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EFFECT OF GIBBERELLIC ACID ON STRAWBERRY (*Fragaria X ananassa* Duch.) MICROPROPAGATION DURING MULTIPLICATION STAGE

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ABSTRACT

The present work was conducted at the tissue culture lab. of the Center of Genetic Engineering and Biotechnology, Fac. Agric., Ain Shams University, Egypt. It aims to reveal the micropropagation possibilities of Festival and Marquez strawberry cultivars using runners meristem culture on different concentrations of gibberellic acid (GA_3); *i.e.,* 0.1, 0.2, 0.3, 0.4 and 0.5 mgl^{-1}) in multiplication stage. Supplementation the media with GA_3 at 0.4 mgl^{-1} gave the highest values for number of shoots per explant and leaf number per shoot, whereas GA_3 at 0.5$mgl^{-1}$for shoot length was found to be the most appropriate concentration. In addition, Festival strawberry cultivar achieved the best results of all features under study than Marquez cultivar. The most favourble interaction treatment for maximizing number of shoots/explant and number of leaves/shoot was MS medium containing 0.1 mgl^{-1} benzyladenine (BA) + 0.4 mgl^{-1} GA_3 with Festival strawberry cultivar.

Key words: Strawberry, cultivars, Festival-Marquez, micropropagation, GA_3.

INTRODUCTION

One of the important goals of the agricultural policy in Egypt is to increase the planted strawberry area to meet the demand of local fresh market, processing and export. Healthy stocks used for propagation through conventional methods are not available. *In vitro* techniques are important tools for modern plant improvement programs to introduce new traits into selected plants, to multiply elite selection and to develop suitable cultivars in the minimum time (Taji *et al*., 2002 ; Rama *et al*., 2012). Several approaches such as shoot tip culture, adventitious shoot regeneration and somatic embryogenesis are being used for *In vitro* micro propagation (Rama *et al*., 2012; Ghasemi *et al*., 2015). The *in vitro* culture of meristem have many advantages, it successful in the mass propagation of strawberry plants which several millions of plants can be produced within a year from a few mother plants and also in case of the introduction of new cultivars (Boxus, 1983; Nehra *et al.,* 1994; Passey *et al*., 2003). Moreover, the storage of tissue culture propagules requires less space than traditional runner plant and it can be initiated at any time during the production cycle (Swartz *et al*., 1981).In the same time, meristem culture is a unique technique to obtain propagules free from various pathogens including viruses viroides, mycoplasma, bacteria and fungi (Pierik, 1989). Benefit of using meristem culture as a means of regeneration is that the incipient shoot has already differentiated to establish a complete plant, only elongation and root differentiation are required (Biswas *et al*., 2007; Ghasemi *et al*., 2015).

In this respect, Biswas *et al*. (2007), Litwińczuk *et al*. (2009), Adak (2011) and Ghasemi *et al*. (2015) reported that strawberry cultivars could be propagated efficiently by meristem culture (runner tips).

Now, a number of substances are known that have a relatively broad spectrum effects such as

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gibberellic acid (GA_3), whose primary morphological effects are associated with cell enlargement and cell division. (Abbas *et al*., 2007). Gibberellic acid was used in micro propagation of strawberry. In general, it was applied at the concentration of 0.1 mgl^{-1} at the proliferation stage (Boxus 1974, 1999; Litwińczuk and Zubel 2005). The influence of GA_3 on strawberry *in vitro* cultures was studied by Adak (2011), Rama *et al.* (2012) and Ghasemi *et al*. (2015). They found that GA_3 at the concentration from 0.1 to 1.0mgl^{-1} improved shoot number of explants as well as shoot development expressed as shoot length and number as well as of leaves/shoot.

Therefore the aim of this study was to evaluate the influence of various GA_3 concentrations in the multiplication medium to increase number of shoots produced from each explant to reduce the long time needed for subculture as well as to enhance the growth of shoots.

MATERIALS AND METHODS

Plant Material and Location

The experiment was carried out at the Tissue Culture Lab., Center of Genetic Engineering and Biotechnology, Fac. Agric., Ain Shams University, Egypt, during the period from 2013 to 2015.

The meristem of two strawberry (*Fragaria x ananassa* Duch.) cultivars; *i.e.,* Festival and Marquez were used as plant materials.

