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Research Article

New Spray Reagent for Detection of Some Plant Hormone on Thin-Layer Chromatography

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Received: December 09, 2015; **Published:** December 28, 2015

Abstract

Plant growth regulators (PGR) are hormone-like chemicals that occur naturally in plants, and play a central role in their growth and development. Five major classes of plant growth regulators have been identified as plant hormones. The five growth hormones include auxins, gibberellins, cytokinins, abscisic acid, and ethylene. Collectively, they regulate many facets of plant growth and development including seed germination, root growth, stem elongation, leaf expansion, flowering, seed development, fruit ripening, and dropping of leaves and fruits. Each of these processes is directly relevant to agriculture. PGR can stimulate human immune cells that boost the body's natural defense against stressors and diseases. Thin layer chromatography (TLC) is an important method for qualitative and quantitative analysis of plant growth regulator because it indicates some advantages comparing to paper chromatography (PC), high performance liquid chromatography (HPLC) and gas chromatography methods (GC). TLC can be used for both partition and absorption chromatography furthermore, it can be used for both preparative and identification. For plant growth regulator detection TLC, silica gel, is the most commonly used support. In this study a new spray reagent, Folin–Ciocalteu reagent, was proposed to detect plant growth regulator either visually be developing color or through the retardation factor (RF) values determination. The advantage of using Folin–Ciocalteu reagent developing color is clear easy to achieve and inexpensive methods lead to direct result.

Keywords: *Plant Hormone Regulator; Folin–Ciocalteu Reagent; The Retardation Factor; Thin-Layer Chromatography (TLC) And Hormone-Like Chemicals*

Abbreviations: TLC: Thin Layer Chromatography; PGR: Plant Hormone Regulator: PC; Paper Chromtogrphy: NAA: Naphthalene Acetic Acid; IAA: Indole acetic acid; GA3: Gibberellic acid

Introduction

Phytohormones are a group of chemically varied compounds known as plant growth regulator. They regulate physiological processes in plant. auxins, cytokinins, ethylene, gibberellins and abscisic acid are the main group of plant hormone regulator in higher plants. Apical dominance is controlled by the concentration gradient of auxins [1-2]. Indole-acetic acid (IAA) is considered to be the only photohormones among the auxins. Synthetic cytokinins are used in plant production and in cultivation processes. Ethylene is involved in flowering, defoliation, fruit ripping and also in the presence of breaking dormancy. Gibberellins have been studied chiefly using gibberellic acid, GA3, which is commercially available. It is involved in stem growth; induction of flowering; development of fruits, seeds and bulbs; and in breaking dormancy. Gibberellins are used to initiate the germination of grain in the production of beer. In conifers the induction of flowering has been of great interest [3]. Abscisic acid plays a role in the abscission of young fruits and also in the ripening of fruits [4]. Like plants, the human body also produces hormones and enzymes. In people and vertebrate animals, hormones act as chemical messengers that are utilized by the endocrine, nervous, and immune systems to convey unseen electrical impulses and chemical signals that would facilitate communication within a cell or between cell and/or among system organs. Hormones and enzymes also regulate body functions, control human behavior and integrate systemic functions – nervous, immune and endocrine systems. Plants cannot benefit from human

hormones; however, plant hormones can do wondrous health effects to human health. Plant hormones can stimulate human immune cells that boost the body's natural defense against stressors and diseases, based on new work from Santina Bruzzone, Elena Zocchi (University of Genova, Genoa, Italy), and colleagues. The authors identify the plant hormone abscisic acid (ABA) as a human cytokine [5-6]. There are three types of plant hormones that have shown promising benefits to us humans - Plant Growth Hormone (PGH), Plant Stress Hormone (PSH) and Plant Immune Hormone (PIH).

Detection of endogenous plant hormones can be technically very demanding because of low levels of hormone in the tissue, closely related but biologically inactive compounds [7] and hormones can be degraded or metabolized during extraction. Moreover, several biological effects of PGR are induced by cooperation of more than one phytohormone.

Thin-layer chromatography was the first technique used for rapid separation and determination of phytohormones [6-7]. It is widely used to detect plant hormones such gibberellin (GA3) and related compounds, Cytokinis and Auxine. TLC of indole derivatives including

auxine has been investigated very well not in the field of plant physiology but also in the field of medical science [8]. Silica gel is the most generally used adsorbent for auxins detection. Although identification of gibberellines are becoming more difficult because of the increasing gibberellines number TLC is still an important technique as a preliminary test for identification [9]. Paper and cellulose- TLC are indispensable for identification and purification of both gibberellines and cytokinins. If compounds are identified as spots on TLC, it can be observed by the TLC as a course of purification without using bioassay. The advantages of using TLC, for plant hormone detection are superior to Pc chromatography in such points: good separation, rapid development, applicability for many kinds of reagent for detection.

In this study a new chromogenic reagent was proposed for easy identification of tested PGR on thin-layer chromatography by developing clear colors. Plant growth regulators are not just substances of control but tools of effective tree management and fruit quality. Therefore, the main aim of this work was to optimize conditions for easy and inexpensive identification. Burrows., *et al*. [10] used TLC of cellulose containing a fluorescent substance to identify cytokines.

