Effect of Furedan, Seven, Glyphosate and Stroby on In Vitro Propagation of Strawberry

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المستخلص:

أجريت دراسه لتأثير اربعة مركبات كيميائيه: ال "فيوريدان"(٠.٥ مليجرام إلى ٤.٥ مليجرام/لتر)، ال "سيفين" (٠.٥ مليجرام إلى ٤.٥ مليجرام/لتر)، ال"قلايفوسيت" (٠.٢ مليجرام إلى ٦.٤ مليجرام/لتر) وال"استروبي" (١.٠ مليجرام إلى ١٢.٠مليجرام/لتر) على نمو وتطور قمم سيقان الفراوله (*Fragaria* x *ananasa* Duch.) صنف "فستيفال" في الانابيب بغرض تحسين كفأءة تكوين السيقان ونموها. أستخدمت قمم سيقان مفصوله من نبيتات منتجة في الانابيب كأجزاء للإستزراع وأستخدم وسط "موراشيجي واسكوج"كوسط اساسي. اوضحت النتائج ان ال "فيوريدان" عند التركيز٠.٥ مليجرام/لتر هو الامثل لكل القياسات المرصوده مقارنةً بالتراكيز الأخرى التي تم إختبارها. وكان تركيز ال "سيفين" ١.٠ مليجرام/لتر هو الافضل من التراكيز الأخرى المختبره لتكوين السيقان وثبط اقل تركيز مختبر من ال "سيفين" إستطالة السيقان مقارنةً بالشاهد، ولم يستجب عدد الاوراق ولا عدد الجذور وإستطالتها لكل التراكيز المختبره من ال "سيفين". وفي إستجابةٍ لإضافة ال "قلايفوسيت" للوسط الغذائي كان تكوين السيقان والاوراق افضل عند التركيز ٠.٨ مليجرام/لتر عن التراكيز الأخرى التي تم إختبارها بينما ثبطت كل تراكيزه المختبره إستطالة السيقان ولم تؤثر على تكوين وإستطالة الجذور.إستجابت قمم السيقان بصورةٍ افضل للتراكيز العاليه نسبياً من ال "ستروبي" حيث تم الحصول على اكبر عدد من السيقان على وسط غذائي يحتوى ٦.٠ مليجرام/لتر "ستروبي" و من ناحيةٍ أخرى، ادت كل تراكيز ال "ستروبي" المختبره إلى إنخفاض معنوي في قيم كل القياسات الأخرى المرصوده مقارنةً بالشاهد.على الرغم من صعوبة تفسير هذه التأثيرات في الوقت الراهن إلا ان نتائج هذه الدراسه اوضحت ان التراكيز المنخفضه نسبياً من هذه المركبات الكيميائيه لها تأثير معنوي على نمو وتكشف قمم سيقان الفراوله صنف "فستيفال" المزروعه في الانابيب مما يشير إلى ان كل المركبات الكيميائيه التي تم إختبارها في هذه الدراسه لديها انشطه مشابهة لمنظمات النمو.

Abstract:

The effect of concentrations of four chemical compounds: Furedan (0.5 mg to 4.0 mg/l). Seven (0.5 mg to 4.0 mg/l), Glyphosate (0.2 mg to 6.4 mg/l) and Stroby (1.5 mg to 12 mg/l) on *in vitro* growth and development of strawberry (*Fragaria* x *ananasa* Duch.) cv. “Festival” shoot tips was

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examined in order to optimize shoot regeneration and growth efficiencies. Shoot tip explants were obtained from *in vitro*-grown plantlets and Murashige and Skoog original medium was used as basal medium. The results showed that Furedan at 0.5 mg/l was optimal for all parameters measured compared with other concentrations tested. Seven at the concentration of 1.0 mg/l was better than other concentrations tested for shoot formation. The lowest concentration of Seven tested inhibited shoot elongation over the control. Leave number and root number and length were, however, largely unresponsive to all levels of Seven tested. In response to inclusion of Glyphosate in the culture medium, shoot and leaf formation were better with 0.8 mg/l than with other concentrations tested. Shoot elongation was inhibited whereas root formation and elongation were largely unaffected by all Glyphosate concentrations tested.The shoot tip explants responded best to relatively high concentrations of Stroby and the largest number of shoots was obtained on the medium containing 6.0 mg Stroby/l. On the other hand, all Stroby concentrations tested resulted in significant decrease in values of all other parameters measured relative to the control. Although it is now difficult to give an explanation for the effects of these chemicals, the results of this study demonstrate that relatively low concentrations of these chemicals have significant influence on growth and development of *in vitro* cultured shoot tips of the strawberry"Festival" cultivar which indicate that all chemical tested, in this study, have varying degrees of growth regulators-like actions.

