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Impact of Biostimulant and Synthetic Hormone Gibberellic acid on Molecular Structure of *Solanum melongena* L.

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Abstract

A comparison study between the application of gibberellic acid (GA3) and *Saccharomyces cerevisiae* as a biostimulant on the growth and molecular structures of Eggplant (*Solanum melongena* L.) has been carried out through a pot experiment. Growth of *Solanum melongena* L. increased with exogenous application of GA3 followed by yeast. Chlorophyll contents of plant were enhanced with yeast treatment compared with GA3 application and control. Activitiy of antioxidant enzymes, catalase and peroxidase was increased with increasing concentration of GA3 and *S. cerevisiae* application particularly with using GA3. HPLC analysis showed the highest concentration of salicylic acid in plant treated with GA3 (104.20 mg) followed by *S. cerevisiae* (70.00 mg) application compared with the untreated plant (57.86 mg). Six common polypeptide bands were observed in treated and untreated *S. melongena* plants, their molecular weights were 16, 17, 34, 90, 120 and 150 KDa. While the untreated *S. melongena* plant is characterized by the presence of 8 polypeptide bands, their molecular weights were 19, 24, 32, 33, 36, 50, 109 and 133 KDa. Yeast treatment increased the number of protein bands to 12 instead of 8 in the control plant with molecular weights 18, 125, 74, 69, 62, 31, 30, 27, 25, 23, 20 and 18 KDa. Three polypeptide bands with molecular weights 25, 72 and 125 KDa were detected in *S. cerevisiae* and GA3 treated plants. PCR analysis showed that total of 16 amplified fragments was visualized in the tested samples. Eight fragments with different molecular weights, four of them are monomorphic bands while the others are polymorphic unique bands. Plant sample sprayed with yeast showed 5 fragments range in molecular weight between 426 to 1766 bp. Only one of these fragments was unique polymorphic fragment. Four monomorphic fragments range in molecular weight from 426 to 1213 bp were showed up in plant sample sprayed with gibberellic acid.

Key words: Biostimulant, gibberellic acid , molecular structure, *Solanum melongena* L.

1. Introduction

Eggplant (*Solanum melongena* L.) is one of the Solanaceae plants and considered as one of the cultivated vegetable crops in many regions of the world, including tropical regions. It is considered as a rich crop in carbohydrates, protein and minerals (Tsao and Lo, 2006). Extensive research is going on throughout the world to find out natural sources to be used as plant growth promoter. In this connection, yeasts have been reported to be a rich source of phytohormones, vitamins, enzymes, amino acids and minerals (Mahmoud, 2001). It was reported about its stimulatory effects on cell division, protein, nucleic acid and chlorophyll synthesis (Castelfranco and Beale, 1983). Also, they have been found to produce auxins including indole-3-acetic acid and indole-3-pyruvic acid, gibberellins and polyamines; albeit induced *in vitro*. So called plant growth promoting yeasts include genera such as *Candida*, *Cryptococcus*, *Rhodotorula*, *Sporobolomyces*, *Trichosporon*, *Williopsis* and *Yarrowia* (El-Tarabily and Sivasithamparam, 2006; Roland et al., 2015). Numerous studies indicate that plant growth may be directly or indirectly promoted by microorganisms as well as yeasts (Xin et al., 2009; Botha 2011; El-Tarabily and Sivasithamparam 2006; Abd El-Ghany et al., 2013; Abd El-Ghany et al., 2015a,b,c). Likewise, Reda and Ismail (2008) stated that foliar application with active yeast extract induced significant enhancement the vegetative growth of river red gum plant (*Eucalyptus camaldulensis Dehn*.). Many studies have found the increase in germination, length and biomass of seedlings after seed treated with yeast strains, enhance plant growth, increase of photosynthesis productivity (Amprayn et al., 2012; Agamy et al., 2013; Hu and Qi 2013). Recently, Kahlel (2015) stated that the addition of bread yeast near the plants roots led to a significant increase in the number of stems, leaf area and fresh and dry weight of the plant compared with the control.