Materials and Methods

1. Standard preparation: 0.1g of indole acetic acid (IAA, Molecular Weight: 175.19-sigma), Indole bytric acide (IBA, molecular weight = 203.43- sigma), Gibbrllic acid (GA3, Molecular Weight: 346.38-sigma) and cytokinine was dis-solved in 10ml distilled water (individually) then spotted in TLC TLC paper.
2. Folin–Ciocalteu reagent: Folin-Ciocalteau reagent 2N, SIGMA (LOC: 4130) used as a spry Chromogenic reagent Ninhydrin solution: dissolve 0.25g of Ninhydrin in 12.5 ml of ethanol (95%).
3. Detection on Plates: Standard plant hormones regulator solutions were spotted on to TLC Chromatography [11], silica Gel, 60 F254 TLC Sheet 20 x 20cm; thickness 0.2.mm. Merck- Germany, by graduated capillary tubes (capacity: 5µL volume-Spectrochem). TLC was air-dried then subjected to the devolving solvent contains butanol- glacial acetic acid and water 12:5:3 (v/v) as mobile phase. After development plates were dried and sprayed with Diluted Folin-Ciocalteau reagent (1V/3V distilled water) and then heated at 110°C for 3 min. in an oven. Colors were observed visually and Rf values were determined.

Results and Discussion

Table (1) shows the calculated Rf, color reaction of tested plant hormone regulator, IAA- NAA- IBY-GA3; Kintine treated with Folin-Ciocalteau reagent. RF value of tested standards were varied, depends on kind of PAG. NAA reacted with Folin-Ciocalteau reagent by developing deep pink color and RF value equal to 0.76 while RF value of IAA and IBA standards were 0.88 and 0.94 with light pink and purple color respectively. Spot of GA3 standard showed clear deep pink color with RF Value 0.91. Also Kinteen standard reacted with Folin-Ciocalteau reagent and it given clear deep pink spot with RF Value 0.29 (Figure 1).

Plant hormones regulator	Color observation	R_F value
Indole Bytric acid (IBA)	purple	0.94
Naphthalene acetic acid (NAA)	Deep pink	0.76
Indole acetic acide (IAA)	Light pink	0.88
Kinteen (Kin.)	Deep pink	0.29
Gibbrelic acid (GA3)	Deep bink	0.91

Table 1: *RF value and Color formation of plant hormones regulator on TLC paper with foline-Ciocalteau reagents.*

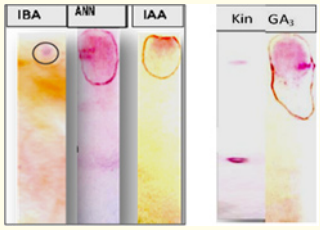

Figure 1: *Formation of color by plant growth regulator standers using foline-Ciocalteau reagents.*

TLC was carried out as convenient methods for identification of plant growth regulator. Studies done by [12, 13] revealed that gibberellins are separated by adsorption thin-layer chromatography in many cases a mixture of GA1 and GA3; a mixture of GA4 and GA7 only separated clearly by partition TLC. Indole derivative including auxin has been investigated very well. Study by Weller., et al. [14] reported the detection of indole-3- actic acid (IAA) in plant extract by paper chromatography. Moreover, he recorded paper chromatographic data of 22 IAA- related compounds. Mothia [15] detected IAA using TLC silica gel and Propanol: Water (8:2) was used as solvent system. TLC. Chromatogram was developed with the Salkowski's reagent. TLC standard and Samples showed pink color spots at RF value 0.57as shown in Figure (2).

Figure 2: *Thin layer chromatogram of bacterially sized IAA detected by Salkowski's reagent compared with standard [15].*

The result of this study showed different RF value for IAA (0.88) comparing to Mothia [15] result. Also color formation for tested PGR differed comparing to [16] results (Table. 2). Study by Barman., *et al*. [17] illustrated that the RF values and color formation may varied depend on purity of solvent, solvent composition, chromogenic reagent composition.

Plant growth regulator	Color Observation	Solvent system	Rf values	Reference
Indole acetic acid (IAA)	pink	butanone-ethyl acetate-ethanol-water (3:5:1:1)	0.92	[15]
Indole acetic acid (IAA)	Crimson	Isopropanol: Ammonia: Water (10:10:1)	0.25	[18]
Indole Bytric acid (IBA)	Yellow- rown	Isopropanol: Ammonia: Water (10:10:1)	0.44	[18]
Gibbrelic acid (GA3)	Green-blue	Phosphate buffer (0.1M; pH 6.3) TLC	0.70	[19]

Table 2: *RF value and Color formation of plant hormones regulator on TLC paper with foline reagents.*

In this study, distinguishable colors have been seen that may due to the presence of either Engvild [20] or glacial-acetic acid in a mobile phase. The advantages of simplicity and sensitivity of folin reaction recommend it for a number of biochemical purposes.

TLC is widely used as technique. Folin reagent were used in this technique were facilitated identification and sensitivity of Plant growth regulator (PGR) in tested standards, and give an indicator to the presence of f PGR in soil and foods for nutritional purposes.

Conclusion

The application of this method would be of value in assessment of plant growth regulators identification for determining their occurrence in the Free State in natural products. Furthermore the chromogenic reagent proposed in this modified reacted with tested standard and gives clear pink color. Thin layer chromatography used in this study greatly contributed to analysis of tested standards. Thus, this study impasses that TLC still used as one of the promising methods of separation and quantitative determination for which GC or HPLC is not suitable unavailable. Presented data indicate the detection progress of selected plant growth regulators investigated by thin-layer chromatography.

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Volume 3 Issue 1 December 2015