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**Research Article**

# Metabolism of volatile metabolites in soybean varieties infected by *Fusarium oxysporum*

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## Abstract

This is the first report of screening for volatile metabolite metabolism in local soybean cultivars affected by Fusarium blight. Volatile metabolites in the aerial and root parts of three local soybean varieties (*Glycine max* (L.) Merr.) infected with the *Fusarium oxysporum* fungus were monitored using gas chromatography. The results showed no significant differences in the volatile metabolites between the above-ground and root parts of the plants. However, as the disease progressed, an increase in the number of similar metabolites was observed in the root parts. Conversely, the quantity of similar volatile metabolites in the aerial parts of the plants decreased as the disease advanced. Notably, 4-aminobenzoic acid was detected in all parts of the samples, with higher concentrations found in the aerial parts than in the root parts.

**Keywords:** *Fusarium oxysporum*, 4-aminobenzoic acid, soybean diseases, volatile metabolites, gas chromatography

## Introduction

A significant challenge facing the soybean variety *Glycine max* (L.) Merr. is the prevalence of diseases caused by Fusarium fungi? Among these, Fusarium blight stands out as a primary concern (Cruz *et al.*, 2019; Arias *et al.*, 2013). This soil-borne plant disease, caused by *Fusarium oxysporum* f.sp. ciceris (Foc), affects soybeans in approximately 33 countries worldwide. The disease significantly impacts crop yields, resulting in losses of 30-50 percent (Soares *et al.*, 2021; Nendel *et al.*, 2023). Currently, six (1A, 2, 3, 4, 5, and 6) of the eight species of this fungus display wilting symptoms, while species 0 and 1B/C exhibit yellowing symptoms (Ortel *et al.,* 2020; Kurbanbaev *et al.*, 2023). Given the soil-borne nature of this pathogen, conventional management strategies, such as crop rotation or chemical treatments, are largely ineffective. The notable resistance and survivability of the fungus underscore the urgent need for developing Fusarium-resistant varieties and implementing them effectively (Lamichhane *et al.,* 2020).

The primary objective of this study is to investigate changes in volatile metabolites in plants following infection by Fusarium fungi. The experimental approach involves analyzing samples from local soybean varieties exhibiting various degrees of infection. For these experiments, samples from the local soybean varieties “Genetik-1”, the hybrid progeny of “Genetik-1” and “Sochilmas”, and “Orzu” showing signs of Fusarium disease were selected. Microbiological analysis confirmed the presence of *F. oxysporum,* the causative agent of *Fusarium wilt*, in these samples, confirming their infection status. Samples were collected from infected soybean cultivars at different stages of the disease. Fresh plant materials were then directly used to extract volatile compounds, obtaining 75 grams from both the aerial and root parts of each plant sample. The samples were extracted three times over 72 hours at room temperature using the organic solvent n-hexane. The resulting extract was concentrated under vacuum, and residual water in the solvent was removed using sodium sulfate. Gas chromatography was employed to analyze the volatile metabolites present in the extracts. The gas chromatographic analysis was performed on a Shimadzu GC-2010 Plus gas chromatograph equipped with an HP-5 capillary column, as described by Salleh et al. (2021). To identify volatile organic components, we used a joint introduction with standards (main components), as well as a comparison of retention indices and mass spectral characteristics from the NIST08 and NIST17 libraries (Asilbekova *et al.*, 2022).

Recent studies have focused on examining the volatile organic metabolites present in various species of vegetable soybean plant. Specifically, gas chromatography was employed to analyze the volatile organic compounds of five distinct types of soybean vegetables. The findings revealed that alcohols and aldehydes constitute the primary substances, which are believed to impart an aromatic fragrance to these plants (Liu *et al.,* 2017). A similar investigation compared the volatile metabolites obtained from six different species of vegetable soybean plants, identifying prevalent metabolites from categories such as aldehydes, alcohols, ketones, and hydrocarbons (Yuan *et al.,* 2021). Recent publications have underscored the identification of metabolites from these categories in the volatile metabolites of vegetable soybean plants (Cai *et al.,* 2022; Yuan et al., 2021; Guo et al., 2022).