Key words: Seven, growth regulator-like activity. Gly, Stroby, *Fragaria* spp., Furidan

Introduction:

Strawberry, (*Fragaria* x *ananassa* Duch.), belongs to the Rosaceae family. It is a perennial stoloniferous, herbaceous species representing the most important soft fruit worldwide that has been commercially cultivated in many countries throughout temperate and sub-tropics regions (Hancock, 1990; Biswas*et al.*, 2008). Information on strawberry production in Sudan is lacking It is not known exactly when and how strawberry was introduced into Sudan. Strawberry is conventionally propagated by rooted runners (Hartmann *et al.*, 2002). This method is not adequate to meet the enormous demand for planting material. Tissue culture propagation has become a practical means for rapid clonal and large-scale multiplication of some fruit species on a continuous year-round basis (Murashige, 1990). Strawberry appears to be amenable to rapid, efficient tissue culture propagation (Nehra, *et al.* 1989;Biswas*et al.*, 2007; Haddadi*et al.*, 2010).

Strawberry cultivation in Sudan is receiving much attention and recognition nowadays as an important fruit crop by government officials and private sectors. The establishment of large-scale commercial strawberry plantation has been limited by the inaccessibility of stock plants of desired known strawberry cultivars. Production of large numbers of clonal nursery strawberry transplants has not yet been described. The importation of nursery transplants of commercially known varieties from abroad is difficult and expensive.There is a pressing and undeniable need to develop rapid and easy propagation techniques to avail strawberry planting material of desired cultivars for large-scale plantations in the country.

The induction of*in vitro* morphogenesis is often controlled by the cytokinin: auxin ratio of the basal medium. Auxins and cytokinins have been used extensively in plant tissue culture to elicit specific morphogenic responses.The scarcity and availability and the virtually prohibitive cost of the natural and

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synthetic auxins and cytokinins have restricted their use in some countries including Sudan, due to political issues with the major source countries. This has promoted several researchers to search for chemical compounds with growth regulators action as alternatives for auxins and cytokinins.

More recently, evidence has shown that some chemical compounds such as inhibitors of seed germination, pesticide, herbicide, fungicide and acaricide may have growth regulators-like action. The successful use of some of these substances as growth regulators under*in vitro* conditions (Jansson and Svensson, 1980; Scora*et al.*,1984; Gowda and Prakas, 1998; Werbrouch*et al*., 1999; Idris*et al*., 2010;Saadalla, 2015) and field conditions (Neuman,1959; Stebbins, 1962; Coupland and Casely, 1975; Welker, 1976; Baur*et al*., 1977; Byers, 1978 Bauer, 1979; Byers, *et al.*, 1982;Olofinboba and Kozlowski, 1982; Rogers and Thompson, 1983; Reddy*et al.*, 1986; Cranshaw and Thorton, 1988; El-Khair,2013).

The effects of sub-lethal levels of some of these chemicals on growth and development of plant verified that they have growth-regulating properties exhibiting cytokinin-like action (Welker, 1976; Scora*et al*., 1984) or auxin-like action (Neuman, 1959; Jansson and Svensson, 1980; Idris*et al*., 2010).
In the last few years, efforts have been directed in our laboratory towards seeking possible alternatives for pure growth regulators that could affect plant growth and development and that are more economical and available. Mohamed (2012) has evaluated the influence of Confidor, Seven, Furedan and Stroby on growth and development of ginger tissue culture and more recently Saadalla (2015) has experimented with Seven, Stroby, Furedan and Glyphosate on *in vitro*growth and development of pineapple and chrysanthemum. The results obtained and the conclusions reached from these studies indicated that each of the chemical compounds tested, exhibits growth regulator-like actions.

Therefore the objective of this study was to evaluate the influence of selected chemical compounds in terms of growth and development of strawberry shoot tip culture. The chemical substances and the concentrations were chosen on the basis of preliminary experiments in our laboratory where various concentrations of several commercial pesticides, fungicides, acaricides and nemacides have been tested for their possible influence on *in vitro* growth and development of a variety of plant species.

