

Discrimination of Different Types Damage of Tomato Seedling by Electronic Nose

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Abstract: The profiles of volatile compounds emitted by plants varies in response to different damage. The potential of electronic nose technology to monitor such changes, with the aim of diagnosing plant health was investigated. An electronic nose (E-nose) was used to analyse tomato seedlings that were subjected to different types of damage (infection by Early blight disease, infection by Gray mold disease, mechanically damage, and undamaged). Principal component analysis (PCA), linear discrimination analysis (LDA), back-propagation neural network (BPNN), and support vector machine (SVM) network were used to evaluate the E-nose data. The results indicated that the E-nose can successfully discriminate between tomato seedling with different types of damage. The results of PCA and LDA showed that clusters of data were divided into 3 groups (ZP, HP, and CP/MP). Samples from groups CP and MP overlapped partially. Back-propagation neural network (BPNN) and support vector machine (SVM) network were used to evaluate the E-nose data. Good discrimination results were obtained using SVM and BPNN. The results demonstrate that it is plausible to use E-nose technology as a method for monitoring damage in tomato seedling.

1 Introduction

Plants increase the amount of volatile compounds that they release when they were damaged by insects or diseases. A number of studies have demonstrated that plants respond to damage with releasing a variety of volatiles [1-3]. Over the past decade, there has been significant progress in plant volatile research as a result of the increasing sensitivity of analytical instrumentation and improvements in molecular and biochemical analyses. However, this method involves several stages which makes it time consuming, especially at the stage of volatile-compound trapping and sample preparation. In addition, the technology is very expensive, the equipment is bulky, and the analysis requires skilled analysts. Therefore, it is not suitable for use in a warning system for plant insects and disease attacks in a commercial plant-growing setting.

An electronic nose (E-nose) system is sensor-based technology that measures total headspace volatiles and creates a unique smell print. An E-nose does not resolve the sample's volatiles into individual components but rather responds to the whole set of volatiles in a unique digital pattern. This pattern is the signature of a particular set of aromatic compounds. For each process or application of interest, a database of such digitized patterns is created, called the training set. When an unknown sample is exposed to the E-nose sensors, the E-nose first digitizes the sample's volatiles, and then

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compares the resulting pattern with the existing training set. Its principle is different from that of chemical analysis methods such as gas and liquid chromatography, mass spectrometry, nuclear magnetic resonance, and spectrophotometry. The E-nose does not resolve the sample's volatiles into its individual components but responds to the whole set of volatiles in a unique digital pattern. E-nose technology has been successfully used in a variety of applications, including food quality and control [5,6,14], environmental monitoring [7,17,18], micro-organism identification (Magan et al., 2000), medical and health detection [15,16] and plant monitoring [9-13]. Recently, more research has been directed towards early detection of insect and disease attack. This technology has been used to differentiate volatile profiles from tomato, cucumber, and pepper leaves damaged by spider mites and beet armyworm [4,8]. The E-nose technology is rapid, sensitive, specific, non-destructive, and easy to use. Unlike GC-MS, the E-nose does not require prolonged pre-concentration intervals and is appealing as a potential technology for monitoring changes in disease-induced plant volatiles signatures. However, little information about E-nose monitoring of damaged tomato seedlings has been reported.

The objective of the present study was to determine the feasibility of detecting tomato seedling damage caused by the Early blight disease, the Gray mold disease, mechanical means damage and undamaged control seedlings utilizing an E-nose (PEN2). The aim was to develop discrimination models of E-nose signals for different types of damage. Principal component analysis (PCA), linear discrimination analysis (LDA), back-propagation neural network (BPNN) and support vector machine (SVM) network were used to verify the classification capacity of the proposed methods.