In the results obtained, samples from different varieties showed varied results compared to the control. Specifically, in the aerial part of the “Genetik-1” variety, where the disease had just begun to manifest, 20 metabolites differing from the control appeared in varying percentages. The most abundant, 4-aminobenzoic acid, accounted for 17.07% of these, as shown in (Table 1). In the root part of the “Genetik-1” variety, which differed from the control, ten metabolites were present in varying percentages, with the highest being 4-aminobenzoic acid at 5.79%.

The mean metabolites of the aerial and root parts of the “Genetik-1” sample differed from the control. In control, aldehydes and ketones were the main substances, as reported in the literature, with an increase in nitrogen-containing substances observed in this cultivar sample (Guo et al., 2022; Jiang et al., 2014). In the aerial part, higher quantities of 4-aminobenzoic acid, 1-nitro-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid diethylamide, and 2,6-dimethyl-N-trimethylsilyl-4-trimethylsilyloxyaniline metabolites were identified, whereas lower quantities were found in the root part. Additionally, nitrogen-containing substances were increased in both parts of the plant. At the early stage of the disease, little change in metabolism was observed in this cultivar sample (Liu et al., 2017; Yuan et al., 2021).

The hybrid progeny of “Genetik-1” and “Sochilmas” soybean varieties, classified as having the second level of disease severity, revealed 22 and 17 different metabolites in the aerial and root parts, respectively, compared to the control. Among these metabolites, the predominant compound in the aerial part was 1-benzylpyrrolidine-3,4-diol, constituting 3.14%, and in the root part, 4-aminobenzoic acid, accounting for 11.89%, as shown in (Table 2). Gas chromatographic analysis of samples from the hybrid plants of the “Genetik-1” and “Sochilmas” varieties detected several volatile metabolites in both the aerial and root parts. An increase in metabolites was observed in the root parts compared to the aerial parts, yet similarities were noted across both. The primary metabolites in these cultivar samples belonged to the carboxylic acid class, consistent with findings reported in the literature (Guo et al., 2022; Yuan et al., 2021). Other substances that differed from the control included primarily nitrogen-containing substances and, at stage 2 of the disease, silicon-containing substances were also synthesized, as detailed in (Table 2).

For the non-diseased “Orzu” variety, which was conditionally defined as having a third degree of disease severity, the main metabolites mainly belonged to the hydrocarbon class (Jiang et al., 2014; Guo et al., 2022). Analysis of changes in volatile metabolites revealed 13 metabolites in the aerial part that differed from the control, with the predominant metabolites being N, N-dimethyl-1-[methyl(propan-2-yloxy) phosphoryl] formamide at 16.08%, 4-aminobenzoic acid at 12.9%, and (2-amino-5-chlorophenyl) phosphonic acid at 10.67%, as shown in (Table 3). In the root part, 21 metabolites were identified, with 2,6-dimethyl-N-trimethylsilyl-4-trimethylsilyloxyaniline being the most abundant at 5.62%.

Moreover, similar metabolites were present in the aerial and root parts of the “Orzu” variety. Several additional metabolites different from the control were synthesized, particularly in the root part, where the total number of metabolites increased as the disease progressed. The

