



Research Article

Use of Chemo-Informatics to Identify Molecular Descriptors of Auxins, Cytokinins and Gibberellins

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Abstract

We have identified those molecular descriptors differentiating four auxins, four cytokinins and four gibberellins. DRAGON software (version 5.5, 2007) and CambridgeSoft ChemOffice (version 12, 2010) including ChemDraw and Chem3D were used to calculate 212 molecular descriptors. Only 49 descriptors showed statistically significant differences among auxins, cytokinins and gibberellins. Of them, the most important differences can be described as follows. Gibberellins contain terminal tertiary C (sp³), terminal quaternary C (sp³), ring secondary C (sp³), ring tertiary C (sp³), and ring quaternary C (sp³) that are not present either in cytokinins or auxins. Gibberellins are also relatively rich in terminal secondary C (sp³) and 10-membered rings which are absent in cytokinins. Cytokinins have 10 times more nitrogen atoms than auxins but this atom is not present in gibberellins. Auxins have 10 times more substituted benzene C (sp²) and 5 times more benzene-like rings than cytokinins but these structures are not in gibberellins. Regarding the numbers of unsubstituted benzene C (sp²), auxins average 4.50, cytokinins 1.25 but they are absent in gibberellins. A dendrogram was generated using data of those molecular descriptors with statistical significant differences (49). The three groups of regulators were correctly classified in three independent branches. The procedure described here may help identify new chemical compounds with potential uses as plant growth regulators.

Keywords

Chemo-informatics; Plant growth regulators; Molecular descriptors

Introduction

Auxins, cytokinins and gibberellins are by far the most important substances for regulating growth and morphogenesis in plant cell, tissue and organ culture [1-3]. Recently, for instance, *in vitro* rooting was enhanced in *Nicotiana benthamiana* by auxin indoleacetic acid [4]. Indolebutyric acid was recommended for shoot and root organogenesis of *Eriosephalus africanus*, a medicinal and aromatic plant species [5]. Naphthalene acetic acid significantly increased the number of bulblets developed on leaf explants of *Scadoxus puniceus* [6]. Callus cultures from leaves and young shoots of *Taxus globosa* were produced with 2,4-dichlorophenoxyacetic acid [7].

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Examples of cytokinin use in plant *in vitro* culture can be found in the following papers. Date palm *Phoenix dactylifera L.* acclimatization was improved with the use of kinetin [8]. Micropropagation by axillary budding of *Quercus ilex* was achieved by culturing shoots with zeatin [9]. A reliable protocol was established for *in vitro* propagation of *Artemisia nilagirica* with N6-isopentyladenine [10]. *Heuchera villosa* petioles were cultured with N6-benzyladenine to induce callus formation [11]. Regarding gibberellins, they have been used to control *in vitro* morphogenesis of potato [12], bromeliads [13] and peony [14].

In spite of the physiological effects of auxins, cytokinins and gibberellins have been frequently studied, their chemical dissimilarities to justify their differential impact on plants require more attention. The present study compared the molecular descriptors of indolebutyric acid, indoleacetic acid, 2,4-dichlorophenoxyacetic acid, 1-naphthaleneacetic acid (auxins), kinetin, zeatin, N6-isopentenyl adenine, N6-benzyladenine (cytokinins), GA1, GA3, GA4 and GA7 (gibberellins) (Figure 1).

Materials and Methods

DRAGON software (version 5.5, 2007) and Cambridge Soft Chem Office (version 12, 2010) including ChemDraw and Chem3D were used to calculate 212 molecular descriptors. All data of this study were statistically evaluated using SPSS (Version 8.0 for Windows, SPSS Inc., New York, NY) to perform One - Way ANOVA and Tukey (p=0.05). The overall coefficients of variation (OCV) were calculated as follows: (standard deviation/average) *100. In this formula, we considered the average values of the three growth regulators compared (auxins, cytokinins, gibberellins) to calculate the standard deviation and average. Therefore, the higher the difference between the three groups of chemicals, the higher is the OCV [15]. A hierarchical cluster analysis using the molecular descriptors for auxins, cytokinins and gibberellins was performed. The dendrogram was built using average linkage (between groups). Variables were standardized to vary from 0 to 1 according to Kantardzic [16].