Materials andMethod

Experiments were carried out at the tissue culture laboratory of the Department of Horticulture, Faculty of Agricultural Studies, Sudan University of Science and Technology, Khartoum North. Primary explants consisting of shoots 1.5-2.0 cm long, were aseptically excised from *in vitro* grown plantlets of the strawberry cultivar "Festival" and inserted (three per vessel, proximal portion down) into Magenta (GA-7-3) culture vessels containing a stock plant medium developed in our laboratory for multiple-shoot induction and plantlet regeneration of a diverse number of herbaceous plant species. This medium consisted of Murashige and Skoog, (1962) inorganic salts plus (per litre) 30 g sucrose, 40 mg *myo*-inositol, 40 mg adenine sulphate, 0.4 mg thiamine-HCl, 0.1 mg naphthalene acetic acid (NAA), 1.0 mg benzyl adenine (BA) and 7 g agar.

Unless otherwise stated, the basal medium used throughout this study consisted of full-strengthMurashigeand Skoog, (1962) inorganic salt; 30 g sucrose; 10 ml/l of a vitamin stock made of (glycine 2 mg/l, thiamine-HCl 1 mg/l, pyridoxine-HCl0.5 mg/l, plus nicotinic acid 0.5mg/l); 100 mg/l *myo*-inositol; 0.1mg /l BA; 0.0 mg/l NAA and 7g /l agar.

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The pH's of all media were set at 5.7 ± 0.1 with 0.1N NaOH and/or 0.1N HCl prior to agar addition. Agar was melted by heating on a stirring hot plate and the medium was dispensed in aliquots of 25 ml into Magenta (GA-7-3) culture vessel and sterilized by autoclaving at 1.06 kg/cm² and 121°C for 15 min.

The primary explants and subsequent cultures were maintained in an incubation room at a constant temperature of 25 ± 2 °C and a 16-h light provided by Phillips cool-white (F- 4D) fluorescent tubes and an 8-h dark cycle.

Proliferating shoot cultures were established by repeatedly sub-culturing the shoot tip of the original *in vitro* produced plantlet on a freshly prepared stock plant medium, after each harvest of the newly formed shoots. Once an abundance of stock plants is available for experimentations, various concentrations of sucrose, *myo*-inositol, and casein hydrolysate were tested, each separately, for determining the optimal concentration of each for optimizing shoot proliferation and growth vigor of *in vitro* cultured shoot tips of "Festival" strawberry cultivar.

Due to plant material limitations, all tests were not conducted at the same time but each test was carried out separately. In the first experiment, the following concentrations of Furedan were tested: 0.0, 0.5, 1.0, 2.0 or 4.0 mg/l. Seven concentrations of 0.0, 0.5, 1.0, 2.0 or 4.0 mg/l were added to the basal medium to determine their effects on shoot proliferation and subsequent growth and development in experiment 2. In experiment 3, Glyphosate in concentrations of 0.0, 0.2, 0.8, 3.2 or 6.4 mg/l were tested. Experiment 4 consisted of the addition of different concentrations of Stroby at 0.0, 1.5, 3.0, 6.0 or 12.0 mg/l to the basal medium.

All experimental treatments were arranged in completely randomized design with each treatment in an experiment replicated 4 times, 3 shoot tips per replication. Hand

sections of new shoots were examined microscopically to determine axillary or adventitious origin. Cultures were evaluated for number and length of shoots and roots and number of leaves after 6 weeks of incubation. Analysis of variances was carried out using M-Stat computer programme and Duncan Multiple Range Test was used to separate treatment means.

Results and Discussion

Furedan concentration

All measured parameters of *in vitro* cultured strawberry shoot tips responded to Furedan concentrations tested. The magnitude of response varies with concentration and the growth variable measured (Table 1). The lowest concentration of Furedan, (0.5 mg/l), tested significantly increased all parameters measured over the control. Higher concentrations than 0.5 mg/l Furedan decreased growth and morphological development with significant differences between treatments. The largest number of shoots (134.0), the longest shoots, (3.2 cm) the highest number of leaves (11.5) the greatest number of roots (20.0) and the longest roots (3.8 cm) were registered on medium containing 0.5 mg/l Furedan. The least values for all parameters measured were obtained on medium containing 4.0 mg/l furedan, the highest concentration of Furedan tested.

Furedan, (Carbo-furan), is an important chemical pesticide. It has been found to exhibit growth regulator-like action at sub-lethal concentrations (Idris*et al.*, 2010). Results herein indicate that the lowest concentration of Furedan tested (0.5 mg/l) significantly improved growth and development of *in vitro* cultured strawberry shoot tips in accordance with the findings of (Idris*et al*., (2010) and Mohamed, (2012) who obtained better growth and development of cultured plant tissues by the inclusion of low concentrations of Furedan in the culture medium. Furedan exhibits considerable potential as an agent for

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stimulating shoot proliferation in shoot tips culture of strawberry indicating that it has cytokinin-like action. Idris*et al*.,(2010), working with ginger tissue culture maintained the same view.