**Table 1.** Metabolites of the “Genetik-1” variety sample.

| № | **Genetik-1** | | | | | |
|---|---|---|---|---|---|---|
| | **Stem and leaf parts** | | | **Root part** | | |
| | **Metabolites** | **RI** | **Area %** | **Metabolites** | **RI** | **Area %** |
| 1 | N, N-dimethyl-1-[methyl(propan-2-yloxy) phosphoryl] formamide | 772 | 4.67 | Nonane | 900 | 2.05 |
| 2 | 4-Aminobenzoic acid, 2TMS derivative | 1836 | 17.07 | 5- [1,4-Dioxa-8-azaspiro [4.5] dec-8-yl]-6-ethyl-2,4(1H,3H)-pyrimidinedion | 893.2 | 1.18 |
| 3 | 3,4,5-Trimethoxybenzonitrile | 1551 | 4.41 | 4-Aminobenzoic acid, 2TMS derivative | 1836 | 5.79 |
| 4 | 2-(4-cyano-1-oxo-5H-pyrido[1,2-a] benzimidazol-3-yl) acetic acid methyl ester | 795.4 | 3.61 | 2-Oxiranylmethyl 4-methylphenylcarbamate | 1422 | 1.52 |
| 5 | 7-Chloro-4-methoxy-3-methylquinoline | 1707 | 3.12 | 3-Amino-7-nitro-1,2,4-benzotriazine 1-oxide | 897.2 | 1.02 |
| 6 | *N*-methyl-*N*-[(*E*)-(2-nitrophenyl) methylideneamino] methanamine | 1653 | 1.49 | 2,6-dimethyl-N-trimethylsilyl-4-trimethylsilyloxyaniline | 1625 | 1.29 |
| 7 | N-Methyl-1-adamantaneacetamide | 1553 | 4.35 | Acetoxyacetaldehyde 2,4-dinitrophenylhydrazone | 898.6 | 1.12 |
| 8 | 4-(4-Methoxyphenyl)-N-methyl-butyramide | 1760 | 1.9 | 2-p-Nitrophenyl-oxadiazol-1,3,4-one-5 | 2111 | 2.12 |
| 9 | 7-Chlorocinchoninic acid | 797.8 | 3.98 | 1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethylamide | 894.8 | 1.7 |
| 10 | 1-Benzyl-pyrrolidin-3,4-diol | 1762 | 3.56 | 2,6-dichloro-4-nitrophenol | 1656 | 1.73 |
| 11 | (2-Amino-5-chlorophenyl) phosphonic acid | 789.3 | 5.33 | | | |
| 12 | 2,3-Dihydroxy-6-nitroquinoxaline | 786.5 | 3.98 | | | |
| 13 | Ethyl 3-methyl-4,5,6,7-tetrahydro-1H-indole-2-carboxylate | 788.1 | 2.64 | | | |
| 14 | 7,9-Diethyl-2,4-bis(dimethylamino) -10-imino-8-thio-1,7,9-triazaspiro [4.5]-1,3-decadiene-6,8-dione | 791.6 | 3.86 | | | |
| 15 | 1,3-dibromo-5-nitrobenzene | 1715 | 1.17 | | | |
| 16 | trans-3-Ethoxy-b-methyl-b-nitrostyrene | 1648 | 2.04 | | | |
| 17 | 2-Chloro-4,6-bis(methylthio)-1,3,5-triazine | 1664 | 1.35 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 18 | 2,6-Dihydroxybenzaldehyde, carbamoylhydrazone | 1577 | 1.85 | | | |
| 19 | 2,6-dimethyl-N-trimethylsilyl-4-trimethylsilyloxyaniline | 1625 | 5.15 | | | |
| 20 | 1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethylamide | 794.7 | 3.58 | | | |