Plant growth is regulated and controlled by different action of the small molecules called plant hormones, which may play either close to or remote from their sites of biosynthesis to mediate genetically programmed developmental changes or responses to stimulation of environment (Colebrook et al., 2014). Gibberellins (GAs, also referred to as gibberellic acid) are a phytohormone chemically belongs to the group of tetracyclic diterpenoids, which produced by angiosperms plant and some fungi (Sun 2011; Colebrook et al., 2014). There are different group of bioactive GAs, however the important once which include GA1, GA3, GA4 and GA7, which derived from a basic diterpenoid carboxylic acid skeleton, and generally have a C3- hydroxyl group (Davière and Achard, 2013). The sparse literature on GAs indicates that this group of phytohormones does contribute to the growth and development of the plants. GAs are generally involved in growth and development; they control seed germination, leaf expansion, stem elongation and flowering (Magome et al., 2004). During the last decade, much progress has been made to understand the mechanism of Gibberellic acid signaling. It is well known that GAs promote plant growth by inducing the degradation of the nuclear family of transcription factors known as DELLA proteins. Thus, DELLA proteins restrain growth, while GAs induce their disappearance (Jiang and Fu, 2007). Exogenous application of GAs to plants causes the increase in the activities of many key enzymes (Aftab et al., 2010.) and enhanced the primary root elongation (Bidadi et al., 2010). However, several studies on impact of GAs on animal indicated that they induce the level of DNA breakage in human blood cells (Abou-Eisha, 2001), hepatocellular carcinomas (El-Mofty and Sakr, 1988), reductions of catalase, superoxide dismutase and glutathione peroxidase (Soliman et al., 2010) in animals. Therefore the aim of the study was to explore the effects of yeast, as an environmentally and healthy safe method on molecular level compared with synthetic plant growth hormone gibberellic acid on Eggplant (*Solanum melongena* L.).

2. Material and Methods

2.1 Plant Material, Hormone and Yeast Treatments

In the present study, Eggplant (*Solanum melongena* L.) seeds were used. Prior to sowing, the seeds were surface sterilized for 10 min with 10:1 water/bleach (commercial NaOCl) solution and then washed few times with distilled water. After the sterilization, the seeds were soaked in distilled water about 5 h and then sowed in pots (20-cm diameter) with 10 seeds per pot. During the period of experiment the moist of the soil were kept at fixed percentage of available water. Seedlings were grown in a growth chamber under controlled environmental conditions for 26 days. The used hormone GA3 obtained from the Sigma-Aldrich Co., USA sprayed twice during the plant life on the leaves of 12 and 18 day-old plants at different concentrations (100, 200 and 400 ppm). Also at the same condition brewers' yeast (*S. cerevisiae*) was applied at three doses including 2, 4 and 6 g/L distilled water. Distilled water was used to spray control plants. Plants were harvested at 22 days and used to measure some biochemical and molecular markers.

2.2 Quantitative Determination of Chlorophylls and Antioxidant Enzymes

Chlorophyll content was determined according to Vernon and Seely (1966) using the following equations:

Chlorophyll a (mg)/ tissue (g) = 11.63 (A 665) -2.39 (A 649).

Chlorophyll b (mg)/ tissue (g) = 2.11 (A 649) -5.18 (A 665).

Where A denotes the reading of the optical density

Antioxidant enzymes including catalase and peroxidase of plant were detected according to Kar and Mishra (1976).