Seven concentration

Seven (carbary: 1-naphyl-N-methyl carbamate), is used chiefly as an insecticide.The effect of Seven on *in vitro* growth and development of strawberry shoot tips is shown in Table 2. Shoot proliferation and shoot elongation responded differently to Seven concentrations tested. The lowest concentration of Seven tested, (0.5 mg/l), significantly increased shoot number, decreased shoot elongation over the control but had little or no effect on the other growth responses measured.The greatest number of shoots was obtained on medium containing 1.0 mg/l, a lesser number on medium containing 2.0 mg/l and the least number of shoots was recorded on medium containing 4.0 mg/l Seven. Leave and root numbers, as well as root length were, however, largely unresponsive to all levels of Seven tested.

Seven has been shown to exhibit growth-regulator-like action on intact plants (Stebbins, 1962; Lee, 1977; Byers*et al*., 1982;Rogers and Thompson, 1983).The current study showed that Seven has a pronounced positive effect on *in vitro* shoot proliferation; doubling the number of shoots of strawberry shoot tips over the control at a concentration of 1.0 mg/l. Shoot elongation was, however, inhibited by the lowest concentration of Seven tested. The results are consistent with those of Mohamed, (2012) who evaluated the influence of Confidor, Seven, Furedan and Stroby on growth and development of ginger tissue culture and found Seven to be the most effective in enhancing shoot proliferation at the lowest concentration tested. The beneficial effects ofSeven on plant growth and development are attributed by (Lee, 1977) to its ability to inhibit the enzymatic degradation of indole-3-acetic acid.

Seven causes a cytokinin-like stimulation of shoot proliferation in *in vitro* cultured shoot tips of strawberry indicating that under certain experimental conditions Seven has a shoot inducing capacity and is able to substitute for an exogenously applied cytokinin.

Glyphosate concentration

Glyphosate, Roundup, is a broad spectrum, non-selective, foliar-applied herbicide having substantial activity in controlling perennial and annual weeds.The lowest concentration of glyphosate, (0.2 mg/l), tested significantly increased shoot and leaves formation and inhibited shoot elongation with significant difference relative to the control (Table 3). The greatest number of shoots (91.2) and of leaves (12.2), were obtained on medium containing 0.8 mg/l Glyphosate. The highest concentration of Glyphosate tested resulted in the least value for shoot number (6.7) and leaf number (3.8). Neither root number nor root elongation differed for all concentrations of Glyphosate examined.

Results herein indicate that addition of 0.8 mg/l Glyphosate in the culture medium resulted in a marked increase in shoot and leaf numbers while inhibiting shoot elongation. It seems that Glyphosate antagonizes apical dominance, resulting in axillary bud-break and subsequent shoot proliferation. Root initiation and elongation were largely unresponsive to all Glyphosate levels tested. Similar results on the beneficial effects of Glyphosate were reported by several investigators(Winata and Harvey, 1980; Frett and Smagula, 1981; Scora*et al*., 1984; Gowda and Prakas, 1998) where the supplementation of the culture media with Glyphosate resulted in better growth and development of *in vitro* cultured plant tissues of a number of plant species.The results indicate that Glyphosate exhibits a cytokinin-like action on axillary bud proliferation and shoot growth, a conclusion that is supported by the findings by others

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(Klosterboer, 1974; Coupland and Casely, 1975; Welker, 1976; Bauer, *et al*., 1977; Fernandez and Bayer, 1977; Bauer, 1979; Lee, 1984; Velini*et al*., 2010) that application of Glyphosate to intact plants result in a loss of apical dominance and formation of branching. The beneficial positive effects of Glyphosateon branching in intact plants (Lee, 1984; Little, 1985), and shoot proliferation in tissue culture (Paterson and Rost, 1981) attested to the cytokinin-like action of Glyphosate.

Stroby concentration

Table 4 illustrates the results of the effects of Stroby concentrations on growth and development of cultured strawberry shoot tip explants. Shoot proliferation was non-significantly increased by the lowest Stroby concentration tested relative to the control. The highest number of shoots (50.2) was obtained with Stroby at 6.0 mg/l and the least number of shoots (14.2) was obtained with Stroby at 12 mg/l, the highest concentration of Stroby tested. However, all Stroby concentrations tested significantly repressed shoot elongation, leave formation, root initiation and elongation over the control.

