Fingerprinting of Volatile Organic Compounds for Quick Assessment of Vigour Status of Seeds

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Introduction

Seed is a fertilized mature ovule, which possesses an embryonic plant. When they dry, mature seeds are subjected to imbibition, they release a wide range of organic substances, which include low molecular weight carbonyl compounds (gases and volatiles) and water-soluble organic substances (enzymes and polysaccharides). The volatile organic compounds (VOCs) are molecules of low molecular weight (300 g mol-1) and high vapour pressure (0.01 k Pa at 20°C) and include diverse chemical compounds. The nature and emission kinetics of volatiles produced from seeds vary, depending on the moisture content of the seeds. Orthodox seeds stored at 'low seed moisture content' undergo seed deterioration, predominantly due to lipid peroxidation, initiated by autoxidation or enzymatic oxidation of unsaturated or polyunsaturated fatty acids. With respect to the seed germination process, exposure of seeds to 'high moisture conditions' leads to increased respiration, triggers glycolysis and mobilization of storage reserves, resulting in the emission of volatile metabolic products. The quantity of VOCs emitted on commencement of metabolic activity in germinating seeds depends on (1) vigour status and (2) amount of storage reserves. Since it has been established that there is a significant difference between high and low vigour seeds with respect to quantity and profile of VOCs emitted, there is great potential for utilizing the VOC profile to obtain a quick and reproducible test of vigour status of crop seeds (Umarani *et al*., 2020).

Sources responsible for emission of VOCs

Emission of volatile compounds is a dynamic process that involves chemical reactions, mobility of molecules within drying cells and sorption/désorption processes. VOCs are emitted by diverse chemical reactions which includes: a. Glycolysis, b. Auto-oxidation or non-enzymatic oxidation, c. Strecker degradation of Maillard reactions.

a. Glycolysis – It is one of the metabolic pathways that converts glucose into pyruvate or in other words one glucose molecule broken down to form two molecules of pyruvic acid and this process occurs in the cytoplasm of plant cells.

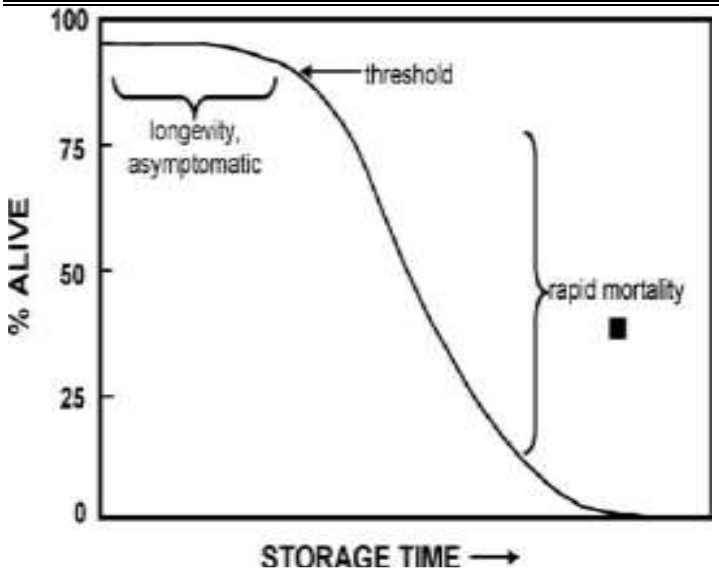
b. Auto-oxidation or non-enzymatic oxidation – It is free radical driven chain reaction in which one of the free radicals induces oxidation of lipids mainly phospholipids containing polyunsaturated fatty acids.

c. Strecker degradation of Maillard reactions – One of the mechanism of seed ageing. It occurs when a non-enzymatic attack on amino group of proteins and nucleic acid protein complexes by reducing sugars or aldehydes. Millard reaction products are associated with loss of seed viability. The modification of macromolecules through Millard reaction affects protein activity particularly in some antioxidant enzymes such as glutathione reductase, ascorbate peroxidase and catalase are sensitive to Millard reactions which causes a decline in the antioxidant capacity and inability to limit oxidative damage during germination resulting in impaired seed vigour and loss of viability.

Lipid peroxidation mechanism and emission of VOCs in dry stored seeds

Orthodox seeds which develop on the mother plant reach maximum seed germination and vigour at physiological maturity; from that point of time seeds undergo physiological and biochemical degenerative changes that lead to progressive seed deterioration, loss of seed vigour and, ultimately, to seed death. Seed deterioration follows a sigmoidal pattern wherein viability remains relatively constant for a period, followed by an abrupt decline in viability, and finally, by a lag period during which a few seeds remain viable. The most visible symptoms of seed deterioration are delayed germination, decreased

tolerance to sub-optimal environmental conditions, lowered tolerance to adverse storage conditions, reduced germinability and increased number of abnormal seedlings. The rate of seed deterioration is influenced by factors such as initial seed quality, genetic background and seed production conditions; however, seed moisture content and temperature of storage atmosphere are the most significant factors which influence seed deterioration.