2.3 Extraction and Quantification of Salicylic Acid

Salicylic acid (SA) was extracted and quantified as described by Malamy et al. (1992). Treated and untreated plants were washed several times with distilled water to remove the remnants of any dusts. Then 25 g of each dried plant were ground in 25 mL of 90% methanol and centrifuged at 6000 rpm for 15 min. The pellet was re-extracted with 3 ml of 100% methanol and centrifuged. Methanol extracts were combined, centrifuged for 10 min and dried at 40 °C under vacuum. For each sample, the dried methanol extract was re-suspended in 5 ml of water at 80 °C, and an equal volume of 0.2 M acetate buffer (pH 4.5) was added and incubated at 37 °C over night. After digestion, samples were acidified with HCl to pH 1 and SA was extracted and back extracted with 2 volumes of cyclopentane/ethyl acetate/isopropanol (50:50:1, v/v/v). The organic extract was dried under nitrogen, re-suspended in 50 ml of 100% methanol and analyzed by HPLC at Regional Center of Mycology and Biotechnology (RCMB), Al Azhar University, Cairo, Egypt.

2.4 DNA and RNA Detection

Determination of nucleic acids DNA and RNA were extracted following the method was similar to that described by Morse and Carter (1949). RNA was estimated colorimetrically by the orcinol reaction as described by Dishe (1953) while DNA was estimated by diphenylamine (DPA) color reaction as described by Burton (1956).

2.5 Induction of indole-3-acetic acid (IAA) Production by Yeast

Yeast was cultured in Conical flasks containing Yeast Extract-Peptone-Dextrose medium supplemented with 0.1 % (w/v) L-tryptophan at pH 6.0 and incubated in the dark on a shaker at 25 °C and 150 rpm for 4 days. One millilitre of the medium was centrifuged at 3000 rpm for 5 min, and 0.5 mL of the supernatant was mixed with 0.5 mL of Salkowski's reagent which consists of 2 mL of 0.5 M iron (III) chloride and 98 mL of 35 % perchloric acid according to Gordon and Weber (1951). After 30 min, the developed red colour of the sample was quantified using a spectrophotometer at 530 nm.

2.6 Protein Gel-Electrophoresis

Five grams of each dried plant was ground in 0.1 mL Sodium dodecyl sulfate (SDS) sample buffer cracking solution. Extracts were added in 1.5 cm eppendorf centrifuge tube according to Laemmli (1970). Homogenates were heated at 95°C for 5 min then briefly centrifuged at 12000 rpm to pellet cellular debris. The resulting supernatants (total protein extracts) were stored at -70 °C until analysis by PAGE. The extract was separated by electrophoresis on 1mm thick 12.5% acrylamide slab gels. Gels were stained with Coomassie blue at RCMB.

2.7 Genetic Characterization of Treated Plant

Plant samples were ground in a mortar using a sterile pestle, and the samples were placed in eppendorf tubes (1.5 ml) DNA extraction was conducted using DNeasy kit (Qiagen-Germany).

2.7.1 DNA Amplification by RAPD-PCR

For PCR amplification, ONE 10-mer random primer was selected; primer 3:(5'-GTAGACCCGT-3'). The amplification was performed in a thermal cycler program:Template DNA was initially denatured at 92 °C for 3 min, followed by 35 cycles of PCR amplification under the following parameters: denaturation for 1 min at 92 °C, primer annealing at 36 °C for 1 min, and primer extension at 72 °C for 2 min. A final incubation for 10 min at 72 °C was performed to ensure that the primer extension reaction proceeded to completion. PCR products were analyzed by gel electrophoresis on 1.5 % agarose and detected by ethidium bromide staining. A 3K-bp DNA ladder (GeneRuler, Fermentas) was used as the molecular standard in order to confirm the appropriate RAPD markers. After washing of gel, the photograph was taken with UV transilluminator. For atcheiving DNA profile, DNA bands on gels were amalgamated using unweighted pair-group average method analysis (UPGAMA) using Statistica for windows, release 4.5f, state Soft, Inc.1993 software. RAPD bands were treated as binary characters and coded accordingly (presence = 1, absence = 0). Euclidean distances (similarity matrix) were used as the distance metric in both as well as dice coefficient as the calculation method. UV visualization, imaging and cluster analysis of the DNA bands were carried out using Quantity one 4.0.3 software of the gel doc.2000 system (BioRad).