Preparation and Sterilization of Explants

Runners (about 10-15 cm in length) were collected from fruity nile on June as a source of explants. Excised runner tips (5.0 cm in length) were washed under running tap water, then washed with sterilized distilled water. Then explants were subjected to chemical sterilization under laminar air flow cabinet using 70% ethyl alchohol for 5 min. and rinsed by sterile double distilled water. Subsequently, the runner tips were sterelized again by 5% sodium hypochlorite (NaOCl) solution with gently shaking for 20 min. followed by 3-5 times washing by sterile double distilled water.

Establishment Stage

The sterilized runner tips of the tested cultivars prepared by napped off the immature leaves as well as leaf primordial and then meristems were trimmed to 0.3-0.5cm.The meristems were quickly cultured in jars containing 30 ml of solidified Murashig and Skoog (MS) medium and supplemental with GA_3 at 0.1$mgl^{-1}$and BA 0.1mgl^{-1} as plant growth regulators. The end of establishment stage is shown in Figs. 1 and 2.

Multiplication Stage

After 21 days of inoculation, the developed meristems of the two cultivars (Festival and Marquez) were cultured on the same Murashige and Skoog (MS) medium (1962) (containing 0.1 mgl^{-1} BA) used through the establishment stage and supplemented with the other different levels of the plant growth regulator GA_3 (0.1, 0.2, 0.3, 0.4 and 0.5 mgl^{-1}).In all cases MS medium was supplemented with 30.0 gl^{-1} sucrose,7.0gl^{-1} agar, 3.0gl^{-1} active charcoal (AC) and the pH was adjusted to 5.7 followed by autoclaving at 121^0C for 20 min (1.06kg/cm^{-2}). Cultures were maintained at 25 ± 2°C under a 16/8 hr., (light/ dark) photoperiod with a light intensity 2000lux provided by white fluorescent lamps and 70-80% relative humidity.

All treatments were conducted in three replicates (five jars of each treatment for each replicate) and five explants were planted in each jar for the two cultivars.

Recorded Data

At 21 days from incubation, number of shoots per explant, number of leaves per shoot and shoot length (cm) were recorded as an average of 5 jars.

The Experimental Design and Statistical Analysis

The trails were laid out in completely randomized design. It included ten treatments which were the combinations between two strawberry cultivars (Festival and Marquez) and five GA_3 concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 mgl^{-1}). Data were statistically analyzed using the SAS software (SAS institute, 2001).When the ANOVA indicated significant effects (5%) based on the F-test, LSD at 0.05 level.

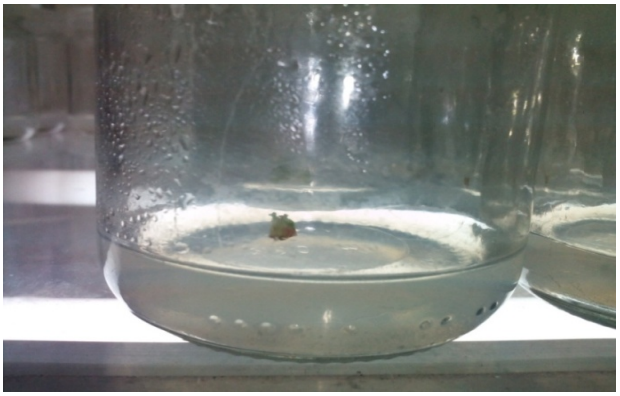

Fig. 1. ***In vitro*** **culture of strawberry cultivar Festival during establishment stage (after five days)**

Fig. 2. ***In vitro*** **culture of strawberry cultivar Marquez during establishment stage (after five days)**

RESULTS AND DISCUSSION

Effect of Cultivars

Data presented in Table 1 show that the two strawberry cultivars Festival and Marquez differed significantly in number of shoots/explant, number of leaves /shoot and shoot length. Generally, Festival cultivar gave the highest value of all the studied traits compared to Marquez cultivar. As for number of shoots /explant and number of leaves /shoot, data in Table 1 reveal that the maximum values were recorded with Festival cultivar (4.63 and 3.69, respectively), while the lowest values in this respect (3.27 and 3.0, respectively) were observed with Marquez cultivar.