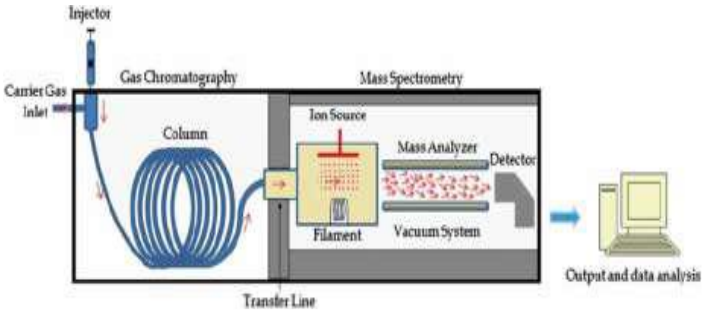
Lipid peroxidation is suggested to play a major role in causing seed deterioration and might occur both in the absence or presence of enzyme catalysis. Lipid peroxidation initiated by autoxidation (atmospheric oxygen) or enzymatic oxidation (lipoxygenase) of unsaturated or polyunsaturated fattyacids, such as oleic and linoleic acids, leads to the generation of free radicals (an atom or a molecule with an unpaired electron), mainly hydrogen free radicals from a methylene group of the fatty acid, adjacent to double bonds. Once these free radicals are initiated, they continue to propagate other free radicals that ultimately combine, terminating the destructive reactions. In this process, unsaturated fatty acids are converted to free radicals and then to hydroperoxides and subsequently these hydroperoxides follow a variety of reactions leading to the formation of more free radicals and hydroperoxides. The final consequence of this chain reaction is the loss of the membrane structure, leakiness and an inability to complete normal metabolism, ultimately resulting in seed deterioration

How fingerprinting is done?

Using gas chromatography (GC) or gas chromatography/mass spectrometry (GC-MS) which is a novel and quick method of seed vigour estimation. Fingerprinting is different for different volatile organic compounds due to its chemical composition.

Gas Chromatography/ Mass Spectrometry (GC/MS)

Gas chromatography/ Mass spectrometry instrument separates chemical mixtures (the GC component) and identifies the components at molecular level (the MS component).



Principle of GCMS: A mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the substances separated substances emerge from the column opening, they flow into the MS, it identifies compounds by the mass of the analyte molecule.

Colville *et al*. (2012) studied on volatile fingerprints of seeds of four species. Comparison of three legume species (*Pisum sativum*, *Lathyrus pratensis* and *Cytisus scoparius*) during artificial ageing at 60 % relative humidity and 50°C revealed variation in the seed volatile fingerprint between species. The volatile compounds are proposed to derive from three main sources: alcoholic fermentation, lipid peroxidation, and Maillard reactions. Lipid peroxidation was confirmed in *P. sativum* seeds through analysis of malondialdehyde and 4-hydroxynonenal. Volatile production by ageing orthodox seeds was compared with that of recalcitrant (desiccation-sensitive) seeds of *Quercus robur* during desiccation. Finally, comparison was made between two methods of analysis; the first used a Tenax adsorbent to trap volatiles, while the second used solid phase micro extraction to extract volatiles. Solid phase micro extraction was found to be more sensitive, detecting a far greater number of compounds.

Rutzke *et al*. (2008) hypothesized that ethanol production in cabbage seeds would be influenced by aging treatment, hydration level, seed integrity (grinding), and oxygen availability. Cabbage seeds were subjected to controlled aging treatments (40°C at 70 % relative humidity for 0, 7, 14, 21, and 28 d. The experiments were performed in ambient oxygen conditions and under nitrogen. Ethanol production was greater in aged than non-aged intact seeds at all water concentrations tested. Non-aged seeds under nitrogen had reduced ethanol production at ≤ 0.54 g of water per gram of seeds, indicating that low seed water concentration limited ethanol production. Non-

aged seeds in ambient oxygen at ≥1.22 g of water per gram of seeds had elevated ethanol production, indicating that a hypoxic environment was created by excess seed water.

Mira *et al*. (2010) studied on characterization of volatile production during storage of lettuce (*Lactuca sativa*) seed. Their study revealed that, over 30 volatile compounds were detected from lettuce seeds during storage at 35ºC at water contents ranging from 0.03 to 0.09 g H2O g-1 dw. Seeds stored at high water content (>0.06 g H2O g-1 dw) emitted molecular species indicative of glycolysis (methanol+ethanol), and evidence of peroxidation was apparent subsequent to viability loss. Seeds containing less water (0.03–0.05 g H2O g-1 dw) produced volatiles indicative of peroxidation and survived longer compared with seeds stored under more humid conditions.

Lee et al., 1997 studied on volatile compounds from soybean (*Glycine max* L. Merrill) and snap bean (*Phaseolus vulgaris* L.) seeds in relation to seed quality and controlled aging treatments. Evolution profiles were similar for both species; aging, but snap bean produced ca. two times the concentration of volatiles as soybeans. Ethanol evolution increased while acetaldehyde decreased as soybean seed aged in an open storage condition. Methanol and ethanol evolution increased with aging period conducted with a range of aging conditions from 0.60 to 0.75 water activity (Aw) and 45 to 35°C in closed packets. Acetaldehyde can non-enzymatically react with proteins, and acetaldehyde-protein adducts (APA) formed in intact seeds were quantified using a competitive ELISA. APA formation coincided with the three phases of germination loss in storage, and was negatively correlated with the percent standard germination.

Conclusion

VOCs, being a major by-product of catabolic reactions that occur both in the dry and imbibed seeds, offer great potential for utilizing them as biomarkers for quick and reproducible assessment of the vigour status of crop seeds. In order to utilize the VOC profile for quick assessment of vigour status of seeds, research has to be carried out to develop standard protocols for fingerprinting of standard volatile biomarker(s) along with retention time, with respect to crop species and vigour status of seeds. This VOC fingerprint-based quick test of seed vigour can be incorporated in the regular quality control programmes of the seed industry. Seed companies can ensure disbursement of high-quality seed lots for sowing in the ensuing season, thereby assuring higher crop productivity and better remuneration to crop growers.

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