3. Results and Discussion

3.1 Plant Growth and Biochemical Characterization

Recent different strategies have been employed to improve crop growth and development with using natural growth promoter like yeasts but very few studies have been carried out to compare the effect of yeast and GA3 applications on endogenous and molecular structures of plants. It is obvious from fig. (1) that exogenous application of GA3 and yeast increased the growth of seedlings of *Solanum melongena* L. compared with the control (untreatment plant), however GA3 application was more effective on plant length compared with yeast application. On the other hand chlorophyll "a" and chlorophyll "b" contents of plant were enhanced with yeast treatment where their content was 6.31 and 2.26 mg/g fresh weight compared with control, 5.68 and 2.11 mg/g fresh weight respectively. Surprisingly chlorophyll"a" and chlorophyll "b" contents were depressed at low concentrations 100 and 200 ppm of GA3 while increased at high concentration 400ppm (Table 1). Chlorophyll a content was enhanced by increase in GA3 concentration up to 250 mg L -1 in *Ficus benjamina* (Salehi sardoei et al., 2014). The obtained results are in agreement with what has been reported (EL-Ghamriny et al., 1999; Sarhan et al., 2011) that spraying bread yeast on the plants increasing the vegetative parameters of plants. Tartoura (2001) explained the role of yeast in increasing the vegetative growth of plants, it may be due to the content of yeast to many important which is necessary for plant biological processers especially photosynthesis and cell division. In addition to, yeast produce growth regulators such as auxin, gibberellin and cytokinin which stimulate biological processes in plants and led to an increase in the vegetative growth of the plant (EL-Ghamriny et al., 1999; Twfiq 2010; Tarek and Hassan 2014). Sarhan et al. (2011) revealed that the

growth and total chlorophyll of *Solanum melongena* L. was significantly increased with dry bread yeast. Recently, Nassar et al. (2016) revealed that leucaena plants grown under salt stress and treated with active yeast extract had better growth behavior than those untreated. Activities of antioxidant enzymes, catalase (CAT) and peroxidase (POX) were increased with increasing dose of GA3 and yeast application particularly with using GA3 (Table 1). In this regard, it can be suggested that exogenous applications of these treatments might enhance plant resistance to stress conditions by inducing antioxidant enzymes activity. The increase in CAT and POX activity in *Solanum melongena* L might lead to accumulation of toxic amount of H_2O_2. Erdal and Dumlupinar (2010) demonstrated that progesterone stimulated antioxidant enzyme activities in germinating seeds of chickpea, maize and bean. Yeast used in the current study was tested to produce IAA with or without addition of tryptophan as precursor of IAA, their productivity increased with addition of tryptophan (Fig. 2). According to Lyudmila et al. (2015) IAA production increased when medium was supplemented with the tryptophan. Recently, Liu et al. (2016) found that almost all *Saccharomyces* yeasts produced IAA when cultured in medium supplemented with the primary precursor L-tryptophan.

Figure 1. Seedlings of *Solanum melongena* L. treated with 6g/L yeast and 400ppm giberellic acid

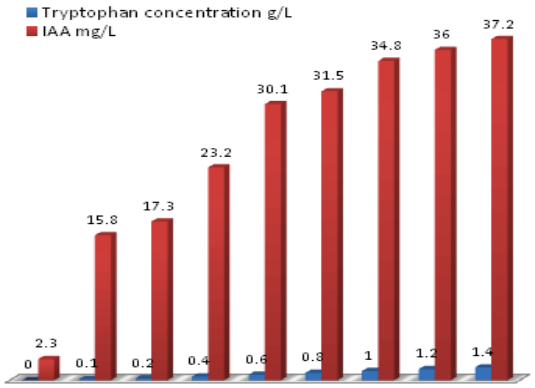

Figure 2. Effect of L-tryptophan concentration on IAA production by yeast.

