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Effect of plant metabolism On Some Qualitative characteristics of Root Exudates among different plants.

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Abstract:

This work is part of a larger research project studying exudates roots of three different plant species, including *Zea mays L.* (C4*), Spinica olereaca L.*, and *Aloe vera L.* (CAM), all of which were applied with three replicates (A‹B and C). The Investigated presence of compounds (Sugars, proteins, phenols, flavonoids, Terpenoids, hormone Gibberellin, hormone Abscisic acid, Amino acid (proline), Tannin, Catalase Enzyme and Enzyme Superoxide Dismutase). The highest percentage of sugars appeared in the exudates of *Zea mays L.* Where it was recorded as 0.638 ml/gm, the highest percentage of proteins appeared in the exudates of *S.olercea* plant, where it recorded 24.53 mg/l, and the highest percentage of phenols appeared in the exudates *of Zea mays L*., where it recorded 56.61 mg/mL, the highest percentage of Flavonoids appeared in the exudates of Zea mays L., where it recorded 6.16 mg / ml. The highest percentage of terpenoids appeared in the exudates of S.olercea, where it recorded 377.33 µg / L. The highest percentage of the hormone gibberellin appeared in the exudates of *Zea mays L*., where It was recorded as 0.057 µg/l. The highest percentage of abscisic hormone, a lion, appeared in the exudates of *Zea mays L*., where it was 0.028 µg/l. The highest percentage of proline concentration appeared in the exudates of *Zea mays L*., where it recorded 49.533 µg/ml. The highest percentage of tannin concentration appeared in the exudates of Zea mays L., where it recorded 16.58 mg/ml. Catelase enzyme appeared in the exudate of *S.olercea* plant, where it was recorded as 0.61 units/ml.

Keywords: exudates, stimulation of root perfusion, metabolism of study plants C4, C3, CAM

Introduction

Exudates are nutrient-rich root secretions which act as afferents to apical symbionts and result in mutual symbiosis (Sasse et al., 2008). Plant roots secrete a variety of primary metabolites, including organic acids, carbohydrates, carbohydrates, amino acids, and amino acids, that either signal the emergence of or obstruct the way it starts to draw you in (Venturi and Keel, 2016, Cardoso et al., 2014). In order to increase root perfusion:

The collection medium's inclusion of organic chemicals and inorganic ions; • Plasma membrane permeability.

Low molecular weight organic compounds such as sugars, organic acids, and amino acids are naturally cycled across the root cell plasma membranes, and any net influx is termed exudation. The concentration of amino acids and sugar in young roots is higher compared to old roots (Nabais et al., 2011). Some plant species can regulate the exudation of organic acids and reduce sugars by opening certain anion chan-nels to facilitate diffusion (Shen and Yan, 2002; Rees et al., 2005). Anions, including depolarization-activated channels, anion conductance correction inwardly activated by membrane hyperpolarization and anion conductance correction externally (flow), or anion channels activated by light or membrane stretch (Schwenke and Wagner, 1992; 2004). Zhang et al., Roberts, 2006).

C4, C3, plants metabolism (CAM) affects root exudates as C4 plants have higher concentrations of amino acids and organic acids in roots than C3 plants, while carbohydrate concentrations are higher for C3 plants (Fougère et al., 1991; Majerowicz et al., 1991). al., 2000; Graham, 2002) endogenous release of carboxylate in plants also varies with plant metabolism, whereas CAM plants buffer malate (as the main carboxylate) as a C source, C4 plants use it as an intermediate transporter from mesophylls to bundle sheaths, and plants release malate C3 citrate and malate are released from the vacuoles during the night for transport into the mitochondria to support respiration (Meyer et al., 2010). The aim of this research is to study the qualitative and quantitative assessment of the compounds present in the root exudates of the different studied plants.