2 Materials and Methods

2.1 Electronic nose

In this study, a PEN2 (Airsense Analytics, Germany) E-nose, equipped with 10 different metal oxide semiconductor (MOS) sensors was used. The PEN2 system consists of a sampling apparatus, a detector unit containing the array of sensors, and pattern recognition software (WinMuster v.1.6) for data recording. The sensor array is composed of positioned in a small chamber. Each sensor has a certain degree of affinity towards specific chemicals or volatile compounds. Table 1 lists all the sensors used and their main application.

Table 1. Sensors used and their main application in PEN2

Number in array	Sensor-name	General description	Reference
S1	W1C	Aromatic compounds	Toluene, 10 ppm
S2	W5S	Very sensitive, broad range sensitivity, react on nitrogen oxides, very sensitive with negative signal	NO ₂ , 1ppm
S3	W3C	Ammonia, used as sensor for aromatic compounds	Propane, 1ppm
S4	W6C	Mainly hydrogen, selectively (breath gases)	H ₂ , 100ppb
S5	W5C	Alkanes, aromatic compounds, less polar compounds	Propane, 1ppm
S6	W1S	Sensitive to methane (environment) ca. 10ppm. Broad range, similar to No.8	CH ₄ , 100ppm
S7	W1W	Reacts on sulfur compounds, H ₂ S 0.1ppm. Otherwise sensitive to many terpenes and sulfur organic compounds, with are important for smell, limonene, pyrazine	H ₂ S, 1ppm
S8	W2S	Detects alcohol's, partially aromatic compounds, broad range	CO, 100ppm
S9	W2W	Aromatic compounds, sulfur organic compounds	H ₂ S, 1ppm
S10	W3S	Reacts on high concentrations >100ppm, sometime very selective (methane)	H ₂ S, 1ppm CH ₄ , 10 CH ₄ , 100ppm

Figure.1 shows a schematic diagram of the PEN2 E-nose measurements during the experiments. Each tomato seedling was enclosed in a cylindrical stainless steel support (400 mm high \times 100 mm internal diameter) covered with a plastic bag over the top. The plastic bag was a colourless and odourless food-grade polyethylene bag (0.08 mm thick). The plant was sealed at the lower stem by 2 hermetic boards with a hole in the middle. This excluded the odour coming from the medium. The plant was kept at room temperature ($28 \pm 2^\circ\text{C}$) for 20 min before static headspace sampling was begun.

Before each measurement, the E-nose system was cleaned with zero-air (air filtered on active carbon). The main purpose of this was to clean the circuit and to return the sensors to their baselines. During the measurement, the headspace gas of a plant was pumped into the sensor chamber at a constant rate of 200 ml min^{-1} through a Teflon tube (3 mm) connected to a needle. When the headspace gas that had accumulated in the bag was pumped into the sensor chamber, the ratio of conductance of each sensor changed. The response of each sensor was expressed as a ratio of conductance (G/G_0 ; G and G_0 are the respective conductivities of the sensors when the seedling gas and the zero gas pass over). The measurement procedure was controlled by a computer program. The measurement time was 65 s, which was sufficient for the sensors to reach stable values. The interval for data collection was 1 s. A computer recorded the response of the E-nose every second. The flush time was set to 40 s. When the measurement was completed, the acquired data was stored for later use.

