the present study, we have used SVM<sup>light</sup> [8], a freely downloadable package of SVM to predict the NAC proteins. A number of parameters can be chosen in this software apart from inbuilt kernel function including linear, polynomial and radial basis function (RBF). To achieve maximum accuracy, we have attempted seven different

compositional and evolutionary approaches based on various features of a protein sequence which are further explained below.

## 2.3. Features and Modules

#### 2.3.1. Amino-acid composition

Amino-acid composition is the fraction of each amino acid which occurs in a protein sequence. The fraction of all 20 natural amino acids was calculated using the following equation:

Fraction of amino acid = 
$$\frac{\text{Total number of amino acid i}}{\text{Total number of amino acids in protein}}$$
 (1)

## 2.3.2. Traditional dipeptide composition

Traditional dipeptide composition gives global information about each protein sequence utilizing the sequence order effects. This representation gives a fixed pattern length of 400 (20x20) which encompasses the information of the amino-acid composition along with the local order of amino acids. The fraction of each dipeptide was calculated according to the equation:

Fraction of dep (i + 1) = 
$$\frac{\text{Total number of dep (i+1)}}{\text{Total number of all possible dipeptides}}$$
 (2)

#### 2.3.3. Tripeptide composition

This composition not only reflects the total amino acid composition but also the sequence order effects [9,10]. This representation gives a fixed pattern length of 8000 (20x400) which encompasses the information of the amino-acid composition along with the local order of amino acids and fraction of each tripeptide was calculated using Equation 3,

Fraction of tripep 
$$(i + n) = \frac{\text{Total number of tripep}(i+n)}{\text{Total number of all possible tripeptides}}$$
 (3)

Where n=2 and tripep (i + n) is one of 8000 tripeptides.

#### 2.3.4. Four parts composition

In this feature extraction method each protein sequence was divided into four equal parts based on length. This type of composition has shown good results as evident from some earlier studies [10, 11]. The occurrence of each amino acid in divided part was calculated separately using Eq. (1) and then a combined fixed pattern length of 80 (20 x 4) was formed in order to gather more information about the protein sequence.

#### 2.3.5. Hybrid SVM module(s)

The prediction accuracy of  $NACSVM_{Pred}$  was further evaluated with various hybrid approaches by combining different features of a protein sequence.

#### Hybrid approach-1

In this approach, we developed a hybrid module by combining amino acid composition and dipeptide composition features of a protein sequence as calculated by using Equation (1) and (2), respectively. This module was provided with an SVM input vector pattern of 420 (20 + 400) *i.e.* 20 for amino acid and 400 for dipeptide composition.

#### Hybrid approach-2

In the second approach we developed another hybrid module by combining amino acid composition and tripeptide composition (i + 2) as calculated using Eq. (1) and (3), respectively.

The SVM input vector pattern thus formed was 8020-dimensional (20 for amino acid and 8000 for tripeptide).

## 2.3.6. Position-specific scoring matrix (PSSM)

PSSM based module was another approach constructed by combining the evolutionary information of a protein sequence stored in the matrix called as PSSM. This information is expressed in a position-specific scoring table called profile which is created from a group of sequences previously aligned by PSI-BLAST against the non-redundant (NR) database at GenBank. It is a method for detecting distantly related proteins by sequence comparison by adopting PSSM matrix extracted from sequence profiles as input data [12]. The PSSM matrix gives the log-odds score for finding a particular matching amino acid in a target sequence (Figure 1). This method differs from other composition methods of sequence comparison because any number of known sequences can be used to construct the profile allowing more information to be used in the testing of the target sequence. The PSSM provides a matrix of dimension n rows and 20 columns for a protein input sequence of n amino acid residues where 20 columns represent the occurrence of each type of 20 amino acids. The PSSM matrix of a protein sequence generated from the profiles of PSI-BLAST was used to generate a 400-dimensional (20 x 20) input vector to the SVM by summing up all rows in the PSSM matrix of the same amino acid in the primary sequence the range of 0-1 by using the function  $\frac{(X-minimum)}{(maximum-minimum)}$  where X is the individual score of each amino acid and maximum, minimum are the corresponding maximum and minimum value of score in each row of the matrix (Fig 1).