Materials and methods of work:

An experiment was conducted in the root exudates of plants in the wooden shed of the Department of Biology / College of Science / University of Al-Qadisiyah in the winter season 2022-2023 to study the qualitative and quantitative assessment of the compounds present in the root exudates of the different studied plants. According to the sequence of steps below:

Preparation of plant models: three types of metabolically different plants were chosen, each one represents a type of metabolic pathway and a type of crop including *Spinacia oleracea L.* (C3, cereal or grain), *Zea mays L*. (C4, cereal or grain) , and *Aloe vera L.* (CAM, ornamental). Aloe Vera L. was obtained as stem cuttings from a local greenhouse while Spinacia Oleracea L. and *Zea mays L.* were obtained as seeds and certified by the Diwaniyah Agriculture Department. Seeds were sown in a 72-cell tray. Each cell was filled with peat moss and watered regularly until the seedlings were ready for transplanting. seedlings at the stage of two true leaves, four weeks after sowing, were transplanted to a 2 kg plastic pot filled with a mixture of soil and peat moss in a ratio of 1:1 The soil of the experiment were analyzed in the laboratory to determine some of its physical and chemical properties where they were as following consequently E.C.= 4.03, pH= 7. 55, Tot.N. =0.5, p=0.32, K= 16.58, organics= 2.90%. The soil was also separated into its components, and it was found that it is composed of 76.588% sand, 16.05% clay, and 7.28% silt.

Determination of total sugar (mg. ml-1) The sulfuric acid phenol colorimetric method was used to measure the concentration of total sugars, according to (Masuko et al., 2005),

Determination of total proteins (mg. ml-1) The (Bradford, 1976) method was used to estimate the total protein concentration using an apparatus Spectrophotometry of American origin type type (TS/800 microplate reader-2018).

Determination of total phenolic content (mg.100 ml-1) Determination of total phenols Folin detector reagent was used to estimate the total phenolic content according to what was mentioned in (Ribarova, 2005) using a spectrophotometry spectrophotometer of American origin type (TS/800 microplate reader-2018).

Estimation of the concentration of flavonoids in the kidneys (mg 100 ml -1). Determination of total flavonoids The method was used of (Sen et al., 2013) **.**

Determination of total terpenoids (mg.100 ml-1)

The concentration of total terpenoids was estimated according to (Ghorai, 2012) using a spectrophotometer of American origin type (TS/800 microplate reader-2018).

Determination of Gibberellins hormone (micrograms. g1-) The amount of GA was estimated according to the method used by (Nuray, 2002).

Determination of Abscisic acid (micrograms. g1-) Abscisic acid (ABA) concentration was estimated according to the method used by (Nuray, 2002).

Determination of prolin concentration (micrograms. g1-) The concentration of proline was estimated according to the method (Bates et al., 1973), to measure the color intensity (optical density) using a spectrophotometer of American origin type (TS/800 microplate reader-2018).

Determination of Tannin concentration Tannin contents were determined according to the method of (Broadhurst et al., 1978), using catechin as the standard compound**.**

Determination of superoxide dismutase activity the activity of the superoxide dismutase enzyme was determined according to the method of (Marklund et al., 1974).

Determination of catalase activity (absorption unit.ml-1)

The activity of the catylaseenzyme was estimated according to the method of (Hadwan et al., 2018).

Results

A-The results of the statistical analysis of the data presented in Table (1) indicated that there was a significant significance among the experimental treatments at $0.05 \geq P$ in the root exudate content of total:

The root exudates of all the study plants contained sugars, the highest percentage was significant in *Z.mays*, where it recorded 0.638 (mg/ml), compared with *S.oleracea L.*, which contained 0.095 (mg/ml) in root exudates , and plant *Aloe vera L.* A.vera contained 0.336 (mg / ml) of sugars, with a comparison factor of 0.130 (mg / ml).

The roots exudates of all the study plants contained proteins, the highest percentage was significant in plant S.oleracea L., where it recorded 24.53 (mg / ml) compared with aloe vera plant L. *A.vera* root exudates contained 23.04 (mg/ml) plant Z.mays L. 322.3 (mg/ml) of proteins with a comparison coefficient of 18.39 (mg/ml).

The root exudates of all the study plants contained phenols, the highest percentage was significant in *Z.mays*, where it recorded 56.61 (mg/ml) compared with *Aloe Vera* plant L. A.vera, whose roots contained 27.09 (mg/ml) and plants *S.oleracea* L. 20.93 (mg/ml) of phenols, with a comparison coefficient of 13.567 (mg/ml).