Table 1. Chlorophyll content (mg/g fresh weight) and antioxidant enzyme of treated plant with different concentrations of gibberellic acid and yeast

Plant Treatment	Concentration	Chlorophyll contents (mg/g fresh weight)		Antioxidant enzymes (U/ml)	
		Chlorophyll "a"	Chlorophyll "b"	Catalase	Peroxidase
Control	0.0	5.68±0.09	2.11±0.32	19.02	0.79
Gibberilic acid	100ppm	4.78±0.10	1.96±0.12	20.87	0.90
Gibberilic acid	200ppm	4.59±0.11	1.82±0.05	21.86	0. 96
Gibberilic acid	400ppm	5.91±0.04	2.89±0.20	22.76	0.97
Yeast	2g/L	5.68±0.02	2.21±0.30	20.54	0.88
Yeast	4g/L	6.31±0.10	2.26±0.12	21.50	0.85
Yeast	6g/L	6.05±0.21	2.12±0.20	21.08	0.83

Salicylic acid (SA) is a phenolic phytohormone known for its primary function as an endogenous signal mediating plant defense responses against pathogens, as well as influencing responses to a biotic stresses and other important aspects of plant growth and development (Vlot et al., 2009). HPLC analysis chromatogram (Fig. 3) approves the presence of highest concentration of SA in plant treated with GA3 (104.209 mg) followed by yeast (70.00 mg) application compared with the untreated plant (57.868 mg). Muhammad and Muhammad (2013) who reported that the priming with GA3 was very effective in enhancing SA concentration in wheat cultivars. During recent years there has been increasing evidence on the role of SA in elicitation of plant defense mechanism in several a biotic stress conditions (Horvath et al., 2007), although information about the onset of defense mechanisms mediated by SA at the level of seed germination is very scarce (Rajjou et al., 2006). The accumulation of SA has been proposed as an endogenous marker for plant resistance (Klessig and Malamy, 1994).

It is clear that in yeast treatment of *Solanum melongena* L. plants, the resultant increase DNA, as well as RNA synthesis must be an important part of the growth response. While DNA and RNA were reduced with GA3 application and there is no difference in the detected amount of DNA and RNA with any concentration of GA3 (Table 2). Mady (2009) stated that yeast as a natural source of cytokinins-stimulates cell division and enlargement, synthesis of protein and nucleic acids. Also, Shalaby and El-Nady (2008) reported that the increase in photosynthetic pigments could be attributed to the role of yeast cytokinins in delaying the aging of leaves by reducing the degradation of chlorophyll and enhancing the protein and RNA synthesis. The present findings in case application of GA3 are generally in agreement with those reported by (Abdel-Hamid and Mohamed 2014) how found that DNA and RNA decreased from 133 to 132 and from 6.20 to 2.65 respectively in *Hordeum vulgare* as a result of GA3 application**.** It may therefore be suggested that yeast exerts its control over plant growth by controlling RNA and hence protein metabolism.