The difference between the two strawberry cultivars in the abovementioned studied characters during multiplication stage might be attributed to the genetic architecture of the variety. These results were confirmed by the findings of Nehra *et al*. (1994) who indicated that the two tested strawberry cultivars responded differently to various free of *in vitro* propagation. In addition, Mozafari and Gerdakaneh (2012) found variation in the studied parameter of strawberry cultivars. This variation in regeneration capacity amongst the strawberry cultivars indicated a strong genetic component determines the success of regeneration (Gerdakaneh *et al.,* 2011).

Effect of GA_3 Concentration

The effect of different GA_3 concentrations (0.1, 0.2, 0.3, 0.4 and 0.5mgl^{-1}) during multiplication stage were presented in Table 2. Initial growth of the cultured meristem started within 6-10 days by increasing in size (vigor) and in colour (greenish). Growth was continued to shoot development, resulting primary establishment of meristem within 3-4 weeks. The obtained results in Table 2 show that cultivars had a significant result with all GA_3 concentrations for number of shoots per explant and number of leaves per shoot as well as shoot length. GA_3 at the concentration of 0.4mgl^{-1} gave the highest values in number of shoots per explant and leaves per shoot but 0.5mgl^{-1} GA_3 was the best concentration for shoot length. The present results are in agreement with earlier observations of Biswas *et al*. (2007), Litwińczuk *et al*. (2009), Alam *et al*. (2010), Adak (2011) and Ndagijimana *et al*. (2014) who reported that the medium is normally supplemented with gibberellic acid with low concentration of other growth regulators like cytokinins and auxins may be beneficial to shoot growth and multiplication shoot formation. In addition, Ghasemi *et al*. (2015) reported that hormonal balance has a key role in regulation of morphological response from cultured explants. In this respect, Abbas *et al*. (2007) and Ndagijimana *et al*. (2014) revealed that the beneficial effect of GA_3 on multiplication stage of tissue culture techniques

Table 1. Effect of strawberry cultivars on shoot proliferation after 21 days of inoculation during multiplication stage

Parameter / Cultivar	No. of shoots/explant	No. of leaves/shoot	Shoot length (cm)
Festavil	4.63 a	3.69 a	2.49 a
Marquez	3.27 b	3.00 b	1.28 b

Values having the same alphabetical letter (s) in each column did not significantly different according to LSD at (0.05) of probability.

Table 2. Effect of different gibberellic acid (GA_3) concentrations on *in vitro* shoot proliferation of strawberry after 21 days of inoculation during multiplication stage

Parameter / GA_3 concenteration	No. of shoots/explant	No. of leaves /shoot	Shoot length (cm)
0.1	3.54 bc	2.83 c	1.77 c
0.2	3.53 c	2.90 c	1.88 bc
0.3	3.66 bc	3.05 c	1.79 c
0.4	5.15 a	4.65 a	1.90 b
0.5	3.86 b	3.30 b	2.15 a

Values having the same alphabetical letter(s) in each column did not significantly different according to LSD at (0.05) of probability.

might be refer to that GA_3 is very potent hormone whose morphological effects are associated with cell enlargement and cell division. In addition, regarding the number of shoots per explant Badawi *et al*. (1990), Alam *et al*. (2010) and Adak (2011) concluded that the most suitable GA_3 concentration in propagation stage were 0.1 and 0.4 mgl^{-1}, the lower concentration of GA_3 induced positive effects on the number of shoots and also the quality. With respect to number of leaves per shoot and shoot length, the obtained results agreed with those reported by Al-Momani *et al*. (1999), Abbas *et al*. (2007) and Ndagijimana *et al*. (2014) on number of leaves per shoot. Sakila *et al*. (2007), Adak (2011) Rama *et al*. (2012) and Ghasemi *et al*. (2015) on shoot length who stated that GA_3 concentrations from 0.1 to 0.5mgl^{-1} increased the two studied features.