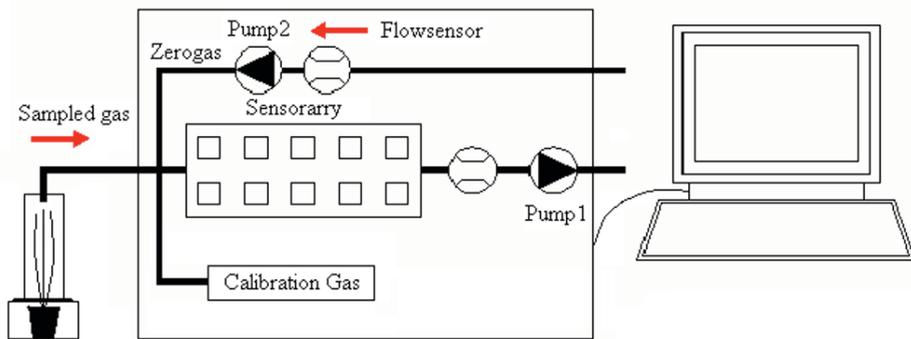


Figure 1. Schematic diagram of the electronic nose measurements of PEN2

2.2 Tomato seedlings

The variety of tomato seedling was ZheZa 809. Pre-germinated seeds were sown at common clay pots (80 mm diameter and 100 mm high) in greenhouse where the temperature was maintained at $28 \pm 2^\circ\text{C}$ and relative humidity (RH) was 70-80%. Plants were managed daily. After 35 days, uniform-tomato seedlings were selected for the experiment.

2.3 Plant treatment

In this study, 4 treatments were tested: (1) damage caused by Early blight disease (EP), (2) damage caused by Gray mold disease (GP), (3) mechanically damage (MP), and (4) undamaged (CP).

First the Pathogenic bacteria of Early blight disease and Gray mold disease were training at laboratory, and then the diseases were vaccinated to 35 day tomato seedlings, respectively. The vaccinated tomato seedlings were kept with high temperature and high RH for 48h.

For the Early blight disease treatment, 4 leaves disease infection were used per tomato seedling. For the Gray mold disease, also 4 leaves disease infection were used per tomato seedling. For the mechanical damage treatment, a single tomato seedling was punched 90 times with a

needle(0.8x26mm).Sixty-four plants were selected for the experiment,and there were 16 replicates for each treatment.

2.4 Data analysis

In this study,the data was subjected to different statistical analyses,including Principal component analysis (PCA), linear discrimination analysis(LDA),back-propagation neural network(BPNN),and support vector machine (SVM) network.All statistical analyses were conducted with SAS v.8 and Matlab 7.0.

3 Results and discussion

3.1 Electronic nose responses to tomato seedling volatiles

Figure. 2 shows the typical responses of 10 sensors during measurements on a tomato seedling. Each curve represents the variation in conductivity of each sensor with time. Electro-value changes when the volatiles from the tomato seedling reach the measurement chamber. The response signal was measured and expressed as, where G and G_0 are the resistance of a sensor in clean air and in a detecting gas, respectively. It is clear that the ratio of G/G_0 was close to 1.0 at the initial period, then increased or decreased gradually,and stabilized after about 60s.The signals of each sensor at response 60s were used in the subsequent analyses.

