Effect of *Spirulina platensis* extract on growth potential of *in vitro* culture pear

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Abstract. Pears are among the most economically important fruits in the world that are grown in all temperate zones. 'Le-Cont' rootstock pear is one of the gene sources used to improve fruit productivity, rootstock resistance, and tolerance to biotic and abiotic stresses. Traditional propagation of pear. Pear is time-consuming and limited by a short growing season and hard winter conditions. Therefore*, in vitro* propagation is a suitable alternative method. Murashige and Skoog medium (MS) and *spirulina platensis* extract at 5,10 and 20 % supplemented with different concentrations (0.25, 0.5 and 1.0 mg/l) of 6-benzyladenine (BA) and kinetin (Kin), individually or in combination with them. Treatments were used for *in vitro* shoot proliferation. Nodal segments were used as explants. MS medium augmented was 3mg/l 6-benzyladenine (BA) plus 20 % Spirulina platensis extraction then used for shootlets proliferation of micro-shoots. A combination of 3 mg/l BA and 20 % Spirulina platensis as a growth media resulted in a significant improvement in shoot proliferation. This combination produced the highest number of shoots (2.8 per explant) and leaves (6.3 per explant) similar with those containing MS media plus 20% extraction (2.9 per explant) and leaves (6.8 per explant). The longest shoots (2.97 cm) were obtained in each previous treatment. However, these shoots were similar with those produced from classical multiplication by MS according to ISSR analysis which scored 89.1 % of mono morphism percentage and 10.1 % polymorphism. The ISSR analysis shows the highest similarity index percentage for P5-P9 0.986301.

1 Introduction

Pear (*Pyrus*) is the third most important temperate fruit in world production, next to grapes and apple, and can be consumed fresh as well as cooked, dried or preserved. Moreover, *Pyrus communis* L considered as the most valuable and compatible rootstock of pear under Egyptian conditions. It can grow in different kind of soils.

The genus Pyrus belongs to the subfamily maloideae of the rosaceae. Pear is considered the most important temperate fruit after grapes and apple in the world. Two main species,

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which are genetically and morphologically different, *Pyrus communis* L., the European pear, which is grown in Europe and America, and *Pyrus pyrifolia* Nakai, the Asian pear or Nashi, which is grown traditionally in Japan, China, Korea and Taiwan, and incrpasingly in Europe and America [11].

Propagation of pears is possible by vegetative means, i.e., cutting, layering, and tissue culture techniques. Among these methods, in vitro propagation allows fruit breeders to quickly multiply a new rootstock in a short time. Micro propagation helps overcome limitations found with traditional propagation, such as variability and seasonal availability of raw materials or fresh material losses [19]. So, the technique of commercial *in vitro* can be employed for micro propagation of pear. Genetic improvements of pear cultivars are possible through two approaches: one is to exploit pre-existing or induced mutations resulting in genetic variability in somatic cells and the other is that of genetic engineering or gene isolation and transfer [16].

The agricultural applications of microalgae have long focused on their use as bio fertilisers and soil conditioners whose effects on crops are mainly attributable to the improvement of physical, chemical, and biological soil fertility [10]. However, in recent years, numerous studies have shown that the variety of physiological responses in plants following the application of these microbial biomasses cannot solely be attributed to the increases in the nutrients bioactive molecules (e.g., phytohormones, amino acids, vitamins, polysaccharides, carbohydrates, polyamines, polyphenols) that are effective on plants at concentrations considerably lower than those of the microelements (such as nitrogen, phosphorus, and potassium) contained in bio fertilizers [16]. The ability of microalgae to produce these bioactive molecules, which plants can absorb and metabolise both foliar and through the root, and the possibility of improving crop productivity using very small quantities of the product compared to bio fertilizers has led the scientific community and companies to take an interest in studying the bio stimulant properties of these microalgae [30]. However, large gaps still exist in fundamental methodologies, including efficient protocols for re-generating plants from matured tissues, especially leaves, of an existing pear cultivar, which could ensure that their genotype is identical to that of the parental material. Most of the previous works on plant regeneration from leaves of pears focused on *P. communis* [26-27].

