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Detection of Water Stress in Khasi Mandarin Orange Plants from Volatile Organic Compound Emission Profile Implementing Electronic Nose

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Abstract: ***Plants in the absence of an innate immune system like animals and being immobile are regularly exposed to a host of stresses, ranging from biotic to abiotic stresses. In response to these, plants have developed a complicated response system like reprogramming gene expressions and emission of secondary metabolites as volatile organic compounds (VOCs) by its various tissues like roots, stems, leaves etc. These VOCs can be used as biomarkers for inspecting plants' in situ health status. This paper address the usefulness of electronic nose (e-nose) system to sense the VOCs emitted by plants' leaves to detect the stresses in it. Standard commercial electronic nose (e-nose) system Alfa Mos Fox 3000 has been used here to identify the stressed and non-stressed plants. Fifteen Mandarin orange plants were considered for the study and were subdivided into three categories. Each one was subjected to a different level of water stress. Leaf samples were collected for e-nose analyses from each plant of all three categories on the 15th day and 30th day of induction of water stresses. Dimensionality reduction techniques like kernel Principal Component Analysis (kPCA), Linear Discriminant Analysis (LDA) and classification algorithms like Support Vector Machines (SVC) and Multi-Layer Perceptron Classifier (MLPC) have been used to classify the three categories of plants. The scores obtained from these analyses reveals the feasibility of using an e nose system in discriminating plants based on the status of water stress in them. This paper analyses the applicability of e nose system in stress diagnosis of agricultural and horticultural crops, which would significantly help in controlling the irrigation regime.***

Keywords**: About four key words or phrases in alphabetical order, separated by commas.***

I. INTRODUCTION

A plant is said to be water stressed when there is insufficient water for it to uptake from the soil (draught stress) or there is a flooding situation resulting in submersion. With the ever diminishing ground water levels in arable land in many parts of the world, draught stress continues to pose a serious and frequent threat to the health and productivity of the plants. According to the international commission on irrigation and drainage almost 40 % of world's population is confronted with periodic droughts, which affect many of the arid and semi-arid countries in the north of Africa, parts of India, China, the Middle East, Mexico, Australia, Middle Asia, Canada, south west of Europe, and the western United States of America[1]. G. Giaouris et al. found that almost one third of potential arable land of the world is suffering from different levels of water stress[2]. In [3] U. Liwani et al. highlighted the severe effect of drought on wheat production. In addition to it, Mortazavian et. al. reported that the growth and development process a plant is adversely affected by the poor water status[4]. This damage depends not only the severity of draught, but also on which developmental stage the plant is at[5]. Irrigation rates playing a definitive role in the health of a plant (Leek), was reported by Kiremit et. al. They observed significant alteration in the height, leaf length, stem length, stem diameter, fresh weight of leaf and stem and dry weight[6]. Hence, it becomes necessary to diagnose a plant of water stress, so that proper management strategies could be applied on a timely basis. There are different diagnostic techniques for detecting water stress in a plant. The density/ dye method, which is a simple and inexpensive method has been in use for measuring leaf water potential[7]. The water content of a leaf is measured in pressure chambers, where controlled pressure is applied on the leaf until the leaf secretes fluid[8], [9]. Application of remote sensing, to determine plant water stress by interacting them with near infrared and middle infrared wave lengths has also been reported[10]. Savage et. al. used thermocouple psychrometry to measure the leaf water potential of citrus jambhiri and compared the results to Scholander's pressure chamber[7]. Time domain reflectometry was used to study the stem water content and stem electrical conductivity due to induced water stress on four species of trees for a period of 220 days[11]. Zheng et.al. in a similar study evaluated the relationship of water stress on the physiological electrical property of corn leaf using four probe method[12]. The possibility of using optical signatures, obtained from hyper spectral imaging of leaves using short wave infrared as an indicator of plant health was reported by Kim et. al.[13]. The response of a plant to a stress is complicated and significant research is being carried out to understand various metabolic pathways that triggers the defence mechanisms of plants. Plants generally reprograms their gene expression profile and metabolic contents.