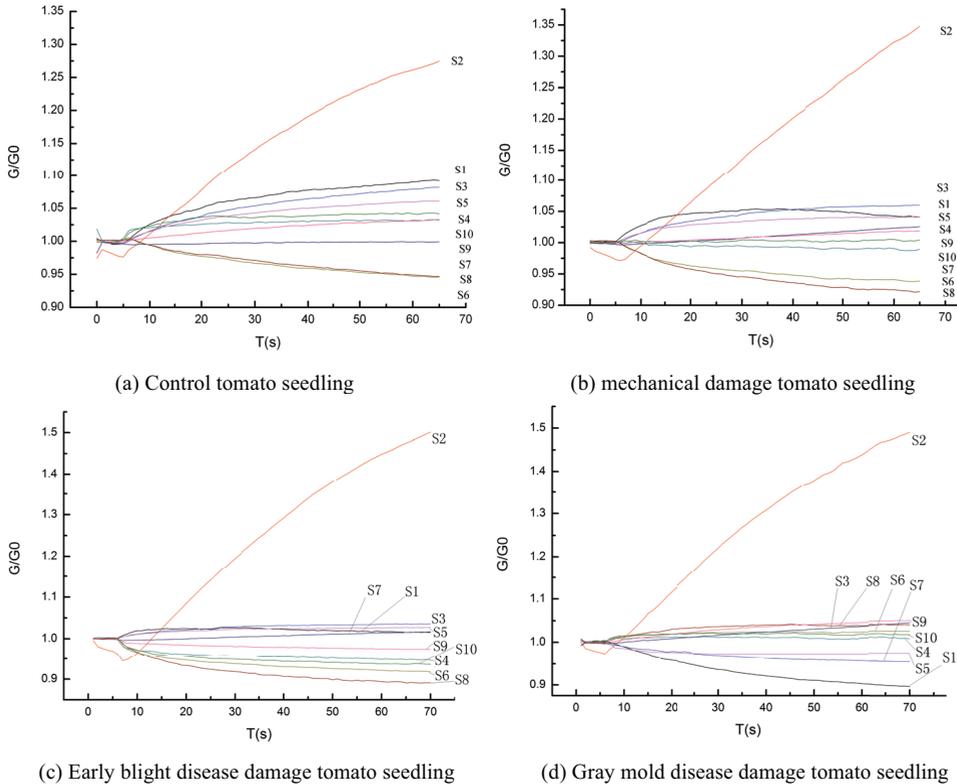


Figure 2. Response curve of 10 sensors to tomato seedling volatiles

3.2 Signal analysis

Figure.3 shows the changes of the sensor signals in response to tomato seedling with different types of damage. Each point represents the mean value of each sensor's response signal to tomato seedlings, linked to the measurements of conductance increases or decreases experienced by the sensors. The E-nose sensor response changed by tomato seedling with 4 different types of treatments. The values of the sensor response signals differed with the different types of damage. The variation in the sensor responses in the CP was small compared to the 3 groups of damaged tomato seedlings (Figure.3). This result is due to the specific changes in the chemical composition in tomato seedlings after the damage.

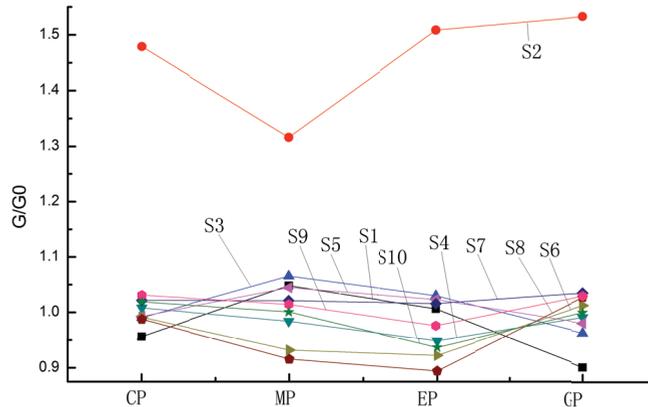


Figure 3. Value of each sensor's response to tomato seedling with different types of damage (CP, control seedlings; MP, mechanically damaged seedlings; EP, seedlings infected by Early blight disease; GP, seedlings infected by Gray mold disease)

3.3 PCA and LDA analysis

PCA and LDA are commonly used techniques for data classification and dimensionality reduction. The data from 64 samples (16 samples from each group) obtained by the E-nose for 4 tomato seedling groups were used for the PCA. Fig.4 shows the results of the PCA. The first component contributed 59.93% of the total variance; the second component, 33.01%. This added up to 92.94% in total. In Fig.4, clusters of data were divided into 3 groups (CP/MP, EP and GP). Samples from groups EP and GP were easily discriminated, whereas samples from groups CP and MP overlapped partially. PCA did not clearly discriminate between these different types of tomato seedlings. Therefore, LDA was used for further separation of the tomato seedling samples.

The results of the LDA are shown in Figure.5. Function1(LD1) contributed 85.83% of the total variance; Function2(LD2), 10.52%, adding up to a total of 96.35%. In the LDA plot, 4 groups of tomato seedling samples could be clearly distinguished. The separation of the samples was better using LDA than using PCA.