# 2.4. Measurement of Performance of NACSVM<sub>Pred</sub>

In statistical prediction, the effectiveness of a predictor is generally examined using either single independent dataset test, cross-validation test or jackknife test. Among these mentioned, the jackknife test is considered to be most rigorous and objective one, as illustrated by a comprehensive review [13]. However jackknife test method takes much longer time to train a predictor based on SVM and therefore, in our present work, only 10-fold cross-validation and independent data set validation techniques were adopted for performance measurement. For 10-fold cross-validation, the relevant dataset was partitioned randomly into ten equally sized sets. The training and testing was carried out ten times with each distinct set used for testing and the remaining nine sets for training. In the independent dataset test, none of the data to be tested occurs in the training dataset used to train the predictor and the selection of data used for the testing dataset could be quite arbitrary.

## **2.5. Evaluation Parameters**

In this work we adopted five frequently considered evaluation criteria's namely accuracy (Ac), sensitivity (Sn), specificity (Sp), precision (Pr) and Mathew's Correlation Coefficient (MCC). Most of the information retrieval papers report precision and accuracy, while bioinformatics, medical and machine learning papers tend to report sensitivity and specificity apart from the MCC. Accuracy (Ac) defines the correct ratio between both positive (+) and negative (-) data sets (Eq. 6). The sensitivity (Sn), sometimes called as recall too, and specificity (Sp) represent the correct prediction ratios of positive (+) and negative data (-) sets of NAC proteins respectively (Eq. 4 and 5).

1. Input protein sequence of the form a1, a2, .....an

2. Run PSI-BLAST with 3 iterations, which generates PSSM matrix of n x 20 dim of the form:

Position Specific Scoring Matrix (PSSM)

$$P_{PSSM} = \begin{bmatrix} E_{1 \to 1} & E_{1 \to 2} & \dots & E_{1 \to j} & \dots & E_{1 \to 20} \\ E_{2 \to 1} & E_{2 \to 2} & \dots & E_{2 \to j} & \dots & E_{2 \to 20} \\ \vdots & \vdots & \dots & \vdots & \dots & \vdots \\ E_{i \to 1} & E_{i \to 2} & \dots & E_{i \to j} & \dots & E_{i \to 20} \\ \vdots & \vdots & \dots & \vdots & \dots & \vdots \\ E_{L \to 1} & E_{L \to 2} & \dots & E_{L \to j} & \dots & E_{L \to 20} \end{bmatrix}$$

3. Normalize each value of the matrix in the range of 0-1 using (x - min)/(max - min)

4. Sum up all rows in the normalized matrix to the same amino acid in the primary sequence

5. Resultant matrix is of the dim 20 x 20.

Figure 1. Flow chart of the algorithm to generate PSSM matrix of 20x20 dimension from the input pattern of nx20 dimension matrix used as input pattern for various models of SVM

Precision is the proportion of the predicted positive cases that were correct (Eq.7). However, when the numbers of positive and negative data differ too much from each other, the Mathew correlation coefficient (MCC) should be included to evaluate the prediction performance of the developed tool (Eq. 8). The MCC is another measure used in machine learning for judging the quality of binary (two-class) as well as multi-labeled classifications and is considered to be the most robust parameter of any class prediction method. The value of MCC ranges from -1 to 1, and a positive MCC value stands for better prediction performance. Among the data with positive hits by NACSVM<sub>Pred</sub>, the real positives are defined as true positives (TP), while the others are defined as false positives (FP).

Sensitivity = 
$$\frac{TP}{TP+FN} \times 100$$
 (4)

Specificity = 
$$\frac{TN}{FP+TN}$$
 x 100 (5)

Accuracy = 
$$\frac{TP+TN}{TP+TN+FP+FN} \ge 100$$
 (6)

Precision = 
$$\frac{TP}{TP+FP} \times 100$$
 (7)

$$MCC = \frac{(TP X TN) - (FP X FN)}{\sqrt{(TP + FP)(TP + FN)(TN + FP)(TN + FN)}}$$
(8)

where TP and TN are truly or correctly predicted positive NAC protein and negative (non-NAC protein), respectively. FP and FN are falsely or wrongly predicted NAC and non-NAC proteins, respectively.