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Emission of secondary metabolites serves as a defence mechanism for priming the plant itself as well as for alerting neighbouring plants for any putative attack. Abiotic stresses (environmental origin) and biotic stresses (pathogen, aphids, herbivory) causing alteration in secondary metabolites (emitted as VOCs) and its affect has been reported amply in many studies[14], [15]. These VOCs are organic compounds having vapour pressure greater than 1mm of Hg and are constituted of a large variety of terpenes, benzenoids, phenylpropanoids, fatty acid derivatives, amino acid metabolites and essential phytohormones like methyl jasmonate (MeJA), and methyl salicylate (MeSA), ethylene, methanol, abscissic acid etc. These emission of metabolites serves as plant to plant, plant to vector, pathogen to host communications besides defence mechanisms. For instance methanol emission due to leaf wounding primes the distant leaves of the same tree as well as neighbouring trees providing anti-bacterial resistance, while facilitating viral spread was reported by Dorokhov et. al.[16]. These emission patterns in a plant in response to different stresses are providing an alternative diagnostic approach for plants under stress. As the water availability in the soil is reduced; plants change the biochemistry and the root structures to retain and absorb maximum possible water for primary metabolic functioning. Plants produce and accumulate compatible solutes like polyols, amino acid and sugars to lower the osmotic potential in the cells, which in-turn facilitates retention of the water within itself. Additionally it produces stress hormone Abscissic Acid (ABA), affecting the growth pattern and stress tolerance[15]. ABA helps in controlling the guard cells of stomatal opening which reduces the transpiration rate. This reduction in the stomatal opening helps the plant in retaining the stored water but also compromises the CO_2 intake leading to decrease in the photosynthesis thereby affecting the growth of the plant[17], [18]. Thus studying the VOC emission profile of a plant in response to water stress by using analytical instruments or electronic nose (e-nose) can provide a diagnostic tool for detecting a plant under stress. The emission pattern of an apple plant studied using GC-MS under varied levels of water stress identified 29 VOCs, out of which emission rates of hexanal, (E)-2-hexenal, (E)-2-hexen-1-ol, hexyl acetate, 1-hexanol, and (E)-2-hexenyl acetate were unregulated in severely stressed plant[19]. Another study reported the variation on VOC emission profile of apple plant due to environmental factors like rainfall, relative humidity and temperature[20]. A study using dynamic enclosure technique for detection of VOCs emitted by citrus sinensis plants revealed down regulation of a sesquiterpene beta-caryophyllene and a mono terpene trans β-ocimene by 6%. These two compounds accounted nearly 82 % of the total terpenoid emitted by the plant's leaves. For the mild stress case the emission of trans β-ocimene decreased whereas beta-caryophyllene showed no responses. They believed this down regulation was due to metabolization of the mono and sesquiterpenes for survival, by the plant under water stress, since there was decrease in photosynthesis due to stomatal closure[21]. Zandalinas et.al studied the metabolomic changes in two citrus genotypes, Carrizo citrange and Cleopatra Mandarin to a combined draught and temperature stress. Carrizo being tolerant to stress, exhibited different metabolite emission pattern. Cleopatra being vulnerable to the combined stress, showed significant alteration in in polar metabolites by up-regulating 68 compounds, while only 8 polar metabolites were up regulated in Carrizo. These up regulation in the case of Cleopatra was to mitigate the energy demand and accumulation of photo protective and antioxidant secondary metabolites to face oxidative stress [22]. The application of e-nose in quality inspection of fruits and disease detection is profuse[23], but their application in water stress is not mature. Implementation of the e-nose technique in water stress detection could provide an affordable alternative to monitor the in situ health status of a plant. This would in turn help in proper management and irrigation scheduling thereby minimizing huge economic losses to farmers. The objectives of this study is to implement e-nose technology for diagnosing artificially induced water stress on Khasi Mandarin orange

II. MATERIAL AND METHODS

Fifteen healthy, shed house grown and belonging to the same age group Khasi Mandarin orange saplings were selected from Citrus Research Station, Tinsukia, Assam. These were then planted in 15 different pots (30cm diameter top, 20 cm diameter base and 28 cm height) inside a greenhouse and each pot was filled with equal amount of soil. Physical parameters such as temperature, humidity, and light exposure were maintained to be constant for all the plants. Saplings were kept in the greenhouse 30 days before starting the experiment so that the same environmental conditions can be imposed equally for all the plants. These 15 plants were further separated into three categories (C1, C2, C3), each subjected to artificially generated water stresses as shown in Fig 1. Drip irrigation was implemented inside the green house (Fig 2) to irrigate the plants in a systematic ways. The amount of irrigated water for each category was controlled using voltage operated solenoid valves.

Fig. 1. Irrigation scheduling pattern of the plants diversified into three cetagoriesC1, C2 and C3

Fig. 2. Irrigation scheduling of the plants

The optimum amount of water to be irrigated for a plant was calculated using the value of crop evapotranspiration (ET_c). The reference evapotranspiration (ET_0) can be calculated from the climatic data using the Penman Monteith method[24]. ET_0 is calculated by putting the daily mean temperature, wind speed, relative humidity and solar radiation as input parameter in the equation given by

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T+273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)} \quad (1)$$

Where, ET_0= reference evapotranspiration [mm day^{-1}], R_n= net radiation at the crop surface [MJ m^{-2} day^{-1}], G= soil heat flux density [MJ m^{-2} day^{-1}],T= mean daily air temperature at 2 m height [^{0}C],U_2= wind speed at 2 m height [ms^{-1}],e_s = saturation vapour pressure [kPa], e_a = actual vapour pressure[kPa], $(e_s - e)_a$=saturation vapour pressure deficit [kPa], Δ = slope vapour pressure curve [kPa $^0C^{-1}$], γ= psychrometric constant [kPa $^0C^{-1}$].