The natural extracts also contain some important amino acids, proteins, carbohydrates, lipids and primary and secondary products which positively influence the growth of micro propagules under *in vitro* conditions. The artificial MS medium, which are mostly used in plant tissue culture, contain all the essential inorganic and organic salt, chelating agents, carbon source, growth regulators and water. But the exact nutritional requirements differ from plant to plant and within the same plant differs from cell to cell. The natural plant extract, which are produced through many complex metabolic pathways, contains several metabolites that may act as potent growth enhancers, which a synthetic medium is completely lacking [8].

The green algae have many nutritional values because its content of a wide range of essential nutrients, such as provitamin, minerals, proteins, polyunsaturated fatty acids like gamma linolenic acid and sulphated polysaccharides [28, 25].

The aim of the research was to use *Spirulina platensis* extract as natural extract for the laboratory propagation of pears and study their impact on growth, chemical content and genetic structure to come up with a suitable growth protocol based on natural materials of *in vitro* culture pear

2 Material and methods

2.1 Preparation explants

At actively growing, the micro nodes of Pear (*Pyrus communis* L) were collected at 3-4 months old and disease-free plant from the Experimental Tropical Fruits Department at the Horticulture Researches Institute, Agricultural Researches Centre, Egypt; during 2022-2023 growth season. The cuttings were washed thoroughly in tap water, soaked in water with a few drops of liquid soap for 1 h, and finally rinsed with sterile distilled water. Then, the washed cuttings were surface-sterilized by immersion in 70% (v/v) ethanol (Al-Nassr Chemical Co., Egypt) for 60 s, followed by (10 and 20) min in (20 and 10%) (v/v) commercial bleach (40 g L−1 active chlorine content) with 0.05% (v/v) and /or $HgCl_2$ at (0.1 or 0.2 %) for (10 or 5 min) with few drops of Tween-20® (Merck KGaA, Darmstadt, Germany). Finally, the cuttings were rinsed 5 times with sterile distilled water. Then, explants of about 20 mm in length were vertically cultured in 25 × 150 mm glass jars with polypropylene caps used to seal the jars. Media and growth regulators employed in this work were purchased from Duchefa Biochemie (Haarlem, Netherlands). The pH of all media was adjusted to 5.75–5.8 with 0.1 M NaOH or 0.1 M HCl before autoclaving for 20 min at 121 ◦C

2.2 Media alternatively compounds

2.2.1 Source of spirulina platensis

The strain was maintained in 500 mL sterilized Erlenmeyer flasks containing 100 mL Zarrouk's medium [40] at 30 ± 2 °C, pH 9 with continuous illumination using cool white fluorescent tubes (2500 Lux) and twice daily shaking by hand.

2.2.2 Isolation of Spirulina platensis

The culture would be picked up from the stock culture with the help of needle and transferred to petri plates and culture tube containing Zarrouk's medium and incubated at 28^0C for 30 days with (600 – 1600 lux) with a continuous light, 12 hrs per day [35].

2.2.3 Optimal concentrations of alternatives

The green algae *Spirulina platensis* was drying and powdered in electronic blinder, the powder weighted at 0.5, 1.0 and 2.0 g/l and adding to the media as showing:
0.5 g/l *Spirulina platensis* powder + agar + 25 g/l sucrose
1.0 g/l *Spirulina platensis* powder + agar + 25 g/l sucrose
2.0 g/l *Spirulina platensis* powder + agar + 25 g/l sucrose

The mixture of algae was dissolved in distilled water with 25 g/l sucrose and stirring with electronic stir to complete homogenate, adjust pH at 5.7 and adding 7 g/l agar. The filtrate of green algae was prepared by boiling optimum weight (5, 10 and 15 %) of algae in 100 ml distilled water at 100 º C for 30 min. and filtered with Wittman filter paper No.1. The filtrate was diluted on culture media at 2 ml/l of 5%, 4 ml/l of 10% and 6 ml/l of 15%. After complete cooked all treatments we dispensed it in 200 ml glass jars and autoclaved at 121ºC with 1.12 bar for 20 min.