**Table 2.** Metabolites of a sample of hybrid variety “Genetik-1” and “Sochilmas.”

| № | Genetik-1 and Sochilmas | | | | | |
|---|---|---|---|---|---|---|
| | Stem and leaf parts | | | Root part | | |
| | **Metabolites** | **RI** | **Area %** | **Metabolites** | **RI** | **Area %** |
| 1 | 2-ethylsulfanyl-1H-pyrimidin-6-one | 682.9 | 2.43 | 4-Aminobenzoic acid, 2TMS derivative | 1836 | 11.89 |
| 2 | 2-Amino-4-(2-methylpropenyl)-pyrimidin-5-carboxylic acid | 685.3 | 3.13 | 2-Amino-4-(2-methylpropenyl)-pyrimidin-5-carboxylic acid | 685.3 | 4.56 |
| 3 | 3,5-dichloro-2-(hydroxymethyl)-6-methyl-1H-pyridin-4-one | 2110 | 1.84 | (5Z)-5-[(2,3-dimethoxyphenyl) methylidene]-2-(4-fluorophenyl) imino-1,3-thiazolidin-4-one | 1396.2 | 1.53 |
| 4 | 7-Hydroxy-7,8,9,10-tetramethyl-7,8-dihydrocyclohepta [d,e] naphthalene | 2151 | 1.08 | (5E)-5-[(Trimethylsilyl)methylene] hexahydro-1(2H)-pentalenone | 1310 | 2.38 |
| 5 | 4-Bromo-2-chlorobenzenamine | 1377 | 1.28 | Corydaldine | 1708 | 3.19 |
| 6 | 1-Benzazirene-1-carboxylic acid, 2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester | 694.1 | 1.05 | N,N-dimethyl-1-[methyl(propan-2-yloxy)phosphoryl] formamide | 825 | 4.19 |
| 7 | 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene | 2233 | 3.08 | 7,7,9,9,11,11-Hexamethyl-3,6,8,10,12,15-hexaoxa-7,9,11-trisilaheptadecane | 1598 | 2.88 |
| 8 | Methyl 2-(4-cyano-1-oxo-5H-pyrido[1,2-a] benzimidazol-3-yl) acetate | 696.1 | 1.22 | 2-Chloro-2,5-dimethyl-5-propyl-2,5-disilaoctane | 825 | 6.33 |
| 9 | 7,7,9,9,11,11-Hexamethyl-3,6,8,10,12,15-hexaoxa-7,9,11-trisilaheptadecane | 1598 | 2.33 | 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene | 2233 | 1.27 |
| 10 | 4-Aminobenzoic acid, 2TMS derivative | 1836 | 2.86 | Trimethylsilyl 2,5,8,11,14-pentaoxahexadecan-16-oate | 1950 | 6.41 |
| 11 | 4-chloro-6-methoxy-2-methylquinoline | 699.78 | 2.39 | Prop-2-yn-1-yl N-(2-ethylphenyl) carbamate | 2438 | 2.06 |
| 12 | 3,5-dinitrobenzonitrile | 2357 | 2.18 | Methyl 2-(4-cyano-1-oxo-5H-pyrido[1,2-a] benzimidazol-3-yl) acetate | 696.1 | 5.23 |
| 13 | Jasmonic acid O-TMS | 1837.26 | 1.31 | 4-(3-Methoxyphenoxy)- | 1389.3 | 4.45 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | 1,2,5-oxadiazol-3-amine | | |
| 14 | 1-benzylpyrrolidine-3,4-diol | 1762 | 3.14 | 1-benzylpyrrolidine-3,4-diol | 1762 | 3.06 |
| 15 | 3,4,5-Trimethoxybenzonitrile | 1771.8 | 1.91 | 3,5-dinitrobenzonitrile | 2357 | 4.26 |
| 16 | Methyl 3-oxo-2H-[1,2,4] triazolo[4,3-a] pyridine-8-carboxylate | 3345 | 0.96 | 2-trimethylsilyloxyethyl 2-trimethylsilyloxybenzoate | 1808 | 4.49 |
| 17 | 2,6-dimethyl-N-trimethylsilyl-4-trimethylsilyloxyaniline | 1690 | 2.74 | Butyl N-methyl anthranilate | 1660 | 3.69 |
| 18 | 7-Chlorocinchoninic acid | 797.8 | 1.5 | | | |
| 19 | 1,3-Dioxolane, 2-(4-methoxyphenyl)-4-methyl | 2234 | 1.15 | | | |
| 20 | 2'-Deoxyadenosine, 3TMS derivative | 2619 | 1.39 | | | |
| 21 | [(E)-(3,5-dimethylphenyl) methylideneamino] thiourea | 1208 | 2.86 | | | |
| 22 | 2-(4-nitrophenyl)-2H-1,3,4-oxadiazol-5-one | 688.6 | 1.35 | | | |

