COMPLETING THE PROCESS OF CREATING VITRO PLANT *IN VITRO* DTLD RED GERBERA JAMESONII BOLUS

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ABSTRACT

Gerbera jamesonii is a popular flower. Currently, the demand for growing gerbera jamesonii pots to decorate offices, campuses and Tet holidays is increasing. Red gerbera jamesonii bolus with black stamens with large flower diameter is very popular in the market. We have carried out study with the aim of finding out the best procedure to generate complete plants *in vitro* DTLD red gerbera jamesonni bolus. Study results show that: The culture medium is MS+0.3 mg/IBA supplemented with 20g/l sugar; using an airtight bag with an opening diameter of 25mm and using LEDs designed to have a red light to blue light ratio of 7:3 for lighting, which is best for the complete plant growth stage of DTLD gerbera jamesonni bolus. The cultivating medium is mixed with coconut husk and peat moss at a ratio of 1:1, added with 15% perlite, which is the most suitable for crowning DTLD gerbera jamesonni bolus. EC starting cultivating medium 1.5 is the most suitable for this DTLD gerbera jamesonni variety.

HOÀN THIỆN QUY TRÌNH TẠO CÂY HOÀN CHỈNH *IN VITRO* GIỐNG HOA ĐỒNG TIỀN LÙN ĐỎ DTLD

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TỪ KHÓA

Đồng tiền lùn
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Giá thể phối trộn
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TÓM TẮT

Hoa đồng tiền *(Gerbera jamesonii)* là loài hoa, được nhiều người ưa chuộng. Hiện nay, nhu cầu trồng đồng tiền chậu để trang trí văn phòng, khuôn viên và dịp lễ Tết tăng cao. Giống đồng tiền lùn màu đỏ nhị đen với đường kính hoa lớn được thị trường rất ưu chuộng. Chúng tôi đã nghiên cứu thực hiện nhằm mục đích tìm ra quy trình tốt nhất tạo cây hoàn chỉnh *in vitro* giống đồng tiền lùn đỏ DTLD. Kết quả nghiên cứu cho thấy: Môi trường nuôi cấy là MS+0.3 mg/IBA bổ sung 20g/l đường; sử dụng túi kín thoáng với đường kính lỗ thoáng 25mm và sử dụng đèn LED được thiết kế để có tỷ lệ ánh sáng đỏ và ánh sáng xanh là 7:3 để thắp sáng là tốt nhất cho giai đoạn tạo cây hoàn chỉnh giống đồng tiền lùn DTLD. Giá thể phối trộn xơ dừa và peat moss tỷ lệ 1:1 bổ sung 15% perlite là thích hợp nhất để ra ngôi giống đồng tiền lùn DTLD. EC giá thể ban đầu 1.5 là thích hợp nhất với giống đồng tiền DTLD này.

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1. Rationale

Gerbera jamesonii is a beautiful flower, rich in shape and color, long lasting flowers and especially the ability to flower all year round, simple cultivation techniques, care, few labor [1]. Therefore, the growing area of Gerbera jamesonii is increasingly expanding, consumption and prices are increasing day by day.

Currently, the demand for decorating homes, offices and shops is increasing, so the consumption of gerbera pots is increasing. Gerbera jamesonii bolus with the characteristic of much flower blooming so it is very popular for potting. Therefore, the demand for seedlings is increasing [2], [3].

To satisfy the demand for quality seedlings for production, we have selected a number of species of beautiful Gerbera jamesonii bolus, meeting some criteria of gardeners such as: beautiful, outstanding colors, fast flowering time, compact stems and flowers, suitable for growing in pots… Among them is the red Gerbera jamesonii bolus with large flower diameter which is very popular in the market [4].

At present, we have successfully bred DTLD gerbera jamesonni bolus. However, due to the rapid multiplication process performed on other cuttings of Gerbera jamesonii, the quality of the complete *in vitro* production stage is not as expected [1], [5].