2.2.4 Combination of algae with MS salts

The optimum weight of dry matter (2 g/l) of green algae was added to the MS (Murashige and Skoog, 1962) salts at combination with the following:

Dry matter 2 g/l green algae
Dry matter 2 g/l green algae + Sucrose
Dry matter 2 g/l green algae + Macronutrients + Sucrose
Dry matter 2 g/l green algae + Micronutrients + Sucrose
Dry matter 2 g/l green algae + Vitamins + Sucrose
Dry matter 2 g/l green algae + MS + Sucrose

2.2.5 Data and parameters tested

a. Morphological characteristics shoot lets for multiplication stage

The culture in this stage was incubated for one month and the parameters were determined:

- Survival percentage = [No. of survived explants / total number of cultured explants] X 100
- Number of shoots = calculated as the number of developed shoots per each explants.
- Shoot length (cm): summation of shoots length as cm / number of shoots.
- Number of leaves per each shoots

b.Chemical analysis

1. Total indoles

The total indoles contents were determined in the methanol extract, using P-dimethyl amino benzaldehyde test "Erlic's reagent" according to Selim *et al*. (1972) [33]

2. Total soluble phenols

Folin – Ciocaltea reagent [1] Optical densities of these samples were measured by colorimeter using wave length 730 nm. Concentrations of total phenols in different samples were calculated in extracted tissue by the application of standard curve. This method was conducted according to Williams *et al.* (1965) [5].

3. Total soluble carbohydrates

Total carbohydrates were determined by using the sulphuric acid with phenol method according to Dubois *et al*. (1956) [12].

4. Total antioxidants

The free radical scavenging activity of methanol extracts was measured by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) using the methods described by Band-Williams *et al*. (1995) [5]. The absorbance was measured at 517nm. Antioxidant activity % = [(Control OD-Sample OD)/ControlOD] x 100

5. Photosynthetic pigments (Ch-A, Ch-B and total carotenoids)

The pigments of chlorophyll-a, chlorophyll-b and carotenoids were determined quantitively as mg/100g F.W. according to the procedure achieved by Saric *et al.* (1967) [32], the colour density was spectrophotometric ally measured at wavelengths of 660,640 and 440 nm for chlorophyll-a, chlorophyll-b and total carotenoids.

6-Genetic evaluation

2.2.6 Isozymes electrophoresis

Native-polyacrylamide gel electrophoresis (Native-PAGE) was conducted to identify isozyme variations among studied *in vitro* explants using two isozyme systems according to Stegemann *et al*. (1985) [37].

2.2.7 Inter simple sequence repeats (ISSRs) procedure

PCR reaction was conducted using 9 primers. Their names and sequences are shown in Table (1).

Table 1. List of the primer names and their nucleotide sequences used in the study for ISSR procedure

No	Primer code	Sequences
1	UBC-825	GACTGCGTACGAATTAAC
2	UBC-809	TGAGTCCAAACCGGAAG
3	UBC-811	GACTGCGTACGAATTGAC
4	UBC-817	TGAGTCCAAACCGGAAT
5	UBC-864	TGAGTCCAAACCGGACC
6	UBC-812	GACTGCGTACGAATTTGA
7	UBC-880	AGA GAG AGA GAG AGAGG
8	HB-15	GAG AGA GAG AGA GAGAA
9	HB-13	GGAGAGGAGAGGAGA

2.2.8 Statistical Analysis

All data obtained during both seasons were subjected to analysis of variance and significant differences among means were determined according to [36].

3 Results and Discussion

3.1. Effect of media salts and spirulina extract concentrations on contamination and browning and survival percentage

Table 2. Effect of spirulina platensis extraction on contamination and browning and survival percentage comparing with MS media free of pear explants

	Media components sources	Contamination %	Browning %	Survival %
MS media	Colorex 20 for 10 min	25.0	50.0	100.0
	Colorex 10 for 20 min	12.5	33.3	100.0
	HgCl2 0.1 % for 10 min	33.33	12.5	50.00
	HgCl20.2 % for 5 min	6.66	12.5	50.00
	LSD 5 %	6.271	10.655	10.233
MS+20%	Colorex 20 for 10	33.33	25.0	100.0
	Colorex 10 for 20	12.50	25.0	100.0
	HgCl2 0.1 % for 10	11.11	12.5	75.00
	HgCl20.2 % for 5 min	6.66	12.5	75.00
MS+10%	Colorex 20 for 10	100	0.00	0.00
	Colorex 10 for 20	100	0.00	0.00
	HgCl2 0.1 % for 10	100	0.00	0.00
	HgCl20.2 % for 5 min	100	0.00	0.00
	LSD 5 %	2.66	2.91	2.88