**Table 3.** Metabolites of the “Orzu” variety sample.

| № | **Orzu** | | | | | |
|---|---|---|---|---|---|---|
| | **Stem and leaf parts** | | | **Root part** | | |
| | **Metabolites** | **RI** | **Area %** | **Metabolites** | **RI** | **Area %** |
| 1 | 3-Phenyl-oxindole | 1255.9 | 6.01 | 2-Amino-4-(2-methylpropenyl)-pyrimidin-5-carboxylic acid | 685.3 | 2.21 |
| 2 | N-formyl-N-methyl-3,4-methylenedioxybenzylamine | 1553 | 9.42 | (2-Amino-5-chlorophenyl) phosphonic acid | 2195 | 2.67 |
| 3 | N,N-dimethyl-1-[methyl(propan-2-yloxy)phosphoryl] formamide | 756.3 | 16.08 | 7,7,9,9,11,11-Hexamethyl-3,6,8,10,12,15-hexaoxa-7,9,11-trisilaheptadecane | 1598 | 3.65 |
| 4 | 1,3-diphenyl-3-trimethylsilylpropan-1-one | 173.6 | 4.21 | 2-(4-nitrophenyl)-2H-1,3,4-oxadiazol-5-one | 688.6 | 4.91 |
| 5 | 2,6-dichloro-4-nitrophenol | 1656 | 3.65 | 2'-Deoxyadenosine | 2619 | 1.75 |
| 6 | 1-(2-benzothiazolyl)-ethanone, | 1008.9 | 4.86 | 2-(9-oxo-10H-acridin-4-yl) acetic acid | 698.5 | 1.33 |
| 7 | 4-Aminobenzoic acid, 2TMS derivative | 1836 | 12.9 | 3,5-Dimethylbenzaldehyde thiocarbamoylhydrazone | 1208 | 1.23 |
| 8 | 2-Amino-4-(2-methylpropenyl)-pyrimidin-5-carboxylic acid | 685.3 | 5.14 | 2,6-dichloro-4-nitrophenol | 1656 | 4.31 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 9 | (Z)-9-Octadecenamide | 2375 | 5.32 | Methyl 2,2,6-trimethyl-1-[(E)-3-oxobut-1-enyl]-7-azabicyclo [4.1.0] heptane-7-carboxylate | 1482.2 | 1.26 |
| 10 | 2,3-Dihydroxy-6-nitroquinoxaline | 786.5 | 2.98 | 4-Aminobenzoic acid, 2TMS derivative | 1836 | 2.76 |
| 11 | (2-Amino-5-chlorophenyl) phosphonic acid | 2195 | 10.67 | 2-Chloro-8-methoxy-3-methylquinoline | 1707 | 2.82 |
| 12 | 4-Formyl-2,5-dimethoxy-6-methyltropone | 1473 | 4.76 | Methyl 2-[(1E)-4,4-dicyano-3-(N-methylanilino) buta-1,3-dienyl] triazole-4-carboxylate | 1789.2 | 1.56 |
| 13 | 2,6-dimethyl-N-trimethylsilyl-4-trimethylsilyloxyaniline | 1690 | 2.83 | Propan-2-yl 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate | 2823 | 1.28 |
| 14 | | | | Phenylglyoxylic acid, TMS derivative | 1459 | 1.51 |
| 15 | | | | Hydrazinecarbothioamide, 2-(1-phenylethylidene)- | 2013 | 0.9 |
| 16 | | | | 2-(4-cyano-1-oxo-5H-pyrido[1,2-a] benzimidazol-3-yl) acetic acid methyl ester | 1791.3 | 0.95 |
| 17 | | | | 3,4-Dihydro-6-nitrocoumarin | 1369 | 1.26 |
| 18 | | | | 2,3-Dihydroxy-6-nitroquinoxaline | 786.5 | 2.39 |
| 19 | | | | 1-(4-Methoxyphenyl)-7-methyl-5-oxo-1,2,3,5-tetrahydroimidazo[1,2-a] pyridine-6-carbonitrile | 1794.8 | 2.1 |
| 20 | | | | Corydaldine | 1795.28 | 1.64 |
| 21 | | | | 2,6-dimethyl-N-trimethylsilyl-4-trimethylsilyloxyaniline | 1690 | 5.62 |

Synthesis of silicon-containing metabolites also increased in the root part. Some of these metabolites were higher in the aerial parts of the plant than in the root parts, and vice versa.

When comparing the aerial and root metabolites of diseased cultivar samples with control plants, it was evident that 4-aminobenzoic acid was present in both the aerial and root parts of all varieties (Jiang et al., 2014; Guo et al., 2022; Liu et al., 2017; Yuan et al., 2021). All parts of the obtained varietal samples also contained metabolites such as 2-amino-4-(2-methyl propenyl)-pyrimidine-5-carboxylic acid and 2,6-dimethyl-N-trimethylsilyl-4-trimethylsilyloxyaniline. The results from gas chromatographic analysis revealed that several more metabolites were found in almost all parts of the plants. Based on our observations and data from the gas chromatographic analysis, synthesizing the main metabolites between the aerial and root parts of the selected varietal samples showed no significant difference. Metabolites such as 4-aminobenzoic acid, N, N-dimethyl-1-[methyl(propan-2-yloxy) phosphoryl] formamide, and (2-amino-5-chlorophenyl) phosphonic acid were synthesized in large amounts in all infected soybean plants (Table 3). Particularly, 4-aminobenzoic acid was found to be synthesized in larger quantities in all infected samples of soybean varieties (“Genetik-1”, hybrid variety “Genetik-1” and “Sochilmas”, and “Orzu”) compared to the control. As the disease progressed, the total amount of silicon-containing substances increased. In summary, it can be inferred that soybean varieties undergoing stress from this pathogenic fungus tend to synthesize these specific metabolites.

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