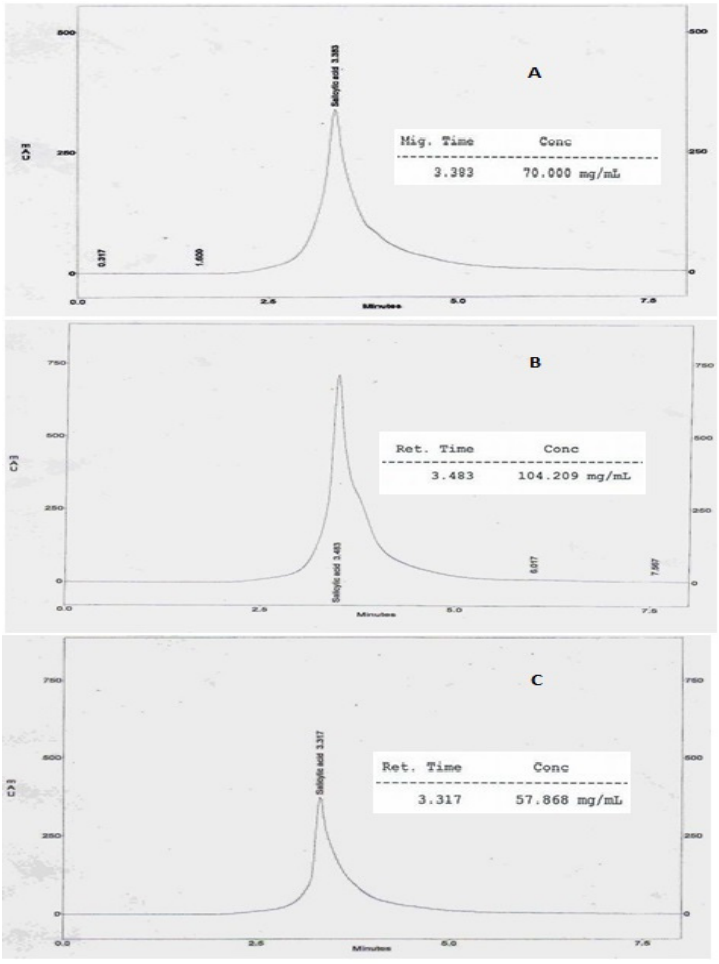

Figure 3. HPLC typical chromatogram of detected Salcylic acid in plant treated with 6g/L yeast (A), 400ppm gibberellic acid (B) and controlwithout treatment (C)

Table 2. DNA and RNA contents (µg g-1 dry weight) of *Solanum melongena* L. seedlings in response to Yeast and Gibberellic acid

Treatment		Nucleic acid content µg g-1 dry weight	
	Concentration	DNA	RNA
Control	0.0	50.56	2.70
Gibberilic acid	100 ppm	48.00	1.89
Gibberilic acid	200 ppm	48.60	1.50
Gibberilic acid	400 ppm	49.68	1.08
Yeast	2g/L	50.56	1.87
Yeast	4g/L	52.08	1.89
Yeast	6g/L	57.00	2.72

3.2 Changes in Protein Profiles

The electrophoretic pattern of *Solanum melongena* plants (Fig.4 and Table 3) was used in the present investigation to differentiate between the treatment with yeast and GA3. Six common polypeptide bands were observed in treated and untreated *S. melongena* plants, their molecular weights were 16, 17, 34, 90, 120 and 150 KDa. While the untreated control *S. melongena* plant is characterized by the presence of 8 polypeptide bands their molecular weights were 19, 24, 32, 33, 36, 50, 109 and 133 KDa. Yeast treatment increased the number of protein bands to 12 instead of 8 in the control plant with molecular weights 18, 125, 74, 69, 62, 31, 30, 27, 25, 23, 20 and 18 KDa. Three polypeptide bands with molecular weights 25, 72 and 125 KDa were detected in yeast and GA3 treated plants. Protein content in plants is an important indicator of reversible and irreversible changes in metabolism, therefore according to Dogra and Kaur (1994), these parameters are considered to be markers of growth and development. Stefanov et al. (1998) reported that sodium dodecyl sulfate polyacrylamide gel electrophoresis of soluble proteins revealed quantitative differences between treated plants with gibberellic acid. In the current study, the appearance of high molecular weight protein (125 KDa) in plant treated with yeast and gibberellic acid may be due to similar effect of this treatment. The SDS-electrophoretic pattern showed that one new band having molecular weight 77.5 KDa that appeared by the effect of biostimulants *Rhizobium* with Phosphobacteria treated seeds of *Vigna mungo* L (Selvakumar et al., 2012). Shehata and EL-Khawas (2003) stated that treated sunflower plant with biostimulant stimulate new protein with low molecular weight 2.1 Kda