## 2.6. Sequence Similarity Search

PSI-BLAST is a method to search for the similarities between a protein query sequence and all the sequences in a protein database. Contrary to BLAST, this search method uses the position specific scoring matrix (PSSM) to score matches between query and database sequences. The advantage of using a profile to search a database is that it often detects close relationships

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between proteins that are structurally or functionally distant. Here we have used PSI-BLAST in place of normal BLAST because of former's capacity to detect remote homology of NAC proteins against Swiss Prot database and result was analysed.

# 2.7. ROC Curves

The performance of a binary classifier can be explained with ROC curve which is a graphical plot drawn by varying threshold values. The analysis of ROC curve helps to characterize the prediction for individual locations [14, 15]. ROC curve is a graph created by plotting the fraction of false positives (FPR) against true positives (TPR) at various threshold settings. The area under the curve (AUC) represented in the ROC curve further measures the classifier accuracy.

# **3. RESULTS AND DISCUSSION**

Here we have carried out 10-fold cross-validation and independent data test validation to evaluate the performance of NACSVM<sub>Pred</sub> (Table 1 and Table 2). Cross validation and independent data test result from the table shows that cross validation have better results for different composition methods compared to independent data test. From Table 1 and 2, it is clear that PSSM method has 100% accuracy with respect to all the three kernels of SVM compared to all the other six composition methods and hence PSSM was selected to be the best model for NACSVM<sub>Pred</sub>.

# 3.1. Statistical Tests of the Best Classifier

In the 10-fold cross-validation test, the best overall sensitivity was achieved from PSSM which has 100% accuracy over the other composition methods. Performance comparison of overall accuracies achieved by various composition methods is represented graphically in Figure 2. This classifier achieved an overall prediction accuracy of 100 % with a high-confidence MCC of 1. Sensitivity and specificity are two competing but nonexclusive measures of quality useful for testing the performance of any classification methods. The MCC provides a balanced measure between the sensitivity and specificity for each class. An ideal classification method should have both sensitivity and specificity values close to 100% and MCC value equal to 1.

# 3.2. Composition Based Modules

The amino-acid composition based module with polynomial kernel achieved a maximum accuracy of 96 % with MCC of 0.93. A module based on traditional dipeptide composition (i + 1) was implemented which gave more information about frequency and local order of residues. This module could achieve a maximum accuracy of 83% with MCC of 0.72. Tripeptide (i + 2) composition based module was also developed to have more comprehensive information on the sequence order effects and this could achieve an accuracy of 95% with MCC of 0.9. The four parts composition method achieved maximum accuracy of 97% with MCC of 0.93. All the above validation test results are based on 10-fold cross-validation. The detailed performance of amino acid, traditional dipeptide, higher order peptide, PSSM and four parts composition based modules with cross validation are presented in Table 2.

On analyzing independent test validation, it could be observed that amino-acid composition achieved an accuracy of 79% with MCC of 0.64, dipeptide composition achieved an accuracy of 93% with MCC of 0.86, tripeptide (i+ 2) composition based module achieved an accuracy of 93% with MCC of 0.87 and four parts composition method achieved maximum accuracy of 79% with MCC of 0.63 for all the three kernels. The detailed performance of amino acid, traditional dipeptide, higher order peptide, PSSM and four parts composition based modules with independent data test validation are presented in Table 1. From Tables 1 and 2, it is clearly evident that best performance results are achieved for 10-fold cross validation for the various composition based modules irrespective of PSSM based module which has an accuracy of 100% for both validation.

#### 3.3. Hybrid Based Modules

In addition to the above composition methods, hybrid methods were applied which was the combination of various features of a protein sequence. The first hybrid method, hybrid 1was developed by combining amino acid feature with dipeptide feature of a protein sequence which achieved an accuracy of 83% with MCC of 0.67 (all three kernels) for independent data test and 95% accuracy for RBF kernel with MCC of 0.91 in the case of 10-fold cross validation test. The second hybrid method, hybrid 2, was developed by combining amino acid and tripeptide composition which also obtained a little less accuracy of 79% and MCC value of 0.64 was obtained for independent data test and 94% for RBF kernel with an MCC value of 0.89 for cross validation data test. The above results shows that for hybrid based modules also cross validation test results has an upper hand over independent test results (Table 1 and 2). The performance comparison of both composition and hybrid based method for cross validation are shown in the figure 2.