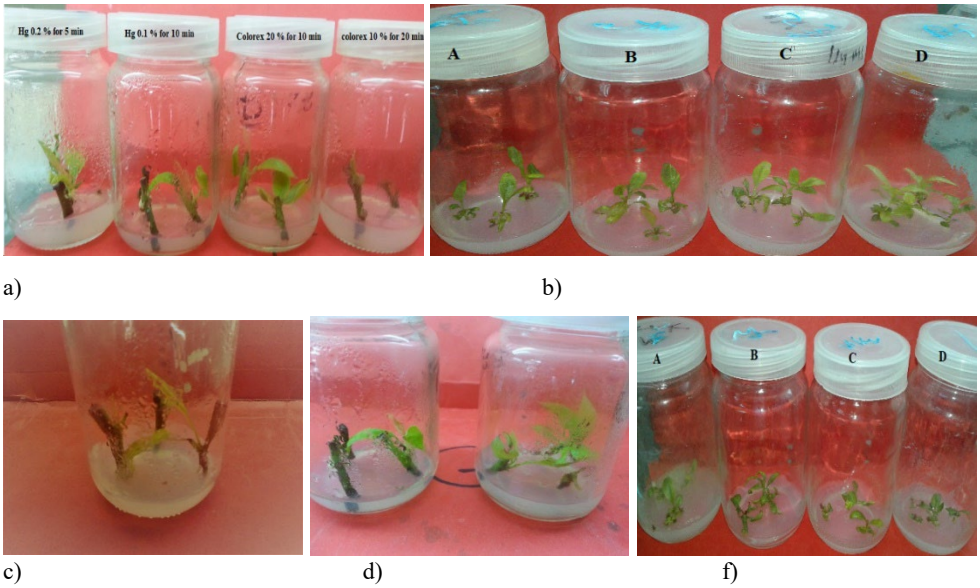

Fig. 1.

The successful explants free of pathogens were cultured on four salts concentration of MS medium and combined at three algae concentrations were illustrated in Table 2. The data showed that the explants cultured on MS medium full strength gave the best results of the survival percentage (100 %). Also, browning percentage 0.0 % for the explants cultured on MS media these results gave the highest sprouting of buds (100 %). On the other hand, spirulina individual concentrations without MS salts scored survival 100 % and 90 % for each 5, 10 and 20 %, respectively with sprouting buds 66 85 and 75 %, respectively. Moreover, applying spirulina extract individual and cultured the explants on it increased browning in the media 25 % of cultured explants scored browning followed by 50 % for 5, 10 and 20 % extractions.

Table 3. Effect of media alternatives components treatments on bud sprouting, browning and survival percentage of pear by in vitro culture

Treatments	Survival %	Browning %	Bud sprouting %
MS Full	100.0	0.00	100.0
MS 3/4	75.00	0.00	100.0
MS 1/2	50.00	0.00	75.00
MS 1/4	50.00	0.00	75.00
Spirulina 50 ml/l without MS	100.0	25.00	66.66
Spirulina 100 ml/l without MS	100.0	50.00	85.00
Spirulina 200 ml/l without MS	90.00	50.00	75.00
LSD 5 %	12.545	11.357	13.578

The data are in agreement with findings of [29] they found that the highest establishment in *Pyrus Patharnakh* (81.76%) and Kainth (78.66%) resulted by using Murashige and Skoog medium. Moreover, [9] compared between the effect of various media (MS 1/2 strength, MS full strength and WPM full strength) and they found MS full strength medium containing BAP (1.5 mg-1) and IBA (0.25 mg-1) gave maximum explant establishment (52.80%) on *Pyrus pashia*.

3 Optimization concentration of spirulina extract

3.1 Optimization with MS, sucrose and BAP 3 mg/l

The successful explants produced from the previous treatments were cultured on three concentrations of extract added to MS medium and combined at sucrose and BAP at 3 mg/l were illustrated in Table 3. From the study, all spirulina combined with 3 mg/l BAP with sucrose increased growth gradually. The highest level of survival was 80 % for 20 % spirulina extraction with sucrose decreased to 78 and 75 % with decrease extraction concentration 10 and 5 %, respectively. Meanwhile, applying algae extraction with MS plus BAP at 3 mg/l gave the same effects which scored 75, 74 and 55 % survival, respectively. the same effects were observed on bud sprouting after exposed explants to the same concentrations of algae extract plus BAP and glucose which scored 83, 80 and 75 % for the concentrations 20, 10 and 5%, respectively. In the observation of growth, the number of shoots development on the base of explants for that cultured on MS plus extraction and BAP gave good growth of shootlets no. shootlets length and leaves number compared with extraction without MS. The shootlets number gave the highest count (2.9 shoot let/explant) for 20 % extract plus MS and BAP, followed by for the same treatment without MS (2.8 shootlet/explant).