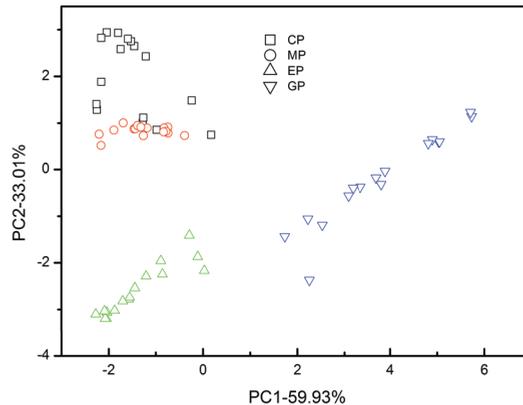


Figure 4. PCA plot for tomato seedlings with different types of damage (CP, control seedlings; MP, mechanically damaged seedlings; EP, seedlings infected by Early blight disease; GP, seedlings infected by Gray mold disease)

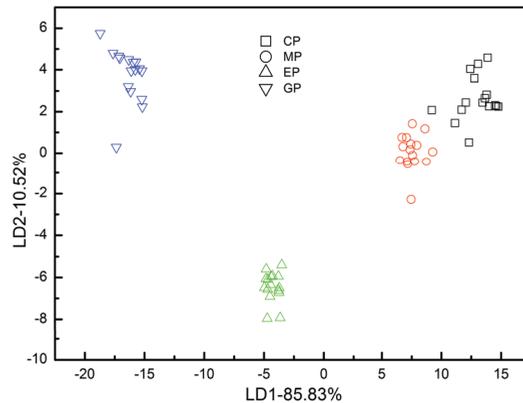


Figure 5. LDA plot for tomato seedlings with different types of damage (CP, control seedlings; MP, mechanically damaged seedlings; EP, seedlings infected by Early blight disease; GP, seedlings infected by Gray mold disease)

3.4 BPNN and SVM

In this study, sixty-four samples (16 duplicates for each of the 4 groups) were divided into 2 groups: 40 samples (10 samples of each group) for the training set and 24 samples (6 samples of each group) for testing set.

For the BPNN, the chosen architecture of the artificial neural network was a 10-15-1 three-layer back-propagation according to Kolmogorov's theorem. The 10 input neurons correspond to the data obtained by 10 sensors, while the 1 output is the different types of damage. The training function is 'traingda' and the training epoch is 2000. The training parameters were chosen a epoch of 0.01 and a goal of 0.001. The result is shown in Table 2. The correct rate of the training set was 100%. The correct rates of the testing set for the CP, MP, EP and GP groups were 100%, 100%, 83.3%, and 100%, respectively.

The SVM network also had 10 and 1 neurons in the input and output layers, respectively. The

kernel function used in this paper was 'radial basis kernel function'. The SVM analysis showed an overall classification success of 100% for the training sets and 95.8% for the testing sets. The result is shown in Table 2. The results indicate that it is possible to use E-nose signals to discriminate between tomato seedlings with different types of damage.

Table 2. Results of BPNN and SVM analyses for tomato seedling with different types of damage

Network style	Correct rate of training set		Correct rate of testing set		
		CP	MP	EP	GP
BPNN	100%	100%	100%	83.3%	100%
SVM	100%	100%	100%	83.3%	100%

Conclusions

To sum up, the results prove that the E-nose PEN 2 can successfully distinguish between tomato seedlings with different types of damage (infection by Early blight disease, infection by Gray mold disease, mechanically damage, and undamaged). The LDA discriminates more effectively between the different groups than the PCA. Good discrimination results are obtained in BPNN and SVM. The results demonstrate that it is plausible to use E-nose technology as a method for monitoring damage in tomato seedling.