## 3.4. PSSM-based Module

A PSSM matrix for each protein sequence was constructed from the generated sequence profiles by PSI-BLAST and was converted into a 400 dimension input information to the SVM. The PSSM based module achieved high accuracy of 100% for all the three kernels of SVM with the different validation techniques (cross validation and independent data test). The result of PSSM based module with three algorithms are presented in Table 1 and 2 which demonstrates that prediction performance can be significantly improved on application of PSSM based module that offers important evolutionary information about proteins than any other composition methods. This shows that PSSM-based classifiers are statistically better than the other modules developed in this study with the best overall accuracy achieved by PSSM- based SVM module alone and which was also statistically significant compared to other modules.



Figure 2. Comparison of performance validation of NACSVM<sub>Pred</sub> with different composition methods

#### 3.5. ROC curves

The ROC curve is a measure which represents the relationship between sensitivity and (1-specificity) for a class. We have plotted the ROC curves based on the independent test performance of the various compositions. From the ROC curve (Figure 3), it is clear that the PSSM composition module represents a perfect classifier since the curve represents an inverted 'L'. This is a desirable characteristic of an ROC curve. The figure also represents the comparison of the performance of various classifiers with respect to different compositions. Each point on the ROC curve was plotted based on different threshold scores. The figure also depicted "excellent classification" area under the curve (AUC = 1) value for PSSM composition and high confidence AUCs for all other compositions [16]. The AUC shows the probability that when one positive and negative samples are drawn at random, the decision function assigns a higher value to the positive than to the negative sample.



Figure 3. ROC curve for different compositions in SVM using independent test results

Composition	Algorithm	Sn	Sp	Ac	Pr	MCC
		(%)	(%)	(%)	(%)	
Amino acid	Linear	100	57	79	71	0.64
	Polynomial	100	57	79	71	0.64
	RBF	100	57	79	71	0.64
Dipeptide	Linear	93	93	93	93	0.86
	Polynomial	93	93	93	93	0.86
	RBF	93	93	93	93	0.86
Tripeptide	Linear	87	100	93	100	0.87
	Polynomial	87	100	93	100	0.87
	RBF	87	100	93	100	0.87
4- parts	Linear	100	57	79	70	0.63
-	Polynomial	100	57	79	70	0.63
	RBF	100	57	79	70	0.63
Hybrid 1	Linear	93	71	83	78	0.67
•	Polynomial	93	71	83	78	0.67
	RBF	93	71	83	78	0.67
Hybrid 2	Linear	100	57	79	71	0.64
-	Polynomial	100	57	79	71	0.64
	RBF	100	57	79	71	0.64
	Linear	100	100	100	100	1
PSSM	Polynomial	100	100	100	100	1
	RBF	100	100	100	100	1

Table 1. Validation of independent data test results of NAC proteins with SVM

Table 2. Comparison of the prediction performance of three kernels of SVM with different composition techniques using 10-fold cross validation

Composition	Algorithm	Sn	Sp	Ac	Pr	MCC
1	U	(%)	(%)	(%)	(%)	
Amino acid	Linear	88	90	89	91	0.79
	Polynomial	98	95	96	96	0.93
	RBF	94	92	93	93	0.87
Dipeptide	Linear	99	63	81	77	0.64
	Polynomial	76	96	83	95	0.72
	RBF	79	84	83	96	0.63
Tripeptide	Linear	100	0	53	53	0.00
	Polynomial	100	0	53	53	0.00
	RBF	95	95	95	96	0.90
4- parts	Linear	90	100	95	100	0.90
	Polynomial	96	95	97	99	0.93
	RBF	91	94	94	97	0.87
Hybrid 1	Linear	93	94	93	94	0.88
	Polynomial	93	94	93	95	0.87
	RBF	94	96	95	96	0.91
Hybrid 2	Linear	93	94	93	94	0.87
	Polynomial	93	94	93	95	0.87
	RBF	94	94	94	95	0.89
PSSM	Linear	100	100	100	100	1
	Polynomial	100	100	100	100	1
	RBF	100	100	100	100	1

## 3.6. Sequence Similarity Search

To summarize evolutionary information about the proteins, the homology of a protein with other related sequences provides a broad range of information about each functional encoded protein and is carried out through sequence similarity search PSI-BLAST. This was used to compare a protein sequence with a created database to generate the homology of the given sequence with other related sequences in the database [17]. This provided a broad range of information about each functional encoded protein. A 10-fold cross-validation was conducted with no significant hits and an average accuracy of 53% was obtained (Table 3). This result suggests that similarity based search tools alone cannot be efficient and reliable as compared to different composition based modules based on computational methods.