Table 4. Effect of spirulina extraction concentrations combined with MS, BAP 3 mg/l and sucrose on some growth characters of pears

Treatments	Survival %	Bud sprouting	Shoot No.	Shoot length	Leaves No.
Spirulina 5 % + MS Free	30.00	45.00	0.7	0.56	3.1
Spirulina 5 % + MS + BAP 3 mg/l	55.00	75.00	2.2	0.76	3.4
Spirulina 10 % + MS Free	33.33	65.00	1.5	1.23	2.3
Spirulina 10 % + MS + BAP 3 mg/l	74.33	72.00	2.1	2.69	3.3
Spirulina 20 % + MS Free	35.00	40.00	1.8	1.77	2.4
Spirulina 20 % + MS + BAP 3 mg/l	75.00	73.33	2.9	2.97	6.8
Spirulina 5 % + Sucrose	55.00	25.00	2.2	1.22	2.7
Spirulina 10 % + Sucrose	40.00	35.00	2.1	0.77	3.3
Spirulina 20 % + Sucrose	30.00	35.00	1.0	0.88	3.0
Spirulina 5 % + BAP 3 mg/l + sucrose	75.00	75.00	1.6	1.62	4.6
Spirulina 10 % + BAP 3 mg/l + sucrose	78.00	80.00	1.6	1.96	5.4
Spirulina 20 % + BAP 3 mg/l + sucrose	80.00	83.00	2.8	2.97	6.3
LSD 5%	3.126	2.391	0.832	0.432	0.261

As shown in table (4) and(5)

The addition of 20 % extract to MS media plus BAP at 3 mg/l also induced shoot let elongation to the highest length 2.97 cm for each treatment followed by 10% extract plus MS media and BAP at 3 mg/l (2.96 cm) compared with 5% extract plus MS free (control) (0.56 cm). The other growth character that is leaves number was increased to the maximum number 6.8 leaves/shootlets for the explant cultured on 20 % extract plus MS media containing 3 mg/l BAP, following by the same treatments but without MS which scored 6.3 leaves/shootlets.

3.2 Interaction with plant growth regulator (PGRs) and spirulina

Effect of interaction between spirulina extraction at 20 % (the best concentration for growth) and some PGRs on shootlets elongation and production were observed in Table 3 which showed that applying BAP at 1.0 mg/l gave the maximum length of explants 1.44 cm compared to control (0.55 cm). In addition, BAP at 1.0 mg/l increased the leaves number to 6,31 leaf/shootlets followed by BAP at 0.5 mg/l 6.25 leaf/shootlet in comparison to control (4.22 leaf/shootlet). Similarly, shootlets production increased to 3.2 shootlets/shoot for the explants cultured on extraction media containing BAP 1.0 mg/l. On the other hand, BAP (1 mg/l) gave a positive effect of shoot number, shootlet length and leaves number by increased the level to (5.66 shootlets/shoot, 2.58 cm and leaves number 11.78 leaves/shootlets) compared with control gave an adverse effect on shoot number, shootlet length and leaves number which decreased the values to (1.11 shootlet/shoot, 1.64 cm and 9.59 leaf/shootlet) respectively.

Table 5. Effect of BAP and kinitin concentrations on some growth characters of pear le-cont *in vitro* culture

Media	**MS**			**Spirulina 20 %**		
PGRs mg/l	**Shoot No.**	**Shoot length**	**Leaves No.**	**Shoot No.**	**Shoot length**	**Leaves No.**
Control	1.11	1.64	9.59	1.0	0.54	4.22
BAP 1.0	5.66	2.58	11.78	3.2	1.44	6.31
BAP 0.5	3.94	2.26	11.44	2.2	1.36	6.25
BAP 0.25	2.0	2.51	11.44	1.5	0.98	5.12
Kin 1.0	1.0	1.55	9.83	1.3	1.2	6.21
Kin 0.5	1.0	1.46	9.389	1.5	1.1	5.35
Kin 0.25	1.0	1.23	8.056	1.4	1.2	6.01
LSD 5%	1.921	0.826	2.115	1.025	0.645	0.954