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References

1. Kessler, I.T. Baldwin. Defensive function of herbivore-induced plant volatile emissions in nature. *Science*, **291**(2001):2141-2144
2. Y.J. Lu, X. Wang, Y.G. Lou, J.A. Cheng. Role of ethylene signaling in the production of rice volatiles induced by the rice brown planthopper *Nilaparvata lugens*, *Chines Science Bulletin*, **51**(2006):2457-2465
3. Y.G. Lou, X.Y. Hua, T.C.J. Turlings, J.A. Cheng, X.X. Chen, G.Y. Ye. Differences in induced volatile emissions among rice varieties result in differential attraction and parasitism of *Nilaparvata lugens* eggs by the parasitoid *Anagrus nilaparvatae* in the field, *Journal of Chemical Ecology*, **32**(2006):2375-2387
4. J. Laothawornkitkul, J.P. Moore, J.E. Taylor, M. Possell, T.D. Gibson, C.N. Hewitt, N.D. Paul. Discrimination of plant volatile signatures by an electronic nose: a potential technology for plant pest and disease monitoring, *Environmental Science and Technology*, **42**(2008):8433-8439
5. A.H. Gomez, J. Wang, G.X. Hu, A.G. Perira. Discrimination of storage shelf-life for mandarin by electronic nose technique, *LWT-Food Science and Technology*, **40**(2007):681-689
6. L. Pang, J. Wang, X. Lu, H. Yu. Discrimination of storage age for wheat by E-nose, *Transactions of the ASABE*, **51**(2008):1707-1712
7. A.C. Bastos, N. Magan. Potential of an electronic nose for the early detection and differentiation between *Stretomyces* in potable water, *Sensors and Actuators B:Chemical*, **116**(2006):151-155
8. N. Magan, P. Evans. Volatiles as indicator of fungal activity and differentiation between species, and the potential use of electronic nose technology for early detection of grain spoilage, *Journal of Stored Products Research*, **36**(2000):319-340

9. M. Kunert, A. Biedermann, T. Koch, W. Boland. Ultrafast sampling and analysis of plant volatiles by a hand-held miniaturized GC with pre-concentration unit: kinetic and quantitative aspects of plant volatile production, *Journal of Separation Science*, **25**(2002):677-684
10. R.M. Jansen, J.W. Hofstee, J. Wildt, F.W. Verstappen, H.J. Bouwmeester, E.J. van Henten. Induced plant volatiles allow sensitive monitoring of plant health status in greenhouse, *Plant Signal Behav.*, **4**(2009):824-829
11. H.M. Zhang, J. Wang. Detection of age and insect damage incurred by wheat with an electronic nose, *Journal of Stored Products Research*, **43**(2007):489-495
12. W.G. Henderson, A. Khalilian, Y.J. Han, J.K. Greeme, D.C. Degemjardt. Detecting stink bugs/damage in cotton utilizing a portable electronic nose, *Computers and Electronics in Agriculture*, **70**(2010):157-162
13. B. Zhou, J. Wang. Use of electronic nose technology for identifying rice infestation by *Nilaparvata lugens*, *Sensors and Actuators B:Chemical*, **160**(2011):15-21
14. S. Cheng, Y. Wang, J. Wang, Z. Wei, Q. Lu. Detection of eggshell crack using electronic nose[J]. *Transactions of the ASABE*, **53**(2010):789-794
15. J. Dummer, M. Storer, M. Swanney, M. McEwan, A.S. Thomas, S. Bhandari, S. Chambers, R. Dweik, M. Epton. Analysis of biogenic volatile organic compounds in human health and disease, *Trends in Analytical Chemistry*, **30**(2011):960-967
16. M. Bernabei, G. Pennazza, M. Santonico, C. Corsi, C. Roscioni, R. Paolesse, C. Di Natale, A. D'Amico. A preliminary study on the possibility to diagnose urinary tract cancers by an electronic nose, *Sensors and Actuators B:Chemical*, **131**(2008):1-4
17. J.H. Sohn, N. Hudson, E. Gallagher, M. Dunlop, L. Zeller, M. Atzeni. Implementation of an electronic nose for continuous odour monitoring in a poultry shed, *Sensors and Actuators B:Chemical*, **133**(2008):60-69
18. S. De Vito, E. Massera, M. Piga, L. Martinotto, G. Di Francia. On field calibration of an electronic nose for benzene estimation in an urban pollution monitoring scenario, *Sensors and Actuators B:Chemical*, **129**(2008):750-757