2. Material, study method and methodology

2.1. Study materal: Red DTLD gerbera jamesonni bolus sample

2.2. Study content

Experiment 1: *Effect of environmental background on the stage of complete in vitro plant formation DTLD gerbera jamesonni bolus*

Formula	Environmental background
CT1	MS
CT2	1/2 MS
CT3	1/3MS
CT4	1/4MS

Experiment 2: *Effect of sugar on the stage of complete in vitro plant formation DTLD gerbera jamesonni bolus*

Formula	Supplemented sugar amount
CT1	30g/l
CT2	25g/l
CT3	20g/l
CT4	15g/l

Experiment 3: *Effect of light on the stage of complete vitro plant formation DTLD gerbera jamesonni bolus*

Formula	Type of light used
CT1	Fluorescent T8 36W Rang Dong
CT2	Rang Dong dedicated 18W white light LED for NCM
CT3	Rang Dong dedicated 18W yellow LED light for NCM
CT4	Duhal's 18W Plain White LED
CT5	LED 18W light ratio 7Red/3Blue

Experiment 4: *Effect of air exchange on the stage of complete in vitro plant formation DTLD gerbera jamesonni bolus*

Formula	Airy hole diameter
CT1	Closed bags
CT2	25mm
CT3	50mm
CT4	100mm
CT5	150mm

Experiment 5: *Determining suitable cultivating medium to bring DTLD gerbera jamesonni bolus seedlings to nursery*

Formula	Cultivating medium used
CT1	Coconut husk
CT2	Peat moss
CT3	Coconut husk/Peat moss ratio 1:1
CT4	Coconut husk/Peat moss ratio 1:1 added with 15% perlite

Experiment 6: *Effect of EC of initial media on DTLD gerbera jamesonni bolus seedlings when bringing to the nursery*

Formula	EC cultivating medium levels
CT1	0.7
CT2	1.0
CT3	1.5
CT4	2.0
CT5	2.5

2.3. Method of data processing

The data are statistically processed using Microsoft Office Excel and IRRISTAT 4.0 software.

3. Results and discussion

3.1. Study on optimizing the stage of complete in vitro plant formation DTLD gerbera jamesonni bolus

3.1.1. Effect of environmental background on the stage of complete in vitro plant formation DTLD gerbera jamesonni bolus

The objectivbe of this stage is to create the best complete *in vitro* plant to ensure the survival rate of seedlings at the nursery, so the quality criteria will be prioritized. According to the results in Table 1, it can be seen that the average root length increases gradually as the amount of nutrients in the medium decreases. In contrast, the number of roots tends to decrease gradually. In which formula 1 with environmental background is MS and formula 2 has environmental background of ½ MS, there is no difference. In formula 1 and formula 2 with fat plants with dark green leaves also ensure the quality so that when the plants are brought to the nursery, they can develop the best.

Table 1. *Effect of environmental background on the stage of complete in vitro plant formation DTLD gerbera jamesonni bolus after 3 weeks of follow-up*

Formula	No. of roots	Average root length (cm)	Quality
MS	4.16	1.49	Fat plant, dark green leaves
1/2 MS	**4.26**	**1.49**	**Fat plant, dark green leaves**
1/3MS	3.51	2.02	Stunted plant, green leaves, thing roots
1/4MS	2.47	2.71	Stunted plant, yellowish-green leaves, thing
CV%	2.10	2.00	
$LSD_{0,05}$	*0.14*	*0.07*	

Meanwhile, in formula 3 (1/3MS) and formula 4 (1/4MS), the completed plants are small, stunted, thin roots, showing signs of nutritional deficiency when leaves turned pale green and yellow. With such quality it is difficult to guarantee the survival rate outside the nursery. From such results with MS and ½ MS giving equivalent results while formula 2 reduced the amount of mineral salts used to prepare the medium by 50%, compared with formula 1. Therefore, formula 2 with the medium background of ½ MS is optimal to use for the complete plant generation phase of this DTLD gerbera jamesonni bolus.

Figure 1. *Completing plants of DTLD gerbera jamesonni bolus on different media after 3 weeks*

3.1.2. Effect of sugar on the stage of complete in vitro plant development DTLD gerbera jamesonni bolus

For *in vitro* plants to adapt well to nursery conditions, it is essential to consider reducing sugars to reduce dependence on heterotrophic carbon sources. Especially in the stage of creating complete plants before putting them into the nursery. However, it is also necessary to consider the appropriate level of sugar reduction to ensure the quality of the plants when exposed to *ex vivo* conditions. This experiment is done to ensure those goals. The experimental results are presented in Table 2 below.