These results are in harmony with findings of Ahmadi *et al.,* (2021) [3] who found that multiplication rate, length, and the number of shoots in plant micropropagation depend, among other factors, on the type of culture medium, the concentration of PGRs, and the cultivar. In the present experiment, a combination of 1 mg/L BA and 20% extraction as a medium was the most effective for shoot multiplication of *P. communis*. Nonetheless, studies on the simultaneous effect of these PGRs on *in vitro* organogenesis are scarce. Generally, BA is the cytokinin of choice for the *in vitro* propagation of many species and cultivars in the genus Pyrus [31]. Aygun and Dumanoglu (2015) [4] reported that the in vitro shoot proliferation of *P. elaeagrifolia* was affected by BA concentration. Similar findings were reported by several other researchers, who used BA at concentrations ranging from 1 to 5 mg/l [39]. In *P. pashia*, the number of shoots increased with the increase in BA level up to 2 mg/l ; however, further elevation of BA levels reduced the number of shoots produced [13]. In *P. communis* 'Bartlett', the highest number of shoots (3.87) was obtained with 2.40 mg/l BA [38]. On the other hand, the pear 'Conference' had the highest shoot proliferation rate with 1 or 2 mg/l BA [27-28]. The optimum level of PGRs for maximum shoot proliferation is different in each species and explant type [2, 19]. For example, successful shoot growth, proliferation, and establishment using 1.5 mg/l BA were reported in Pyracantha coccinea [11]. This might be due to the different content of endogenous phytohormones in plant cells and tissues [19].

4 Chemical analysis

Table 6. Effect of stevia extract on phytochemical analysis of pear (pyrus commenus)

Treatments	T. Phenols	T. Indoles	T. Flavonoids	Carbhy.	Antioxid.	Ch A	Ch B	Caroteno id.
Spirulina 20 % + Agar	0.11	0.35	22.35	0.46	0.542	0.29	0.028	0.012
Spirulina 20 % + Sucrose	0.31	0.54	45.75	0.42	0.112	0.27	0.021	0.013
Spirulina 20 % + MS Free	0.18	0.25	65.57	0.71	0.313	0.21	0.031	0.011
Spirulina 20 % + BAP 1 mg/l	0.09	0.56	13.34	0.71	0.091	0.21	0.023	0.016
Spirulina 20 % + MS + BAP 1 mg/l	0.26	0.43	32.21	0.61	0.421	0.27	0.023	0.02
LSD 5%	0.061	0.32	1.071	0.023	0.18	0.025	0.021	0.0103

1. Total soluble phenols
The data in Table (6) showed that total phenol decreased with applying spirulina 20% with BAP 1 mg/l to lowest level 0.09 % while this level increased to 0.31 % with the extraction plus sucrose.
2. Total soluble indoles
According to the data in table (6) indoles increased to the highest level 0.56 % for the explants cultured on extraction 20 % plus BAP 1 mg/l followed by the same concentration of extract plus sucrose (0.54 %).
3. Total flavonoids
Applying extraction with MS raised flavonoids contents to the maximum level 65.57 % followed by those exposed to extraction plus sucrose 45.75 % compared with the same treatment with BAP 1 mg/l.
4. Total antioxidants
The antioxidants increased to the maximum level 0.542 % as shown in Table (6) for the explants cultured on extraction 20 % plus agar followed by the same concentration plus MS and BAP at 1 mg/l (0.421%) compared with extraction plus BAP 1 mg/l (0.091 %).
5. Total carbohydrate
The total carbohydrates contents in the explants cultured in all treatments gave a non-significant effect.
6. Total antioxidants
The antioxidants increased to the maximum level 0.542 % as shown in Table (5) for the explants cultured on extraction 20 % plus agar followed by the same concentration plus MS and BAP at 1 mg/l (0.421%) compared with extraction plus BAP 1 mg/l (0.091 %).
7. Total carbohydrate
The total carbohydrates contents in the explants cultured in all treatments gave a non significant effect.
8.The photosynthetic pigments
The data in Table (6) showed non-significantly effect of all treatments for chlorophyll-a, chlorophyll-b and total carotenoids.