Results and Discussion

Even though 49 (out of 212) molecular descriptors showed statistically significant differences among auxins, cytokinins and gibberellins, based on the OCVs in (Table 1), only the numbers of terminal tertiary C (sp³), terminal quaternary C (sp³), ring secondary C (sp³), ring tertiary C (sp³), ring quaternary C (sp³), nitrogen atoms, substituted benzene C (sp²), benzene-like rings, terminal secondary C (sp³), 10-membered rings, and unsubstituted benzene C (sp²) were classified as "High" OCVs (116.94-173.21%). They indicated a remarkable distinction among these three groups of regulators.

Gibberellins contain terminal tertiary C (sp³), terminal quaternary C (sp³), ring secondary C (sp³), ring tertiary C (sp³), and ring quaternary C (sp³) that are not present either in cytokinins or auxins. Gibberellins are also relatively rich in terminal secondary C (sp³) (4.4 times more than auxins =5.50/1.25) and 10-membered rings (4 times more than auxins=1.00/0.25) which are absent in cytokinins.

Cytokinins have 10 times more nitrogen atoms than auxins (5.00/0.50) but this atom is not present in gibberellins. Auxins have 10 times more substituted benzene C (sp²) and 5 times more benzene-

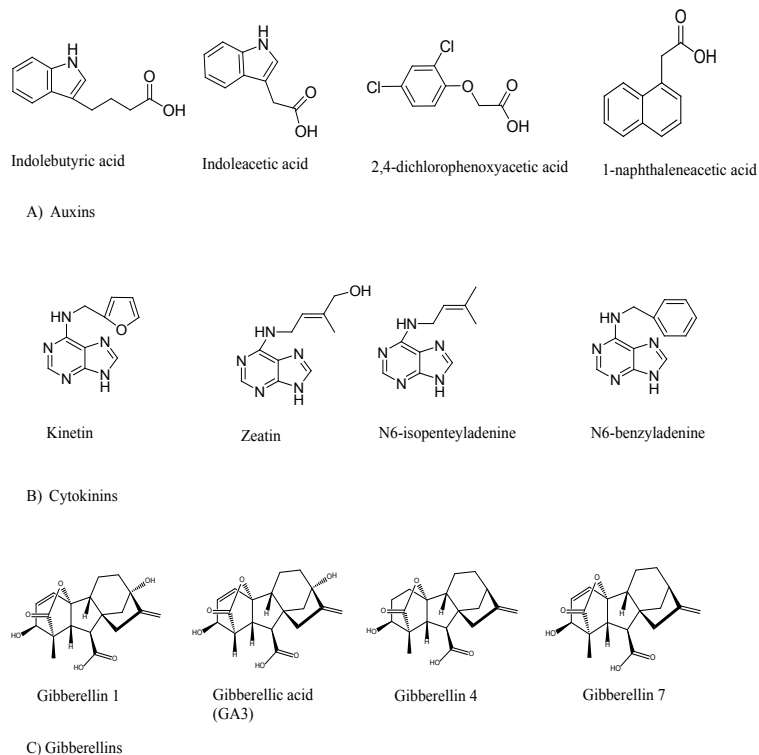


Figure 1: Auxins, cytokinins and gibberellins compared.