Effect of Interaction

With regard to the effect of interaction between cultivars and GA_3 concentrations on shoot parameters, it is obvious from data in Table 3 that Festival cultivar supplemented with GA_3 at 0.4mgl^{-1} achived the highest values of number of shoots (6.26 shoots/explant) and number of leaves (5.11 leaves/shoot) in comparison with Marquez cultivar which gave 4.03 shoots /explant and 4.18 leaves /shoot when both cultivars grown on MS medium supplied with 0.4mgl^{-1} GA_3. In addition, the presented data in Table 3 and Figs. 3 and 4 indicated that the highest values of shoot length recorded by Festival cultivar (2.81cm) comparing with Marquez cultivar (1.50 cm) when both cultivars were cultured on MS medium amended with 0.5mgl^{-1} GA_3. Regarding to the differences in response of the two strawberry cultivars to gibberellic acid concentrations in (MS medium) on shoot characteristics might be due to genetic variability among the varieties and culture medium including plant growth regulators and their combinations. Amer (2013) and Ghasemi *et al*. (2015) came to similar results.

Table 3. Effect of the interaction between strawberry cultivars (Festival and Marquez) and the different concentrations of GA_3 after 21 days of inoculation during multiplication stage

Cultivar × GA_3 (mgl^{-1})	Parameter	No. of shoots/explant	No. of leaves /shoot	Shoot length (cm)
Festival	0.1	4.21 c	3.25 cd	2.43b
	0.2	4.26 bc	3.26 cd	2.45b
	0.3	4.26 bc	3.35 cd	2.36b
	0.4	6.26 a	5.11 a	2.41b
	0.5	4.13 c	3.48 c	2.81a
Marquez	0.1	2.86 e	2.41 f	1.11d
	0.2	2.80 e	2.53 ef	1.16d
	0.3	3.06 e	2.75 e	1.21d
	0.4	4.03 cd	4.18 b	1.40c
	0.5	3.60 d	3.13 d	1.50c

Values having the same alphabetical letter(s) in each column did not significantly different according to LSD at (0.05) of probability.

Fig 3. *In vitro* culture of strawberry 'Festival" at 0.4 mgl^{-1} GA_3 during multiplication stage (after 10 days)

Fig. 4. *In vitro* culture of strawberry 'Marquez" at 0.4mgl^{-1} GA_3 during multiplication stage (after 10 days)

Conclusion

It could be concluded from this investigation that the best cultivar for giving the highest number of shoots per explant and shoot characteristics (number of leaves and shoot length) was Festival during multiplication stage when MS containing 0.1mgl^{-1}BA medium and supplemented with 0.4$mgl^{-1}GA_3$. In this respect, the findings of the present work can be used for increasing the number of shoots produced from each runner meristem to reduce time needed for multiplication subculture and this consequently, save the required time and costs.

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تأثيـــر حمـض الجبـرلين علـــى الإكثـــار الدقيـق للفـراولة أثنـــاء مرحلـــة التضاعف

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أجريت هذه الدراسة بمعمل زراعة الأنسجة بمركز الهندسة الوراثية والتكنولوجيا الحيوية – كلية الزراعة - جامعة عين شمس بشبرا الخيمة بهدف الوصول لأفضل تركيز من الجبرلين أثناء مرحلة التضاعف لكل من الصنفين فيستفال وماركيز باستخدام وزراعة القمم المرستيمية للمدادات مع استخدام تركيزات مختلفة من حمض الجبرلين (٠,١-٠,٢-٠,٣-٠,٤-٠,٥ ملليجرام / لتر) خلال مرحلة التضاعف، من خلال النتائج، لوحظ أن حمض الجبرلين عند التركيز ٠,٤ ملليجرام / لتر لكل من عدد النبيتات لكل منفصل نباتى وعدد الأوراق لكل نبيتة بينما ٠,٥ ملليجرام /لتر لطول النبيتة هى التركيزات الأكثر ملائمة لإكثار الصنفين حيث أعطت هاتان المعاملتان أعلى القيم، وبالنسبة للأصناف؛ فقد حقق صنف الفراولة فيستفال أفضل النتائج لكل الصفات محل الدراسة مقارنة بالصنف ماركيز، وكانت أفضل معاملات التفاعل لإعطاء أعلى عدد للأفرع وعدد الأوراق لكل نبتة هى إمداد بيئة الزراعة المحتوية على بنزيل ادنين بتركيز ٠,١ملليجرام /لتر + الجبرلين بتركيز ٠,٤ ملليجرام / لتر خلال مرحلة التضاعف.

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