The relationship between the ETc and ET_0 is given by

$$ET_c = K_c \times ET_0 \quad (2)$$

Where, K_C is the crop coefficient

The calculations for finding the value of ET_0 was done using the software CROPWAT 8.0, which works on the basis of Penman Monteith method. The temperature of the green house was controlled at a set temperature of 20°C, with a hysteresis of 1.2°C and relative humidity at 40 %. The plants were exposed to light for 8 hours per day. The ET_c value calculated from the equation was found to be 2.3035 mm/ day. The area to be irrigated for a plant was 0.070695 m^2 .Thus the estimated optimum amount of water (W_0) required for irrigation for each plant was found out to be 162.85ml/day. Hence W_0 was considered as a reference for generating the artificial stress in each category of plants (Fig. 1). The category C1 was supplied with the optimum amount of water W_0 per day, while the categories C2 and C3 were daily supplied with 80% of W_0 (130 ml) and 60% of W_0 (98ml) approx. respectively as shown in Table I.

Table I: Irrigation scheduling for the plants

Time of sample collection from 0th day of stress induction	Irrigation		
	C1(W_0)	*C2(80% of W_0)*	*C3(60% of W_0)*
15thday (Experiment 1)	1	2	3
30th day (Experiment 2)	4	5	6

Fifteen fully expanded leaves, weighing 10 grams were collected from each plant for e-nose analysis. The samples were collected on the 15th (experiment 1) and 30th day (experiment 2) after introducing the calculated stresses. The leaf samples were thoroughly cleaned with 75% (V/V) ethanol for removing traces of dirt and superficial microorganisms. Standard commercial e-nose system, Alpha-MOS Fox 3000 was used for the e-nose analysis, which consisted of an array of 12 metal oxide gas sensors ((LY2/LG, PA/2, T70/2, P40/1, P10/2, P10/1, T30/1, LY2/gcT, LY2/gcTL, LY2/GH, LY2/AA, LY2/G). The samples were fed to the sample holder and contamination free dry air was used for carrying the sample odor to the sensor chambers. The system acquired the sensor response data from each sample for 2 minutes and a brief 20 minute recovery time was given to the system between two subsequent analyses. The system having 12 sensors (features) recorded data for every second there by creating a data matrix of dimension 120 ×12 for each samples. Fig 3 shows the plots of the responses of each sensors for the 120 second duration for two samples belonging to two different categories C1 and C3. These data acquired by the e nose system was further analyzed with machine learning algorithms as described below.

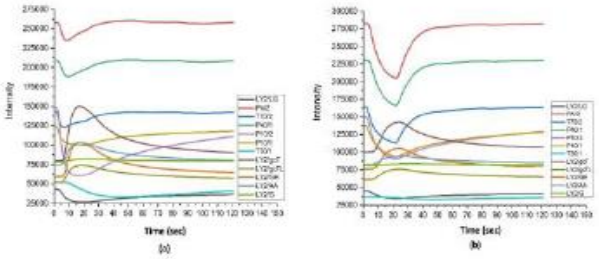

Fig 3. Sensor responses of the e nose system for category C1 and C3

III. DATA ANALYSES AND VALIDATION

The data acquired by the system was pre-processed (normalized) and analyzed using two classifier algorithms viz. Multi-Layer Perceptron Classifier (MLPC) and Support Vector Classifier (SVC). Bootstrap aggregation (Bagging) ensemble was employed for the two classifier to boost the performance and also reduce over fitting if any. The three categories of plants C1, C2 and C3 were considered as the three classes to be classified by the classifier algorithms. For classification the dataset was divided into 80% training data and 20% testing data. 10 fold cross validation was employed for training the classifier and parameter tuning. During each fold in the cross validation process, the trained classifier was tested with the untouched 20% testing data, and the performance scores were computed. The mean of the scores obtained during the cross validation was considered for estimating the performance of the classifier. The scores used for evaluating the performance of the classifiers are accuracy, Cohen's Kappa index, precision, recall, and f1 score. Dimensionality reduction techniques like Kernel Principal Component Analysis (kPCA) and Linear Discriminant Analysis (LDA) was used for visualization of the data patterns. LDA reduces the original number of features (12) in the dataset into C-1 features (where C is the number of classes) in order to maximize the class separability. These new feature set was then fitted to the classifiers used and predictions were plotted to visualize the decision boundaries of each classifier used.