Stroby, a fungicide/acaricide has a profound effect on shoot number when added to culture medium at a concentration of 6.0 mg/l. It is worth mentioning that Furedan, Seven and Glyphosate tested, exert their beneficial effects on shoot proliferation at relativelylow concentrations of 1.0 mg/l or less. The results showed thatStroby exerted its beneficial effects on shoot tip culture of strawberry at relatively high concentrations supporting previously reported findings by Mohamed, (2012) that indicate that best responses for shoot proliferation in ginger tissue culture were obtained on medium containing 5.0 mg/l Stroby, a relatively higher concentration than the concentrations of Confidor, Seven or Furedan found optimal for the same respective parameter.

In a more or less similar manner El-Khair, (2013) in his unpublished thesis, examined the influence of BA, Furedan, Stroby and Seven on the percentage of scion graft take in mango (*Mangiferaindica* L.). The results show that Stroby consistently gave higher percentage of scion graft-take than the other chemicals tested attributing that to loss of apical dominance and subsequent axillary bud-break.

Cytokinins are known to stimulate cell division and induce shoot formation and proliferation (Paterson and Rost, 1981; Little, 1985) and to retard root formation (Ben-Jaacov*et al*., 1991).Stroby exhibits similar cytokinin-like effects on axillary bud proliferation and shoot and root growth of strawberry shoot tips. The beneficial effects of Stroby on strawberry shoot proliferation and shoot growth and development would appear to be a result of loss of apical dominance. The promoting effect of Stroby on shoot proliferation and its inhibitory effect on shoot elongation, leaf formation, root initiation and elongation testify to the cytokinin-like action of Stroby.

Histological observations showed that shoot formation resulted from axillary bud growth and development and not from basal callus tissues, thus ensuring the clonal status of regenerated plantlets.

It could, therefore, be concluded, that Furedan, Seven, Glyphosate and Stroby, each tested separately, exhibit considerable potential as agents for increasing shoot proliferation of strawberry shoot tips. Our research did not attempt to compare differences in the effectiveness of these chemicals on growth and development of strawberry shoot tips. Plantlets produced were morphologically normal with no apparent injury. However, other types of chemicides, application methods, and the economics and environmental impact, of the application of the results of this study to commercial strawberry nursery operation merit further

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study. Among the chemical compounds tested, Furedan seems to be a strong candidate for use as a growth promoting chemical compound for shoot proliferation in strawberry since it consistently gives high number of shoots at relatively low concentrations. Caution should be exercised so that too generalized conclusions are not drawn from limited experimental data of this type.

Table 1: Effect of Furedan on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period

Furedan conc. (mg/l)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
0.00	69.50c	1.98d	9.33b	3.67c	1.83c
0.5	134.00a	3.17a	11.50a	20.0a	3.82a
1.0	71.50 c	2.83b	9.83b	15.67b	3.08b
2.0	103.00b	2.65c	8.17c	14.17b	2.82b
4.0	43.50d	2.65c	8.11c	11.00c	2.02c

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan Multiple Range Test.

Table 2: Effect of Seven on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period

Seven conc. (mg/l)	Shoot No.	Shoot length (cm)	Leaf No.	Root No.	Root length (cm)
0.00	16.50d	2.40a	7.33a	2.00a	0.22a
0.5	22.00c	1.48c	7.67a	2.00a	0.22a
1.0	37.67a	1.50c	8.10a	2.00a	0.22a
2.0	29.50b	1.70b	8.00a	2.00a	0.22a
4.0	4.67e	1.38d	7.33a	2.00a	0.22a

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan Multiple Range Test.

Table 3: Effect of Glyphosate (Gly) on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period

(Gly) conc. (mg/l)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
0.0	10.83d	2.03a	7.00b	1.33a	0.47a
0.2	30.83c	1.63b	7.00b	1.33a	0.50a
0.8	91.17a	1.71b	12.17a	1.33a	0.45a
3.2	39.33b	1.27c	5.00c	1.33a	0.45a
6.4	6.67e	1.17c	3.83c	1.33a	0.45a

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan Multiple Range Test.

Table 4: Effect of Stroby on growth and development of strawberry shoot tips cultured *in vitro*, after 6 weeks of incubation period

Stroby conc. (mg/l)	Shoot No.	Plant height (cm)	Leaf No.	Root No.	Root length (cm)
0.00	23.67c	2.00a	8.167a	2.17a	1.97a
1.5	26.50c	1.33c	6.83b	1.33b	0.97b
3.0	36.17b	1.83c	5.50c	1.33b	0.60c
6.0	50.17a	1.58c	6.50bc	1.00b	0.57c
12.0	14.17d	1.20c	5.50c	1.00b	0.47c

Means in a column followed by the same letter are not significantly different at P = 0.05, according to Duncan Multiple Range Test.

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