Test	No. of sequences	Correctly	Accuracy
	given	predicted	(%)
1	20	10	50
2	20	10	50
3	20	10	50
4	20	8	40
5	20	10	50
6	20	11	55
7	20	10	50
8	20	10	50
9	15	11	73.3
10	15	10	66.7
Average			53.5

Table 3: Prediction result of NAC proteins with similarity search (10-fold cross validation)

## 3.7. Comparison of NACSVM<sub>Pred</sub> with 'All Plant' method

To strengthen the fact that a species-specific predictor(s) is much more advantageous than 'All Plant' method, we trained a corresponding method using the same encoding method as used in NACSVM<sub>Pred</sub> on a dataset derived from all the plant species, and then compared the performance of two methods on the rice independent dataset. To train the all-plant method a dataset consisting of six plants, namely Arabidopsis, soybean, wheat, poplar, maize and cotton were downloaded from Uniprot knowledgebase consisting of 95 sequences in total. As we wanted to compare the performance of rice independent dataset on both the NACSVM<sub>Pred</sub> and 'All Plant' method and to obtain fair result we made sure that All Plant training dataset were independent of rice sequences. Thus we made sure that both the NACSVM<sub>Pred</sub> as well as the 'All Plant' classifier had not been trained from any of the sequences in the rice independent dataset.

Finally, the traditional amino acid composition based classifier for 'All Plant' dataset was developed using the independent dataset approach and rice independent dataset was tested on the model files generated from the 'All Plant' classifier. Statistical parameters were calculated as done in NACSVM<sub>Pred</sub> and these were compared with the amino acid based classifier of NACSVM<sub>Pred</sub>. Though the best classifier obtained in NACSVM<sub>Pred</sub> was from PSSM matrix using evolutionary information of a protein sequence, but for comparison we have used simpler composition method for 'All Plant' module. Results are presented in Table 5 which shows that species specific tools are much superior to 'All Plant' tool.

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Method	Algorithm	Sn	Sp	Ac	Pr	MCC
		(%)	(%)	(%)	(%)	
	Linear	100	50	76	68	0.58
All-Plant	Polynomial	93	57	76	70	0.55
	RBF	100	50	76	68	0.58
NACSVM <sub>Pred</sub>	Linear	88	90	89	91	0.79
	Polynomial	98	95	96	96	0.93
	RBF	94	92	93	93	0.87

Table 4. Comparison of performance of NAC proteins with All-Plant tool and NACSVM<sub>Pred</sub>

## 3.8. Description of web server

A web server has been implemented on the World Wide Web as a dynamic web server 'NACSVM<sub>Pred</sub>'. The tool was developed in Perl and web interface in PHP and HTML to assses user queries. It allows users to submit/paste their protein sequences through the standard FASTA format or allows uploading of sequence through a file. The prediction result will be displayed in a user friendly format on the screen within few seconds (Figure 4). NACSVM<sub>Pred</sub> uses PSSM-based module for prediction. The overall architecture of the NACSVM<sub>Pred</sub> web server is shown in the Figure 5.



Figure 4. An overview of the submission form of NACSVM<sub>Pred</sub>



Figure 5. Schematic diagram showing the flow of steps in developing the web based tool

# 4. CONCLUSIONS

Finding new protein-coding genes is one of the most important goals of genome sequencing projects, especially for genomes with little experimental data. There is a dearth of accurate gene prediction programs with respect to various functionalities in rice. Thus, the availability of systems/tools that can predict location from sequence is essential to the full characterization of expressed proteins. Computational tools provide faster and accurate access to predictions for any organism.