The root exudates of all the study plants contained flavonoids, the highest percentage was significant in *Z.mays*, where it recorded 6.16 (mg/ml), compared with spinach *S.oleracea L.*, which contained 3.84 (mg/ml) in root exudates , and the plant *Aloe vera L.* contains 3.68 (mg / ml) of flavonoids, with a coefficient of comparison of 2.47 (mg / ml).

The root exudates of all the study plants contained Terpenoids, the highest percentage was significant in spinach plant *S.oleracea* L., where it recorded 377.33 (mg / ml) compared with plant L. *A.vera root* exudates contained 190.66 (µg/l) and maize Z.mays L. 145.67 (mg/ml) of Terpenoids with a comparison coefficient of 38.03 (mg/ml).

The root exudates of all the the study plants contained the Amino acid (proline), the highest percentage of which was significant in *Z.mays*, where it recorded 49.533 (mg/ml), compared with plants, *S.oleracea L.*, which contained 47.186 (mg/ml), and *Aloe vera L.* which contained 47.453 (mg / ml) of proline, with a coefficient of comparison of 11.86 (mg / ml).

The root exudates of all the study plants contained Tannin, the highest percentage of which was significant in *Z.mays*, where it recorded 16.58 (mg/ml) compared with *Aloe vera L.*plant root exudates contained 9.65 (mg/ml) and plants *S.oleracea L.* 6.0 (mg/ml) of Tannin with a comparison factor of 1.59 (mg/ml).

Table (1) Average content of root exudate's content of secondary metabolites for plant, *Zea mays L.(C4), Spinacia oleracea L.(C3) and Aloe vera L.(CAM)* (mg. 100 ml-1) .

No.	**Plant metabolism**	**Total sugars**	**Total proteins**	**Total phenols**	**Total flavonoids**	**Total terpanoids**	**Total proline**	**Total tannin**
1	**C4**	0.638	22.33	56.61	6.16	145.67	49.533	16.58
2	**C3**	0.095	24.53	20.93	3.84	377.33	47.186	6.0
3	**CAM**	0.336	23.04	27.09	3.68	190.66	47.453	9.65
	L.S.D	0.210	1.15	21.83	1.95	188.29	3.63	5.92

B-The results of the statistical analysis of the data presented in Table (2) indicated that there was a significant significance among the experimental treatments at $0.05 \geq P$ in the root exudate content of total;

The root exudates of all the study plants contained the Gibberellin hormone, the highest percentage was significant in the plant *Z.mays*, as it recorded 0.057 (mg / L-1) compared with the plant L. *Aloe Vera*, which contained 0.042 (mg/L-1) roots and plants, *S.oleracea L.* 0.036 (mg/L-1) of Gibberellin hormone , with a comparison coefficient of 0.014 (mg/L-1).

The root exudates of all the study plants contained Abscisic hormone, the highest percentage was significant in *Z.mays*, where it recorded 0.028 (mg/ml) compared to *S.oleracea L.*, which contained

0.026 (mg/ml) in root exudates . and Aloe vera plant which contained 0.25 (mg / ml) of the essential Abscisic hormone, with a comparison factor of 0.017 (mg / ml).

The root exudates of all the study plants contained the enzyme SOD, which reached the highest percentage in plant *Z.mays*, where it recorded 5.67 (mg / ml) compared with plant *A.vera* root extracts contained 4.83 (mg/ml) and plants *S.oleracea* L. 2.25 (mg/ml) of SOD Enzyme with a comparison coefficient of 0.47 (mg/ml).

The root exudates of all the study plants contained the enzyme catalase, which reached the highest significant percentage in plant *S.oleracea L*., where it recorded 0.61 (mg / ml), compared with my plant and *Z.mays L*., which contained 0.51 (mg / ml) in root exudates and plant *A.vera* 0.33 (mg / ml) of Catalase enzyme with a coefficient of comparison of 0.028 (mg / ml).

Table (2) Average content of root exudate's content of Hormones and Enzymes for plant, *Zea mays L.(C4), Spinacia oleracea L.(C3) and Aloe vera L.(CAM)* (mg. 100 ml-1) .