Table 2. *Effect of added sugar on complete plant growth stage DTLD gerbera jamesonni bolus after 3 weeks of follow-up*

Formula	No. of roots	Average root length (cm)	Plant quality
30g/l	4.17	1.59	Fat plant, dark green leaves
25g/l	4.31	1.82	Fat plant, dark green leaves
20g/l	**4.21**	**1.93**	**Fat plant, dark green leaves**
15g/l	3.03	2.30	Thin plant, light green leaves
CV%	1.90	1.50	
$LSD_{0,05}$	0.14	0.05	

Figure 2. *Completing plant DTLD gerbera jamesonni bolus on different sugar concentrations after 3 weeks*

The amount of sugar used in this stage of complete plant growth doesnot significantly affect the number of roots and the quality of the plants when in 3 formulas: 30g/l, 25g/l and 20g/l, the complete plants are of good quality, with fat plants dark green leaves and 4.17 roots, respectively; 4.31; 4.21 there is no statistical difference at 5% significance level; Only formula 4 with sugar content of 15g/l in medium does not guarantee the growth of the plant during this period, so the complete plant is thin and light green, and the number of roots is only 3.03. Meanwhile, the average root length increased from 1.59cm to 1.9cm when the amount of sugar used decreases by 30g, 25g, 20g and 15g, respectively. With such results, formula 3 with the amount of sugar used at 20g/l medium ensures the complete quality of the plants and optimizes the cost, for this period.

3.1.3. Effect of light on the stage of complete in vitro plant formation DTLD gerbera jamesonni bolus

Increasing plant adaptability *in vitro* is not only a reduction in dependence on heterotrophic carbon sources for plants, but also a matter of light use for plants because natural light will have a greater intensity and the light spectrum will also have many differences compared to the light used in the breeding room. Therefore, using a light source that is closest to natural light can help plants better adapt to *ex vivo* conditions.

With the light sources used, LEDs are designed to have a ratio of red light to blue light of 7:3, giving a completely better effect than other light sources, with most average roots reaching 4.2, the longest average root length is 2.09 and the seedlings are fat, with dark green leaves. In formulas 1, 2 and 3, when using fluorescent lamps of Rang Dong company T8 36W and 2 types

of Rang Dong company white and yellow LEDs specialized for tissue culture, the plants have equivalent quality in terms of quality for all three parameters of root count, average root length and plant quality. Meanwhile, duhal's LEDs give the worst results. This shows that LEDs are only used for general lighting and are not suitable for use in micropropagation processes at both the rapid multiplication stage as well as the complete plant growth stage. As such, LEDs designed to have a red light to blue light ratio of 7:3 are best suited for the complete vegetative stage of DTLD gerbera jamesonni bolus in the micropropagation process.

Table 3. *Effect of light on the stage of complete plant formation DTLD gerbera jamesonni bolus after 3 weeks of follow-up*

Formula	No. of roots	Average root length (cm)	Plant quality
Fluorescent T8 36W Rang Dong	4.00	1.84	Fat plants, dark green leaves
Rang Dong dedicated 18W white light LED for NCM	3.87	1.65	Fat plants, dark green leaves
Rang Dong dedicated 18W yellow LED light for NCM	3.87	1.48	Fat plants, dark green leaves
Duhal's 18W Plain White LED	3.57	1.56	Thin plants, light green leaves
LED 18W light ratio 7Red/3Blue	**4.20**	**2.09**	**Fat plants, dark green leaves**
CV%	*2.10*	*3.70*	
$LSD_{0,05}$	*0.15*	*0.11*	

Figure 3. *Complete plant DTLD gerbera jamesonni bolus grown under different light conditions after 2 weeks*

3.1.4. Effect of air exchange on the stage of complete in vitro plant formation DTLD gerbera jamesonni bolus

Cultivating the flowers in conditions with air exchange helps plants have more CO2 to perform more photosynthesis, which makes it easier for plants to adapt outside the nursery. At the same time, the air exchange also reduces the humidity in the culture vessel, which makes the *in vitro* plants less succulent (vitrified) and helps the plants to avoid moisture shock when given nursery conditions.