IV. RESULTS AND DISCUSSION

As shown in table I, the entire experiment was repeated two times, Experiment 1 and Experiment 2. Fig 4 shows the plots of kPCA plotted using two principal components, an extension of PCA utilizing the kernel technique. The cosine kernel showed the best separation among the classes in the plots. Although there was a distinct pattern for each classis in both the cases, slight overlaps was observed in the plots.

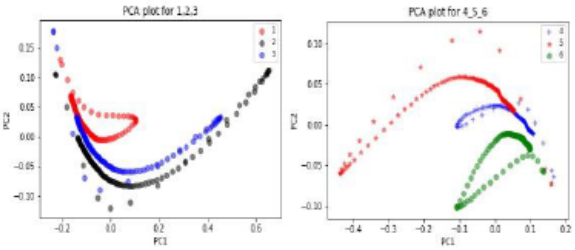

Fig. 4. kPCA plots for each categories of samples for two different experiments performed on the same plants but on different times (plot for 1, 2, 3 is at 15th day and plot for 4, 5, 6 is at 30th day).

Table II shows the scores of the base classifiers used for the classification as well as their ensemble counterparts for the first experiment. The accuracy scores in the case of the MLPC classifier was low, and was enhanced by the bagging ensemble of the classifier. SVC being a stable classifier did not reveal a large boost in the performance of the classifier. The F1 score which is a weighted average between the precision and recall follows a similar pattern. The Cohen's Kappa score signifying the inter rater agreement between two raters (the classifier predictions and the true values) is greater than 80%, except for the MLPC classifier. Agreement greater than 80% in this case is regarded as an acceptable score.

Table II: Scores of the base classifier and their bagged classifier for samples after 15 days of stress.

Score in %	MLPC		SVC(Linear)	
	MLPC	*Bagging*	*SVC*	*Bagging*
Accuracy	76.14	82.56	83.56	84.93
F1-Score	76	83	84	85
Cohen's Kappa	70	80	82	84

The results of the second experiment (samples 4, 5, 6) are shown in Table III. The performance of the same classifiers in this experiment outclassed the previous experiment, as this experiment was done after a comparatively longer period of stress induction.

These results were visualized by the decision boundaries of the classifiers (Fig. 5) showing the class separation. The figures shows the decision boundaries of the bagging ensemble classifiers used in classifying the data from the three categories of stress induced plants in the two experiments. Much like the comparatively lower scores in table II and table III, there are misclassified data points in the first experiment (Fig 5(a) and Fig 5(b)). The decision boundary in the second experiment perfectly classifier the three categories / classes of plants subjected to different stresses (Fig 5(c) and Fig 5(d)).

Table III: Scores of the base classifier and their bagged classifier for category 2 (sample 4, 5, and 6)

Score in %	MLPC		SVC(Linear)	
	MLPC	*Bagging*	*SVC*	*Bagging*
Accuracy	94.49	96.33	98.63	98.63
F1- Score	94	96	99	99
Cohen's Kappa	94	96	99	99

V. CONCLUSION

The study reveals the usefulness of e-nose technique for successful classification of Khasi Mandarin orange under artificially induced water stress. Fifteen orange plants, subdivided into three categories, are considered for this experiment and kept in controlled environment inside a greenhouse. Each categories are subjected to different levels of artificially generated water stress. E-nose technique was employed to diagnose the plants under water stress and machine learning algorithms were employed to classify the categories of plants. Analysis of the samples after 30 days of stress subjection was found to have better performance than that of the 15 day analysis, which was expected. The Cohen's Kappa value showed perfect agreement for the former, while moderate to strong agreement for the latter case. The e-nose technique used could successfully differentiate plants under water stress from optimal water fed plants, thus providing a possible usage of the technology in monitoring large scale orchards in a non-invasive way.

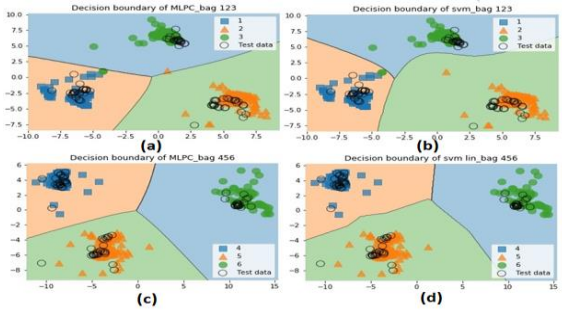

Fig. 5. MLPC and SVM-Bag plots for both the experiments

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