The content of total phenols, total indoles, total flavonoids and total antioxidants in explants *cultured on MS media and spirulina extraction* were similar of their chemical contents. Hassimott *et al*. (2005) evaluated the antioxidant activity of different frozen fruits and vegetables in Brazil, reporting that commercial pulp of *A. muricata* contains 120 ± 8 mg of gallic acid /100 g FW [18]. Formagio *et al*. (2013) reported that methanol extract of *Annona dioica* leaves had high levels of total phenols and flavonoids with values of 187.77

mg/g and 733.20 mg/g of dry plant, respectively [14]. On the other hand, Luján-Hidalgo *et al.* (2015) [21] reported the content of total phenols and flavonoids in a range of concentrations from 94.9-193.1 GAE mg/g dw and 29.39 to 44.17 QE mg/g dw, in plants of *A. purpurea* cultivated in the soil and fertilized with vermicomposting and rock phosphoric. The difference obtained in the concentration of the metabolites produced by *in vitro* culture is due to the fact that in plant cell cultures, substances are not always produced qualitatively and quantitatively equal to those elaborated by the mother plants. The production and the profile of chemical compounds can be unstable, because within the whole plant the plant cells have a different biochemical and physiological environment than the cells that grow in culture media [23].

5 Genetic variation

5.1 ISSR studies

ISSR markers are the direct reflection of abundances and distribution of microsatellite repeat in the genome. Out of 25 screened ISSR primers, 9 anchored ISSR primers produced 74 clear bands with two DNA samples of micropropagated plantlets cultured on extraction 20% plus 1 mg/l BAP and control cultured on MS (Fig.2). The number of amplified fragments varied from 3 to 11, with an average of 7.30 bands per primer (Table 7). Among 74 bands, 8 bands were polymorphic and the average polymorphic percentage was 10.8 % across all the plantlets. Highest percentage of polymorphism (33.3 %) was found with the primer numbers HB-13 followed by UBC811 and UBC864 which scored (23.0%) and lowest percentage (0 %) was found all other primers.

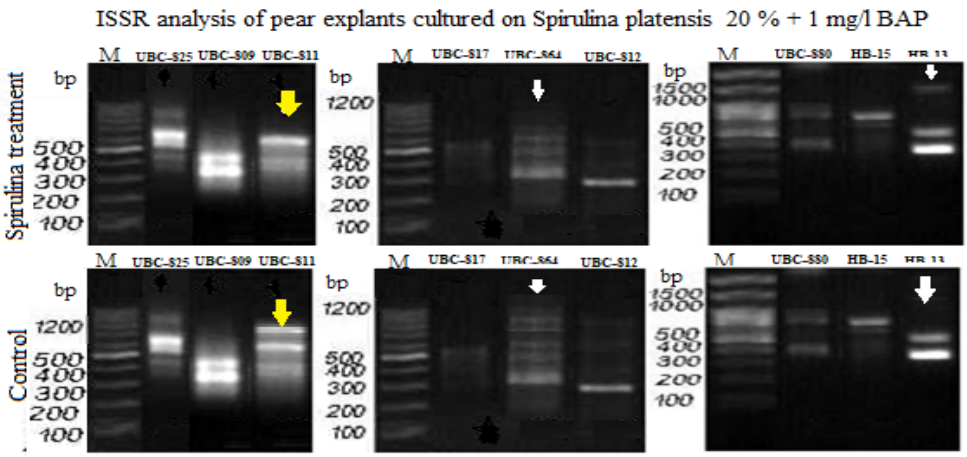

Fig. 2. ISSR Products of two types cultured pear amplified along with ISSR marker. A ISSR Primer-825. B ISSR Primer- 809. C ISSR Primer-811. D ISSR Primer-817. E ISSR Primer-864. F ISSR Primer-812. G ISSR primer -880. F. ISSR primer-HB15. I. ISSR primer-HB-13.