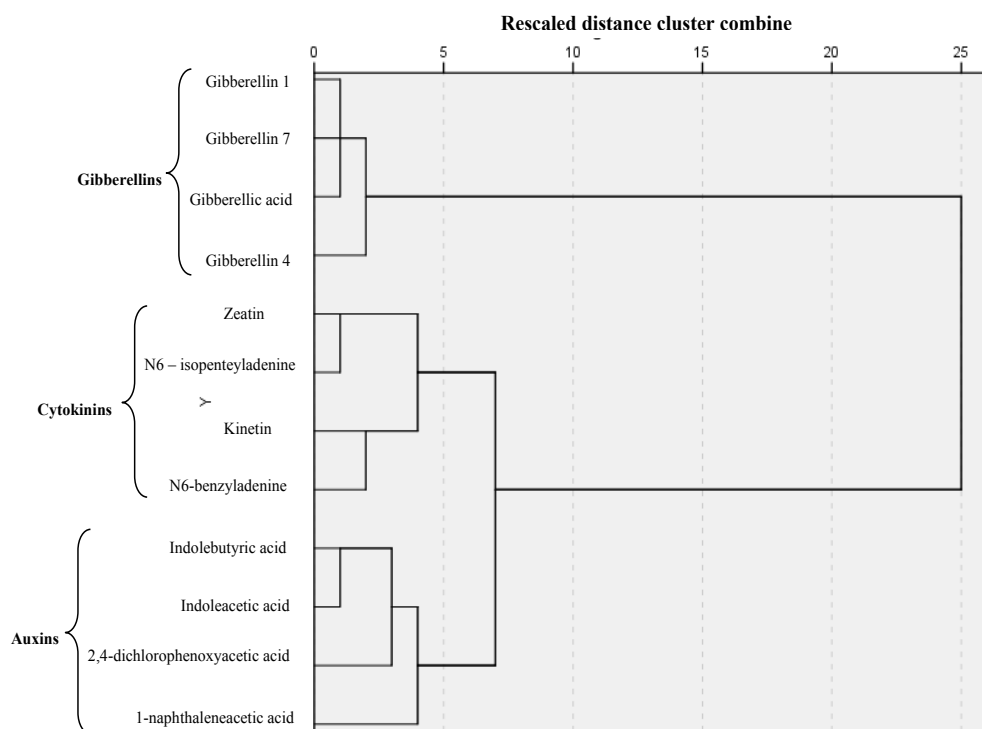


Figure 2: Hierarchical cluster analysis using the molecular descriptors for auxins, cytokinins and gibberellins. Only those descriptors with statistical significant differences among auxins, cytokinins and gibberellins were included (Table 1). The dendrogram was built using average linkage (between groups). Variables were standardized to vary from 0 to 1 according to Kantardzic [16].

like rings than cytokinins (2.50/0.25 and 1.25/0.25, respectively) but these structures are not in gibberellins. Regarding the numbers of unsubstituted benzene C (sp²), auxins average 4.50, cytokinins 1.25 but they are absent in gibberellins.

“Medium” OCVs (60.67 to 116.94%) remarkably distinguished gibberellins from auxins and cytokinins (Table 1). Gibberellins have 3 times more aliphatic secondary C (sp²) and 2 times more aliphatic tertiary C (sp²) than cytokinins (1.50/0.50; 1.00/0.50; respectively) but these types of atoms are not in auxins. Gibberellins are also rich in double bonds (7.5 times more than cytokinins=3.75 /0.50 and 3.75 times more than auxins=3.75/1.00), circuits (4.3 times more than cytokinins=15.0/3.5 and 6 times more than auxins=15.0/2.5), and oxygen atoms (11 times more than cytokinins=5.50/0.50 and 2.4 times more than auxins=5.50/2.25).

The numbers of hydroxyl groups, 5-membered and 9-membered rings are also higher in gibberellins: 10 times more hydroxyl

groups than cytokinins (2.50/0.25) and 2.5 times more than auxins (2.50/1.00); 2.4 times more 5-membered rings than cytokinins (3.00/1.25) and 6 times more than auxins (3.00/0.50); and 2 times more 9-membered rings than cytokinins (2.00/1.00) and 4 times more than auxins (2.00/0.50). Contrastingly, gibberellins do not have either aromatic bonds (cytokinins: 12.75; auxins: 9.25) or aromatic C (sp²) (cytokinins: 7.50; auxins: 8.00). Aromatic ratio is zero in gibberellins while 0.72 in cytokinins and 0.63 in auxins. On the other hand, the rotatable bond fraction is lower in gibberellins (0.02) compared to auxins (0.12) or cytokinins (0.11). It is important to note the squared Ghose-Crippen octanol-water partition coefficient is remarkably higher in auxins (5.96) in comparison with cytokinins (1.69) and gibberellins (1.31).

Descriptors shown in Table 1 were used to generate the dendrogram shown in Figure 2 which correctly classified the three groups of regulators in three independent branches. Molecular descriptors have

Table 1: Comparison of molecular descriptors for auxins, cytokinins and gibberellins. IBA: Indolebutyric acid; IAA: Indoleacetic acid; 2,4-D: 2,4-dichlorophenoxyacetic acid; NAA: 1-Naphthaleneacetic acid; KIN: Kinetin; ZEA: Zeatin; ZIP: N6 – isopentenyladenine; BA: N6-benzyladenine; GA1: Gibberellin 1; GA3: Gibberellin 3; GA4: Gibberellin 4; GA7: Gibberellin 7.