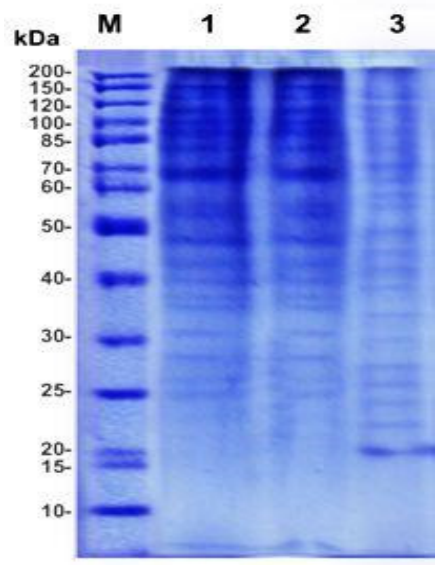

Figure 4. Protein electrophoresis of *Solanum melongena* plant after 26 days from sowing. M, Lane Marker represents Genei molecular marker (200–10 kDa); 1 untreated plant; 2 treated with 6g/L yeast; 3 treated with 400 ppm gibberellic acid

Table 3. Molecular weight (KDa) of separated protein in of *Solanum melongena* plant treated with 400 ppm gibberellic acid and 6g/L yeast after 26 days from sowing

BANDS (KDa)	Control	Yeast	GA3	BANDS (KDa)	Control	Yeast	GA3
155KD	-	-	-	40KD	-	-	-
150KD	+	+	+	39KD	+	+	-
142KD	-	+	-	38KD	-	-	-
133KD	+	-	-	37KD	-	-	+
125KD	-	+	+	36KD	+	-	-
120KD	+	+	+	35KD	-	-	-
109KD	+	-	-	34KD	+	+	+
104KD	+	+	-	33KD	+	-	-
100KD	-	-	+	32KD	+	-	-
93KD	-	-	-	31KD	-	+	-
90KD	+	+	+	30KD	-	+	-
85KD	-	-	+	29KD	+	+	+
80KD	+	+	-	28KD	-	-	+
78KD	-	-	+	27KD	-	+	-
74KD	-	+	-	26KD	-	-	-
72KD	-	+	+	25KD	-	+	+
69KD	-	+	-	24KD	+	-	-
66KD	-	-	+	23KD	-	+	-
62KD	-	+	+	22KD	-	-	+
60KD	-	-	+	21KD	-	-	-
57KD	+	+	-	20KD	-	+	-
56KD	-	-	-	19KD	+	-	-
54KD	-	-	+	18KD	-	+	-
52KD	+	+	-	17KD	+	+	+
51KD	-	-	+	16KD	+	+	+
50KD	+	-	-	15KD	+	-	+
49KD	-	+	-	14KD	+	-	-
48KD	-	+	+	13KD	+	-	-
46KD	-	-	-	12KD	+	+	-
45KD	+	+	+	11KD	+	+	+
43KD	-	+	-	10KD	-	+	-
42KD	-	+	+	9KD	-	+	+
41KD	-	-	-	7KD	+	-	-