Identification of NAC proteins from sequence databases is difficult due to poor sequence similarity. In this work, we present a new method for NAC prediction based on SVM, a simple machine learning technique. The performance was found to be highly satisfactory. Comparison between different kernels of SVM was also carried out to validate the accuracy. Very high prediction accuracies for the validation tests show that NACSVM<sub>Pred</sub> is a potentially useful tool for the prediction of NAC proteins.

# REFERENCES

- [1] Y. Fang, J. You, K. Xie, W. Xie & L. Xiong, (2008) "Systematic sequence analysis and identification of tissue-specific or stress-responsive genes of NAC transcription factor family in rice", *Molecular Genetics and Genomics*, Vol. 280, No. 6, pp 547-563.
- [2] J. L. Riechmann, J. Heard, G. Martin, L. Reuber, C. Jiang, J. Keddie, L. Adam, O. Pineda, O. J. Ratcliffe, R. R. Samaha, R. Creelman, M. Pilgrim, P. Broun, J. Z. Zhang, D. Ghandehari, B. K. Sherman & G. Yu, (2000) "Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes", *Science*, Vol. 290, No. 5499, pp 2105-2110.
- [3] G.A. Wray, M.W. Hahn, E. Abouheif, J. P. Balhoff, M. Pizer, M. V. Rockman & L.A. Romano, (2003) "The evolution of transcriptional regulation in eukaryotes", *Molecular Biology and Evolution*, Vol. 20, No. 9, pp 1377-1419.
- [4] C. Cortes & V. Vapnik, (1995) "Support vector networks", Machine Learning, Vol. 20, No. 5, pp 273–297.
- [5] V. Vapnik, (1995) The Nature of Statistical Learning Theory, Springer, New York.
- [6] B. E. Boser, I. M. Guyon, & V. N. Vapnik, (1992) "A training algorithm for optimal margin classifiers", in *5th Annual ACM Workshop on COLT*, D. Haussler, Ed. Pittsburgh, PA: ACM Press, pp 144–152.
- [7] W.S. Noble, (2004) "Support vector machine applications in computational biology", in *Kernel Methods in Computational Biology*, B. Sch olkopf, K. Tsuda & J. P. Vert, Eds. Cambridge, MA: MIT Press, pp 71–92.

- [8] T. Joachims, (1999) "Making large-scale SVM learning practical", in Advances in Kernel Methods : Support Vector Learning, B. Sch olkopf, C. Burges & A. Smola, Eds. Cambridge, MA: MIT Press, pp 41–56.
- [9] A. Garg, M. Bhasin & G. P. S. Raghava, (2005) "Support vector machine-based method for subcellular localization of human proteins using amino acid compositions, their order, and similarity search", *Journal of biological Chemistry*, Vol. 280, pp 14427–14432.
- [10] M. Wang, A. Li, D. Xie, Z. Fan & H. Feng, (2005) "Improving prediction of protein sub-cellular localization using evolutionary information and sequence-order information", 27th Annual International Conference of the IEEE-EMBS, pp 4434–4436.
- [11] D. Xie, A. Li, M. Wang, Z. Fan & H. Feng, (2005) "LOCSVMPSI: a web server for subcellular localization of eukaryotic proteins using SVM and profile of PSI-BLAST", *Nucleic Acids Research*, Vol.33, pp 105–110.
- [12] D. T. Jones, (1999) "Protein secondary structure prediction based on position-specific matrices", *Journal of Molecular Biology*, Vol. 292, pp 195-202.
- [13] K. C. Chou & C. T. Zhang, (1995) "Prediction of protein structural classes", *Critical Reviews in Biochemistry and Molecular Biology*, Vol. 30, pp 275–349.
- [14] J. A. Swets, (1988) "Measuring the accuracy of diagnostic systems", Science, Vol. 240, pp 1285– 1293.
- [15] M. H. Zweig & G. Campbell, (1993) "Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine", *Clinical Chemistry*, Vol. 39, pp 561–577.
- [16] D.W. Hosmer & S. Lemeshow, (2000) "Applied Logistic Regression", Ed. 2, John Wiley and Sons, New York, pp 156–164.
- [18] S. F. Altschul, T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller & D.J. Lipman, (1997) "Gapped Blast and PSI-Blast: a new generation of protein database search programs", *Nucleic Acids Research*, Vol. 25, pp 3389–3402.

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