No.	Plant metabolism	Total Gibberellins	Total Abscisic acid	Total SOD	Total catalase
1	**C4**	0.057	0.028	5.67	0.51
2	**C3**	0.036	0.026	2.25	0.61
3	**CAM**	0.042	0.025	4.83	0.33
	L.S.D	0.007	0.010	1.36	0.14

Discussion

Root exudates play an important role in phytoremediation processes as a green technology, which are plant metabolites exuded from plant roots to improve nutrient absorption and reduce response to environmental stress (Luo et al., 2014), and their chemical composition and quantity depend on the biological nature of the plant such as plant type and Its growth cycle and the environment in which it lives (Lou et al., 2017). Zhu et al., 2005 showed that exudates release involves two mechanisms: passive influx of organic anions and active proton pumping via H+ ATPase activity. And through the permeability of the plasma membrane of root cells by plant hormones and enzymes that increase or decrease the permeability of plasma membranes (Jones et al., 2005; Cara et al. 2002).

The previous results showed a significant and clear increase in the root exudates in the study plants. Table (1, 3, 4, 6) revealed a significant increase in the root exudates of the yellow corn plant (sugars, phenols, flavonoids, and the hormone gibberellin). Table (1) revealed an increase Significant in the sugars of the exudates of *Zea mays L*. roots and these results are consistent with the findings of Kuzyakov and Domanski, 2000) in the release of sugars such as fructose, glucose and ABA hormone from maize roots and Arabidopsis thaliana, the reason is due to cold stress that increases the permeability of membranes and salinity stress that increases permeability Plasma membrane of C4 plant root cells while oxygen stress reduces membrane permeability in C3 plant (López-Pérez, 2009; Fan et al., 2011), And by increasing the effectiveness of the ATPase enzyme that regulates and absorbs sugars by maintaining the electrochemical potential across the plasma membranes, the type of sugar, the concentration and the pH value (Jimenez et al., 2011) in Table (6) the results revealed that the Gibberellin hormone has a high level In the exudates roots of the *Zea mays L*. plant, this may be attributed to the difference in the type of plant and the path of photosynthesis, the influence of environmental factors such as temperature, light and dark, or the length of the day, pH, soil properties, and plant hormones such as abscisic acid ABA and auxin IAA, or it may be attributed to a difference The content of plant tissues of calcium ions Ca + 2, or may be attributed to the nature of microbial communities and their density in the rhizosphere, (Eidhuset et al., 2007), The previous results also showed a significant and clear increase in the secretion of the root exudates of *Zea mays L*. plants, represented by the hormone (absic acid ABA, the amino acid (proline), theanine and the enzyme super oxide dismutase (SOD). Table (7) revealed a significant increase in the hormone ABA in the exudates of the roots of the *Zea mays L*. plant, and the reason is due to the increase in the absorption of abscisic acid in acidic soils, as its action is related to the flow of K ions, and it works to regulate the secretion of organic acids and reduce sugars by opening certain anion channels to facilitate diffusion (Rees et al. .,2005), Table (8) revealed a significant increase in the roots exudates of the amino acid (proline) of the *Zea mays L*. plant. The reason is due to the presence of amino acids in the mitochondria and the cytosol, which may accumulate in vacuoles or be released into the apoplastic cell passage and by specific transport proteins. Exchange occurs between the root cells and the soil (López-Bucio et al, 2000). The results revealed in Table (9) an increase in the content of teanines in the roots exudates of the *Zea mays L*. plant. The reason is due to the fact that they are present in leaves and stems and were also found in corn grains, all of which are above-ground plant parts (Luna-Cavazos et al., 2016). and can play roles in interactions between microbes and plants (Pereira et al. 2021). The results in Tables (4-10) also recorded a significant increase in the activity of SOD enzyme and flavonoids in the clear roots exudates of the *Zea mays L*. plant because it maintains optimal levels of reactive oxygen species (ROS), which are a number of molecules that derive from molecular oxygen (O2). Such as the negative ion of oxygen -O2, hydrogen peroxide H2O2, and the hydroxyl radical OH- (Turrens, 2003), and these molecules are produced secondary in cells that are not exposed to stress when oxygen is reduced to water during the respiration process (Chen et al., 2012), and the process begins with reduction Oxygen with a single electron leads to the production of -O2, which is the 'starting compound' of most (Borisova et al., 2012) ROS molecules. This anion is short-lived and chemically unstable. It is rapidly converted to hydrogen peroxide by the SOD enzyme) and Tripathi, 2011. Bhatt, which is transformed in the presence of iron and through the Haber-Weiss reaction to the hydroxyl radical OH-, which is the most effective type of ROS and responsible for most of the toxicity of ROS in plants (Jones et al., 2011) (However, the concentrations of these ROS compounds exceed certain limits due to Vital and abiotic stresses, including salinity, outweigh the plant's ability to remove scavenging of these compounds, so the plant is exposed to a state of stress called oxidative stress, which is a state of imbalance between stimuli and antioxidants in favor of oxidative stress (Seis, 2015).