Table 7. ISSR primers with their structures and type of band

SiNO.	Primer code	sequence	Total bands	Total poly bands	Total mono bands	MP %
P1	HBC-825	GACTGCGTACGAATTAAC	10	0	10	0
P2	HBC -	TGAGTCCAAACCGGAAG	4	0	4	0
P3	809	GACTGCGTACGAATTGAC	13	3	10	23.1
P4	HBC -	TGAGTCCAAACCGGAAT	6	0	6	0
P5	811	TGAGTCCAAACCGGACC	13	3	10	23.1
P6	HBC -	GACTGCGTACGAATTTGA	8	0	8	0
P7	817	AGA GAG AGA GAG AGAGG	8	0	8	0
P8	HBC -	GAG AGA GAG AGA GAGAA	6	0	6	0
P9	864	GGAGAGGAGAGGAGA	6	2	4	33.3
	HBC - 812					
	HBC - 880					
	HB-15					
	HB-13					
	total		74	8	66	89.1

Shoots were similar with those produced from classical multiplication by MS according to ISSR analysis which scored 89.1 % of monomorphic percentage and 10.1 % polymorphism. The ISSR analysis shows the highest similarity index percentage for P5-P9 0.986301.

Table 8. Similarity matrix of two culture types of in vitro plantlets cultured on 20 % spirulina extract and MS plant of Pear

	P1	P2	P3	P4	P5	P6	P7	P8	P9
P1	1.000000				1.000000				
P2	0.643836	1.000000			0.76712	1.00000			
P3	0.616438	0.616438	1.000000		3	0			
P4	0.753425	0.726027	0.534247	1.000000	0.73972	0.68493			
P5	0.835616	0.671233	0.808219	0.534247	6	2			
P6	0.328767	0.712329	0.136986	0.821918	0.49315	0.35616			
P7	0.808219	0.739726	0.739726	0.657534	1	4	1.000000		
P8	0.657534	0.767123	0.520548	0.630137	0.98630	0.54794	0.287671	1.000000	
P9	0.273973	0.205479	0.410959	0.434521	1	5	0.438356	0.164384	1.000000

The genetic similarities among two types of culture of *pear* were estimated according to the ISSR data. Jaccard's coefficient showed that there were two closely related accessions, i.e. P5-P9 with the highest similarity index 0.986301, and two distance-related accessions, i.e. P3-P6 with the lowest similarity index 0.1369863 respectively (Table 8).

In contrast, genetic instability occurs in the in vitro regenerated plants (somaclonal variation) due to use of hyper-optimum potency of growth regulators and continuous sub culturing. ISSR marker was used in order to detect genetic stability of in vitro plantlets cultured two culture media types. Allelic composition of tissue culture raised plantlets on MS control and those cultured on 20 % extraction were monomorphic. This result suggested no somaclonal variation in pear, which interfered with the integrity of the regenerated clonal plantlets. Somaclonal variation amid in vitro propagation may occur from pre-existing variations, the type of explant accustomed, the concentration and type of growth regulator in the medium, the number and duration of subcultures, effect of stress, genotype and the method of propagation espoused. The concentration of synthetic plant

growth regulators in the medium is also associated with somaclonal variation and some nutrient extraction [22]. Moreover, the preparation of many explants from only one donor plant augments the chances of variation in cultures [20].

The results of the ISSR marker system in the present study revealed no genetic variability among the in vitro culture plantlets of pear PCR contours of explants cultured on two types of media for 9 ISSR primers exhibit monomorphic among two explants treated with MS individual or 20 % extraction. Estimated clonal variation among the Gerbera plants by using ISSR marker regenerated from leaf explants [7]. The exact cause of variation might be due to explant source or due to mode of plant regeneration [16], media composition, or culture conditions and sub- or supra-optimal levels of plant phytohormones [6]. Still, somaclonal variation was reported for a numerous of in vitro derived plant species, such as Prunus persica [17]), grapevine cv. Crimson Seedless [15].

Unlike our findings, genetic stability was reported in several cases, micro propagated shoots of *Pinus thunbergii* Parl [6].

6 Conclusion

It can be concluded that various Spirulina platensi yest extract affected in vitro growth Le-Cont' rootstock pear. A combination of MS+ 3 mg/l BA and 20 % Spirulina platensis improvement in shoot proliferation, longest shoots, the highest number of shoots and leaves. Shoots of tissue culture were similar with those produced from classical multiplication by MS according to ISSR analysis which scored 89.1 % of mono morphism percentage and 10.1 % polymorphism. The ISSR analysis shows the highest similarity index percentage for P5-P9 0.986301.

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