	Auxins				Cytokinins				Gibberellins				Auxins ¹	Cytokinins ²	Gibberellins ³	OCV ⁴	Classification of OCV ⁵
	IBA	IAA	2,4-D	NAA	KIN	ZEA	ZIP	BA	GA1	GA3	GA4	GA7					
Number of terminal tertiary C (sp ³)	0	0	0	0	0	0	0	0	5	6	5	5	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	5.25 ± 0.25 ^a	173.21	High
Number of terminal quaternary C (sp ³)	0	0	0	0	0	0	0	0	2	1	2	2	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	1.75 ± 0.25 ^a	173.21	High
Number of ring secondary C (sp ³)	0	0	0	0	0	0	0	0	5	5	7	5	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	5.50 ± 0.50 ^a	173.21	High
Number of ring tertiary C (sp ³)	0	0	0	0	0	0	0	0	5	6	5	5	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	5.25 ± 0.25 ^a	173.21	High
Number of ring quaternary C (sp ³)	0	0	0	0	0	0	0	0	2	1	2	2	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	1.75 ± 0.25 ^a	173.21	High
Number of nitrogen atoms	1	1	0	0	5	5	5	5	0	0	0	0	0.50 ± 0.29 ^b	5.00 ± 0.00 ^a	0.00 ± 0.00 ^b	150.21	High
Number of substituted benzene C (sp ²)	2	2	3	3	0	0	0	1	0	0	0	0	2.50 ± 0.29 ^a	0.25 ± 0.25 ^b	0.00 ± 0.00 ^b	150.21	High
Number of benzene-like rings	1	1	1	2	0	0	0	1	0	0	0	0	1.25 ± 0.25 ^a	0.25 ± 0.25 ^b	0.00 ± 0.00 ^b	132.29	High
Number of terminal secondary C (sp ³)	3	1	0	1	0	0	0	0	5	5	7	5	1.25 ± 0.63 ^b	0.00 ± 0.00 ^b	5.50 ± 0.50 ^a	128.14	High
Number of 10-membered rings	0	0	0	1	0	0	0	0	1	1	1	1	0.25 ± 0.25 ^b	0.00 ± 0.00 ^b	1.00 ± 0.00 ^a	124.9	High
Number of unsubstituted benzene C (sp ²)	4	4	3	7	0	0	0	5	0	0	0	0	4.50 ± 0.87 ^a	1.25 ± 1.25 ^{ab}	0.00 ± 0.00 ^b	121.19	High
Number of aliphatic secondary C (sp ²)	0	0	0	0	0	1	1	0	2	2	0	2	0.00 ± 0.00 ^b	0.50 ± 0.29 ^{ab}	1.50 ± 0.50 ^a	114.56	Medium
Number of double bonds	1	1	1	1	0	1	1	0	4	4	3	4	1.00 ± 0.00 ^b	0.50 ± 0.29 ^b	3.75 ± 0.25 ^a	100	Medium
Number of aliphatic tertiary C (sp ²)	0	0	0	0	0	1	1	0	1	1	1	1	0.00 ± 0.00 ^b	0.50 ± 0.29 ^{ab}	1.00 ± 0.00 ^a	100	Medium
Number of circuits	3	3	1	3	4	3	3	4	15	15	15	15	2.50 ± 0.50 ^b	3.50 ± 0.29 ^b	15.00 ± 0.00 ^a	99.23	Medium
Number of oxygen atoms	2	2	3	2	1	1	0	0	6	6	5	5	2.25 ± 0.25 ^b	0.50 ± 0.29 ^c	5.50 ± 0.29 ^a	92.26	Medium