The data results in Table 4 show that the larger the diameter of the airy hole, the stronger the *in vitro* plant, even the older plant for bags with diameters of 100mm and 150mm. Meanwhile, in the closed bag condition, the plant still showed signs of succulent. With such plants, when being brought to the nursery, if the optimum air humidity is not ensured, it will cause the plants to lose water supply, affecting the survival rate and the ability to develop outside the nursery later. Regarding the number of roots, formula 2 with a airy hole diameter of 25mm will give the

highest average number of roots, reaching 3.95 roots/plant, which is superior to formula 1 when using sealed bags. When increasing the diameter of the vesicles in treatments 2, 3 and 4 to 50mm, 100mm and 150mm, respectively, the average number of roots also decreased to 3.49 roots/plant, 3.29 roots/plant and 3.18 roots/plant, respectively. It can be seen that the diameter of the openings increases, causing the plant to age quickly and grow poorly. Therefore, the use of a 25mm diameter air bag is most suitable for complete plant formation with DTLD gerbera jamesonni bolus.

Table 4. *Effect of air exchange on the stage of complete plant formation DTLD gerbera jamesonni bolus after 3 weeks of follow-up*

Airy hole diameter	No. of roots	Average root length (cm)	Plant quality
Túi kín	3.03	1.25	Fat plant, dark green leaves, slightly succulent
25mm	**3.95**	**1.44**	**Fat plant, dark green leaves, sturdy**
50mm	3.49	1.72	Fat plant, dark green leaves, sturdy
100mm	3.29	1.62	Thin plant, dark green leaves, old
150mm	3.18	1.32	Thin plant, dark green leaves, old
CV%	*1.60*	*2.50*	
$LSD_{0,05}$	*0.10*	*0.06*	

Figure 4. *Complete plant of DTLD gerbera jamesonni bolus using bags with different pore sizes after 3 weeks*

3.2. Study on optimization of nursery stage DTLD gerbera jamesonni bolus

3.2.1. Determining suitable medium to introduce seedlings of DTLD gerbera jamesonni bolus to the nursery

Cultivating medium used should also ensure seedling quality, that is, the uniformity and stability of the seedlings. Peat moss and coconut husk are two types of medium moss selected for their ability to retain good moisture, industrially produced, so the quality is uniform, less altered compared to traditional mediums such as organic humus, rice husk hum or alluvial soil… In addition, perlite is mixed in formula 4 to increase the aeration of DTLD medium to monitor the growth of gerbera jamesonii bolus seedlings.

From the table data of 5 plants, the mixed huskpeat medium is mixed with the ratio of 1:1 huskpeat moss + coconut husk, adding 15% per cent per cent, which is the best for producing dwarf gerbera plants like DTLD when the highest survival rate is 90. %, the average number of leaves is 5.21 leaves/plant, the average height is 5.64cm. In formula 2, when using the whole medium of peat moss, the best average plant height is 5.79cm but the lowest survival rate is only 71%; with formula 1, when using completely coconut husk, the survival rate reached 81%, but the quality of the plant is somewhat inferior compared to other formulas.

Table 5. *Effect of medium used on DTLD gerbera jamesonni bolus seedling growth after 8 weeks of follow-up*

Formula	Surviving ratio (%)	Actual number of leaves (leaves/plant)	Plant height (cm)	Plant quality
Coconut husk	81%	4.39	5.23	Strong, a little stunted
Peat moss	74%	4.84	5.79	Fat, strong
Coconut husk/Peat moss ratio 1:1	86%	4.51	5.50	Fat, strong
Coconut husk/Peat moss ratio 1:1 added with 15% perlite	**90%**	**5.21**	**5.64**	Fat, strong
CV%		*9.80*	*5.7*	
$LSD_{0,05}$		*0.89*	*0.6*	