The previous results also revealed a significant and clear increase in the root exudate secretion of proteins, terpenoids and the enzyme catalase of the *Spinica olereaca L.* plant variety. Both quantitatively and qualitatively (Watt et al., 2006; Badri and Vivanco, 2009) and these results agree with (Zhang et al. 2017) that the proteins present in the epiphytes of Medicago truncatula of the legume family were able to cause changes in expression Genetic in Sinorhizobium meliloti, a plant symbiont of M. truncatula and in response to shared salt stress, enzymes involved in osmotic adaptation, and its ability to communicate between plants and soil microorganisms (Kuźniar et al.,2017) and can act as a source of nitrogen for microorganisms in soils and contribute to nutrient cycling in soil ecosystems (Huang et al., 2014).

Table (5) reveals a significant increase in the terpenoids in the *Spinica olereaca L.* plant exudates of roots, and the reason is due to their effective effect on the activity of soil microbes involved in the carbon and nitrogen cycle, for example, terpenoid α-pinene was found to stimulate the activity of the urease enzyme, which participates in in the nitrogen cycle, in soil samples (Huang et al., 2015). In a previous study conducted by (Sharaf-Eldin et al. 2008) it was found that terpenoids in the exudates of the roots of the aloe vera plant stimulate the growth of beneficial root bacteria and inhibit the growth of pathogenic fungi. and production of terpenoid β-sitosterol in *Aloe vera L.* roots that showed antimicrobial activity against several pathogenic microorganisms (Khafagy et al.2019) Table (11) revealed a significant increase in the activity of the catylase enzyme in the *Spinica olereaca L.* plant exudates of roots. The reason is that it plays an important role in protecting plants from oxidative stress by breaking down hydrogen peroxide into water and oxygen, as oxidative stress causes damage to cellular components and the vital and physiological processes within cells through its effect It affects major biomolecules such as proteins, fats, and carbohydrates, as well as inhibiting the action of many enzymes, affecting membrane properties, and leading to cell death (Carol and Dolan, 2006). The significant decrease in the exudate content in the roots of *Aloe vera L*. plant compared to *Zea mays L*. and *Spinica olereaca L.*, and the reason is due to rotting of the roots from time to time during the growth stages (Li PD, 2010), and may be attributed to the role of the exudates in resistance to salinity, the type of plant, its variety, its growth stage, and the nutritional status of the soil. plants, the degree of soil interaction, environmental conditions such as temperature and humidity, soil texture, vital activity of soil microbes, soil properties, and micronutrients (Paterson et al., 1999), and this has been confirmed by many studies, as it has been shown that the exudates of the roots are affected by many Factors including plant type, cultivar, stage of development, environmental conditions, nutritional status of the plant, soil properties, abundance and quality of microbes in the rhizosphere (Brimecombe et al., 2007)

Conclusion

clear that our understanding of root-mediated processes has moved beyond the classical be- lief that the sole functions of roots are an- chorage and uptake of water and nutrients. The concept now is that the roots secrete exudates that are nutrient-rich substances and outlets for beneficial microbes, leading to mutual coexistence, facilitating contact between the plant and other soil organisms such as organic acids, sugars, amino acids, etc. in the soil surrounding the roots. They can vary depending on the plant type and stage of plant development. The highest rates of exudates appeared in the roots of Zea mays L. and Spinacia oleracea L.plants, and the lowest percentages in the Aloe vera plant ,The quantity and quality of exudates varies with metabolism .

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