3.3 RAPD- PCR Analysis of Plant Treated with Yeast and Gibberellic Acid

Plants sprayed with yeast extract and gibberellic acid showed morphological and/or physiological changes when compared to control samples. These changes might be a result of physiological effect or genetic modifications. Hence, Random amplified polymorphic DNA analysis were carried out for control (untreated) and treated plants with 6 g/L yeast and gibberellic acid (Fig.5 and Table 4). DNA was extracted and purified from the samples and their amplification was carried out using Single primer 3: (5'-GTAGACCCGT-3'). Total of 16 amplified fragments were visualized in the tested samples. Eight fragments with different molecular weights, four of them are monomorphic bands while the others are polymorphic unique bands. Monomorphic fragments could be plant markers. Control sample produced 7 fragments with molecular weights range from 426 bp to 3063 bp. Three of these are polymorphic unique fragments that don't show up in the treated samples. Plant sample sprayed with yeast showed 5 fragments range in molecular weight between 426 to 1766 bp. Only one of these fragments was unique polymorphic fragment. Four monomorphic fragments range in molecular weight from 426 to 1213 bp were showed up in plant sample sprayed with gibberellic acid. Generally in all cases, polymorphisms were due to the loss and/or gain of amplified bands in the treated samples compared with the control. DNA polymorphisms can become useful markers in general fingerprinting (Kawakami et al., 1999; Shehata & EL-Khawas, 2003). Also, Yang and Quiros (1993) postulated that quantitative changes could be explained on the basis of alterations of some DNA sequences. Increases in SA levels have been correlated to changes in gene expression. Precisely how SAR leads to resistance is not completely understood, but several of the pathogenesis-related genes (PR genes) expressed during the development of resistance are antagonistic to pathogens (Navarrea & Mayoa, 2004). In the present study, the appearance and disappearance of some bands may be attributed to the structural genes.

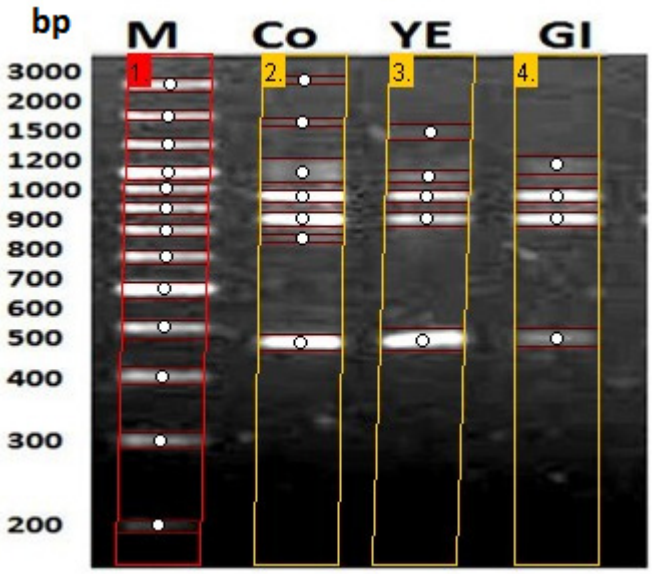

Figure 5. PCR-amplification Lane 1: Marker; Lane 2: control plant ; Lane 3: plant treated with 6g/L yeast; Lane 4: plant treated with 400 ppm gibberellic acid

Table 4. Amplification products polymorphism of *Solanum melongena* L. treated with yeast and giberellic acid

MW	Control	Yeast	Giberellic acid	Polymorphism
3063	+	-	-	poly-unique
1953	+	-	-	poly-unique
1766	-	+	-	poly-unique
1213	+	+	+	mono
972	+	+	+	mono
813	+	+	+	mono
709	+	-	-	poly-unique
426	+	+	+	mono

4. Conclusion

From the above mentioned results it can be concluded that, the application of the biostimulants has been effected on several metabolic processes, enhances plant growth and development via the increasing of photosynthesis, endogenous hormones salicylic acid. Similar proteins bands with the same molecular weights were detected with yeast and gibberellic acid treatments; this indicates the yeast plays the same role of gibberellic acid in plant development. PCR showed16 amplified fragments visualized in the tested samples. Eight fragments with different molecular weights, four of them are monomorphic bands while the others are polymorphic unique bands. Monomorphic fragments could be plant markers. Control sample produced 7 fragments with molecular weights range from 426 bp to 3063 bp. Three of these are polymorphic unique fragments that don't show up in the treated samples.

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To King Abdulaziz university, Jeddah, Saudi Arabia and to Regional Center of Mycology and Biotechnology, Al Azhar University, Cairo, Egypt.

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