Number of hydroxyl groups	1	1	1	1	0	1	0	0	3	3	2	2	1.00 ± 0.00 ^b	0.25 ± 0.25 ^b	2.50 ± 0.29 ^a	91.65	Medium
Number of aromatic bonds	10	10	6	11	15	10	10	16	0	0	0	0	9.25 ± 1.11 ^a	12.75 ± 1.60 ^a	0.00 ± 0.00 ^b	89.83	Medium
Aromatic ratio	0.625	0.714	0.462	0.73	0.833	0.588	0.625	0.842	0	0	0	0	0.63 ± 0.06 ^a	0.72 ± 0.07 ^a	0.00 ± 0.00 ^b	87.16	Medium
Number of aromatic C (sp ²)	8	8	6	10	9	5	5	11	0	0	0	0	8.00 ± 0.82 ^a	7.50 ± 1.50 ^a	0.00 ± 0.00 ^b	86.74	Medium
Squared Ghose-Crippen octanol-water partition coefficient. (log ^A P)	7.156	3.107	7.907	5.653	1.186	0.235	2.483	2.869	0.174	0	2.8	2.246	5.96 ± 1.06 ^a	1.69 ± 0.60 ^b	1.31 ± 0.71 ^b	86.45	Medium
Number of 5-membered rings	1	1	0	0	2	1	1	1	3	3	3	3	0.50 ± 0.29 ^b	1.25 ± 0.25 ^b	3.00 ± 0.00 ^a	81.03	Medium
Rotatable bond fraction	0.138	0.087	0.158	0.08	0.111	0.133	0.103	0.1	0.02	0.021	0.019	0.02	0.12 ± 0.02 ^a	0.11 ± 0.01 ^a	0.02 ± 0.00 ^b	65.65	Medium
Number of 9-membered rings	1	1	0	0	1	1	1	1	2	2	2	2	0.50 ± 0.29 ^b	1.00 ± 0.00 ^b	2.00 ± 0.00 ^a	65.47	Medium
Number of rings	2	2	1	2	3	2	2	3	5	5	5	5	1.75 ± 0.25 ^b	2.50 ± 0.29 ^b	5.00 ± 0.00 ^a	55.19	Low
Number of multiple bonds	11	11	7	12	15	11	11	16	4	4	3	4	10.25 ± 1.11 ^a	13.25 ± 1.31 ^a	3.75 ± 0.25 ^b	53.46	Low
Ghose-Crippen octanol-water partition coefficient (logP)	2.675	1.763	2.812	2.378	1.089	0.485	1.576	1.694	0.417	0.001	1.673	1.499	2.41 ± 0.23 ^a	1.21 ± 0.28 ^{ab}	0.90 ± 0.41 ^b	52.92	Low
Number of rotatable bonds	4	2	3	2	3	4	3	3	1	1	1	1	2.75 ± 0.48 ^a	3.25 ± 0.25 ^a	1.00 ± 0.00 ^b	50.63	Low
Number of hydrogen atoms	13	9	6	10	9	13	13	11	22	20	24	22	9.50 ± 1.44 ^b	11.50 ± 0.96 ^b	22.00 ± 0.82 ^a	46.84	Low
Number of acceptor atoms for H-bonds (N.O.F)	2	2	3	2	5	5	4	4	6	6	5	5	2.25 ± 0.25 ^b	4.50 ± 0.29 ^a	5.50 ± 0.29 ^a	40.77	Low
Number of bonds	29	23	19	25	27	30	29	30	51	48	52	50	24.00 ± 2.08 ^b	29.00 ± 0.71 ^b	50.25 ± 0.85 ^a	40.5	Low
Number of atoms	28	22	19	24	25	29	28	28	47	44	48	46	23.25 ± 1.89 ^b	27.50 ± 0.87 ^b	46.25 ± 0.85 ^a	37.85	Low
Sum of atomic Sanderson Electronegativities (scaled on Carbon atom)	28.06	22.29	20.16	24.07	25.6	29.37	28.04	28.16	47.68	44.8	48.24	46.36	23.65 ± 1.67 ^b	27.79 ± 0.79 ^b	46.77 ± 0.77 ^a	37.66	Low
Number of non-H bonds	16	14	13	15	18	17	16	19	29	28	28	28	14.50 ± 0.65 ^c	17.50 ± 0.65 ^b	28.25 ± 0.25 ^a	36	Low
Number of carbon atoms	12	10	8	12	10	10	10	12	19	18	19	19	10.50 ± 0.96 ^b	10.50 ± 0.50 ^b	18.75 ± 0.25 ^a	35.95	Low
Sum of Kier-Hall electrotopological states	36.67	33.67	38.89	35.17	35.67	37.67	32.17	36.17	65.83	63.92	58.92	59.92	36.10 ± 1.11 ^b	35.4 ± 1.16 ^b	62.15 ± 1.64 ^a	34.2	Low
Sum of atomic polarizabilities (scaled on carbon atom)	18.48	14.96	14.12	16.72	17.01	18.53	18.07	19.31	30.1	28.34	30.41	29.65	16.07 ± 0.97 ^b	18.23 ± 0.48 ^b	29.63 ± 0.46 ^b	34.18	Low
Sum of atomic Van der Waals volumes (scaled on carbon atom)	17.6	14.41	13.33	16.01	16.68	17.87	17.36	18.76	28.65	27.05	28.73	28.13	15.34 ± 0.93 ^a	17.67 ± 0.44 ^b	28.14 ± 0.39 ^b	33.46	Low
Topological polar surface area using N.O polar contributions	53.09	53.09	46.53	37.3	79.63	86.72	66.49	66.49	104.06	104.06	83.83	83.83	47.50 ± 3.74 ^b	74.83 ± 5.03 ^a	93.95 ± 5.84 ^a	32.38	Low
Topological polar surface area using N.O.S.P polar contributions	53.09	53.09	46.53	37.3	79.63	86.72	66.49	66.49	104.06	104.06	83.83	83.83	47.50 ± 3.74 ^b	74.83 ± 5.03 ^a	93.95 ± 5.84 ^a	32.38	Low
Numb of non-N atom	15	13	13	14	16	16	15	17	25	24	24	24	13.75 ± 0.48 ^c	16.00 ± 0.41 ^b	24.25 ± 0.25 ^a	30.71	Low