When mixing coconut husk and peat moss with a 1:1 ratio in formula 3, the survival rate when crowned is increased to 86%. Based on the properties of these mediums, it is possible to use very good mediums to explain the results as follows: Because Peat moss has very good capacity to hold water, resulting in difficulty to control medium humidity when watering, so, when taking care of cultivating medium, it is easy to get too wet, making the roots not grow and killing the plant at the early stage. However, since this cultivating medium has good nutrition supplement, the surviving plants have nutrients to grow, so the plant height when using this medium is the best. Coconut husk makes it easier to control moisture, but the downside is no additional nutrition so seedling quality is affected. Mixing these two cultivating mediums in combination with the perlite can solve the problem of using each medium in combination with the perlite, help increase the airy, so the roots grow better, the seedlings grow faster. Thus, it can be concluded that coconut husk medium, which is a blend of coconut husk and peat moss at a ratio of 1:1, combined with the addition of 15% perlite, is the most suitable medium to crown DTLD gerbera Jamesonni bolus.

3.2.2. Effect of EC DT medium initially on seedlings of gerbera jamesonni bolus when taking to the nursery

In order to better see the effect of initial nutritional supplementation, as well as to provide the best initial nutrient intake to crown DTLD Gerbera Jamesonii bolus, this experiment was conducted. The amount of dissolved salts, including all mineral ions in the cultivating medium or ionic medium, when moistened with nutrient medium or nutrient solution is measured indirectly through the electrical conductivity (EC) of the cultivating medium. Therefore, this experiment is based on different EC values of the original cultivating medium for evaluation. The EC level will be adjusted by the addition of MS mineral solution when humidifying the medium to reach the EC required by the experiment. The experimental results are presented in Table 6.

Table 6. *Effect of initial EC sedum medium on seedlings of DTLD gerbera jamesonni bolus at nursery after 8 weeks of follow-up*

EC cultivating medium levels	Surviving ratio (%)	Actual number of leaves (leaves/plant)	Plant height (cm)	Plant quality
0.7	88%	5.19	5.82	Fat, strong plants
1.0	88%	5.57	6.07	Fat, strong plants
1.5	**92%**	**5.97**	**6.06**	**Fat, strong plant, thick, black green leaves**
2.0	75%	4.66	4.99	Fat, strong plants
2.5	63%	4.14	4.71	Fat, strong plants
CV %	-	2.20	2.90	
$LSD_{0,05}$	-	0.89	0.6	

Figure 6. *Effect of initial EC sedum medium on seedlings of DTLD gerbera jamesonni bolus at nursery after 8 weeks of follow-up*

According to the results from Table 6, the best initial EC for DTLD gerbera jamesonni seedlings out of the nursery is 1.5. At that time, the survival rate is 92% with the average number of leaves reaching 5.97 leaves/plant, the average height reaching 6.06 after 8 weeks in the nursery with the shape of the plant being fat, strong, thick and dark green. In formula 1, no nutrition is added with an initial EC of 0.7; formula 2 added with nutrients to EC to reach 1.0, the survival rate is still very high at 88% with the average number of leaves reaching 5.19 and 5.57 leaves/plant, the plant height reaching 5.82 and 6.07 cm; this can be understood because the initial nutrient content in 5 cultivating medium is not as low as that of these 2 formulas, so the quality of the plants is the same as that of formula 3. Formula 4 and the formula 5, with higher nutrient content, but due to initial stage when *In vitro* plant is still very weak, so with such high nutrient content, this will cause poisoning and plant death, so the survival rate in these two treatments decreased to only 75% and 63%, respectively. The surviving plants also developed worse due to initial nutritional shock, it took time to adapt, so the growth is slower, with the average number of leaves reaching only 4.66 and 4.14 leaves/plant and the height reaching only 4.99 and 4.71 cm.

4. Conclusion

The culture medium is MS+0.3 mg/IBA, supplemented with 20g/l sugar; using an airtight bag with an opening diameter of 25mm and using LEDs designed to have a red light to blue light ratio of 7:3 for lighting, is the best for the DTLD gerbera jamesonni bolus complete plant stage.

Cultivating medium mixing coconut husk and peat moss at a ratio of 1:1 and adding 15% perlite is the most suitable for crowning DTLD gerbera jamesonni bolus. The original EC medium 1.5 is the best for this DTLD gerbera jamesonni variety.

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