Molecular weight	203.26	175.2	221.04	186.22	215.24	219.28	203.28	225.28	346.41	332.38	332.43	330.41	196.43 ± 10.03 ^b	215.77 ± 4.65 ^b	335.41 ± 3.70 ^a	30.21	Low
Number of 6-membered rings	1	1	1	2	1	1	1	2	2	2	2	2	1.25 ± 0.25 ^b	1.25 ± 0.25 ^b	2.00 ± 0.00 ^a	28.87	Low
Unsaturation index	3.585	3.585	3	3.7	4	3.585	3.585	4.087	2.322	2.322	2	2.322	3.47 ± 0.16 ^a	3.81 ± 0.13 ^a	2.24 ± 0.08 ^b	26.03	Low
Ghose-Crippen molar refractivity	57.654	48.452	48.366	53.816	57.686	61.575	59.8	65.295	86.414	81.913	84.078	84.887	52.07 ± 2.26 ^c	61.09 ± 1.61 ^b	84.32 ± 0.94 ^a	25.28	Low
Sum of conventional bond orders (H-depleted)	22	20	17	21.5	25.5	23	22	27	33	32	31	32	20.13 ± 1.13 ^c	24.38 ± 1.14 ^b	32.00 ± 0.41 ^a	23.6	Low
Mean electrotopological state	2.44	2.59	2.99	2.51	2.23	2.35	2.14	2.13	2.63	2.66	2.45	2.5	2.63 ± 0.12 ^a	2.21 ± 0.05 ^b	2.56 ± 0.05 ^a	9.1	Low
Mean atomic van der Waals volume (scaled on carbon atom)	0.63	0.65	0.7	0.67	0.67	0.62	0.62	0.67	0.61	0.61	0.6	0.61	0.66 ± 0.01 ^a	0.65 ± 0.01 ^a	0.61 ± 0.00 ^b	4.4	Low
Mean atomic polarizability (scaled on carbon atom)	0.66	0.68	0.74	0.7	0.68	0.64	0.62	0.69	0.64	0.64	0.63	0.64	0.70 ± 0.02 ^a	0.60 ± 0.02 ^{ab}	0.64 ± 0.00 ^b	4.4	Low

¹Average information of indolebutyric acid, indoleacetic acid, 2,4-dichlorophenoxyacetic acid and 1-naphthaleneacetic acid.

²Average information of kinetin, zeatin, N6 – isopentenyladenine and N6-benzyladenine.

³Average information of gibberellin 1, gibberellin 3, gibberellin 4 and gibberellin 7. Results with the same letter are not statistically different (One-Way ANOVA, Tukey, p=0.05).

⁴Overall coefficient of variation=(Standard deviation/Average)*100. To calculate this coefficient, average values of auxins, cytokinins and gibberellins were considered. The higher the difference among these three averages, the higher the overall coefficient of variation.

⁵Classification of OCVs: "Low" from 4.40 to 60.67%; "Medium" from 60.67 to 116.94% and "High" from 116.94 to 173.21%.

been applied to describe biological activities, in many studies showing their applicability as an attractive tool for efficient (e.g.) drug design process [17-19].

To end we would like to emphasize the effectiveness of the chemo-informatics procedure described here to differentiate auxins, cytokinins and gibberellins and also in the search for new plant growth regulators with potential applications in modern *in vitro* culture and agriculture. Molecular descriptors of new chemical compounds can be determined and included in the dendrogram shown in Figure 2. If new chemicals are located, for instance, near auxins they can be regarded as potential auxin-like compounds, although this should be later tested experimentally.

Author Contribution

I.A., D.G., L.P. and J.C.L. designed the research, analyzed the data and wrote the paper. J.C.L. had primary responsibility for the final content. All authors have read and approved the final manuscript.

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